Renin-Angiotensin-Aldosterone System: Double-Edged Sword in COVID-19 infection

Jana S. Burchfield, Ph.D.

Division of Radiation Oncology, The University of Texas MD Anderson Cancer Center

Mailing Address: 1515 Holcombe Blvd, Houston, TX 77030

Email: jburc138@gmail.com

Abstract

The role of the Renin-Angiotensin-Aldosterone System (RAAS) in Corona Virus Disease 2019 (COVID-19) infection has become a controversial topic of discussion. RAAS inhibitors, such as Angiotensin Converting Enzyme (ACE) inhibitors and Angiotensin II receptor blockers (ARBs), which are used to treat cardiovascular diseases, have been implicated in potentially increasing cell surface levels of ACE2. ACE2 is the host receptor for COVID-19 that was discovered in Wuhan, China in December 2019. Since December, COVID-19 has transmitted rapidly across the world and has become a global pandemic. COVID-19 is similar to the Middle East respiratory syndrome coronavirus (MERS-CoV) with the first case reported in Saudi Arabia in September 2012. COVID-19, also known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is also similar to SARS-CoV, which first infected humans in the Guangdong province of southern China in 2002, and caused an epidemic between November 2002 and July 2003. Both SARS-CoV and COVID-19 use ACE2 to enter host cells. ACE2 is primarily expressed in the mouth, lung, heart, esophagus, kidney, bladder, and intestines, and is a component of RAAS, which serves to maintain vascular tone and blood volume. Inhibition or activation of other components of RAAS has been shown to directly increase or decrease the expression and/or activity of ACE2. Furthermore, RAAS-targeting therapeutics, such as ACE inhibitors and ARBs, have also been shown to regulate the expression and/or activity of ACE2, albeit in animal models. Although these changes in ACE2 have been demonstrated only in animal models, there is no evidence that administration of RAAS-targeting therapeutics to humans for the treatment of hypertension, diabetes, and other cardiovascular diseases (e.g., myocardial infarction and heart failure) causes changes in ACE2 expression. Nor is there clinical evidence that RAAS-targeting therapeutics augment COVID-19 infection, morbidity, or mortality. However, clinical evidence does suggest that ACE2 expression may protect against respiratory distress caused by a variety of noxious agents. This review attempts to provide a balanced overview of the potential role of RAAS in regulating ACE2, and the role of ACE2 during COVID-19 infection. Evidence is provided to show that the expression of ACE2 may mediate both positive and negative outcomes, depending on the timing of ACE2 expression.

Keywords

Clinical Characteristics of COVID-19-infected Patients

Since the outbreak of Corona Virus Disease 2019 (COVID-19), also known as Severe Acute Respiratory Syndrome 2 (SARS-CoV-2), in Wuhan, China that is now spreading throughout the world, the clinical characteristics of patients and their risks for major complications and death have been reported from several clinical studies.[1, 2] After COVID-19 infection, patients commonly present with fever (88.5%), cough (68.6%), myalgia or fatigue (35.8%), expectoration (28.2%), and dyspnea (21.9%).[3] In addition, some patients experience gastrointestinal symptoms.[4] Although many patients present with mild symptoms, some patients suffer severe complications and have an increased risk of death. A recent, comprehensive analysis of six clinical studies, which comprised 783 patients,[5] revealed patient fatality was approximately 2%, primarily due to acute respiratory distress syndrome (ARDS), acute kidney injury, or myocardial injury, and the major complications leading to patient fatality appear to be related to pre-existing cardiovascular diseases. One of the earliest studies in Wuhan that assessed 41 COVID-19-infected patients, who had been infected and identified before January 2, 2020, revealed that of the 41 COVID-19 patients, eight had diabetes (20%), six had hypertension (15%), and six had unspecified cardiovascular disease (15%).[1] In a study of 26 patient fatalities, 53.8% of these patients had hypertension, 42.3% diabetes, and 19.2% coronary heart disease, and these major comorbidities were associated with an increased risk of mortality.[6] In a retrospective study of COVID-19 patients, who were hospitalized in Wuhan Jinyintan Hospital from January 1, 2020 to January 20, 2020 and followed up on January 25, 2020, 50 (51%) of the 99 patients had chronic diseases, including cardiovascular diseases, and 17 (17%) of patients developed ARDS, with subsequent rapid decline of 11 (11%) to multiple organ failure and death.[7] Death also occurred in 44 (52.4%) of the 84 COVID-19-infected ARDS patients who were hospitalized at the Wuhan Jinyintan Hospital between December 25, 2019 and January 26, 2020 and followed up on February 13, 2020. Of those 84 ARDS patients, a significant number of patients had comorbidities, such as hypertension (27.4%; 23 of 84 patients) and diabetes (19%; 16 of 84 patients).[8] Precisely why these cardiovascular comorbidities are associated with increased complications and/or increased mortality rates for patients infected with COVID-19 is not known. As COVID-19 is highly transmissible and exceptionally pathogenic and, now, threatening the world population, there is an urgent need in understanding these mechanisms in order to expedite the development of effective treatments for COVID-19 infections.

