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REVIEW

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Angiotensin-converting enzyme 2: The old door for new severe acute respiratory syndrome coronavirus 2 infection

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Summary

Coronavirus (CoV) disease 2019 (COVID-19) is an ongoing pandemic caused by severe acute respiratory syndrome CoV 2 (SARS-CoV-2). The highly contagious SARS-CoV-2 belongs to the genus *Betacoronavirus*, and it is phylogenetically closely related to SARS-CoV, a human CoV that caused an outbreak back in 2002 to 2003. Both SARS-CoV-2 and SARS-CoV enter human cells via the interactions between viral crown-like spike protein and human angiotensin-converting enzyme 2 (ACE2) receptor. Here, we aim to review the involvement of ACE2 in human CoV infections by discussing the roles of ACE2 in CoV evolution, cross-species transmissibility, and COVID-19 susceptibility. We also provide our perspectives on COVID-19 treatment and prevention.

KEYWORDS
ACE2, COVID-19, SARS-CoV-2

1 | INTRODUCTION

The recent outbreak of a novel coronavirus (CoV) disease 2019 (COVID-19) caused by severe acute respiratory syndrome CoV 2 (SARS-CoV-2; formally 2019-nCoV) has emerged as a global health and economic crisis. The World Health Organization has officially declared COVID-19 a pandemic on March 11, 2020. As of April 28, 2020, the extremely contagious SARS-CoV-2 had already spread to all continents except Antarctica, infected more than 3 million people and caused at least 210 000 deaths worldwide.

The CoVs are enveloped, positive-sense single-stranded RNA viruses with genomes ranging from 26 to 32 kb, the largest among all known RNA viruses.[1] They belong to the subfamily Orthocoronavirinae (a.k.a. Coronavirinae) in the family Coronaviridae, and can be classified into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*.[2] CoVs are commonly found in mammals, vertebrates, and other wild animals.[3,4] Studies have shown that humans and other mammals are primarily infected by the CoVs of *Alphacoronavirus* and *Betacoronavirus* genera, whereas vertebrates are usually infected by *Gammacoronavirus* and *Deltacoronavirus*.[5]

To date, scientists have identified seven strains of CoVs that infect humans. Some strains such as HCoV-229E, HCoV-NL63,

Abbreviations: ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; CoV, coronavirus; COVID-19, coronavirus disease 2019; cryo-EM, cryogenic electron microscopy; MERS-CoV, Middle East respiratory syndrome coronavirus; RAS, renin-angiotensin system; RBD, receptor-binding domain; rhACE2, recombinant human ACE2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCGA, The Cancer Genome Atlas; TMPRSS2, transmembrane protease serine 2.

HCoV-OC43, and HCoV-HKU1 are regularly circulating in the human population and are responsible for respiratory infections.[6,7] People infected with these CoVs generally show mild symptoms of upper respiratory disease such as fever, sore throat, and coughing, and only on rare occasion, they may cause lower respiratory tract infections and severe pneumonia.[8] However, certain human CoV strains, such as SARS-CoV-2, SARS-CoV, and Middle East respiratory syndrome CoV (MERS-CoV), are associated with higher fatality rates. SARS-CoV and MERS-CoV have previously claimed over a thousand lives in total during the CoV outbreaks in 2002 to 2003 and 2012, respectively.[9,10] In addition to human CoVs, animal CoVs also could cause a significant loss to the animal husbandry. One of the most recent examples is the swine acute diarrhea syndrome CoV outbreak in 2017 to 2018.[11] All these incidences signify that the CoVs have continually posed a serious threat to the well-being of humans and animals.[9,10]

Genome sequencing and phylogenetic analysis of the newly emerged SARS-CoV-2 has placed it under the genus *Betacoronavirus*, making it a close relative to another two notorious CoVs: SARS-CoV and MERS-CoV.[4] The *Betacoronavirus* also contains a range of bat CoV strains, including several strains that show high % sequence similarity to the SARS-CoV-2, suggesting that SARS-CoV-2 is likely originated from the bats.[12-14] However, the intermediate reservoir of SARS-CoV-2 remains unclear.

The surface of all CoVs has a characteristic crown-like structure known as the spike protein (commonly referred to as S protein). The spike protein can bind to a specific cell membrane receptor and it is the key mediator for CoV entry into host cells.[15] Previously, angiotensin-converting enzyme 2 (ACE2) was identified as the target receptor for SARS-CoV.[16-18] Current studies have indicated that the novel SARS-CoV-2 also uses the ACE2 receptor.[14,19] Here, we review the involvement of ACE2 in human CoV infections by discussing the significances of ACE2 in relation to CoV evolution, cross-species transmissibility, and COVID-19 susceptibility. Lastly, we provide our perspectives on COVID-19 treatment and prevention. Also, since COVID-19 is an ongoing pandemic, some of the first-hand data discussed in this review are sourced from non-peer-reviewed preprints.

