5 Relationship Between COVID-19 and Angiotensin-Converting Enzyme 2: A Scoping Review

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Abstract

Following the outbreaks of SARS-CoV in 2002 and MERS-CoV in 2012, the COVID-19 pandemic caused by the SARS-CoV-2 virus has become an increasing threat to human health around the world. Numerous studies have shown that SARS-CoV-2 appears similar to the SARS-CoV as it uses angiotensin converting enzyme 2 (ACE2) as a receptor to gain entry into cells. The main aims of this scoping review were to identify the primary hosts of coronaviruses, the relationship between the receptor binding domain of coronaviruses and ACE2, the organ specificity of ACE2 expression compared with clinical manifestations of

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P. C. Guest (ed.), *Clinical, Biological and Molecular Aspects of COVID-19*, Advances in Experimental Medicine and Biology 1321, https://doi.org/10.1007/978-3-030-59261-5_5

the disease, and to determine if this information can be used in the development of novel treatment approaches for the COVID-19 pandemic.

Keywords

SARS-CoV · SARS-CoV-like · SARS-CoV-2 · COVID-19 · Angiotensin converting enzyme 2 · ACE2 · Spike protein · Receiver connection range · Bat-SARS-CoV

5.1 Introduction

Nidovirales encompasses three viral families known as Coronaviridae, Arteriviridae, and Roniviridae. Although these have common genomic characteristics and use the same strategy for replication inside hosts, they differ in morphology. The main pathogenic forms to humans involve two genera known as coronavirus and torovirus. Coronaviruses are spherical enveloped viruses with a diameter of 100–120 nm and contain a single-core RNA genome with positive polarity. They gained the "Corona" nomenclature due to their spike proteins having a similar appearance to a crown in electron micrographs (Fig. 5.1). These viruses also contain significantly more RNA than most other viruses at 27–32 kilobytes in length. The fast multiplicity of coronaviruses confers their high recombination capacity [1]. This makes them highly infectious in avian and mammalian species.

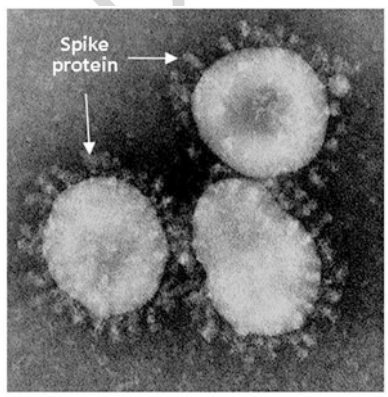

Fig. 5.1 Electron micrograph of SARS-CoV

Based on genomic sequences, coronaviruses can be divided into four groups known as, alpha (HCoV-229E, HCoV-NL63), beta (HCoV-OC43, HCoV-HKU1, MHV, SARS-COV, MERS-COV), gamma (IBV), and delta (pdCoV) [2–4]. The alpha and beta forms are infective in mammals, the gamma forms appear specific for birds, and the delta form is less defined. The Severe Acute Respiratory Syndrome epidemic of November 2002 to July 2003 was caused by a beta-coronavirus (SARS-CoV). This first erupted in the Guangdong province of China in November 2002 and spread to approximately 30 countries or territories, such as Hong Kong, Taiwan, Canada, Singapore, Vietnam, USA, and the Philippines [5]. Within 9 months, no new cases were reported but a total of 8098 people had been infected and, of these, 774 had died. Thus, the death rate of SARS-CoV was almost 10% of the infected population. An eruption of another beta-coronavirus known as Middle East Respiratory Syndrome (MERS-Cov) began in September 2012 with the majority of cases occurring in Saudi Arabia and some spreading to other countries, such as United Arab Emirates, Jordan, Qatar, and South Korea [6].

Late in 2019, a novel coronavirus erupted in the city of Wuhan of the Hui province of China. This virus was named SARS-CoV-2 and the World Health Organization (WHO) named the disease COVID-19 (for coronavirus disease 2019) [7, 8]. The recurrence and fulminant spreading of SARS-CoV-2 indicated that it was a potential threat to health around the world. The genome of SARS-CoV-2 is more similar to other beta-coronaviruses such as those from bats, as well as SARS-CoV and MERS-CoV. Early manifestations of the disease are fever, fatigue, dry coughing, myalgia, and dyspnea. Some patients may report headache, vertigo, stomach ache, diarrhea, nausea, and vomiting. In addition, some cases may progressively develop respiratory distress leading to alveolar injury and death [9].

