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Review Article

Characterization of the angiotensin-converting enzyme 2 (ACE2), the main receptor for the SARS-CoV-2 virus

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Abstract: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes Coronavirus Disease 2019 (COVID-19), one of the deadliest medical difficulties to affect people in more than a century. The virus has now spread to many countries worldwide, posing a big challenge to the health status of people in affected populations. Gaining more knowledge about the different aspects of this virus will lead us to better control and treatment methods. In this paper, we discuss the SARS-CoV-2 structure and the mechanism of this virus's entry into host cells through angiotensin-converting enzyme 2 (ACE2), the main receptor for the SARS-CoV-2 virus. The main connection between SARS-CoV-2 and ACE2 is Spike protein. Other topics are also included, like ACE2 structure, functions, and physiology. For instance, ACE2 is involved in the renin-angiotensin-aldosterone system, Angiotensin A/ACE2/Alamandine/MAS-Related GPCR D (MrgD) Axis, the Kinin-Kallikrein System. It also acts as Chaperone Protein for the Amino Acid Transporter, BOAT1, and has a connection with Apelin Peptides. Since ACE2 plays a primary role in COVID-19 pathogenesis, scientists have discovered some SARS-CoV-2 therapy methods based on ACE2 targeting. Tissue expression in different genders and ages, polymorphisms, and host epigenetics, the role of ACE2 in hypertension, and cytokine storm are explained separately.

Keywords: Angiotensin-converting enzyme 2, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19), pathophysiology, renin-angiotensin-aldosterone system (RAAS), treatment

Introduction

Coronaviruses are a wide range of viruses that can pass on a disease to a variety of animals while causing respiratory illnesses in humans with different levels of severity. Two highly pathogenic coronaviruses with a zoonotic origin, inclusive of SARS-CoV and MERS-CoV (Middle East respiratory syndrome coronavirus), separately appeared in humans and created lethal respiratory disease in 2002 and 2012, bringing coronaviruses to the center of attention of public health in the 21st century [1]. A new coronavirus was discovered in the respiratory epithelium of patients with unknown pneumonia in December 2019 [2, 3]. Researchers have recently come to the conclusion that SARS-CoV was seen weeks or even months before Wuhan in different areas, including Europe. However, for months before Wuhan, this new virus was dormant because it did not have the necessary conditions to become a pandemic [4]. This novel coronavirus infection in general mentioned as COVID-19, has been distributed quickly over the globe because of its high transmissibility [5, 6]. On March 11, 2020, the World Health Organization confirmed Covid-19 as a pandemic [7]. According to the PubMed database, more than 200,000 articles have been written on the issue of COVID-19 until now. Based on *WHO reports,* the overall number of confirmed cases and mortality till now-March 30, 2022-is about 480 M and 6.1 M, respectively. As the number of verified infections and deaths continues to rise daily, we must learn more about virus transmission mechanisms and epidemiology [8].

After an incubation period of about 5.2 days, COVID-19 infection symptoms arise. Fever, cough, and exhaustion are among the symptoms, while sputum production, headache, haemopty-

sis, diarrhea, dyspnea, and lymphopenia are also present [9-13]. Viral infections rely on the virus entering the cell and using the host cells' replication machinery to produce numerous viral copies, which are then excreted by the cell [14]. SARS-CoV consists of 4 structural proteins including nucleocapsid (N), membrane (M), envelope (E) and spike (S) proteins together with 16 nonstructural proteins and 5-8 accessory proteins. Attachment to the host cell membrane and fusion - carried out by glycoprotein S. The N protein is packaged into the viral genomic RNA within the virion, and the structural proteins S, E, and M are incorporated into the virion membrane. E and M proteins assist virus gathering and budding by interacting with other viral proteins [15, 16]. Cell entrance receptors are unquestionably important in determining virus tropism and altering the severity of infection [17]. Various host cell receptors are used by SARS-CoV proteins to enter host cells including Integrins, angiotensin-converting enzyme 2 (ACE2), sialic acid receptor, dipeptidyl peptidase 4 (DPP4), and glucose-regulated protein 78 (GRP78) [18]. The researchers also reported that SARS-CoV-2 could infect cells expressing ACE2, but it does not have an effect on ACE2 lacking or cells expressing other SARS-CoV-2 receptors, such as aminopeptidase N and dipeptidyl peptidase 4 (DPP4) [19]. Based on these results, the primary cell entrance receptor for SARS-CoV-2 has been recognized as angiotensin-converting enzyme 2 (ACE2) or angiotensin-converting enzyme homolog (ACEH) [20, 21]. The viral attachment action is continued by priming the spike protein S2 subunit by the host transmembrane serine protease 2 (TMPRSS2). This helps cell entrance and subsequent viral replication endocytosis with the assembly of virions. It was discovered for the first time in 2003 as the SARS-CoV receptor [22]. The structure, physiology, interactions with the COVID-19 virus, and treatments based on the metallocarboxyl peptidase angiotensin receptor ACE2 will be discussed in this paper. Understanding the involvement of ACE2 in various paths will be crucial in determining the effect of SARS-CoV-2/ACE2 binding on organismal physiology and in developing better therapeutics and diagnostic techniques.

Material and methods

We conducted a review of studies that have investigated the characteristics of ACE2 and its function in the pathogenesis of the SARS-CoV-2 virus. Databases such as PubMed, Google Scholar, Scopus, Ovid-Medline and Web of Science were searched up to June 2022. There were no restrictions on the course, language or type of paper. This was done through analysis of cases, clinical studies and original research. We used free text as well as Medical Subject Heading (MeSH) terms "angiotensin-converting enzyme 2 (ACE2)", "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)", "coronavirus disease (COVID-19)", "ACE2 structure", "ACE2 functions and physiology", "ACE2 expression", "host epigenetics and SARS-CoV-2 infection", "ACE2 targeting". Some studies based on inclusion and exclusion criteria were excluded from our review. All studies written in English and indexed in PubMed, Scopus, Web of Science (ISI), MEDLINE, and EMBASE journals were included in the study. Studies in non-English language and studies published before 2000 were excluded.

Epidemiological studies reporting ACE2 characteristics and its role in SARS-CoV-2 pathogenesis were included. Reviews, in vivo studies, letters to the editor, case series, case reports, and epidemiological studies without data on ACE2 characteristics and its role in SARS-CoV-2 virus pathogenesis were excluded.

SARS-CoV-2 structure

The classification of human coronaviruses is as follows: The kingdom Riboviria, order Nidovirales, family Coronaviridae, and subfamily Orthocoronavirinae [23]. Alphacoronavirus (α-CoV), Betacoronavirus (β-CoV), Gammacoronavirus (γ-CoV), and Deltacoronavirus (δ-CoV) are four members of the Coronaviridae family [24]. Alphacoronaviruses include human coronaviruses 229E (HCoV-229E) and human coronaviruses NL63 (HCoV-NL63), while Betacoronaviruses include, Coronavirus human OC43 (HCOV-OC43), human coronavirus HKU1 (HCoV-HKU1), Severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), Middle East respiratory syndrome coronavirus (MERS Coronaviruses, both Alpha and Beta, can communicate a disease to types of mammals, including humans) [25]. Coronaviruses are single-stranded, enveloped RNA viruses. Some coronaviruses of the genus betacoronavirus, such as SARS-CoV, MERS-CoV and SARS-CoV-2, have caused human infections in recent years.