2 | PROPERTIES AND BIOLOGICAL FUNCTIONS OF ACE2

ACE2, a homologue of ACE, was firstly described 20 years ago.[20,21] Both ACE2 and ACE are zinc metalloproteases that play crucial roles in the renin-angiotensin system (RAS), a system that regulates blood pressure, fluid, and electrolyte homeostasis.[22,23]

Human ACE2 is a protein with 805 aa encoded by the *ACE2* gene (HGNC: 13557) while ACE is a larger protein consists of 1306 aa encoded by the *ACE* gene (HGNC: 2707). ACE2 and ACE share approximately 40% identity and 61% similarity in their aa sequences.[21] Despite the similarity, ACE and ACE2 do not share the same substrate specificity.[24] Also, ACE inhibitors that commonly used for treating high blood pressure or cardiovascular and kidney diseases, such as captopril, enalaprilat, and lisinopril, are ineffective against ACE2.[24] In the RAS, ACE2 acts as a potent counter-regulator against ACE.[25] Physiologically, ACE converts inactive decapeptide angiotensin (Ang) I into vasoconstrictor Ang II and degrades vasodilator bradykinin, leading to increased blood pressure.[20] ACE2, on the other hand, decreases blood pressure by competing with ACE to hydrolyze Ang I into the nonapeptide Ang-(1–9), and at the same time degrades Ang II into Ang-(1–7) and promote the release of vasodilator bradykinin.[20,26]

ACE2 and ACE are mainly expressed in the cell membrane of vascular endothelial cells found in various organs. Generally, ACE is more widespread than ACE2 with highest levels of expression observed in, but not limited to, gastrointestinal tract, kidney, and lung.[21,27] For ACE2, gallbladder, gastrointestinal tract, heart, kidney, and testis are the primary organs of expression.[27,28] Both ACE2 and ACE can be secreted from the cell surface into the circulation or urine.[20,29,30] Aberrant expression of ACE or ACE2 is associated with many diseases, including hypertension, lung injury, and cardiovascular, renal, and liver diseases.[31-33] ACE2 is also known to be involved in human- and animal-CoV infections. The high-resolution cryogenic electron microscopy (cryo-EM) structure of full-length human ACE2 was recently revealed, and its interactions with SARS-CoV or SARS-CoV-2 were determined.[34]

3 | ACE2 AND CORONAVIRUS PATHOGENESIS

The interactions between spike protein and host receptor are critical for CoV pathogenesis. The spike protein is a crown-shaped class I viral membrane fusion protein distributed throughout the surface of all CoVs.[35] It is made up of a short intracellular tail and a large ectodomain connected by a single-pass transmembrane anchor.[36] The ectodomain consists of two subunits: three S1 subunit heads resting above a trimeric S2 subunit stalk.[37] The S1 subunit is responsible for host receptor-binding while the S2 subunit is accountable for creating an entrance for the viral genomes to invade the host cells by fusing the viral and host membranes.[35,38] Structural studies on the S1 subunit have revealed two receptor-binding domains (RBDs) that can interact with a variety of receptors. Specifically, the N-terminal domain mainly binds sugar receptors and CEACAM1 in mouse hepatitis CoV[39-42] whereas the C-terminal domain appears to bind protein receptors (eg, APN, ACE2, and DDP4) more exclusively.[38,42-46] In order to bind a host-cell receptor, the RBD undergoes hinge-like conformational movements that either buried (lying state; receptor-inaccessible state) or exposed (standing state; receptor-accessible state) its receptor-binding regions.[47] Some CoVs, such as lineage A *Betacoronavirus*, have a shorter spike-like envelope-associated hemagglutinin-esterase that acts as a receptor-destroying enzyme.[48,49] Structures and functions of CoV spike proteins are reviewed extensively in Li.[2]

So far, three CoVs (HCoV-NL63, SARS-CoV, and SARS-CoV-2) have shown to utilize human ACE2 receptor. However, the RBDs of these three ACE2-utilizing human CoVs do not share identical sequences. Previously, the interactions between ACE2 and S1 domain

of SARS-CoV spike protein was firstly reported by Li et al,[16] and the structure of ACE2-spike protein complex had been determined.[50] Research indicated that the ACE2 protein could bind directly with the extended tyrosine-enriched loop on the RBD (residues 424-494) of SARS-CoV spike protein[50,51] and that the distantly-related ACE protein did not have the same function as ACE2.[16] Interestingly, although HCoV-NL63 also interacts with ACE2, its RBD shares no sequence and structural similarity with the SARS-CoV.[52] In fact, unlike SARS-CoV that binds the ACE2 through a continuous subdomain, HCoV-NL63 binds the receptor with three discontinuous beta-loops.[52] Research has indicated that both HCoV-NL63 and SARS-CoV bind overlapping regions of ACE2 and that mutations in the ACE2 protein generally resulted in the same binding properties of these two strains.[17] However, some mutations in the ACE2 protein only hinder the interactions of either HCoV-NL63 or SARS-CoV, indicating that both strains engage ACE2 differently.[17,53]