The first step that occurs during a viral infection relies on the ability of the virus to enter the cells of the host via recognition and attachment to

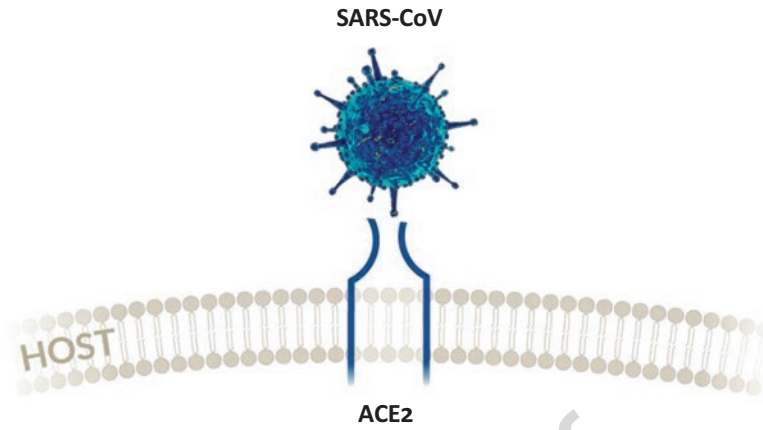

Fig. 5.2 Scheme showing the interaction between SARS-CoV and ACE2 to gain entry into host cells

a specific receptor [10]. Many studies have reported that the SARS-CoV receptor is angiotensin converting enzyme 2 *(*ACE2) (Fig. 5.2) [11, 12]. As the pandemic progressed, more studies were carried out on this topic and these confirmed that the novel coronavirus also uses ACE2 to gain entry into host cells [13–16]. ACE2 is homologous to ACE that regulates blood pressure, fluid and electrolyte balance, and systemic vascular resistance [17]. In this pathway, renin converts angiotensinogen to angiotensin 1 (AGT-I) and ACE converts AGT-I to AGT-II. In turn, AGT-II acts on the adrenal gland, causing it to release aldosterone. ACE2 converts AGT-I to AGT (1–9) and AGT-II to AGT (1–7) which bind to the mitochondrial assembly receptor (MAS), leading to antagonism of a wide variety of the effects of AGT-II. In general, ACE2 acts as a counter- regulatory enzyme that decreases the local concentration of AGT-II [18].

There are also two types of ACE2 with respect to functional characteristics. ACE2 contains a trans-membrane domain that connects its extracellular domain, which can act as a receptor for coronavirus spike proteins [11–16]. ACE2 is expressed in many cell types, especially pulmonary pneumocytes, myocardium cells, cholangiocytes, proximal tubules of the kidney, surface enterocytes of the intestines, cholecyst cells, lymphatic endothelial cells, epithelial cells of the bladder, corporeal cytotrophoblasts, and syncytiotrophoblasts, and it is also found in the eyes, epithelial cells of the mouth cavity, monocytes and macrophages, parietal cells of the stomach, the external layer of the adrenal glands, pancreatic islet cells, acidophilic cells of parathyroid glands, epithelial cells of sweat glands, and acidophilic cells of the pituitary [17, 18].

The spike proteins of SARS-CoV-2 provide the mechanism that allows it to enter cells in a manner similar to that used by the SARS coronavirus [13–16]. The spike protein contains two domains known as S1 and S2, and the receptor binding domain (RBD) is the main functional determinant within the S1 region that plays a crucial role in binding to ACE2 [19]. Species like civets, horseshoe bats, ferrets, golden Syrian hamsters, rabbits, turtles, monkeys, cows, sheep, pigs, weasels, and raccoon dogs are potential hosts for SARS-CoV-2 due to their inherent ACE2 receptors [20]. Studies of the RBD amino acid sequences of coronaviruses and the ACE2 attachment site have led to some information on severity of infections as well as the identity of potential intermediate hosts [11–16, 19, 20]. In general, a more comprehensive understanding of ACE2 expression regarding cells, tissues, organs and host species, as well as on the evolution and adaptability of the coronavirus spike proteins, may aid our development of effective treatments.

With this in mind, the aims of this review were to: 1) identify the primary reservoirs and intermediate hosts of coronaviruses; 2) explore the interaction between the coronavirus spike proteins and ACE2; 3) determine if any relationship exists

between ACE2 tissue expression and the clinical manifestations of coronavirus infection; and 4) use this information to provide potential insights into novel treatment strategies against COVID-19.

5.2 Methods

This scoping review focused on the probable relationship between the novel COVID-19 coronavirus, SARS-CoV-2, and the ACE2 receptor. The selection process followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria (Fig. 5.3).

5.2.1 Data Sources and Research Strategies

All published and unpublished (gray literature) works up to the 21st of March 2020 were investigated. At first, suitable and related keywords were defined by the research team then the fields of title, abstract, keywords, topic, title/abstract were examined using the English language databases of Scopus, Web of science, ProQuest, Embase, and PubMed. Medical Subject Headings (MeSH) databases were also assessed and related synonyms were applied to increase the comprehensiveness of the study and minimize attrition. In addition unique Boolean syntax and operators related to each database were applied to extend the scope of the search (Table 5.1).