Binding of COVID-19 to ACE2

COVID-19 is a beta coronavirus that is homologous to SARS-CoV, which was identified in 2003 and caused an epidemic in 26 countries. Similar to SARS-CoV, COVID-19 binds to host angiotensin-converting enzyme 2 (ACE2),[9, 10] but with much higher affinity.[11] ACE2 is expressed in the mucosa of the oral cavity, specifically enriched in epithelial cells of the tongue[12] as well as lung, heart, esophagus, kidney, bladder, and intestinal ileum.[13] SARS-CoV infection of human airway epithelia is associated with ACE2 expression,[14] and in autopsy samples from patients who died of SARS, SARS-CoV RNA and protein could be detected only in cells that expressed ACE2.[15] SARS-CoV cleaves ACE2 into the cell, where it is subsequently degraded.[16] Furthermore, the expression pattern of ACE2 seems to correlate with cellular susceptibility of SARS-CoV infection,[17] and in mice deficient of ACE2, infection by SARS-CoV was abrogated.[18] Susceptibility of infection by the coronavirus NL63 also correlates with ACE2 expression;[19] however, whether ACE2 expression levels and patterns similarly correlate with COVID-19 infection has not been confirmed.

Role of ACE2 within the Renin-Angiotensin-Aldosterone System

ACE2 is a component of the renin-angiotensin-aldosterone system (RAAS), which functions to maintain blood volume and vascular tone. RAAS is frequently targeted by therapeutics used to treat hypertension, diabetes, and other cardiovascular diseases (e.g., acute myocardial infarction and heart failure). During a normal, physiological response to low blood pressure, renin is released from the kidney into the blood cleaving its target angiotensinogen into Angiotensin (Ang) I. Angiotensin-converting enzyme (ACE) cleaves Ang I to physiologically active Ang II that signals to type 1 angiotensin II receptor (AT1R) or type 2 angiotensin II receptor (AT2R) to mediate vasoconstriction. Ang II also stimulates the release of aldosterone, a steroid hormone, which increases salt reabsorption for increased blood volume. In addition, Ang II serves as a substrate of ACE2. Although ACE2 shares 44% homology with ACE, ACE inhibitors do not inhibit ACE2. ACE inhibitors (e.g., aptopril, enalapril, lisinopril) decrease the generation of Ang II, thereby reducing the amount of Ang II available for AT1R binding and ultimately, reducing blood pressure. Angiotensin receptor blockers (i.e., AT1R), such as olmesartan, valsartan, losartan, also inhibit Ang II-mediated vasoconstriction. Aldosterone inhibitors (e.g., spironolactone, eplerenone, amiloride) control vascular tone by affecting sodium channels for sodium reabsorption in the kidneys to maintain blood volume. RAAS-targeted therapeutics are becoming major topics for discussion surrounding the COVID-19 pandemic.

These RAAS-targeting therapeutics have become cost-effective and are commonly used in the management of hypertension, diabetes, acute myocardial infarction, and heart failure throughout the world. Pre-existing and persistent use of these RAAS-targeting therapeutics by COVID-19 patients before infection may be associated with poor clinical outcomes. Eighty-four patients (41.8%) of 201, who were infected with COVID-19, developed ARDS, and 44 (52.4%) of these patients died.[8] Of the 84 patients who developed ARDS, 23 of 84 [27.4%] patients had pre-existing hypertension and 16 of 84 [19.0%] patients had diabetes. However, 117 of the 201 patients did not develop ARDS and had hypertension or diabetes (16 of 117 [13.7%] patients and 6 of 117 [5.1%] patients, respectively). It is not known whether these patients with hypertension or diabetes were treated and managed with different classes of drugs before COVID-19 infection. Specifically, it is not known whether the COVID-19-infected patients in China who had major complications had previously received aldosterone inhibitors, ACE inhibitors, or AT1R blockers.