The engagement of SARS-CoV-2 with ACE2 is similar to SARS-CoV, and important features of the spike protein in SARS-CoV-2 and its most related CoVs are compared and summarized in Andersen et al.[54] Virus infectivity study has indicated that the SARS-CoV-2 is able to utilize ACE2 of human, Chinese horseshoe bats, civet, and pig but was not able to use mouse ACE2.[14] The cryo-EM structure of SARS-CoV-2 spike protein was recently determined.[55] Results indicated that the spike proteins of SARS-CoV and SARS-CoV-2 were highly homologous and structurally similar, with minor differences in the position of their RBDs in receptor-inaccessible states: RBD of SARS-CoV packs tightly against the N-terminal domain of the neighboring protomer whereas RBD of SARS-CoV-2 angles closer to the central cavity of the trimer.[55] Notably, the affinity between ACE2 and the spike protein of HCoV-NL63 is weaker compared to SARS-CoV and SARS-CoV-2, and although SARS-CoV and SARS-CoV-2 share similar spike protein and RBD sequences, the affinity between the spike protein of SARS-CoV-2 and ACE2 appears to be 10 to 20 folds higher than SARS-CoV and ACE2, suggesting that SARS-CoV-2 has a much higher human-to-human transmissibility compared to SARS-CoV and HCoV-NL63.[6,52,53,55] In addition, both SARS-CoV and SARS-CoV-2 use the same host transmembrane protease serine 2 (TMPRSS2) and possibly other proteases (eg, cathepsin B and cathepsin L) to cleave their spike protein and enhance viral entry.[56,57] Therefore, other host factors in addition to ACE2 are likely also contributed to human CoV pathogenesis and required further study.

4 | ACE2 AND CORONAVIRUS EVOLUTION

Sequences in the spike proteins determine the tissue and species tropism of CoVs. Mutations in the RBD of spike protein not only could affect the virulence of CoVs, but also could alter viral host spectrum, allowing cross-species infection to happen.[36,37,51] RNA viruses can rapidly evolve and adapt to new environments due to their high mutation rates associated with the infidelity of RNA polymerase.[58] This feature of RNA viruses increases the probabilities of CoVs to invade a new host population. In fact, a substantial number of emerging pathogens that caused major epidemics in the past few decades are by RNA viruses.[59]

Recognizing the origin and host of CoVs is extremely critical for disease control and prevention. Understanding the underlying molecular mechanism of human receptor usage by different CoV strains is equally important. Bats are likely the ancestors of five out of seven human CoVs.[3,15,60,61] They are the natural reservoirs for CoVs and many other viruses such as Ebola, Hendra, Marburg, and Nipah.[62] Studies have suggested that the diverse viral inhabitants within and between bat species may perhaps promote co-evolution of these zoonotic viruses, further increasing the chances of cross-species transmission.[62-64] The relationships of *Betacoronavirus* RBDs between bat- and human-CoVs are reviewed in Cui et al[3] and Lu et al.[65] A review article from Fan et al has postulated that China is a hotspot for future bat-orientated CoV outbreaks due to multiple reasons, including the track record of bat CoV outbreaks in human and animals, high population density, great wildlife diversity, and coexistence of diverse viruses in bats.[61] However, CoV outbreaks could likewise happen anywhere in the world since mutations of CoVs in bats or other wild animals are occurred by chance.

Despite overwhelming data pointed toward bats as the progenitor of most human CoVs, the evidence of bat CoVs to cross-transmit directly to humans is still lacking.[60] Studies have suggested that all human CoVs have or require intermediate reservoirs before cross-transmitting from bats into humans.[3] For instance, the potential intermediate hosts of bat-orientated strains HCoV-229E, SARS-CoV, and MERS-CoV are identified as camelids,[64,66] palm civets,[67] and dromedary camels,[68] respectively. HCoV-NL63 is also originated from the bats, but the intermediate host remains unclear.[64] Native hosts of HCoV-OC43 and HCoV-HKU1 are likely rodents, and cattle might be the intermediate host for HCoV-OC43.[8,52] As for SARS-CoV-2, the phylogenic analysis indicated that this new CoV strain is also of a bat origin (≈96% similarity),[12-14] and smuggled Malayan pangolins in China's border are currently the prime suspect of being its intermediate host.[69] The spike protein RBD of pangolin CoV strains showed ≈99% similarity to SARS-CoV-2, with only one aa difference. The binding capacity of pangolin RBD was tested, and it showed that the spike proteins of pangolin CoV and SARS-CoV-2 could potentially bind to both pangolin and human ACE2.[69] However, despite the high % similarity in the RBD region of pangolin CoV and SARS-CoV-2, they shared only a mere ≈90% similarity of their overall genomes.[69] A few more similar studies also analyzed CoV strains isolated from pangolin in China, and although pangolin CoVs are considered phylogenetically closer to SARS-CoV-2, the % genome similarity was only 92.4% at best.[70-72] Since closely related CoVs should have higher genome similarity, like those observed between SARS-CoV and CoVs isolated from its potential intermediate host palm civet (up to 99.8% similarity), whether pangolin is truly the intermediate host for SARS-CoV-2 is still in question.[73]

All three human CoVs (HCoV-NL63, SARS-CoV, and SARS-CoV-2) that utilized ACE2 are originated from bats. Human CoV strains appeared to engage human ACE2 differently, mainly through targeting different positions of the ACE2 protein (reviewed in Li et al[17]).