5.2.2 Study Selection

In the first stage of the search, all English-language studies were tracked considering title and abstracts, and papers addressing the key points were included. This included studies reporting on angiotensin converting enzyme 2 (ACE2) or SARS-like coronavirus in any hosts, studies covering any relation between the spike protein residues of coronaviruses and amino acid sequences of ACE2, as well as studies related to

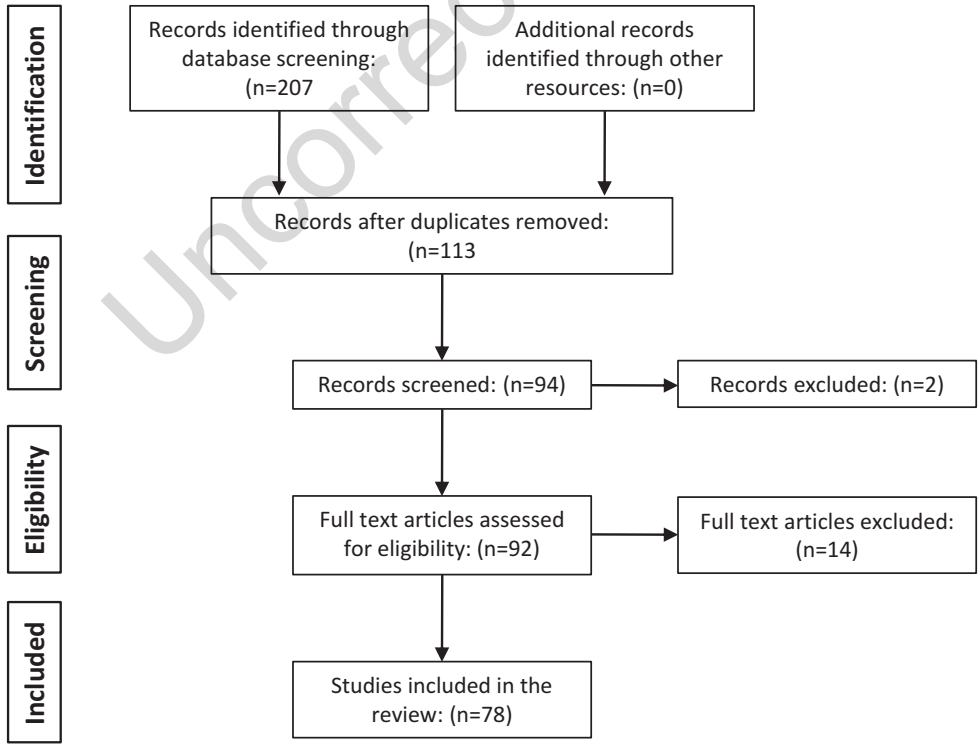

Fig. 5.3 Scheme showing the study selection procedure

Table 5.1 Search terms and databases

Scopus: 74 TITLE ("angiotensin converting enzyme 2" OR ace2) AND TITLE ("SARS CoV" OR coronavirus OR covid OR "SARSr CoV" OR "MERS CoV" OR ncov)
PubMed: 72 ("angiotensin converting enzyme 2" [title] OR ace2 "angiotensin converting enzyme 2" [title] OR ace2 [title]) AND ("SARS CoV" [title] OR coronavirus [title] OR covid [title] OR "SARSr CoV" [title] OR "MERS CoV" [title] OR ncov [title])
ProQuest: 171 ("angiotensin converting enzyme 2" OR ace2) AND ti ("SARS CoV" OR coronavirus OR covid OR "SARSr CoV" OR "MERS CoV" OR ncov)
Web of science: 65 ("angiotensin converting enzyme 2" OR ace2) AND TITLE: ("SARS CoV" OR coronavirus OR covid OR "SARSr CoV" OR "MERS CoV" OR ncov)
EMBASE: 79 ("angiotensin converting enzyme 2":Ti OR ace2:Ti) AND ("sars cov":Ti OR coronavirus:Ti OR covid:Ti OR "sarsr cov':Ti OR "mers cov":Ti OR ncov:Ti)

Table 5.2 Inclusion and exclusion criteria for selected articles

Inclusion criteria	Exclusion criteria
Published in English	Not written in English
Published between January 2003 and March 2020	Literature that did not include empirical data (letters, editorials, news, etc.)
Focus on relationship between ACE2 and SARS-coronaviruses	Articles found not be relevant

expression of ACE2 in cells, tissues, and body organs. The refining process was done by all the research team members to increase accuracy. The inclusion and exclusion criteria are summarized in Table 5.2.

5.2.3 Listing and Exploring Data and Analyzing the Studies

The format for listing data followed the Joanna Briggs Institute (JBI) approach as an accepted methodology for scoping reviews. The research team decided how to search questions. After discussions and investigations, the following topics were explored: 1) the country (or countries) in which the study was carried out, 2) study type, 3) the aims, and 4) the main findings. To increase accuracy, two external reviewers checked and explored the results separately. General conformity was obtained by discussions in cases of disagreement between the team members or between the team members and external reviewers. Kendall's coefficient of concordance was acceptable between the research team ($r = 0.95$; $p < 0.0001$) and between the team and reviewers ($r = 0.93$; $p < 0.0001$).