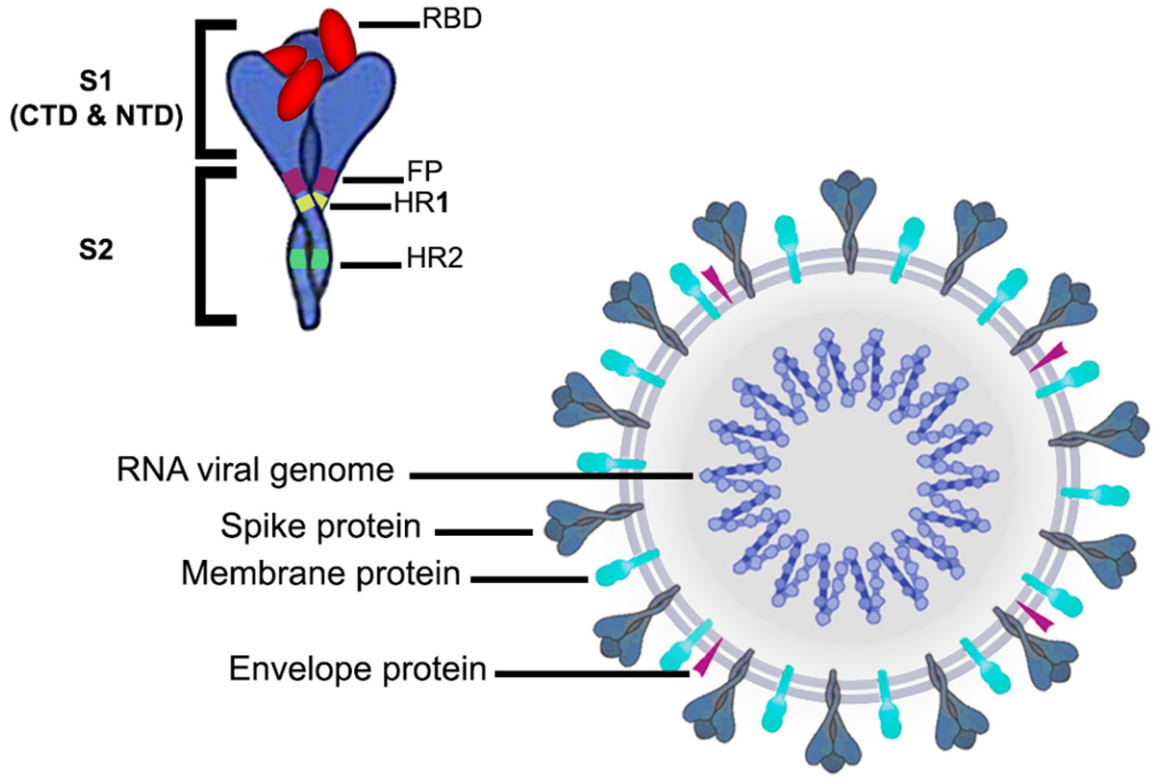

Figure 1. The structure of SARS-CoV-2 virus. Coronavirus contains a single-stranded RNA genome. There are different proteins on the surfuce of each particle including envelope protein, membrane protein, and spike protein. The spike protein is divided into two subunits: S1 and S2. S1: subunit S1, CTD: carboxy-terminal (C-terminal) domains, RBD: receptor-binding domain (RBD), HR: heptad repeat (HR), FP: fusion peptide (FP) domain.

SARS-CoV-2 contains a 30 kb single-stranded positive-sense RNA genome with 80% sequence identity to SARS-CoV. In its genomic RNA (gRNA), SARS-CoV-2 contains 14 open reading frames (ORFs). Two-thirds of the genome is covered by ORF1a and ORF1b, which overlap with a ribosomal frame shift and are translated into the polyproteins pp1a and pp1ab, respectively [26]. The translation product of ORF1ab is cleaved by proteases encoded by SARS-CoV into 16 non-structural proteins (nsps). These include key enzymes such as papain-like protease(s) (PLpro), chymotrypsin-like protease (3CLpro), RNA-dependent RNA polymerase (RdRp) and helicase (Hel) [27]. Coronaviruses have four structural proteins: the nucleocapsid protein (N) forms a helical capsid to accommodate the viral genome. A lipid envelope, consisting of S (spike), E (envelope) and M (membrane) proteins, surrounds the entire structure (**Figure 1**). The membrane and envelope proteins are required for virus assembly, while the S protein is essential for virus entry and recognition by the host cell [28, 29]. Almost all coronaviruses have two primary subunits, S1 and S2, and the spike protein has both N- and C-terminal domains [30].

SARS-CoV-2 entry

First, the receptor binding domain (RBD) region of the S protein binds specifically to the peptidase domain of ACE2 [28, 29, 31-33]. The coronavirus entry mechanism usually requires two S-protein cleavages. The first is near the S1-S2 boundary, while the second is near the S2' position in the S2 subunit, performed by furin and TMPRSS2, respectively (**Figure 1**) [34]. Although TMPRSS2 tends to activate SARS-CoV-2, cathepsins, particularly cathepsin L, can also cleave the S2' site [22]. If the target cells do not produce enough TMPRSS2 or if a virus-ACE2 complex does not contact TMPRSS2, the ACE2-bound virus is internalized into the late endolysosome via clathrin-mediated endocytosis, where cathepsins cleave the S2' site [35, 36].