Deleterious role of the Renin-Angiotensin-Aldosterone System in COVID-19 Infection

ACE inhibitors and AT1R blockers have been shown to increase ACE2 activity and/or expression in kidneys,[20] hearts,[21, 22] and intestines[23] of animal models, and aldosterone antagonists upregulate ACE2 expression in renal disease.[24] This increase in ACE2 expression may lead to a greater density of ACE2 molecules on the host cell surface available for COVID-19 binding as well as an increase in ACE2 on different cell types (e.g., endothelial cells, cardiac myocytes, lung epithelial cells, intestinal epithelial cells, etc.). This increase in ACE2 availability could enhance the efficiency of COVID-19 host cell membrane fusion and explain how COVID-19 might affect seemingly similar hosts differently. Indeed, expression levels of ACE2 were shown to mediate acute lung injury from the influenza H7N9 virus.[25] Furthermore, aggregates of viruses can infect the same cell. This can increase cellular multiplicity of infection, expedite the early stages of infection, which can provide short-term evolutionary fitness of a virus, and release multiple viral genomes into one cell.[26] Although not yet proven, it has been suggested that two different strains of COVID-19 can infect the same cell and undergo genetic

recombination,[27] which could lead to the acquisition of new drug-resistance or drug-susceptibility traits, a stealthier strain of COVID-19, or a strain undetectable by current diagnostics tests. It is plausible that the cardiovascular diseases of COVID-19-infected patients in China, and elsewhere, had been managed with RAAS-targeting therapeutics that may have altered ACE2 expression and/or activity, and subsequently the pathogenesis and disease severity.

Genetic Variations in ACE2

The increased risk of major complications and mortality in some patients may also be contributed to genetic variations in ACE2 that may lead to differences in ACE2 expression in certain human populations. In one study of only eight normal and healthy human lung samples, single-cell RNA sequencing suggested that the sample from the only Asian individual had more lung cells expressing ACE2 than samples from Caucasians and African Americans.[28] Interestingly, this study revealed that ACE2-expressing lung cells also highly express viral infectious cycle genes. Conversely, another study analyzing RNA sequencing and microarray datasets showed no differences in ACE2 gene expression between Asians and Caucasians, but found differences in nonsmoker samples compared to smoker samples and former smoker samples.[29] In a rat model of smoke exposure-induced ARDS, ACE2 expression was augmented.[30] Whether the lungs of smokers of electronic nicotine delivery systems also have changes in ACE2 expression is not known, but nicotine, itself, alters RAAS components,[31] and whether smoking is associated with COVID-19 infection and clinical outcomes is not yet clear.[32]

Investigation of allele frequency differences between East Asians, Europeans, Africans, South Asians, and Mixed Americans found that diverse genetic backgrounds could affect ACE2 function in different populations. Using genetic analysis of expression quantitative trait loci, the authors demonstrated that the genotypes of *ACE2* gene polymorphisms may be associated higher expression levels of ACE2 in East Asians.[33] These data would suggest that the COVID-19 patients treated in Wuhan, China are more susceptible to COVID-19 infection, morbidity, and mortality. In an analysis of susceptibility to SARS-CoV infection, single-nucleotide polymorphisms in ACE2 were measured, but there was no association with common genetic variants of ACE2 and SARS-CoV susceptibility or outcomes.[34] Furthermore, the current, high rate of mortality from COVID-19 infection in Italy compared with Wuhan, China would suggest that ACE2 genotype is not be the only factor in determining the lethality of COVID-19. Unfortunately, the genetic analysis by Cao Y et. al. did not find any natural resistant mutations in ACE2 that would inhibit or limit COVID-19 binding.[33]

ACE2 expression in Cardiovascular Diseases

Alternatively, differences in ACE2 expression levels may be dependent on pre-existing pathological health conditions, regardless of ACE2 genetic polymorphisms. Complications and morbidity from COVID-19 infection have been associated with pre-existing cardiovascular diseases, and differences in ACE2 expression have been shown in these diseases. ACE2 expression is increased in failing human heart.[35] This cardiac ACE2 expression in mice can mediate infection of the heart upon pulmonary infection of human SARS-CoV.[36] ACE2 has been shown to be important in cardiac contractility. In animal models of diabetes, kidney ACE2 activity concurrently increases along with ACE2 protein expression.[37] [38] Interestingly, endothelial cells, which express high levels of ACE2 , have been shown not to be infected by SARS-CoV. These findings suggest that ACE2 can be upregulated in chronic cardiovascular conditions, and thus, may contribute to major complications in COVID-19-infected patients.