5.3 Results

5.3.1 Search Outcomes

The PRISMA flow chart was used to illustrate the study selection process and results (Fig. 5.3). Across the five databases a total of 207 studies were retrieved. After removal of duplicates, 94 titles and abstracts were screened for relevance and two were removed. The remaining 92 full-text articles were screened for eligibility and 78 articles were considered directly related to the research questions and included for the synthesis.

5.3.2 Article Information

Among the 78 studies, 73 used laboratory methods [22–31, 33–37, 38–42, 44–47, 48, 49, 50, 52–97], 2 were reviews [21, 98], 1 was a title of book [51], 1 was correspondence [99], and 1 was a perspective [32].

The included studies originated from different countries and, based on frequencies, 26 were from China [25, 27, 30, 31, 33, 39–42, 44–46, 53, 56, 57, 61, 62, 65, 73, 74, 76, 77, 82, 86, 94, 97], 18 from the USA [21, 22, 24, 26, 28, 36, 37, 38, 47, 49, 55, 58, 68, 69, 72, 79, 84, 99], 15 were carried out as multinational collaboration [29, 32, 35, 50, 51, 54, 59, 66, 70, 75, 88, 90–92, 98], 5 were from Japan [34, 48, 64, 78, 87], 4 from Germany [38, 71, 85, 96], 3 from Poland [52, 83, 89], 3 were from Taiwan [63, 66, 67], 2 Holland [23, 60], 1 Israel [81], and 1 from South Africa and Tunisia [80].

5.3.3 Narrative Summary of Studies

The main topics that the 78 studies focused on were: 1) the primary or intermediate reservoirs of coronaviruses; 2) the relationship of spike protein of the viruses and ACE2 as the related receptor; 3) the expression of ACE2 in various body organs; and 4) the recommended medical strategies based on the relationship of the spike protein and ACE2.

5.3.4 Studies Addressing the Primary Reservoir and Intermediate Hosts of Coronaviruses

Fifteen relevant studies are summarized below:

1. Li et al. (2006) addressed the following questions [98]:

(a) If bats are a reservoir of SARS-CoV-like viruses, when and in which species did these viruses acquire a spike protein capable of using palm civet and human ACE2?

(b) Are changes in the spike protein which enhanced human-to-human transmission a likely consequence of incubation in palm civets and other animals or is it a unique event not likely to recur?

(c) Did SARS-CoV gain the use of ACE2 through recombination and, if so, with what virus?

(d) What changes in other viral proteins were necessary for SARS-CoV to infect humans efficiently?

This paper described the emergence of dangerous variants of common pathogens including HCoVNL63 and animal equivalents and discussed coping strategies of viruses such as recombination.

2. Heller et al. reported that both mink and palm civet had 83/87 amino acid identity/similarity with human ACE2 [22]. This study suggested mink as a potential reservoir of SARS coronavirus in North America and established it as a suitable animal model to study this virus.
3. Zamoto et al. showed that ferret ACE2 acts as a SARS-CoV receptor with similar efficiency as human ACE2 and with greater efficiency than mouse ACE2 [48].
4. In 2007, a study by Fukushi et al. showed that SARS-CoV needs to bind via the RBD in the spike protein to ACE2 [78].
5. Chen et al. reported that, in comparison to human ACE2, 38 nonsynonymous changes exist in Chinese rhesus-ACE2, but this is just as effective as the human homolog in supporting viral entry [77]. The study also highlighted a natural mutation of tyrosine to asparagine at position 217 that can lead to downregulation of human-ACE2 and reduce viral entry.
6. Guo et al. reported that the number of amino acid differences between human-ACE2 and cat, civet, mouse, and rat ACE2 was 3, 8, 9, and 11, respectively [76]. Since there is no difference in the binding ability of cat ACE2 to the SARS-CoV spike protein, the possibility of zoonotic transmission of SARS-CoV from animals to humans is supported and, of the species tested, the cat ACE2 sequence was evolutionarily the closest.
7. Xu et al. stated there are six amino acid differences in raccoon dog ACE2 compared with human ACE2 and concluded that the raccoon dog may serve as a critical intermediate host for SARS-CoV and may have played a key role in SARS-CoV outbreaks [25].
8. In 2010, Hou et al. pointed out that two bat species, *Myotis daubentoni* and *Rhinolophus sinicus*, are likely to be susceptible to SARS-CoV and may be candidates as the natural host of the SARS-CoV progenitor or virus [75].
9. The study run by Demogines et al. reported that ACE2 utilization preceded the emergence of SARS-CoV-like viruses from bats [47]. Their results were consistent with a model in which an ACE2-utilizing bat coronavirus infected civets and/or other intermediate hosts, or possibly even humans directly.
10. Li et al. noted that human, civet, mouse, cat, golden Syrian hamster, and horseshoe bat support infection of SARS-CoV [45]. Therefore, comprehensive surveillance of these animals is suggested when SARS or SARS-like CoVs reemerge in the human

population in the future. This study also reported that rabbits and horseshoe bats are animal carriers of SARS-CoV.