Generally, higher ACE2 affinity is correlated with higher infectibility of CoVs, and those strains with a higher ACE2 affinity may eventually be the dominant circulating strains in human. Few months into the COVID-19 outbreak, more than a hundred mutations were already detected in the SARS-CoV-2 genomes isolated from 103 COVID-19 patients.[74] These SARS-CoV-2 strains could be subdivided into two major types: the highly contagious and faster-growing L-type that found in ≈70% of samples and the evolutionarily older and less aggressive S-type that found in ≈30% of samples.[74] Similarly, SARS-CoV strains previously isolated from SARS patients showed vary ACE2 affinities: some strains had a moderate affinity for human ACE2 whereas some strains, especially those isolated during the late phase of the SARS-CoV outbreak, had a higher affinity.[75,76] SARS-like CoV strains isolated from palm civets during and after SARS-CoV outbreak showed high affinity for civet ACE2 but lower affinity for human ACE2, and consequently, higher infectivity in civet cells compared with human cells.[73,77] The spike protein RBDs of SARS-CoV and civet CoV only differ by two residues, and these differences are enough to cause SARS-CoV to bind human ACE2 more effectively than civet CoV.[78] Thus, the host range, tissue tropism, and receptor-binding property of CoVs can change dramatically simply due to a few mutations on the spike protein RBD. Previously, aa positions of human ACE2 that may play a role in host range and cross-species infection of CoVs were identified: K31, E35, D38, Y41, M82, and K353.[10]

Chinese horseshoe bats are considered the native host for SARS-CoV even though the overall genetic similarity between SARS-CoV and SARS-like civet CoVs is greater than that between SARS-CoV and SARS-like bat CoVs.[79,80] SARS-like bat CoVs share identical genome

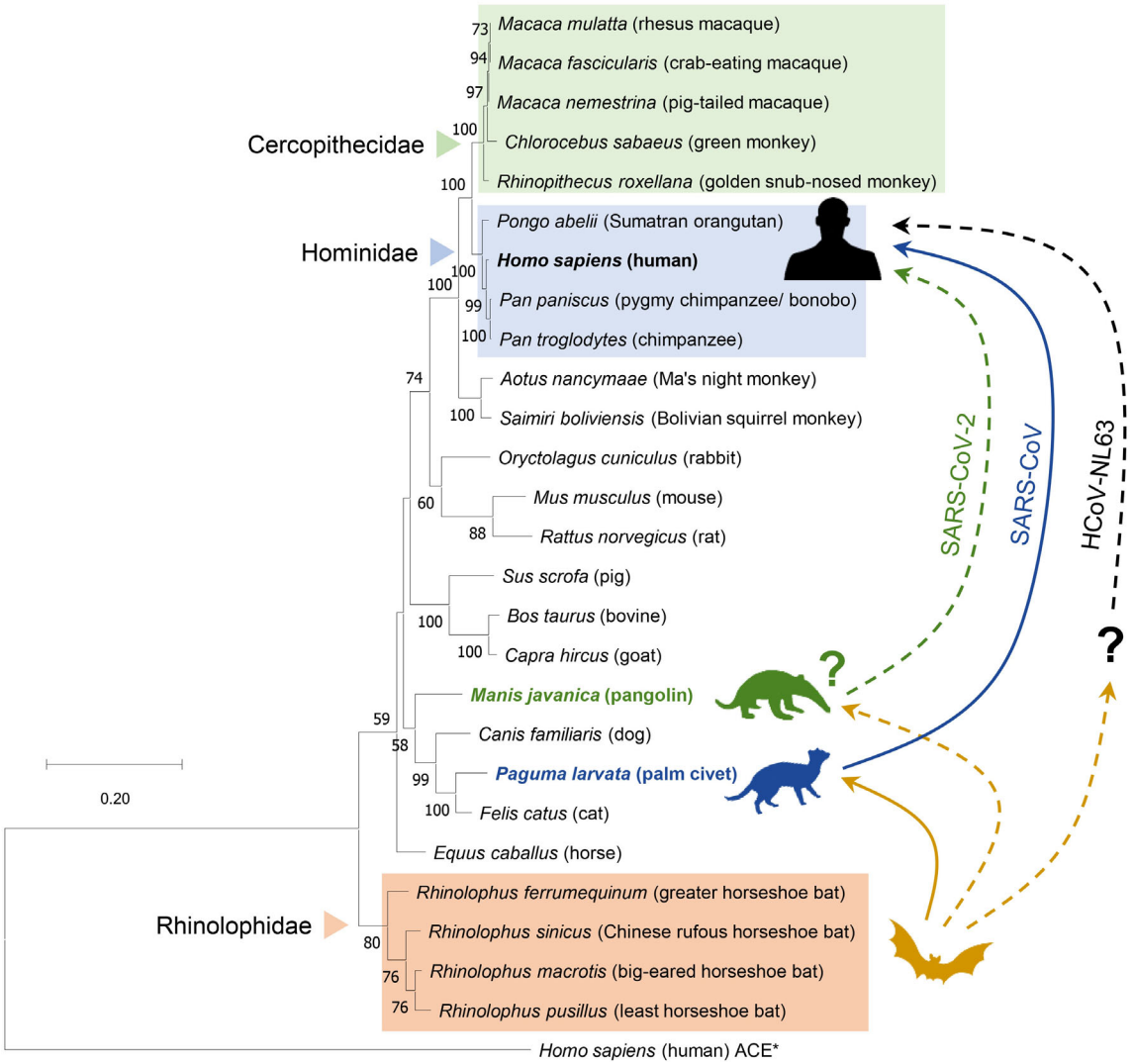

FIGURE 1 Phylogenetic tree of human and animal angiotensin-converting enzyme 2 (ACE2). Amino acid sequences were obtained from UniProt or National Center for Biotechnology Information and aligned by ClustalW using MEGA X (v10.1.7).[88] Maximum likelihood tree was constructed using Jones-Taylor-Thornton model with 100 bootstrap replications. Numbers at the branches represent bootstrap probability values ≥50%. The known and suspected hosts of human coronaviruses that utilized human ACE2 receptors (HCoV-NL63, SARS-CoV, and SARS-CoV-2) are shown. Solid arrows represent interspecies transmission with substantial evidence; broken arrows represent suspected interspecies transmission. *Human ACE, a homologue of ACE2, was used as an outgroup