Conversely, in untreated hypertension and after myocardial infarction, ACE2 appears to be downregulated. Preclinical studies have shown that 4-week infusion of Ang II downregulates ACE2 activity[39] and leads to ACE2 cellular internalization and degradation via ATR1.[40] Ang II can also downregulate ACE2 gene expression in a time- and dose-dependent manner via ATR1.[41] This suggests that the balance of the generation of Ang II by ACE and the utilization of Ang II by ACE2 to generate Ang(1-7) is crucial for normal physiological conditions. In the pathological condition of hypertension, the ACE to ACE2 ratio is higher in human renal tissue from subjects with hypertension compared to those without hypertension.[42] The ability of Ang II to upregulate ACE, but directly downregulate ACE2 gene expression may be a mechanism for the downregulation of ACE2 protein in the kidneys of several hypertensive animal models.[43] In animal models of myocardial infarction, ACE2 activity and mRNA levels were also decreased in hearts,[44] but this was prevented by the ACE inhibitor enalapril.[44] However, this prevention of ACE2 downregulation may be dependent on the specific RAAS-targeting therapeutic, because a different ACE inhibitor ramipril had no effect on ACE2 gene expression in a similar model.[45] Administration of telmisartan, an AT1R blocker, to a spontaneously hypertensive rat model reduced aortic ACE2 levels.[46] However, both losartan and olmesartan, two other ATR1 blockers, increased ACE2 gene expression.[22] These findings suggest that ACE2 may serve as a protective response to tissue injury or disease and the use of RAAS-targeted therapeutics may reduce or eliminate the beneficial effects of ACE2. Identifying which therapeutics result in changes in ACE2 expression could be important in lieu of the COVID-19 pandemic.

COVID-19 infection results in fatality rates that strongly associate with age. Fatality rates due to COVID-19 infection are less than 0.4% for individuals less than 49 years of age, but increase to 1.3% for individuals between the ages of 50 to 59, 3.6% (60 to 69), 8% (70 to 79), 14.8% (≥80).[47] Advanced age is also a major risk factor for developing cardiovascular diseases. Although the associations between age and ACE2 expression have not been shown in humans, ACE2 expression decreases with aging in rats and mice.[48] [49] Upon SARS-CoV infection of aged mice, genes of the immune system, cytokines, and genes associated with ARDS were induced, and the mice that died had decreased levels of ACE2.[50] A decrease in ACE2 expression in elderly and middle-aged COVID-19-infected patients would seem counterintuitive if the levels of ACE2 are associated with major complications and higher mortality rate in COVID-19-infected patients. However, the major complications and higher mortality rate may be due, in part, to when ACE2 is expressed on cells. Increased ACE2 expression on cell surfaces at the time of COVID-19 infection may lead to worse clinical outcomes, unless higher levels of ACE2 in COVID-19-affected organs after the initial onset of illness are present to protect patients and improve outcomes. Thus, in the elderly or middle-aged, wherein ACE2 levels may be lower, the protective role of ACE2 during disease progression may be lost.

Protective role of the Renin-Angiotensin-Aldosterone System in COVID-19 Infection

Numerous studies have implicated a protective role for ACE2 in respiratory illnesses. ACE2 mRNA and protein levels have been shown to increase during the early stages of pulmonary hypoxia,[51] and ACE2 has been shown to protect against acute lung injury in several animal models of ARDS.[52] Therefore, promoting ACE2 expression seems reasonable; however, upon SAR-CoV infection, in which COVID-19 infection is likely to follow similar mechanisms, ACE2 is cleaved and undergoes shedding. Therefore, its protective effects may be lost. Indeed, decreased ACE2 expression correlates with the lethal outcome of SARS-CoV infection-induced

lung injury.[50] It has been postulated that a soluble form of ACE2 may compete for binding with SARS-CoV;[53] thus, a recombinant human ACE2, APN01, has been designed to compete with cell membrane-bound ACE2 and is now being tested to treat COVID-19-infected patients. However, if COVID-19 has an incubation period of approximately 5 days before symptoms begin, a competitive ACE2 receptor would unlikely be administered at the time of COVID-19 infection, when it may be most effective.