11. Ge et al. identified two coronaviruses from Chinese horseshoe bats, RsSHC014 and Rs3367, which had the highest similarity to SARS-CoV, compared to other bat coronaviruses [29]. The similarity was highest in the RBD of the spike protein, supporting the case that these bat species as natural reservoirs of SARS-CoV.
12. Recently Cao et al. found that East Asian populations have higher allele frequencies in expression of quantitative trait loci variants associated with higher ACE2 expression in tissues [74]. This may indicate different susceptibilities or responses to SARS-CoV-2 infection in different populations.
13. Li et al. emphasized potential interspecies transmission of SARS-CoV-2 and the need for further surveillance in animal populations [46]. They found that the ACE2 amino acid positions 30–41, 82–84, and 353–357 are important in the interaction with SARS-CoV and amino acids 31, 35, 38, 82, and 353 are critical. As humans and nonhuman primates (gibbon, monkey, macaque, orangutan, and chimpanzee) showed identical sequences over these regions, this makes them potential hosts of SARS-CoV-2.
14. Another study by Liu et al. confirmed that other than pangolins and snakes, turtles are also potential intermediate hosts for transmission of SARS-CoV-2 to humans [73].

5.3.5 Studies Addressing the Interaction between Coronavirus Spike Proteins and ACE2

We found 27 studies which addressed this point:

1. Kuhn et al. reported ACE as a receptor for coronaviruses [21]. The paper stated that studying the receptor in detail is needed to progress in development of anti-viral drugs, vaccines, and animal models to survey pathogenesis of SARS-CoV. He concluded that the major questions that still need to be answered are the following: 1) Is ACE2 the only cellular factor permitting SARS-CoV cell entry or are co-receptors involved? 2) Does the inflammatory response to SARS-CoV infection lead to upregulation of ACE2 expression in lung tissue?
2. Hofmann et al. pointed out the central role of ACE2 in SARS-CoV infection and a minor contribution of the ACE2 cytoplasmic domain to receptor function [71].
3. Prabakaran et al. identified a deep channel on the top of the ACE2 molecule that contains the catalytic site and negatively charged ridges surrounding the channel that may provide a possible binding site for the positively charged receptor-binding domain of the spike protein [72]. He also noticed hydrophobic patches around the charges that could contribute to binding and the lack of carbohydrates at the top of the molecule could enable high-affinity binding.
4. Wong et al. stated that a 193-amino acid fragment of the spike protein (residues 318–510) bound to ACE2 more efficiently than did the full S1 domain (residues 12–672) [49]. In addition, smaller spike protein fragments, expressing residues 327–510 or 318–490, did not bind ACE2.
5. In their study, Zhang et al. reported that a SARS-CoV spike protein S1 residue (arginine 453) and an ACE2 residue (lysine 341) appear to be involved in the binding of SARS-CoV to ACE2 [50].
6. Li et al. carried out a study which found that the lower affinity of three SARS-CoV spike proteins from the less severe 2003–2004 outbreak could be enhanced by altering specific residues within the spike protein-binding site of human ACE2 to those of civet ACE2, or by altering spike protein residues 479 and 487 to those that were present in the more severe 2002–2003 outbreak. This study suggested that the reason for the low prevalence and intensity of SARS 2003–2004 outbreak was due to lower affinity of the spike protein of this coronavirus to bind ACE2 [93].