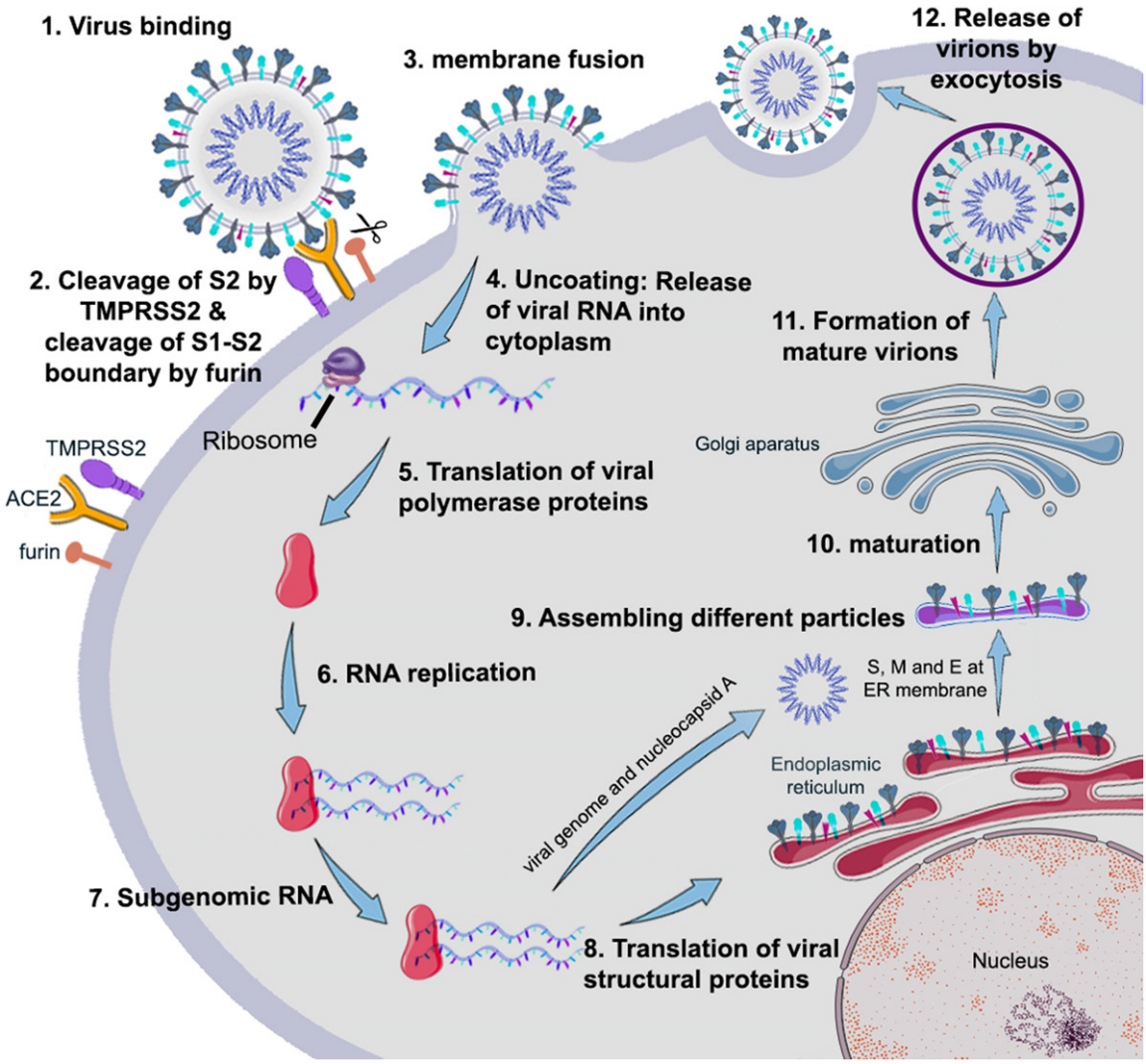

Figure 2. The replication cycle of SARS-CoV-2 virus. All stages of virus replication depend on its entry through ACE2 receptor. TMPRSS2: transmembrane serine protease 2, ACE2: angiotensin-converting enzyme 2.

The RBD alternates between a standing and lying state. This allows it to bind to receptors and evade the immune system [37, 38]. The FPPR (fusion-peptide proximal region), 630 loops and CTD2 (carboxy-terminal (C-terminal) domains) are essential elements of the S fusion machinery that, according to recent structural analyses, appear to control the fusogenic structural rearrangements of the S protein. The FPPR shift exposes the S2' site near the fusion peptide to proteolytic cleavage when ACE2 captures the RBD-up configuration, removing both the 630 loop and the FPPR from their positions in the closed S trimer structure. Due to the cleavage of the S1-S2 boundary of the SARS-CoV-2 S protein by furin, detachment of the 630 loop from the hydrophobic surface of CTD2 may destabilise this domain and release the N-terminal region of S2 from S1, probably releasing S1 altogether. Following S1 dissociation, a series of refolding events would occur in the metastable pre-fusion S2, allowing the fusogenic transition to a stable post-fusion structure [22]. In parallel with these transitions, the thrust of HR1 unfolding forces the fusion peptide into the target cell membrane [39, 40]. The fusion peptide and transmembrane regions of HR2 are folded back to the similar end of the molecule, causing the membranes with which they act together to bend towards each other, resulting in membrane fusion [22]. Several steps happen after virus entry into host cell (**Figure 2**).

ACE2

ACE2 structure

ACE2 is a type I integral membrane protein consisting of 805 amino acids and two functional domains. The first is a 17-amino-acid N-terminal signal peptide containing a peptidase domain (PD) (residues 19-615) with its HEXXH zinc-binding metalloprotease motif, and the second is a C-terminal collectrin-like domain (CLD) (residues 616-768) including a ferredoxin-like folded neck domain (615-726) terminating in a hydrophobic transmembrane hydrophobic helix region of 22 amino acid residues followed by an intracellular segment of 43 amino acid residues. Collectrin is a regulator of renal amino acid transport and insulin. The consensus zinc-binding motif sequence HEXXH is found in zinc metalloproteinases, where two histidine residues chelate a catalytic zinc ion. The HEXXH histidine motif, which is known to be a major component of a large number of zinc-dependent metalloproteases, consists of five residues, a histidine at the beginning followed by a conserved glutamic acid, the two variable amino acids and a histidine at the end [41-43].

The ACE2 protein has two domains in its extracellular part: the zinc metallopeptidase domain and the C-terminal domain. The metallopeptidase domain of ACE2 is divided into two subdomains (I and II) that form the two sides of a long and deep canyon. The bottom of the active site cleft, consisting of a prominent α-helix (helix 17, residues 511-531), is the only place where these two catalytic subdomains meet. The deeply recessed and protected proteolytic active site of ACE2 exists to prevent hydrolysis of properly folded and functional proteins. The zinc-binding site is approximately half the length of the large active site cleft (subdomain I side). In the native structure, His374, His378, Glu402 and a water molecule coordinate the zinc. These residues form the HEXXH + E motif conserved in the zinc metallopeptidase clan MA in the zinc-binding site of ACE2. Clan MA (glutenin family) and clan MB (several families of zinc metallopeptidases with the HEXXH + E and HEXXH + H zinc-binding motifs) are the two most prominent metallopeptidase clans. ACE2 binds to a chloride (Cl-) ion coordinated by Arg169, Trp477 and Lys481 in subdomain II [42, 44-47].

There is a single carboxypeptidase active site in ACE2 [42], omiting only one amino acid from the peptide C-terminus [48]. The catalytic site is located in a deep channel on the top of the molecule, which is an important feature of the ACE2 structure. Crystal structure analysis has suggested the presence of multiple hinge regions and N-glycosylations. Ridges consist of loops, helices, and a part of a β-sheet surrounding the channel. The RBD acts like a surface with a cavity(s) that connects a ridge(s) near the catalytic site in a deep channel [49].

There are essentially two types of ACE2. In full-length ACE2, a structural transmembrane domain links the extracellular domain to the plasma membrane. This version of ACE2 has a PD at the N-terminus and a CLD at the C-terminus, terminating in a single transmembrane helix and an intracellular fragment of about 40 residues. The extracellular domain been shown to be the receptor for the SARS-CoV S protein, and lately, for the SARS-CoV-2. The membrane anchor is absent in the soluble form of ACE2 (sACE2), which circulates in low concentrations in the bloodstream. It is thought that this soluble version may act as a competitive interceptor for SARS-CoV and other coronaviruses by blocking the binding of viral particles to full-length ACE2 on the cell surface [50, 51].

ACE2 functions and physiology

ACE2 and the renin-angiotensin-aldosterone-system (RAAS): Renin-angiotensin-aldosterone system is the most important mainstream in maintaining blood pressure homeostasis besides fluid and salt balance. The cardiovascular system, electrolyte and water balance are also regulated by this system. The primary enzymatic component of this system is ACE2. Ang 1-9 and Ang 1-7 are being created by this enzyme and through the process of cleaving a single residue from angiotensin I (Ang I) and angiotensin II (Ang II), respectively. Ang 1-7 antagonizes the effects of vasopressor ANG II By inactivating Ang II, ACE2 acts as a negative regulator of the renin-angiotensin system [14, 52, 53].