As targeting ACE2 for COVID-19 infection is still early in development, it would be more expeditious to repurpose already approved RAAS-targeting therapeutics.[54] Based on recent evidence that higher levels of plasma Ang II may be associated with viral load and lung injury in COVID-19-infected patients, it was suggested that RAAS-targeted therapeutics could be used to treat COVID-19 infection.[55] The balance between ACE and ACE2 is critical for regulating Ang II levels, and both ACE and ACE2 play a significant role in ARDS.[56] AT1R blockers also have been shown to protect against acute lung injury after infection.[18, 52] In some, but not all studies, administration of ACE inhibitors or AT1R blockers at the time of pneumonia onset was shown to reduce the risk of complications and mortality in patients with community-acquired pneumonia.[57 58 59] However, whether ACE inhibitors or AT1R blockers would mitigate COVID-19-mediated development of ARDS is not known, and their efficacy may be dependent on the specific noxious agent. Studies using RAAS-targeting therapeutics for the treatment ARDS have been reviewed elsewhere.[60]

The efficacy of RAAS-targeting therapeutics may be dependent on timing of expression and/or modulation of RAAS components. In an animal model of pneumonia, caused by the bacteria *Pseudomonas aeruginosa*, pre-existing and persistent ACE2 activity decreased neutrophil infiltration into the lungs,[61] thus, compromising the host response to the bacteria. In this bacterial pneumonia model, the timing of ACE2 activity and ACE2 mRNA and protein expression was dynamic. ACE2 activity and ACE2 protein expression dramatically decreased on day 1 after bacteria inoculation and ACE2 mRNA expression decreased at day 2, whereas ACE2 activity, mRNA, and protein levels increased on day 3 after bacteria inoculation. This dynamic variation in ACE2 was required to induce the correct timing of neutrophil infiltration into the lungs. Timing of a host immune response is dictated by aging and has been shown to be important in the host response against SARS-CoV.[50] In young mice, a rather late immune response (72 hours post inoculation) to SARS-CoV infection was observed compared to aged mice (24 hours post inoculation). This study suggested that an early and robust host immune response (e.g., cytokine storm) against SARS-CoV was detrimental, which may be similar to the responses observed in patients with COVID-19 infection. Therefore, the timing of administration of RAAS-targeted therapeutics may be key in modulating a sufficient versus exacerbating immune response to COVID-19. It is plausible that persistent, long-term administration of RAAS-targeted therapeutics before COVID-19 infection may reduce or eliminate the beneficial effects of the ACE2 pathway after COVID-19 infection. Perhaps administering RAAS-targeted therapeutics within a therapeutic time window after COVID-19 infection may help modulate and temper the immune response, especially in older COVID-19-infected patients.

In conclusion, although targeting ACE2 either directly or indirectly is a reasonable approach to treating COVID-19 infection, ACE2 may not be the only molecule involved in COVID-19 infection. In addition, for SARS-CoV, and perhaps for COVID-19, other tissue-specific virus receptors or co-receptors may be required for infection. SARS-CoV, as well as COVID-19, may

even infect cells without ACE2 expression.[62] Regardless, careful assessment of the timing of administration of our currently available RAAS-targeting therapeutics is warranted and may be the fastest path to win the fight against the COVID-19 pandemic.

Conflict of interest
Author declares no conflict of interests

References

1.   Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J and Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395:497-506.

2.   Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, Li SB, Wang HY, Zhang S, Gao HN, Sheng JF, Cai HL, Qiu YQ and Li LJ. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. *Bmj*. 2020;368:m606.

3.   Li LQ, Huang T, Wang YQ, Wang ZP, Liang Y, Huang TB, Zhang HY, Sun WM and Wang YP. 2019 novel coronavirus patients' clinical characteristics, discharge rate and fatality rate of meta-analysis. *J Med Virol*. 2020.

4.   Xiao F, Tang M, Zheng X, Liu Y, Li X and Shan H. Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology*. 2020.

5.   Jiang F, Deng L, Zhang L, Cai Y, Cheung CW and Xia Z. Review of the Clinical Characteristics of Coronavirus Disease 2019 (COVID-19). *Journal of General Internal Medicine*. 2020.

6.   Deng SQ and Peng HJ. Characteristics of and Public Health Responses to the Coronavirus Disease 2019 Outbreak in China. *J Clin Med*. 2020;9.