7. Lambert et al. showed that ADAM metallopeptidase domain 17 (ADAM17) is the protease responsible for ACE2 shedding [92].
8. Huang et al. reported on two coronaviruses (SARS-CoV, HCoV-NL63) that both utilize the ACE2 receptor, but enter cells through distinct mechanisms [24]. Specifically, only SARS-CoV utilized the enzymatic activity of the cysteine protease cathepsin L to infect ACE2-expressing cells.
9. Smith et al. reported that although the spike glycoprotein of HCoV-NL63 shares only 25% amino acid sequence identity with that of SARS-CoV, both viruses used ACE2 as a receptor [52]. This suggested that both viruses evolved separately to bind to the same receptor.
10. Pöhlmann et al. described how the ACE2 receptor was used for viral entry by CoV-NL63 despite little homology between this coronavirus and SARS-CoV [51].
11. The study run by Inoue et al. concluded that SARS-CoV mainly utilizes the clathrin-mediated endocytosis pathway for its entry into target cells and the cytoplasmic tail of ACE2 is not required for the penetration of SARS-CoV into cells [34].
12. The study of Li et al. noted that the spike proteins of SARS-CoV and HCoV-NL63 bind overlapping regions of ACE2 that include a critical loop between beta-strands IV and V [91]. In addition, changes to ACE2 residue 354, at the boundary of the SARS-CoV binding site, markedly inhibited utilization by HCoV-NL63 but not by SARS-CoV spike proteins.
13. Glende et al. in their study highlighted that cholesterol-rich micro-domains provide a platform facilitating efficient interaction of the SARS-CoV spike protein with ACE2 [90].
14. Mathewson et al. showed that the NL63 coronavirus spike protein has a weaker interaction with ACE-2 than the SARS-CoV spike protein [89].
15. Lin et al. reported that the NL63 coronavirus receptor binding domain binds to human ACE2 more efficiently than its full-length counterpart, with a binding efficiency comparable to the S1 or receptor binding domain of SARS-CoV [88].
16. Yoshikawa et al. reported that both AC70 and AC22 transgenic mice expressing the human ACE2 receptor were permissive to SARS-CoV infection, and caused elevated secretion of many inflammatory mediators within the lungs and brains, although infection was more intense with higher immunosuppression in AC70 than in AC22 mice, especially in the brain [26].
17. Haga et al. identified multiple ACE2-truncated variants that lost the SARS-CoV spike protein-induced shedding of ACE2 and TNF-α production in lung tissue [87].
18. A study by Chen et al. showed that the viral spike protein led to upregulation of fibrosis-associated chemokine ligand 2 (CCL2) and production of virus-like particles, and this was mediated by extracellular signal-regulated kinase 1 and 2 (ERK1/2) and the activator 1 protein (AP-1) transcription factor but not by the IκBα-NF-κB signaling pathway [86].
19. Glowacka et al. reported that SARS-CoV but not NL63 coronavirus replicated efficiently in ACE2-positive cells and reduced ACE2 expression [85].
20. The study of Wu et al. noted that binding to the same hot spot on human ACE2 was likely to be an outcome of convergent evolution by NL63-CoV and SARS-CoV [84].
21. Dijkman et al. showed that decreased ACE2 expression is dependent on the efficiency of NL63 coronavirus replication, and that NL63-CoV and SARS-CoV both affect cellular ACE2 expression during infection [83].
22. The study of Heurich et al. resulted in transmembrane protease serine 2 (TMPRSS2) but not ADAM17 protease promotion of SARS-CoV entry by two separate pathways: 1) ACE2 cleavage, which might promote viral uptake; and 2) SARS spike protein cleavage, which activates this protein for membrane fusion [96].
23. Song et al. showed that the spike glycoprotein retains the pre-fusion trimer structure

after trypsin cleavage and low-pH treatment [82]. Also, binding with the host cell receptor ACE2 promotes the release of S1 subunits from the S trimer and triggers the pre- to post-fusion conformational transition.

24. Brielle et al. described the evolution of coronaviruses (SARS-CoV, SARS-CoV-2, and NL63-CoV) towards host recognition [81].
25. Lan et al. suggested that SARS-CoV-2 is similar to SARS-CoV and reported that the similarities in structure and sequence of these two coronaviruses argue for convergent evolution towards improved binding to ACE2 [44].
26. Othman et al. reported that the interface segment of the spike protein RBD might have been acquired by SARS-CoV-2 via a complex evolutionary process rather than mutation accumulation [80].
27. Yan et al. showed that SARS-CoV-2 recognizes an ACE2 dimer that complexes with a membrane protein, and drugs which disrupt this interaction may be effective in reducing infection [31].

5.3.6 Studies Investigating the Relationship between ACE2 Expression and Clinical Manifestations of COVID-19 Infection

Seventeen studies addressed this topic:

1. To and Lo found that although ACE2 is expressed at high levels in pneumocytes and surface enterocytes of the small intestine, the tissue responses in these two organs are different [27]. They also found that the presence of ACE2 is not enough for coronavirus infection and that other receptors or cofactors may be required in some tissues.
2. Hamming et al. studied expression of the ACE2 protein on lung alveolar epithelial cells and enterocytes of the small intestine [60]. This revealed that ACE2 was present in arterial and venous endothelial cells, and arterial smooth muscle cells in all organs studied.
3. Mossel et al. reported that the human colon epithelial line CaCo-2 was the only human cell type out of 13 tested that supported efficient SARS-CoV replication [28].
4. The study by Jia et al. showed that ACE2 was more abundantly expressed on the apical surface of polarized epithelia, and well-differentiated cells support viral entry and replication [37].
5. Ren et al. showed that ACE2 is localized on the apical plasma membrane of polarized respiratory epithelial cells and mediates infection from the apical side of these cells [59].
6. Li et al. noted that both SARS-CoV receptors (ACE2 and CD209L) are expressed in organ/tissue-derived endothelial cells. The expression of the ACE2 receptor was highest in human lung microvascular endothelial cells, and expression of CD209L was higher in lymphatic endothelial cells [43].
7. Tseng et al. showed that pre-inflammatory mediators and viral titer were high in lung and brain of transgenic mice expressing ACE2 [58].
8. Yang et al. showed that SARS-CoV replicated more efficiently in lungs of ACE2 transgenic mice than in those of wild-type mice. Similar signs (vasculitis, degeneration, and necrosis) were also seen in other organs [57].
9. Dong et al. reported the mRNA of human ACE2 was expressed efficiently in normal lung tissue, but not in cartilage and cancellous bone under the weight-bearing area of the femoral head [56].
10. Netland et al. found that neurons are a susceptible target for SARS-CoV and that only the absence of host cell receptors prevents severe murine brain disease [55].
11. A study by Oudit et al. focused on myocardium showed that that SARS-CoV can mediate inflammation and damage associated with downregulation of the myocardial ACE2 system, which may be responsible for