As recently explained by Zheng et al., ACE2 is not an aminopeptidase (48). It is classified as carboxypeptidase because it is responsible for catalysing the removal of the COOH-terminus phenylalanine residue from ANG II, resulting in the reduction of ANG II by this single catalytic

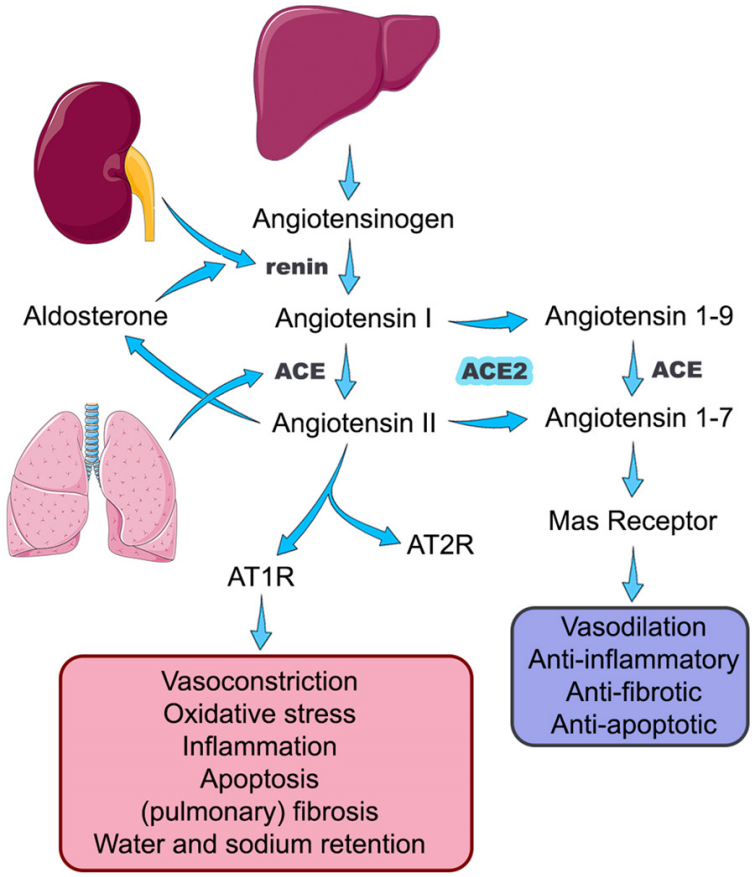

Figure 3. Renin-angiotensin aldosterone system. ACE2 plays a key role in RAAS system by converting angiotensin I and II to angiotensin 1-9 and 1-7 respectively. ATR1R: angiotensin II type I receptor.

event. Ang II is considered a key effector of the RAAS, contributing to hypertension (HTN) by reducing baroreceptor sensitivity (BRS) to control heart rate and promoting vasoconstriction, sodium retention, reactive oxygen species (ROS), inflammation and fibrotic scarring, as well as increasing the bioactive peptide Ang 1-7, which opposes the ANG II-ANG II type 1 (AT1) receptor axis through its anti-inflammatory and antifibrotic actions, as well as increasing BRS. As a result, the ACE2 peptidase pathway is a critical inflexion point in the RAAS processing pathway. An overall higher Ang II and lower Ang 1-7 tone may be the result of ACE2 reduction [14]. Moreover, in response to ANG II, the angiotensin receptor subtype AT1 induces apoptosis in the alveolar epithelium (**Figure 3**) [54].

Angiotensin A/ACE2/Alamandine/MAS-related GPCR D (MrgD) axis: It is classified as carboxypeptidase because it is responsible for catalysing the removal of the COOH-terminus phenylalanine residue from ANG II, resulting in the reduction of ANG II by this single catalytic event. Ang II is considered a key effector of the RAAS, contributing to hypertension (HTN) by reducing baroreceptor sensitivity (BRS) to control heart rate and promoting vasoconstriction, sodium retention, reactive oxygen species (ROS), inflammation and fibrotic scarring, as well as increasing the bioactive peptide Ang 1-7, which opposes the ANG II-ANG II type 1 (AT1) receptor axis through its anti-inflammatory and antifibrotic actions, as well as increasing BRS. The main difference between alamandine and Ang-(1-7) is an alanine residue at the amino terminus of Ang A instead of an aspartate residue. Alamandine has similar physiological effects to angiotensin-(1-7), such as vasodilation, antifibrosis, antihypertensive and critical outcomes. In addition to being a Mas agonist, Ang-(1-7) is a weak agonist of the Mas-related receptor, MrgD. As a result, it has been proposed that alamandine may be an endogenous ligand for MrgD. However, according to the findings of Vera et al., Mas does not appear to be a potential alamandine receptor. In conclusion, alamandine is not a Mas agonist, although its vascular properties are identical to those of Ang-(1-7) [53, 55].

ACE2 and the kinin-kallikrein system (KKS): The precursor kininogen, proteolytic kallikrein enzymes, and effector peptides bradykinin (BK-1-9 or BK) and its active metabolite [des-Arg9]-BK (BK-1-8 or DABK) make up the kinin-kallikrein system (KKS). These peptides bind to two G protein-coupled receptors: B1 receptor (BKB1R) which has DABK as its main agonist, while the B2 receptor (BKB2R) has BK as its ligand. BKB1R is a heptahelical protein that differs from BKB2R in that its expression is highly sensitive to inflammatory mediators including LPS and interleukins. LPS-induced hypotension

is attenuated in BKB1R-deficient mice, and the amount of polymorphonuclear leukocytes that concentrate in inflamed tissues is unusually low. Indeed, the BKB1R may be a beneficial pharmaceutical target for the treatment of pulmonary inflammatory diseases. In the human airway epithelia, DABK is a substrate of ACE2. Cleaving a single amino acid residue of the carboxyl terminus of the DABK, which is then inactivated, ACE2 blocks BKB1R/DABK activation. ACE2 activity is reduced in response to infectious or inflammatory stimuli like COVID-19 disease, making the DABK/BKB1R axis more active. This encourages airway epithelial cells to produce and release chemokines like C-X-C motif chemokine 5 (CXCL5), which bind to receptors on neutrophils such as the C-X-C motif chemokine receptor 2 (CXCR2). As a result, these chemokines attract neutrophils from the BM (bone marrow) or other peripheral reservoirs to the lung. The development of acute lung inflammation is aided by increased neutrophil infiltration of the lungs [56-63].

ACE2 and apelin peptides: Apelin is a peptide hormone that may be found in various tissues and fluids. It is an endogenous peptide that binds to the apelin receptor, originally identified as an orphan G-protein-coupled receptor (GPCR), APJ, or AR [64]. In the process of apelin production, a preproprotein of 77 residues is the first molecule, which is then truncated to proapelin of 55 residues and then to apelin-13 to -36, which act as the bioactive isoforms. Spontaneous cyclization of the N-terminal Gln leads to the pyroglutamate-modified form (Pyr-apelin-13) of apelin-13. Although all apelin isoforms bind to the apelin receptor and have identical physiological effects, their potency, efficacy and receptor recycling rates differ. In addition, research has shown that the production of apelin isoforms is tissue specific [65].