7.   Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X and Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395:507-513.

8.   Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y, Song J, Wang S, Chao Y, Yang Z, Xu J, Zhou X, Chen D, Xiong W, Xu L, Zhou F, Jiang J, Bai C, Zheng J and Song Y. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med*. 2020.

9.   Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, Si H-R, Zhu Y, Li B, Huang C-L, Chen H-D, Chen J, Luo Y, Guo H, Jiang R-D, Liu M-Q, Chen Y, Shen X-R, Wang X, Zheng X-S, Zhao K, Chen Q-J, Deng F, Liu L-L, Yan B, Zhan F-X, Wang Y-Y, Xiao G-F and Shi Z-L. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579:270-273.

10.   Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W and Tan W. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020;395:565-574.

11.   Chen Y, Guo Y, Pan Y and Zhao ZJ. Structure analysis of the receptor binding of 2019-nCoV. *Biochem Biophys Res Commun*. 2020.

12.   Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, Li T and Chen Q. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci*. 2020;12:8.

13.   Zou X, Chen K, Zou J, Han P, Hao J and Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med*. 2020.

14.   Jia HP, Look DC, Hickey M, Shi L, Pewe L, Netland J, Farzan M, Wohlford-Lenane C, Perlman S and McCray PB, Jr. Infection of human airway epithelia by SARS coronavirus is associated with ACE2 expression and localization. *Adv Exp Med Biol*. 2006;581:479-84.

15.   He L, Ding Y, Zhang Q, Che X, He Y, Shen H, Wang H, Li Z, Zhao L, Geng J, Deng Y, Yang L, Li J, Cai J, Qiu L, Wen K, Xu X and Jiang S. Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2+ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. *J Pathol*. 2006;210:288-97.

16.   Glowacka I, Bertram S, Herzog P, Pfefferle S, Steffen I, Muench MO, Simmons G, Hofmann H, Kuri T, Weber F, Eichler J, Drosten C and Pöhlmann S. Differential Downregulation of ACE2 by the Spike Proteins of Severe Acute Respiratory Syndrome Coronavirus and Human Coronavirus NL63. *Journal of Virology*. 2010;84:1198-1205.

17.   Nie Y, Wang P, Shi X, Wang G, Chen J, Zheng A, Wang W, Wang Z, Qu X, Luo M, Tan L, Song X, Yin X, Chen J, Ding M and Deng H. Highly infectious SARS-CoV pseudotyped virus reveals the cell tropism and its correlation with receptor expression. *Biochem Biophys Res Commun*. 2004;321:994-1000.

18.   Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C and Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med*. 2005;11:875-9.

19.   Hofmann H, Pyrc K, van der Hoek L, Geier M, Berkhout B and Pohlmann S. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proc Natl Acad Sci U S A*. 2005;102:7988-93.

20.   Ferrario CM, Jessup J, Gallagher PE, Averill DB, Brosnihan KB, Ann Tallant E, Smith RD and Chappell MC. Effects of renin-angiotensin system blockade on renal angiotensin-(1-7) forming enzymes and receptors. *Kidney Int*. 2005;68:2189-96.

21.   Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, Diz DI and Gallagher PE. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation*. 2005;111:2605-10.

22.   Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB and Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension*. 2004;43:970-6.

23.   Vuille-dit-Bille RN, Camargo SM, Emmenegger L, Sasse T, Kummer E, Jando J, Hamie QM, Meier CF, Hunziker S, Forras-Kaufmann Z, Kuyumcu S, Fox M, Schwizer W, Fried M, Lindenmeyer M, Gotze O and Verrey F. Human intestine luminal ACE2 and amino acid transporter expression increased by ACE-inhibitors. *Amino Acids*. 2015;47:693-705.

24.   Kong EL, Zhang JM, An N, Tao Y, Yu WF and Wu FX. Spironolactone rescues renal dysfunction in obstructive jaundice rats by upregulating ACE2 expression. *J Cell Commun Signal*. 2019;13:17-26.

25. Yang P, Gu H, Zhao Z, Wang W, Cao B, Lai C, Yang X, Zhang L, Duan Y, Zhang S, Chen W, Zhen W, Cai M, Penninger JM, Jiang C and Wang X. Angiotensin-converting enzyme 2 (ACE2) mediates influenza H7N9 virus-induced acute lung injury. *Sci Rep*. 2014;4:7027.