the myocardial dysfunction and adverse cardiac outcomes in patients with SARS [54].
12. Chai et al. showed that SARS-CoV-2 might directly bind to ACE2 positive cholangiocytes but not necessarily to hepatocytes [53].
13. Deng et al. showed expression of ACE2 and TMPRSS2 in human kidney proximal tubules, indicating that the kidney is a potential target organ of SARS-CoV-2 infection [42].
14. Ji et al. showed that after triggering functional changes in ACE2, an imbalance in the steady-state cytokine regulatory axis involving the renin–angiotensin system and IP-10 leads to a cytokine storm [94].
15. Li et al. reported that the SARS-CoV-2 receptor ACE2 was widely spread in specific cell types of the maternal–fetal interface [41].
16. Lin et al. showed high ACE2 gene expression in all subtypes of kidney proximal tubule cells and low expression in bladder epithelial cells [39].
17. Xu et al. reported ACE2 expression on the mucosa of the oral cavity and epithelial cells of tongue [30].

5.3.7 Studies Investigating New Treatment Strategies for COVID-19 Infection

Twenty studies regarding new treatment approaches are summarized below:

1. Han et al. showed that a peptide derived from ACE2, which consisted of two discontinuous parts of ACE2 (amino acids. 22–44 and 351–357), was a good candidate for the treatment of coronary heart disease [69].
2. Li et al. described ACE2 as a functional receptor for SARS-CoV and showed that a solution form of ACE2 rather than ACE1 could block the spike S1 domain [36]. This suggested the potential use of ACE2 antibodies as a treatment for SARS infection, which may also be applicable to COVID-19 cases.
3. The findings of Moore et al. were in line with those of Li [70].
4. Batlle also recently reported that a soluble recombinant form of ACE2 appeared to neutralize SARS-CoV-2 in vitro [99].
5. Hoffmann et al. showed that SARS-CoV-2 uses ACE2 as a receptor and TMPRSS2 for spike protein priming [38]. This study supported that case that TMPRSS2 inhibitors might be a treatment option. The study also showed that sera from convalescent SARS-CoV patients cross-neutralized viral entry and could therefore provide a treatment and/or a vaccination strategy for patients with COVID-19.
6. Lei et al. generated a fusion protein containing the RBD of the SARS-CoV spike protein linked to the Fc portion of human IgG1 and found that this could be internalized into SARS-CoV-susceptible cells with ACE2 [61]. This may also have some implications for vaccine development [61].
7. Ho et al. also showed in their study that peptides derived from the spike protein, especially the use of amino acid residues 668–679, can compete with the ACE2-coronavirus interaction and prevent infection [67].
8. Kuba et al. found that recombinant spike IgG-Fc proteins can block coronary artery disease associated with SARS-CoV [35]. This study also introduced the idea of using ACE2 inhibitors as a way to reduce injury and pulmonary edema.
9. Zhang et al. showed that recombinant spike S1 subunit proteins (amino acid residues 388 to 496) can induce protective neutralizing antibodies against SARS-CoV [65].
10. Wang et al. also found that a SARS-CoV-RBD-IgG-Fc protein could bind to ACE2, again suggesting this as a potential vaccine approach [62].
11. de Lang et al. reported that the anti-inflammatory cytokines interferon-γ and interleukin (IL)-4 could reduce effects of coronary artery disease via reduced ACE2 expression [23].
12. He et al. showed that infection caused by coronaviruses can cause pro-inflammatory cytokines (MCP-1 and TGF-β1, TNF-α,

IL-1β, IL-6) in pneumocystis and macrophages of the lungs and bronchi, which can lead to acute lung damage [68]. This supports the use of anti-inflammatory cytokines as a therapeutic strategy.

13. Haga, S attributes the production of inflammatory cytokines, especially TNF-α, to the stimulation of the 2019-nCoV spike and the cytoplasmic tail of ACE2. This is a multifaceted interaction between the production of pre-inflammatory cytokines, protein spike SARS-CoV, and ACE2 [64].
14. Yan et al. showed that an siRNA approach can effectively prevent viral replication by targeting the ACE2 gene or viral nucleocapsid protein [66].
15. Lu et al. also showed that downregulation of ACE2 expression using an siRNA approach could effectively reduce the proliferation of SARS-CoV [63].
16. Wang et al. also showed that reducing expression of ACE2 by siRNA, makes ACE2 a therapeutic target [33].
17. Wu et al. suggested four potential treatment options for coronavirus infections: 1) the use of ACE2 recombinant proteins; 2) use of ACE2 inhibitors such as lisinopril; 3) use of ACE2 blockers such as losartan; and the use of angiotensin (7-1) to activate the MAS receptor for ACE2 neutralization [84].
18. Zhang et al. also provided treatment strategies for COVID-19 infection based on the role of ACE2, which included: 1) the use of vaccines against the spike protein; 2) the use of serum protease inhibitors against TMPRSS2; 3) blockade of ACE2 with small molecules; and 4) use of the ACE2 soluble form that binds competitively to the SARS-CoV spike protein [32].
19. Ho et al. reported on a number of small molecules that disrupted the SARS-CoV – ACE2 interaction and could therefore be promising leads for development of novel treatments for COVID-19 disease [67].