Apelin has Several functions, including being a strong cardiac inotrope, modulating blood pressure, having great therapeutic ability to treat obesity and cardiovascular diseases, hypothalamic regulation of water intake and the endocrine axis, regulating vascular homeostasis, angiogenesis and fluid balance, therefore performing an important function in vascular diseases [65-67].

Similarities in sequence and mRNA expression distribution were discovered between apelin and angiotensin II, indicating that they have comparable physiological functions [68]. ACE2 can also metabolize apelin-13, in addition to the molecules mentioned previously [69, 70]. According to Wang et al., ACE2 is a basic enzyme that controls the amplitude and duration of native apelin peptide activity in the cardiovascular system. Although the half-lives of the apelin peptides were prolonged in an ACE2-deficient condition, they were still degraded at a relatively high pace, indicating that additional proteases play a crucial role. In total, these findings suggest that ACE2 has a proclivity for cleaving the peptide amide bond, which is defined by proline-phenylalanine as the penultimate and C-terminal residues, respectively [67].

Apelin increased ACE2 promoter function in vitro and increased ACE2 expression in failing hearts in vivo via activation of its receptor, APJ. These findings demonstrate that ACE2 links the RAS to the apelin system, providing a theoretical basis for the apelin-ACE2-angiotensin 1-7 axis as a therapeutic target for cardiovascular disease [71].

ACE2 as a chaperone protein for the amino acid transporter, B0AT1 (SLC6A19): Hartnup amino acid transporter B0AT1 (SLC6A19) is the main luminal sodium-dependent neutral amino acid transporter of small intestine and kidney proximal tubule [72]. The ACE2-B0AT1 complex is formed as a dimer of heterodimers, with homodimerization mediated by the collectrin-like domain of ACE2 [51]. Independently of the RAS system, ACE2 regulates intestinal amino acid homeostasis, expression of antimicrobial peptides, and the ecology of the gut microbiome [73]. The association of B0AT1 with collectrin (Tmem27), a protein homologous to the membrane-anchoring domain of ACE2, has lately been demonstrated to regulate its expression in the kidney. Different analyses employing wild-type and ACE2-null mice revealed that ACE2 is required for B0AT1 expression and activity in the small intestine. The transport rate and cell surface expression of the transporter were boosted when the two accessory proteins, ACE2 and collectrin, were coexpressed. Camargo et al. thus show that ACE2 is required for the expression of the Hartnup transporter in the intestine [72]. Another coronavirus receptor, aminopeptidase N, has also

been shown to interact with B0AT1 (APN or CD13). Yan et al. thus show that B0AT1 may be involved in the regulation of some enteric infections of some coronaviruses [51].

ACE2 expression

Tissue distribution of ACE2

The tropism of virus contamination is specified by the tissue expression of the receptor, which has fundamental implications for the comprehension of its pathogenesis and the creation of therapeutic approaches [74]. While the central symptoms of COVID-19 disease are appeared in the respiratory tract [75, 76], ACE2 is expressed in a variety of human organs besides the lungs, suggesting that SARS-CoV-2 might infect other tissues as well [77]. Furthermore, some studies have shown that human transmission of SARS-CoV-2 can take place by routes outside of the respiratory tract [78].

The research compared the ACE2 expression rates across across 31 normal human tissues [77]. They found that ACE2 expression was highest in the small intestine, testes, kidneys, heart, thyroid and adipose tissue, and lowest in the blood, spleen, BM, brain, blood vessels and muscle. In the lung, colon, liver, bladder and adrenal gland, ACE2 expression was moderate [77, 79]. Two studies have shown that in the normal human lung, ACE2 is mainly expressed by alveolar type II (AT2) and alveolar type I (AT1) epithelial cells [80-82]. The cytoplasm of bronchial epithelial cells also showed weak positive ACE2 staining. Tissues of the upper respiratory tract, such as oral and nasal mucosa and nasopharynx, did not show ACE2 expression on the surface of epithelial cells, which may indicate that these tissues are not the primary site of entry for SARS-CoV. The prominent presence of ACE2 in the epithelia of the human lung and small intestine suggests that SARS-CoV may enter by these routes. The abundance of ACE2 expression on endothelial and smooth muscle cells in virtually all organs shows that once in circulation, SARS-CoV can easily move throughout the body. Another study suggests that although ACE2 is expressed in the lung, liver, stomach, ileum, kidney and colon, its expression rates are quite low, particularly in the lung [82].

Hamming et al. found that ACE2 was widely expressed in enterocytes from all areas of the small intestine, including the duodenum, jejunum and ileum, but not in enterocytes from the colon and stomach. In enterocytes, staining was restricted to the brush border [82]. In addition, Liu et al. discovered ACE2 expression in the pancreas of normal people. SARS-CoV-2 may bind to ACE2 in the pancreas and create pancreatic damage, as the pancreas had a little higher expression than the lungs [83].

Based on Immunohistochemistry techniques, ACE2 protein is found in Clara cells, type II cells, and endothelium and smooth muscle of small and medium vessels in the mouse lung [84]. Skin-derived epithelial cells have been found to express ACE2 [85]. ACE2 was present in the epidermal basal cell layer extending to the hair follicle basal cell layer. ACE2 was also present in the smooth muscle cells surrounding the sebaceous glands. Sebaceous gland cells showed weak cytoplasmic staining. In eccrine gland cells, there was a clear granular staining pattern for ACE2 [80].

Immune cells such as B and T lymphocytes and macrophages were consistently negative for ACE2 in the spleen, thymus, lymph nodes and BM. Because ACE2 is consistently absent from immune cells in all haematolymphoid organs, direct viral infection is unlikely to be the cause of these symptoms [80]. Nonetheless, SARS-CoV-2, like MERS-CoV and SARS-CoV, can infect immune cells [86].

Similar to observations in other organs, only endothelium and smooth muscle cells were stained in the brain [80]. A study used immunohistochemistry to identify ACE2 expression levels in 12 brain areas and found ACE2 in both endothelial and non-vascular cells [87]. The brain has been shown to have ACE2 receptors in glial cells and neurons, making it a potential target for COVID-19. Diffusion of COVID-19 into the systemic circulation or across the cribriform plate of the ethmoid bone during early or late stages of infection may lead to brain involvement, as previously documented in SARS-CoV patients [88].

ACE2 expression in different genders

Comparing the ACE2 expression levels in males and females has led to different and sometimes contradictory results. Although ACE2 is positioned on the X chromosome and has been shown to evade X inactivation, it is believed to

have a sex- and tissue-specific bias, with reduced expression in female lungs versus male lungs [89, 90]. In 99 cases of SARS-CoV-2 infection, researchers discovered that there were more men than women [76], Like MERS-CoV and SARS-CoV, in which males were infected at a higher rate than females [91, 92]. According to a recent study, Asian males had more ACE2-expressing lung cells [74], which explains the more vulnerability of males to SARS-CoV-2 infection comparing to females.

Contrary to the above findings, another study examined ACE2 expression in 31 normal human tissues and found that men and women had similar levels of ACE2 [77]. This conclusion is confirmed by Li et al. as well [93]. In both males and females, ACE2 expression levels were favorably associated with immune signatures in the skin, digestive system, brain and blood vessels. In the lungs, the relationships between ACE2 expression and immune signatures varied between sexes and between ages, demonstrating that different host immune responses to COVID-19 disease may explain why males and females, and young and old people infected with this virus, have significantly different levels of disease severity [77].