organizations and high sequence similarity with SARS-CoV, and these SARS-like CoVs can be found in diverse bat populations but not in other mammals.[81,82] Despite being phylogenetically related, the spike protein RBDs of SARS-like bat CoVs were not identical to SARS-CoV, and bat CoVs were generally not able to utilize human ACE2 or any ACE2 ortholog tested.[83] On the other hand, SARS-CoV was able to infect palm civet but was inefficient against bats.[83,84] However, in other studies, several bat CoV strains that had the ability to utilize human ACE2 were identified, suggesting that the direct spillover of novel CoVs from bat to human is certainly possible.[12,85,86]

The relationships between human CoVs and ACE2 of other different animal species have not been systematically assessed. Previously, SARS-CoV was not able to bind rat ACE2, but it could interact with mouse ACE2 with low affinity.[10,84] Comparing the ACE2 sequences of human and other mammalians may give us some clues on cross-species receptor usage of SARS-CoV-2.[87] Here, we performed a phylogenetic analysis of ACE2 from a range of mammals and found that human ACE2 are most closely related to other species in the same family Hominidae (chimpanzee and orangutan; Figure 1).[88] Furthermore, evolutionary inference suggests that species from the family Cercopithecidae (macaque and monkey) also have ACE2 similar to human ACE2 (Figure 1). Previously, human CoV strain OC43 was detected in fecal samples of macaque and chimpanzee, signifying the potential of CoV spillovers among human, macaque, chimpanzee, and monkey.[89,90] Unfortunately, research regarding CoVs in the above-mentioned primates is scarce, and further studies are required to examine if one of these species could be the intermediate host for SARS-CoV-2. Furthermore, there is a rising concern regarding the transmission of COVID-19 in companion pets such as cats and dogs. Although so far there is no evidence that household pets can spread COVID-19, it appears that some animals, especially those in the family Felidae (eg, cat and Malayan tiger), may contract SARS-CoV-2 from humans.[91,92] At present, the roles of ACE2 in domestic animals in connection to their infection with SARS-CoV-2 plausibly transmitted from humans have remained unclear.

5 | ACE2 EXPRESSION AND COVID-19 SUSCEPTIBILITY

The roles of ACE2 expression in SARS-CoV-2 pathogenesis and human COVID-19 susceptibility are largely unknown. Because SARS-CoV-2 uses ACE2 as the receptor, it is logical to assume that all human cells that express ACE2 are the potential targets for SARS-CoV-2. Therefore, the following questions regarding ACE2 expression in relation to COVID-19 susceptibility can be asked: (a) Which parts of the body express ACE2? (b) Which ACE2-expressing organs are prone to SARS-CoV-2 infection? (c) Is higher expression level of ACE2 equal to greater COVID-19 susceptibility?

ACE2 is expressed in a tissue- and species-specific pattern.[93-95] In human, ACE2 is expressed in virtually all organs, with gastrointestinal tract, heart, and kidney among the highest expressed organs.[27,28,96] Lung is the main target for SARS-CoV-2, but the overall expression of ACE2 in human lung vary significantly according to different studies—some studies indicated that lung expressed high levels of ACE2[94] while some suggested otherwise.[27,97] Using single-cell RNA sequencing, Zhao et al showed that the lung ACE2 explicitly expressed in a small population of type II alveolar cells, and these ACE2-expressing cells also highly expressed certain genes that involved in promoting viral reproduction and transmission.[98] Additionally, they found that age or smoking was not associated with ACE2-expressing lung cell number but revealed that Asian had higher lung ACE2 expression than white and African American, suggesting Asian might be more susceptible to COVID-19.[98] However, the above study only tested on eight samples (with only one Asian). In another study, 224 samples were collectively analyzed for ACE2 expression in healthy lungs, and results indicated that lung ACE2 expression was not significantly associated with age, gender, or race.[99] This study, however, did find that current and former smokers had higher ACE2 expression compare with nonsmokers. Additionally, ACE2 was specifically expressed in different lung cell types of smokers and nonsmokers, suggesting that not only smokers might be more susceptible to COVID-19, but the viral infection path could be different between smokers and nonsmokers.[99] A recent study also found increased ACE2 expression in lower airways of smokers and individuals with chronic obstructive pulmonary disease.[100] Further investigation is required to verify if higher lung ACE2 expression is associated with greater COVID-19 susceptibility.

Individuals with pre-existing disease may be more prone to COVID-19. In vitro study showed that human lung cells exposed to inflammatory cytokines, rhinovirus, H1N1 influenza, or CoV (SARS-CoV or MERS-CoV) resulted in increased expression of ACE2.[101] These data suggest that people with viral infection and/or high inflammatory cytokine levels could be more susceptible to COVID-19. Also, increased cytokines during virus infection may further accelerate COVID-19 infection.