5.4 Discussion

To the best of our knowledge, this is the first scoping review on the SARS-CoV-2 which aims to integrate the existing knowledge on the primary hosts of coronaviruses, the relationship between the receptor binding domain of coronaviruses and the likely host cell receptor ACE2, the organ specificity of ACE2 expression compared with clinical manifestations of the disease, and whether or not this information can be used for development of novel treatment approaches.

In the case of the SARS-CoV, exotic marketplace animals were probably the immediate origin of the virus [100]. These animals included palm civets as the likely carriers since SARS-CoV could be isolated from these animals. In addition, the infections which occurred coincided with the preparation and consumption of palm civet meat products in restaurants. SARS-CoV infections of other marketplace species have also been observed such as the cat, red fox, and badger. Although these species may be an immediate source of SARS-CoV infections in humans, it is likely that they serve as a conduit of the virus from another reservoir species. The most likely of these reservoirs includes certain bat species such as the horseshoe bat [100].

For SARS-CoV-2, 6 amino acids in the RBD of spike protein amino have been found to be critical for ACE2 binding and host determination [101]. Interestingly, 5 of these amino acids differ between SARS-CoV-2 and SARS-CoV which seems to confer a higher affinity of SARS-CoV-2 to ACE2 in humans, cats, ferrets, and other species. As many early cases of SARS-CoV-2 infection were linked to the Huanan market in Wuhan, it is likely that bats served as the primary reservoir given the high genomic similarity of the RaTG13 bat coronavirus with SARS-CoV-2. In addition, illegally imported Malayan pangolins contain coronaviruses similar to SARS-CoV-2 especially within the RBD domain. This suggests that the SARS-CoV-2 spike protein was most likely optimized for binding to human-like ACE2 receptors by natural selection.

Taken together, this study provides insights into the spike protein of SARS-CoV-2 in relation to the probable host cell receptor, ACE2, in COVID-19 disease. Due to the diversity of coronavirus species transmission and the internal and intergenerational diversity of these viruses, the reservoir and intermediate host of SARS-CoV-2 is still not certain. However, as stated above, it is likely that the bat is the main animal reservoir and the results of a recent study are consistent with the pangolin being the intermediate host [102]. This latter study carried out molecular and phylogenetic analyses and showed that a pangolin coronavirus (pangolin-CoV-2020) is genetically related to SARS-CoV-2 and a group of bat coronaviruses and may therefore be natural hosts of betacoronaviruses. Thus, steps taken to minimize human exposure of humans to such wildlife will be important to reduce the risks of coronaviruses spreading from animals to humans.

In addition, it is still not clear if the interaction between ACE2 and the SARS-CoV-2 spike protein evolved separately or if they coevolved to permit the high infectivity of this coronavirus [103]. Recent studies have suggested that this could be due to the higher affinity of the SARS-CoV-2 spike protein receptor binding domain for ACE2 compared with other coronaviruses, such as SARS-CoV [104].

Although the clinical manifestations of COVID-19 disease are varied, at least some of these appear to be due to the targeting of ACE2 in different tissues and organs of the body. Although the virus likely enters the body at the level of the respiratory system due to the high levels of ACE2 expression there, the virus can spread out and cause damage to other vital organs and tissues expressing ACE2, triggering a wide spectrum of pathophysiological effects and symptoms, including digestive [105], neurological [106], and cardiovascular complications [107].

5.5 Conclusions and Future Perspectives

There is currently no proven effective treatment for COVID-19 disease and development of a safe and effective vaccine could take from 6 months to one and half years. However, since the virus gains access to the respiratory system through the cell surface ACE2 protein, a number of strategies are currently being explored to target this interaction [108–112]. One incredible feature of the COVID-19 pandemic has been the worldwide efforts to develop new treatments and vaccines to halt its spread and to raise our awareness of the dangers of pandemics due to such viruses and other pathogens. The emergence of COVID-19 highlights the critical importance of establishing a systematic coronavirus surveillance network. In addition, the current pandemic has instilled in all of us the value of setting in place a worldwide coronavirus surveillance network to prevent such events from reaching the dangerous levels that this one has and to manage outbreaks more effectively in the future.

Acknowledgement Thanks to guidance and advice from the "Clinical Research Development Unit of Baqiyatallah Hospital".

Ethics Approval and Consent to Participate IR.BMSU.REC.1399.011.

Competing Interests The authors have no conflicts of interest.

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