Chen et al. discovered that ACE2 expression is high in Asian females and young individuals, who are considered to be less prone to severe or fatal consequences. Meanwhile, it is low in males and reduces even more with age and type II diabetes (T2D), people who are most vulnerable to negative consequences, demonstrating an inverse relationship between ACE2 expression and COVID-19 severity. These results show that estrogen may play a role in Asian females having higher ACE2 expression than Asian males. Applying estrogen/androgen therapy for transgender males for one year had caused a considerable increase in ACE2 expression levels and ACE2 expressing cells in their testis Sertoli cells [94].

ACE2 expression during aging

One study found that ACE2 expression decreased significantly or slightly with age in all ethnic groups and in both sexes. The statistics showed a substantial association between ages, sex, ethnic group, body mass index (BMI) and ACE2 expression in different tissues when the other four variables were controlled, with the strongest association being with age [94]. Consistent with this, ACE2 levels were found to be significantly reduced in old rats of both sexes. Although rats of all sexes and ages had similar levels of ACE2, male rats appeared to have a greater age-related decline in lung ACE2 expression than female rats [95]. It has also been stated that in humans, ACE2 activity does not change with age in men, but significant changes occur in women with age [96, 97].

In contrast to the above findings, one study showed that ACE2 expression rates did not change significantly between younger and older individuals. This indicates that the susceptibility to SARS-CoV-2 and SARS-CoV may not be significantly associated with sex, age or race. In fact, like SARS-CoV, SARS-CoV-2 can affect both sexes and infect different age groups equally. However, the risk of death from SARS-CoV-2 and SARS-CoV infection appears to be remarkably associated with sex and age, with older people more likely to be infected [77].

ACE2 and SARS-CoV-2

After SARS-CoV-2 enters the lungs and airways via respiratory droplets, the viral life cycle begins. RBD domain of SARS-CoV-2 has a strong tendency to bind to the ACE2 receptor [9]. This high binding affinity of the SARS-CoV-2 RBD domain to bind to ACE2 receptors may be responsible for the virus transmission between different species [98]. In the process of membrane fusion, some transmembrane enzymes such as disintegrin metallopeptidase domain 17 [ADAM17], TMPRSS2, and TNF-α converting enzyme (TACE) and some effective proteins such as vimentin and clathrin are needed [99]. After binding to a specific region within the SARS-CoV spike protein to ACE2 receptors in host cells, membrane fusion is activated and this leads to the release of viral RNA in the cytoplasm and causes infection [100]. The interaction of ACE2 with SARS-CoV-1 and with SARS-CoV-2 and subsequent downstream effects are very similar to each other [31]. Downregulation of ACE2 by SARS-CoV-2 can impair Ang II clearance and lead to exacerbated tissue damage. Moreover, we can consider that the downregulation of ACE2 by SARS-CoV-2 reduces the chance for further invasion of the virus, which leads to slow down the spread of the virus [101]. In their study on Ace2-knockout mice,

Kuba and his colleagues showed the important role of this receptor in the pathogenesis of COVID-19 [102]. Therefore, it is very important to understand the exact way of communication between ACE2 receptors and SARS-CoV in order to control the spread of the disease and find possible treatment methods. Also, more studies are needed to understand more precisely the downstream pathways of communication between these receptors and the virus.

COVID-19 therapy methods based on ACE2 targeting

Currently, there are no specific antiviral drugs for the treatment of COVID-19 disease, making it difficult to control and contain the virus. There are two different therapeutic approaches to COVID-19 treatment, clinical and pharmacological strategies. Symptomatic management and oxygen therapy are the mainstay of clinical treatment, including mechanical ventilation for patients with respiratory failure. Remdesivir is one of several antiviral drugs under investigation, although none has been specifically approved for COVID-19. In addition, vaccine development and approaches that directly target the virus or block viral entry, as well as treatments that address the immunopathology of infection, have become a major focus [103]. Vaccines, monoclonal antibodies, oligonucleotide-based therapies, peptides, interferon therapies, small-molecule pharmaceuticals, or natural remedies (e.g. traditional Chinese medicine [TCM]) are all possible pharmacological COVID-19 treatments [104].

ACE2 receptors of alveolar type 2 (AT2) cells in the lung are one of the important targets for SARS-CoV-2. Because the viral entrance is controlled by receptor-mediated endocytosis, AP2 (activator protein 2)-associated protein kinase 1 (AAK1), a recognized regulator of endocytosis, could be a viable target to stop the virus from entering the cell. The Janus kinase inhibitor baricitinib, which binds to the cyclin G-associated kinase (endocytosis regulator), is sufficient to block AAK1. Two cancer drugs Sunitinib and Erlotinib, have been demonstrated to decrease viral infection of cells by inhibiting AAK1. These chemicals, on the other hand, have substantial adverse effects and cannot be called a safe therapeutic drug [30].

By reducing the expression of TMPRSS2, SARS-CoV enters cells via an endosomal pathway. Cathepsin L, which activates the fusogenicity of the spike proteins, may play a key role in this pathway. In HeLa cells, an immortal cell line used in scientific research, a commercial serine protease inhibitor (camostat) has been shown to moderately limit infection by SARS-CoV and human coronavirus NL63 (HCoV-NL63). It expresses the ACE2 and TMPRSS2 receptors. In human Calu-3 airway epithelial cells, co-treatment with Camostat and EST [trans-epoxysuccinyl-L-leucylamindo-3-methylbutane ethyl ester], a cathepsin inhibitor, effectively blocked both cell entry and multi-step development of SARS-CoV. The dual blocking of entry from the cell surface and through the endosomal pathway may be responsible for this effective suppression. These findings point to camostat as a potential antiviral medication for preventing or suppressing SARS-CoV infection caused by TMPRSS2 [105]. In cell lines, SARS-CoV can use the endosomal cysteine proteases cathepsin B and L (CatB/L) and the serine protease TMPRSS2 for S protein priming, and both proteases must be inhibited for strong viral entry blocking. In the infected host, though, only TMPRSS2 action is required for viral transmission and pathogenicity, whereas CatB/L activity is not required [31].

ACE2 polymorphism and susceptibility to COVID-19

The ACE2 gene was detected on chromosome Xp22 and spans 39.98 kbp of genomic DNA, with 20 introns and 18 exons. Genetic polymorphism is frequent in the ACE2 gene [106]. There is growing evidence that the ACE2 gene polymorphism can affect the interaction between ACE2 and the S protein of SARS-CoV-2, impacting viral entrance into the host cell thus inhibiting COVID-19 lung and systemic damage [107].

A study evaluated over 290,000 samples from public genetic datasets covering over 400 population groups and discovered numerous ACE2 protein-altering variations. They detected natural ACE2 variations that may change virus-host interaction and therefore host vulnerability. S19P, I21V, E23K, K26R, T27A, N64K, T92I, Q102P, and H378R are among the variations anticipated to increase vulnerability. K31R, N33I, H34R, E35K, E37K, D38V, Y50F, N51S, M62V, K68E, F72V, Y83H, G326E, G352V, D355N, Q388L, and D509Y were expected to be defensive variants with reduced S-protein binding. When compared to wildtype ACE2, bio-

chemical studies revealed that K31R and E37K variants had decreased affinity for S-protein, while K26R and T92I variants had enhanced affinity. Soluble ACE2 K26R and T92I were more efficient in inhibiting the entry of S-protein pseudotyped virus, implying that ACE2 variations can affect SARS-CoV-2 susceptibility [108].