Another two potential risk factors for COVID-19 are obesity and cancers.[102] Research has shown that human adipose tissues express high levels of ACE2.[102] In diabetic mice, the ACE2 expressions in the serum, liver, and pancreas were up-regulated.[103] By utilizing online databases (HCCDB, UALCAN, and GEPIA2), Jia et al showed that tumor tissues of specific types of cancers appeared to have higher levels of ACE2 compared with their adjacent noncancerous tissues.[102] Here, we analyzed ACE2 expression in 41 types of cancers obtained from The Cancer Genome Atlas (TCGA) database,[104] and found that for those that showed significant differences in their ACE2 expression between tumor and reference samples, almost all were up-regulated and only four samples (out of 482) from the papillary thyroid carcinoma showed down-regulated ACE2 (Figure 2A). In summary, it seems like tumor tissues tend to have up-regulated ACE2 expression, especially in neuroendocrine prostate cancer and ovarian serous cystadenocarcinoma. Our findings in Figure 2A may have clinical implications for COVID-19. Indeed, increased risk of COVID-19 and poorer prognosis was observed in cancer patients.[105] In addition, using similar methods as described previously,[106] we compared the ACE2 expression in lung cancer patients of various smoking statuses.

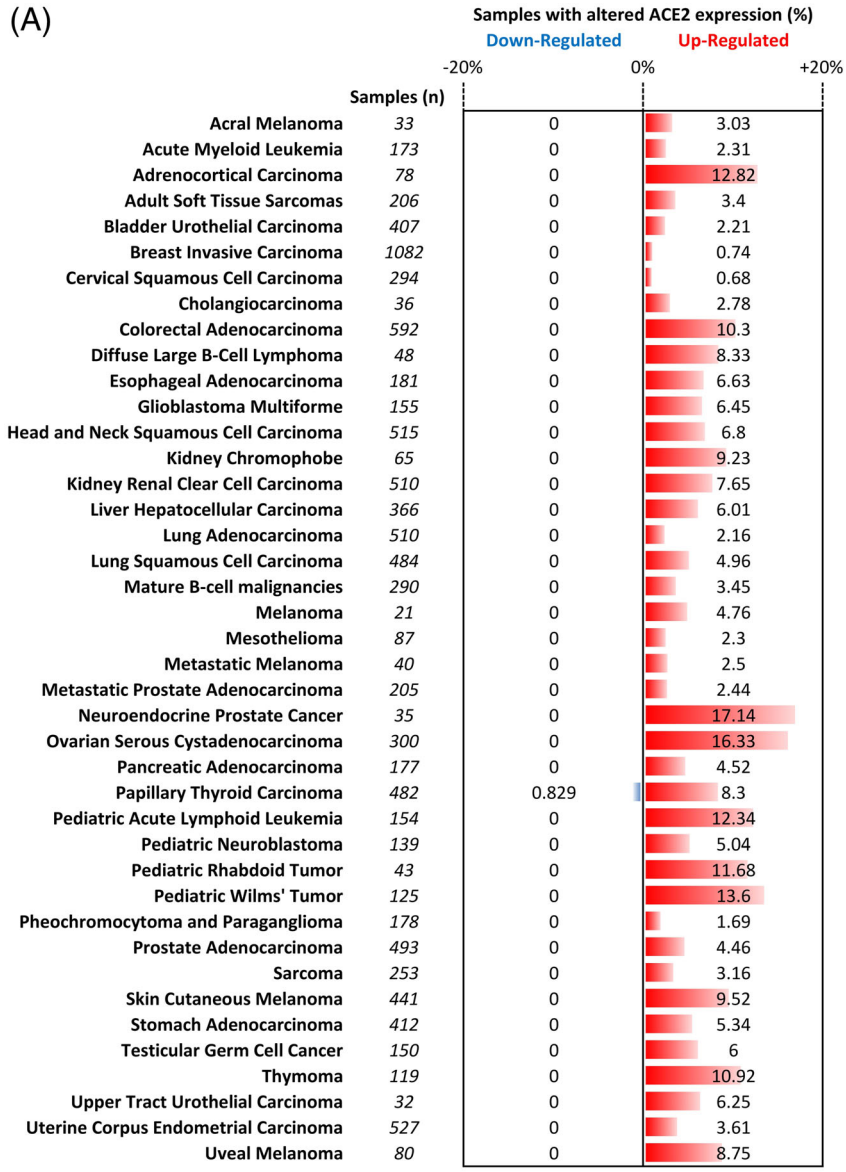

FIGURE 2 Cancer tissues and the lungs of smokers generally contained higher levels of angiotensin-converting enzyme 2 (ACE2), and these tissues can be potentially more susceptible to COVID-19. A, ACE2 expression data of 41 types of cancers obtained from The Cancer Genome Atlas (TCGA) database[104]; mRNA expression z-score threshold was set at ±1.5. B, ACE2 expression in lung cancer patients with different smoking histories analyzed using similar methods as described previously[106]

We found that smokers, including ex-smokers, generally have up-regulated ACE2 (Figure 2B). These results are to some extent in agreement with Cai's and Leung's studies[99,100] that smoking may increase lung ACE2 expression.