26. Andreu-Moreno I and Sanjuan R. Collective Viral Spread Mediated by Virion Aggregates Promotes the Evolution of Defective Interfering Particles. *mBio*. 2020;11.

27. Yi H. 2019 novel coronavirus is undergoing active recombination. *Clin Infect Dis*. 2020.

28. Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y and Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. *bioRxiv*. 2020:2020.01.26.919985.

29. Cai G. Tobacco-Use Disparity in Gene Expression of ACE2, the Receptor of 2019-nCov. *Preprints* 2020.

30. Yilin Z, Yandong N and Faguang J. Role of angiotensin-converting enzyme (ACE) and ACE2 in a rat model of smoke inhalation induced acute respiratory distress syndrome. *Burns*. 2015;41:1468-77.

31. Oakes JM, Fuchs RM, Gardner JD, Lazartigues E and Yue X. Nicotine and the renin-angiotensin system. *Am J Physiol Regul Integr Comp Physiol*. 2018;315:R895-r906.

32. Cai H. Sex difference and smoking predisposition in patients with COVID-19. *Lancet Respir Med*. 2020.

33. Cao Y, Li L, Feng Z, Wan S, Huang P, Sun X, Wen F, Huang X, Ning G and Wang W. Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE2 in different populations. *Cell Discovery*. 2020;6:11.

34. Chiu RWK, Tang NLS, Hui DSC, Chung GTY, Chim SSC, Chan KCA, Sung Y-m, Chan LYS, Tong Y-k, Lee W-s, Chan PKS and Lo YMD. ACE2 Gene Polymorphisms Do Not Affect Outcome of Severe Acute Respiratory Syndrome. *Clinical Chemistry*. 2004;50:1683-1686.

35. Goulter AB, Goddard MJ, Allen JC and Clark KL. ACE2 gene expression is up-regulated in the human failing heart. *BMC Med*. 2004;2:19.

36. Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM and Butany J. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur J Clin Invest*. 2009;39:618-25.

37. Wysocki J, Ye M, Soler MJ, Gurley SB, Xiao HD, Bernstein KE, Coffman TM, Chen S and Batlle D. ACE and ACE2 Activity in Diabetic Mice. *Diabetes*. 2006;55:2132-2139.

38. Danilczyk U and Penninger JM. Angiotensin-converting enzyme II in the heart and the kidney. *Circ Res*. 2006;98:463-71.

39. Bai F, Pang XF, Zhang LH, Wang NP, McKallip RJ, Garner RE and Zhao ZQ. Angiotensin II AT1 receptor alters ACE2 activity, eNOS expression and CD44-hyaluronan interaction in rats with hypertension and myocardial fibrosis. *Life Sci*. 2016;153:141-52.

40. Deshotels MR, Xia H, Sriramula S, Lazartigues E and Filipeanu CM. Angiotensin II mediates angiotensin converting enzyme type 2 internalization and degradation through an angiotensin II type I receptor-dependent mechanism. *Hypertension*. 2014;64:1368-1375.

41. Gallagher PE, Chappell MC, Ferrario CM and Tallant EA. Distinct roles for ANG II and ANG-(1-7) in the regulation of angiotensin-converting enzyme 2 in rat astrocytes. *Am J Physiol Cell Physiol*. 2006;290:C420-6.

42. Wakahara S, Konoshita T, Mizuno S, Motomura M, Aoyama C, Makino Y, Kato N, Koni I and Miyamori I. Synergistic expression of angiotensin-converting enzyme (ACE) and ACE2 in human renal tissue and confounding effects of hypertension on the ACE to ACE2 ratio. *Endocrinology*. 2007;148:2453-7.

43. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, Oliveira-dos-Santos AJ, da Costa J, Zhang L, Pei Y, Scholey J, Ferrario CM, Manoukian AS, Chappell MC, Backx PH, Yagil Y and Penninger JM. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*. 2002;417:822-8.

44. Ocaranza MP, Godoy I, Jalil JE, Varas M, Collantes P, Pinto M, Roman M, Ramirez C, Copaja M, Diaz-Araya G, Castro P and Lavandero S. Enalapril attenuates downregulation of Angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. *Hypertension*. 2006;48:572-8.

45. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, Tikellis C, Grant SL, Lew RA, Smith AI, Cooper ME and Johnston CI. Myocardial infarction increases ACE2 expression in rat and humans. *Eur Heart J*. 2005;26:369-75; discussion 322-4.