Another study designed in silico molecular docking to identify the impact of ACE2 missense mutations on SARS-CoV-2 spike protein interaction. Six ACE2 missense variants (I21T, A25T, K26R, E37K, T55A, E75G) were discovered in HDOCK and FireDock simulations as having a greater affinity for RBD than wild type ACE2 plus 11 variations with decreased affinity (I21V, E23K, K26E, T27A, E35K, S43R, Y50F, N51D, N58H, K68E, M82I). This finding supports the theory that the ACE2 genetic background is the initial "genetic gateway" throughout disease development [107].

In a separate paper, they planned to perform an in silico study of the widely studied ACE2 gene variations and identify the effects of the variants on mRNA secondary structure and cellular factor binding affinity. A total of 14 ACE2 SNPs (single nucleotide polymorphisms) were selected and studied. The RNAsnp database was used to test all the variations and the results showed that three of them, rs233574, rs2074192 and rs4646188, had a significant effect. According to their assessments, these three SNPs can cause significant changes in the secondary structure of RNA. Based on the spliceAid2 database, only the wild-type variant of the ACE2 gene can bind to proteins. The database (spliceAid2) predicted that 5 out of 14 SNPs cause a change in the ACE2 gene so that only the wild-type form can bind to proteins. In two of the fourteen, only the mutant form can bind to proteins. The remaining two SNPs result in a dual form in which certain proteins bind to either the wild type or the mutated sequence. In its wild-type form, rs233574 showed splicing sequence formation and secondary RNA modification upon nucleotide change (**Table 1**) [109].

ACE2 and cytokine storm

The production of vast amounts of pro-inflammatory cytokines and chemokines by immune effector cells leads to a lethal, uncontrolled systemic inflammatory response [110]. In people infected with COVID-19, cytokine storm syndrome (CSS) is reported at a shockingly high frequency (10-20%) and can lead to significant morbidity, including multi-organ failure and mortality [111].

When the cytolytic activity of lymphocytes is impaired, whether due to genetic problems or acquired conditions, NK (natural killer) and cytolytic CD8 T cells may be unable to function. As a result, these cells are unable to lyse infected and activated antigen-presenting cells (APCs), leading to prolonged and amplified interactions between innate and adaptive immune cells. In this situation, many pro-inflammatory cytokines such as TNF, interferon, IL-1, IL-6, IL-18 and IL-33 are produced in an uncontrolled manner, resulting in a cytokine storm [112].

Coronavirus infection decreases ACE2 expression by activating enzymes that degrade ACE2 [102], which includes a disintegrase and metalloprotease 17 (ADAM17) [113, 114]. The extracellular domain of ACE2 is cleaved from the surface of lung epithelial cells by ADAM17, reducing the protective ACE2-dependent signaling, resulting in a negative feedback loop of increased lung inflammation. In addition, ADAM17 is required for the release of pro-inflammatory cytokines and generates the active form of TNF-α. As a result, the cytokine storm, another symptom of severe COVID-19 that causes excessive neutrophil recruitment, is exacerbated [115-117]. The contribution of ADAM17 in the progression of severe COVID-19 has not been investigated experimentally [118].

The important point is, ACE2 is endocytosed with SARS-CoV, causing a reduction in ACE2 on cells and a rise of serum Ang II [119].

The transcription of inflammatory genes is regulated by NF-kappa B (NF-κB). This transcription factor plays an important role in lymphocyte responses to antigens and in cytokine-induced gene expression. I kappa B, the inhibitor of NF-kappa B, keeps NF-kappa B inactive in resting cells. Phosphorylation of I kappa B causes it to be degraded by proteases, freeing NF-kappa B for nuclear translocation [120, 121]. Ang II is a vasoconstrictor that also works as a pro-inflammatory cytokine through the AT1R receptor. NF-κB and ADAM17 are also

Table 1. ACE2 variants and their effect on ACE2-S protein binding affinity

Variants in ACE2 protein	Position	Effect on affinity	Ref.
rs775181355	chrX: 15573391 (GRCh38.p13)	decrease	Benetti et al., 2020a
rs41303171	chrX: 15564175 (GRCh38.p13)	decrease	Gibson et al., 2020
rs4646116	chrX: 15600835 (GRCh38.p13)	decrease	Gibson et al., 2020
		increase	Procko, 2020; Stawiski et al., 2020b
rs146676783	chrX: 15600803 (GRCh38.p13)	decrease	MacGowan and Barton, 2020 Procko, 2020; Stawiski et al., 2020b
		increase	Gibson et al., 2020
rs781255386	chrX: 15600833 (GRCh38.p13)	increase	Gibson et al., 2020 Procko, 2020; Stawiski et al., 2020b
rs143936283	chrX: 15581305 (GRCh38.p13)	increase	Gibson et al., 2020
rs1299103394	chrX: 15600836 (GRCh38.p13)	increase	
rs1447927937	chrX: 15600783 (GRCh38.p13)	decrease	
rs759579097	chrX: 15581314 (GRCh38.p13)	decrease	Gibson et al., 2020 Procko, 2020; Stawiski et al., 2020b
		increase	MacGowan and Barton, 2020
rs766996587	chrX: 15594944 (GRCh38.p13)	decrease	Gibson et al., 2020
rs370610075	chrX: 15581236 (GRCh38.p13)	decrease	MacGowan and Barton, 2020 Procko, 2020; Stawiski et al., 2020b
rs961360700	chrX: 15581228 (GRCh38.p13)	decrease	MacGowan and Barton, 2020 Procko, 2020; Stawiski et al., 2020b
rs73635825	chrX: 15600857 (GRCh38.p13)	increase	Procko, 2020; Stawiski et al., 2020b
rs778030746	chrX: 15600851 (GRCh38.p13)	increase	
rs756231991	chrX: 15600845 (GRCh38.p13)	increase	
rs781255386	chrX: 15600833 (GRCh38.p13)	increase	Gibson et al., 2020 Procko, 2020; Stawiski et al., 2020b
rs1199100713	chrX: 15594998 (GRCh38.p13)	increase	Procko, 2020; Stawiski et al., 2020b
rs763395248	chrX: 15594915 (GRCh38.p13)	increase	Procko, 2020; Stawiski et al., 2020b
rs1395878099	chrX: 15594885 (GRCh38.p13)	increase	
rs142984500	chrX: 15578253 (GRCh38.p13)	increase	
rs758278442	chrX: 15600819 (GRCh38.p13)	decrease	
rs1348114695	chrX: 15600809 (GRCh38.p13)	decrease	
rs1192192618	chrX: 15600763 (GRCh38.p13)	decrease	
rs1569243690	chrX: 15600760 (GRCh38.p13)	decrease	
rs1325542104	chrX: 15600728 (GRCh38.p13)	decrease	
rs755691167	chrX: 15594988 (GRCh38.p13)	decrease	
rs1256007252	chrX: 15594976 (GRCh38.p13)	decrease	
rs759134032	chrX: 15594940 (GRCh38.p13)	decrease	
rs751572714	chrX: 15578223 (GRCh38.p13)	decrease	
rs142443432	chrX: 15589423 (GRCh38.p13)	increase	Chen et al., 2021
rs372272603	chrX: 15589385 (GRCh38.p13)	increase	
rs762890235	chrX: 15578220 (GRCh38.p13)	decrease	
rs776328956	chrX: 15575706 (GRCh38.p13)	increase	
rs191860450	chrX: 15575706 (GRCh38.p13)	increase	
rs140473595	chrX: 15573407 (GRCh38.p13)	increase	

activated by the Ang II-AT1R axis, resulting in the maturation of epidermal growth factor receptor (EGFR) ligands and TNFα, which are two NF-κB stimulators [119].