SARS-CoV was previously shown not only able to affect the lung but also other organs, including brain, gastrointestinal tract, kidney, liver, and more.[107,108] There is no doubt that the lung is the main target for SARS-CoV-2. However, many COVID-19 patients also showed

other symptoms in addition to respiratory symptoms, suggesting that SARS-CoV-2 could perhaps infect other organs (Figure 3). SARS-CoV-2 might infect other ACE2-expressing tissues via blood circulation as a recent report showed that SARS-CoV-2 RNA had been detected in the blood of some COVID-19 patients.[109] Intriguingly, it is recently indicated that higher circulating ACE2 is associated with milder symptoms of COVID-19, suggesting the protective role of secreted ACE2, probably by minimizing the direct contact between SARS-CoV-2 and ACE2-expressing tissues.[110] This may also explain why women and children with confirmed COVID-19 generally respond better to the disease because they appear to have higher levels of ACE2 in the blood.[110]

With a great proportion of COVID-19 patients developed renal abnormalities and acute kidney injury, the kidney seemed to be a major organ affected by the disease.[97,111,112] A consecutive cohort study of COVID-19 patients carried out by Cheng et al showed that older patients and/or those with kidney impairments had higher risks of in-hospital death.[113] The liver is another organ that may be prone to SARS-CoV-2 infection as studies have shown that ACE2 is enriched in the cholangiocytes, and liver injury is observed in some of the COVID-19 patients.[114,115] It is worth noting that although kidney and liver generally have higher ACE2 expressions than the lung, there is so far no evidence that the SARS-CoV-2 can directly infect the liver and kidney cells in the human body.

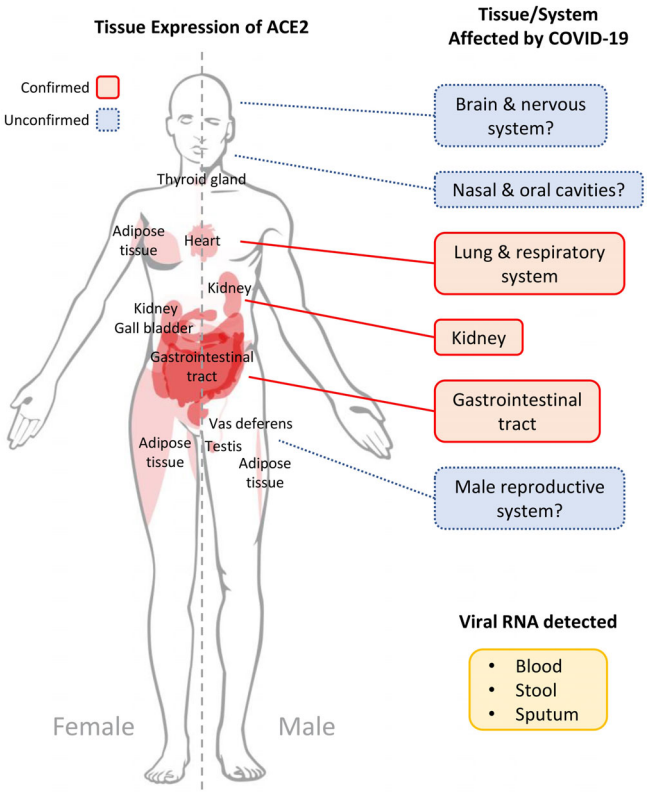

FIGURE 3 Tissue distribution of angiotensin-converting enzyme 2 (ACE2) expression and potential COVID-19 susceptibility. Organ highlighted in red represents positive ACE2 expression. Human images and ACE2 expression data were obtained from The Human Protein Atlas (http://www.proteinatlas.org)[27,96]

The gastrointestinal tract, especially the duodenum and small intestine, expressed high levels of ACE2.[27,96] Since about up to 10% of COVID-19 patients have shown gastrointestinal symptoms such as diarrhea, it is possible that SARS-CoV-2 can infect the gastrointestinal tract.[116] Clinically, the detection of viral RNA based on oral swab was used for confirmation of COVID-19 because the sputa of COVID-19 patients contained SARS-CoV-2 RNA during infection (before the onset of symptoms).[117] Interestingly, it was shown that in some cases, viral RNA could be detected in the sputum even after 2 weeks of clinical recovery.[118] In addition to sputum, SARS-CoV-2 RNA has been detected in the stools of a COVID-19 patient,[119] representing the possibility of fecal-oral transmission of SARS-CoV-2.[120] However, further tests are required to check if infectious SARS-CoV-2 is present in the stools, as well as the blood, sputa, and body fluids.

Reports regarding how COVID-19 could affect testis and brain have also emerged. Studies have suggested that COVID-19 may negatively impact male fertility[112,121] and may cause neurological symptoms and brain injury.[122,123] Furthermore, olfactory and taste disorders have recently been reported in some COVID-19 patients,[124] and whether such symptoms are consequences of neurological/brain damage or direct effects on the epithelial cells of nasal and oral mucosa by SARS-CoV-2 infection have yet to be answered. Latest studies have shown that ACE2 was highly expressed on the tongue and nasal epithelial cells, suggesting that the nasal and oral cavities could be susceptible to SARS-CoV-2 infection.[125,126]

6 | PERSPECTIVES: TREATMENT AND PREVENTION

Since the world has now become more interconnected than ever before, more frequent and faster-moving pandemics are anticipated. The ongoing COVID-19 pandemic has reminded us that the CoVs, with their rapidly evolving nature, continue to pose a serious global health threat. To combat the emerging COVID-19, several strategies have been used or proposed. However, most of the potential treatment options for COVID-19 discussed in this section have either not been assessed by randomized controlled clinical trials or are currently under clinical trials.