46. Zhong JC, Ye JY, Jin HY, Yu X, Yu HM, Zhu DL, Gao PJ, Huang DY, Shuster M, Loibner H, Guo JM, Yu XY, Xiao BX, Gong ZH, Penninger JM and Oudit GY. Telmisartan attenuates aortic hypertrophy in hypertensive rats by the modulation of ACE2 and profilin-1 expression. *Regul Pept*. 2011;166:90-7.

47. Team NCPERE. Vital Surveillances: the epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19). *China CDC Weekly*. 2020.

48. Xie X, Chen J, Wang X, Zhang F and Liu Y. Age- and gender-related difference of ACE2 expression in rat lung. *Life Sci*. 2006;78:2166-71.

49. Yoon HE, Kim EN, Kim MY, Lim JH, Jang IA, Ban TH, Shin SJ, Park CW, Chang YS and Choi BS. Age-Associated Changes in the Vascular Renin-Angiotensin System in Mice. *Oxid Med Cell Longev*. 2016;2016:6731093.

50. Rockx B, Baas T, Zornetzer GA, Haagmans B, Sheahan T, Frieman M, Dyer MD, Teal TH, Proll S, van den Brand J, Baric R and Katze MG. Early upregulation of acute respiratory distress syndrome-associated cytokines promotes lethal disease in an aged-mouse model of severe acute respiratory syndrome coronavirus infection. *J Virol*. 2009;83:7062-74.

51. Zhang R, Wu Y, Zhao M, Liu C, Zhou L, Shen S, Liao S, Yang K, Li Q and Wan H. Role of HIF-1alpha in the regulation ACE and ACE2 expression in hypoxic human pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol*. 2009;297:L631-40.

52. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui CC, Hein L, Uhlig S, Slutsky AS, Jiang C and Penninger JM. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. 2005;436:112-6.

53. Batlle D, Wysocki J and Satchell K. Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? *Clinical Science*. 2020;134:543-545.

54. Gurwitz D. Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. *Drug Dev Res*. 2020.

55. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, Wang Z, Li J, Li J, Feng C, Zhang Z, Wang L, Peng L, Chen L, Qin Y, Zhao D, Tan S, Yin L, Xu J, Zhou C, Jiang C and Liu L. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci*. 2020;63:364-374.

56. Kaparianos A and Argyropoulou E. Local renin-angiotensin II systems, angiotensin-converting enzyme and its homologue ACE2: their potential role in the pathogenesis of chronic obstructive pulmonary diseases, pulmonary hypertension and acute respiratory distress syndrome. *Curr Med Chem*. 2011;18:3506-15.

57.   Etminan M, Zhang B, Fitzgerald M and Brophy JM. Do angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers decrease the risk of hospitalization secondary to community-acquired pneumonia? A nested case-control study. *Pharmacotherapy*. 2006;26:479-82.

58.   Rafailidis PI, Matthaiou DK, Varbobitis I and Falagas ME. Use of ACE inhibitors and risk of community-acquired pneumonia: a review. *Eur J Clin Pharmacol*. 2008;64:565-73.

59.   Mortensen EM, Restrepo MI, Anzueto A and Pugh J. The impact of prior outpatient ACE inhibitor use on 30-day mortality for patients hospitalized with community-acquired pneumonia. *BMC Pulm Med*. 2005;5:12.

60.   Wang D, Chai XQ, Magnussen CG, Zosky GR, Shu SH, Wei X and Hu SS. Renin-angiotensin-system, a potential pharmacological candidate, in acute respiratory distress syndrome during mechanical ventilation. *Pulm Pharmacol Ther*. 2019;58:101833.

61.   Sodhi CP, Nguyen J, Yamaguchi Y, Werts AD, Lu P, Ladd MR, Fulton WB, Kovler ML, Wang S, Prindle T, Zhang Y, Lazartigues ED, Holtzman MJ, Alcorn JF, Hackam DJ and Jia H. A Dynamic Variation of Pulmonary ACE2 Is Required to Modulate Neutrophilic Inflammation in Response to Pseudomonas aeruginosa Lung Infection in Mice. *The Journal of Immunology*. 2019;203:3000-3012.

62.   To KF and Lo AW. Exploring the pathogenesis of severe acute respiratory syndrome (SARS): the tissue distribution of the coronavirus (SARS-CoV) and its putative receptor, angiotensin-converting enzyme 2 (ACE2). *J Pathol*. 2004;203:740-3.