In several IL-6R-negative non-immune cells, including fibroblasts, endothelial cells and epithelial cells, ADAM17 activation also converts the membrane version of IL-6R to the soluble version (sIL-6R), followed by activation of STAT3 by the sIL-6R-IL-6 complex [119]. Many cytokines, including IL-6, initiate intracellular signaling via members of the signal transducers and activators of the transcription (STAT) family of proteins [122]. The major activator of STAT3 in vivo, particularly during inflammation, is IL-6, while there are nine other components of the IL-6 family of cytokines that can activate STAT3, at least in vitro. As a result, SARS-CoV-2 contamination of the respiratory tract can stimulate both NF-κB and STAT3, which can then activate the IL-6 amplifier (IL-6 Amp), a mechanism for hyperactivation of NF-κB by STAT3, leading to a variety of inflammatory and autoimmune diseases [123]. In a positive feedback loop, the IL-6 Amp generates different pro-inflammatory cytokines and chemokines, which include IL-6, and recruits lymphoid and myeloid cells in the lesion, including activated T cells and macrophages, to enhance the IL-6 Amp. Because IL-6 is a prominent practical indicator of cellular senescence, the age-dependent augmentation of the IL-6 Amp could be linked to a rise in COVID-19 mortality [119].

ACE2 and hypertension

The RAS has been implicated in the modulation of hypertension and severe lung damage induced by viruses such as SARS [102, 124]. RAS inhibition is an effective antihypertensive treatment approach [125].

There are two axes in the RAS. These include the ACE/Ang II/AT1R and ACE2/Ang (1-7)/Mas receptor pathways [126]. ACE2 expression is decreased by SARS-CoV infections, leading to an imbalance between the two pathways [102]. A new treatment method for hypertension is to target the ACE/Ang II/AT1R axis [125]. The ACE/Ang II/AT1R system is inhibited by angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II type 1 receptor blockers (ARBs), which are commonly used in patients with high blood pressure [125]. New research reveals that COVID-19 individuals with hypertension are more likely to grow acute cases [127]. As a result, it is important to discover how RAS inhibitors affect COVID-19 patients suffering hypertension [125].

Host epigenetics and SARS-CoV-2 infection

Post-translational chemical modifications in chromatin, RNA and DNA, such as primary methylation, acetylation, phosphorylation, ubiquitination and sumoylation, are all involved in the epigenetic regulation of gene expression. By altering the function of the gene locus without altering the underlying DNA sequence, this type of regulation links genotype and phenotype [128].

ACE2 transcript levels are regulated at the post-transcriptional level by microRNAs (hsa-miR-125a-5p, miR-200 family) that target the 3' untranslated region of the RNA [129]. H5N1 and H7N9 viruses cause downregulation of ACE2 protein by increasing the miR-200c-3p expression [130]. The expression of microRNAs is controlled by epigenetic mechanisms. Lysine demethylase 5B (KDMfB) demethylates H3K4-me3 and inhibits the expression of miR-125a and miR-200 family members [129]. As a result, KDM5B regulates ACE2 transcript levels indirectly [131].

The ACE2 gene has two upstream regulatory areas, proximal and distal [132]. The proportional usage of the two promoters differs within tissues [132]. In pancreatic -cells and embryonic kidney cells, HNF1A controls the production of ACE2 [132, 133]. One study found that the three putative HNF1 binding regions in the proximal promoter region are responsible for the responsiveness of ACE2 expression to HNF1B [132]. However, chromatin immunoprecipitation sequencing (ChIP-Seq) experiments in the liver cancer cell line HepG2 demonstrate that HNF1A binds to the ACE2 proximal and distal upstream promoter regions [134]. MYBL2, USF1, TAED4, EP300, SP1, HNF4A, CEBP, MAFF and GATA3 were also found to bind to the ACE2 distal upstream promoter region by ChIP-Seq analysis [131].

The promoters of transcribed genes are areas free of nucleosomes, which can be detected using a number of different approaches [135, 136]. According to single-cell ATAC-Seq results, type 2 pneumocytes possessed functional promoters. The nucleosome-free regions at the ACE2 locus in alveolar type 1 and 2, secretory, multi-ciliated, ionocytes, and neuroendocrine cells had transcription factor motifs for IRF1, STAT1/2, FOXA1, and FOXD2 [131].

ACE2 expression can be induced by a variety of factors. In human cardiac fibroblasts, angiotensin II stimulates the production of ACE2 [137]. In various human airway tissues, including tracheal cells, bronchial epithelial cells, small airway cells, large airway epithelial cells and primary human nasal epithelial goblet cells, ACE2 expression was induced by IFN-α/β [90, 138-140]. ChIP-Seq data demonstrate that STAT1, STAT3, IRF8, and IRF1 bind to a region -1500 to 500 bp from the ACE2 transcription start site, indicating that ACE2 is an interferon-induced gene [138]. As the SARS-CoV-2 virus load rises, the expression of interferon-responsive genes, including ACE2, increases [141].

A typical hallmark of malignancy is hypoxia [142]. Many epigenetic modifying enzymes, namely the ten-eleven translocation enzyme involved in DNA demethylation and the Jumonji C domain-containing histone demethylases, require oxygen as a substrate cofactor. Epigenetic dysfunction is the result of reduced function of these enzymes. In human hepatocellular carcinoma Huh7 cells, ACE2 expression is increased by hypoxia [143].

Conclusion

ACE2 plays a significant role in numerous physiological and pathophysiological fields, including Renin-Angiotensin-Aldosterone-System, MAS-Related GPCR D (MrgD) Axis, Kinin-Kallikrein System, and Covid 19 pathogenesis. Coronavirus uses the ACE2 receptor to enter host cells. Therefore, accurate identification of the structure, mechanism, and function of these receptors can play an important role in Covid management. For example, AP2 (activator protein 2)-associated protein kinase 1 (AAK1) can prevent the virus from entering the cell. Various studies have also shown that polymorphisms in the ACE2 gene interfere with the pathogenesis of SARS-CoV-2 and result in multiple systemic damages. In this study, we reviewed the structural and functional characteristics of ACE2. Further studies should be performed to more accurately identify the mechanism by which the virus enters the cell, to target these receptors to prevent the virus from entering, to examine the genetic variation of ACE2, and its physiological effects. Understanding the exact mechanism of the mutual effects of ACE2 and SARS-CoV may lead to finding new possible treatment methods based on preventing the virus from entering the host cell and thus preventing its spread and controlling the infection. Also, more studies should be done in relation to the new strains of this virus and how it interacts with cell receptors such as ACE2.

Disclosure of conflict of interest

None.

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