Hundreds of clinical trials targeting COVID-19 have been rapidly set up around the world amid COVID-19 outbreak. More than a few dozens of drugs are being tested, and some of them that attract the greatest attention include broad-spectrum antiviral remdesivir, anti-HIV protease inhibitors lopinavir/ritonavir, antimalaria chloroquine/hydroxychloroquine, and traditional Chinese medicine.[127,128] Notably, on February 15, 2020, the Chinese health authority has approved Favilavir (formerly known as fapilavir) to be used in COVID-19 treatment, making it the first official anti-COVID-19 drug. Nevertheless, the efficiencies of all COVID-19 drugs have to be properly assessed by randomized double-blind placebo-controlled studies.[129]

A healthy lifestyle is essential to keep the immune system active, and a balance host immune response is important to overcome virus infection.[1] Supplementations such as vitamin A and vitamin D could be used to strengthen the immune system and provide benefits in defending virus infection.[130,131] Specifically, regular intake of vitamin D is correlated with higher protection against respiratory tract infections.[132] Moreover, it is shown that zinc, an essential micronutrient, can inhibit viral RNA-dependent RNA polymerase of SARS-CoV and other viruses.[133] Selenium, another essential micronutrient involved in RAS and immune system,[134] has been shown to increase the immune responses of Se deficient patients and animal models upon CoV and other viral infections.[135,136]

In the century-long of war against viruses, vaccination is considered one of the most effective proactive measures to prevent the spread of viral diseases. Previously, multiple attempts have been made to create CoV vaccines, but these vaccines mainly only focused on SARS-CoV or MERS-CoV and have not yet undergone clinical trials.[56,137] However, in just less than 2 months after COVID-19 is declared a global pandemic, a few SARS-CoV-2 vaccines are already being trialed in humans as scientists in different countries raced to create the first effective COVID-19 vaccine. Spike protein of CoV is a major inducer of host immune responses and the main target of neutralizing antibodies during infection.[37] Recently, potential T- and B-cell epitopes of SARS-CoV-2 for vaccines and antibody-based neutralization have been identified using structural bioinformatic and machine learning techniques—the spike protein RBD region of 494-508 appear to be the best vaccine candidate.[138] DNA/RNA vaccines will likely be the most cost-effective form of vaccination against the fast mutating CoVs, if successfully developed. The fundamental idea of DNA- or RNA-based vaccine is to induce host primary immune response by injecting DNA/RNA that encodes a viral protein (eg, spike protein of CoV) into the body, and let the body cells produce the mimic protein for antibody stimulation and immunological memory.[139,140]

Based on the past success with HIV-1 treatment, gene therapy-based strategy to combat COVID-19 could also be considered.[141] For instance, lung cells that targeted by SARS-CoV-2 could be gene-edited to become COVID-19 resistant by knockdown or knockout of SARS-CoV-2 pathogenesis-related host gene(s) or permanent gene disruption of the CoV genome. Also, peptide mimetics or small-molecule inhibitors have been developed to block viral entry or to suppress viral infection, and similar strategies can also be applied to COVID-19.[142] Particularly, targeted immunotherapy using small-molecule inhibitors to prevent cytokine storm syndrome has emerged as a potent treatment option for COVID-19 patients.[143] The ultimate objective of targeted immunotherapy is to optimize the cytokine productions and inflammatory responses upon viral infections.[1] Previously, ACE2 inhibitors have been tested in SARS-CoV and HCoV-NL63, but their antiviral activity against ACE2-utilizing CoVs has remained unknown.[144,145] Although it is possible to create an ACE2 inhibitor that targeting the ACE2 binding region of CoVs or create a recombinant human ACE2 (rhACE2) to trap SARS-CoV-2, the effects of these inhibitors or rhACE2 on the RAS should also be evaluated carefully. Studies showed that the use of serine protease inhibitors to block TMPRSS2 could increase survival in mice upon SARS-CoV infection and reduce the entry of SARS-CoV and SARS-CoV-2 from entering the human cells.[57,146] However, inhibiting host proteases will likely lead to adverse side effects, and therefore, this approach is considerably less attractive than targeting viral proteases. Recently, the crystal structure of SARS-CoV-2 main protease was determined, and this would facilitate the development of viral protease inhibitors specifically targeting SARS-CoV-2.[147] Lastly, reports have suggested that convalescent plasma collected from recovered COVID-19 patients could be used to treat patients in critical conditions, and this method should be further explored.[148]

To conclude, there are still many unknowns regarding COVID-19. Under the existing health emergency, critical knowledge of COVID-19 such as length of SARS-CoV-2 incubation period, route of transmission, viability outside a host, and intermediate host are urgently needed. Since there is currently no effective vaccine or treatment for COVID-19, several precautions should be taken to minimize the spread of this highly contagious virus. For individuals: avoid crowded places, keep distance with and wear fitted mask when sick people are around, maintain personal hygiene, wash hands regularly, avoid touching eyes, nose, and mouth, and stay isolated and seek medical assistance if show suspicious symptoms or happen to get in close contact with sick people; for relevant authorities: identify the source of infection and potential threats, provide sufficient diagnostic tools, offer accessible medical support, advertise proper public health knowledge, rapid update on epidemic information, and drastic measures such as temporary community lock-down may be necessary. Lastly, contact with wild animals should be regulated to prevent future SARS-like outbreaks.

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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