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REVIEW

Lessons from SARS: control of acute lung failure by the SARS receptor ACE2

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Abstract Angiotensin-converting enzyme 2 (ACE2), a second angiotensin-converting enzyme (ACE), regulates the renin–angiotensin system by counterbalancing ACE activity. Accumulating evidence in recent years has demonstrated a physiological and pathological role of ACE2 in the cardiovascular systems. Recently, it has been shown that severe acute respiratory syndrome (SARS) coronavirus, the cause of SARS, utilizes ACE2 as an essential receptor for cell fusion and in vivo infections in mice. Intriguingly, ACE2 acts as a protective factor in various experimental models of acute lung failure and, therefore, acts not only as a key determinant for SARS virus entry into cells but also contributes to SARS pathogenesis. Here we review the role of ACE2 in disease pathogenesis, including lung diseases and cardiovascular diseases.

Keywords Angiotensin-converting enzyme 2 · Renin–angiotensin system · Severe acute respiratory syndrome · SARS coronavirus · Acute respiratory distress syndrome · Knockout mice

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Introduction

The renin–angiotensin system (RAS) plays a central role in the control of cardiovascular functions by maintaining the

physiologic homeostasis of blood pressure and electrolyte balance. Abnormal activation of the RAS has been associated with the pathophysiology of cardiovascular diseases such as hypertension, myocardial infarction, or heart failure [1–3]. For many years, the angiotensin-converting enzyme (ACE) has been known as the key enzyme in the regulation of the RAS [4, 5].

In the year 2000, a novel homologue of ACE was cloned, termed angiotensin-converting enzyme 2 (ACE2) [6, 7]. Despite the sequence similarity in their metalloprotease catalytic domains, ACE and ACE2 differ in their substrate specificity. This difference has important physiological consequences. Whereas ACE cleaves the decapeptide angiotensin I (ANG I) into an octapeptide angiotensin II (ANG II) [4, 5], ACE2 functions as a carboxypeptidase, cleaving a single residue from ANG I, generating angiotensin-(1–9) [6, 7], and a single residue from ANG II to generate angiotensin-(1–7) [7–9] (Fig. 1). Targeted disruption of murine ACE2 resulted in increased systemic ANG II levels, impaired cardiac contractility in aged mice, and upregulation of hypoxia-induced genes in the heart [10]. These genetic data for the first time demonstrated that ACE2 functions as a negative regulator of ANG II levels within the RAS in the heart.

Recently, ACE2 has been identified as a functional receptor for the severe acute respiratory syndrome (SARS)-coronavirus (SARS-CoV) in vitro [11, 12] and in vivo [13]. During several months in 2003, a newly identified illness, termed SARS, spread rapidly throughout the world, causing more than 800 deaths and disrupting travel, economics, and social life [14–16]. A novel coronavirus, termed SARS-CoV, was identified as the causative SARS pathogen [17, 18]. The death rate following infection was ~10% due to the development of atypical pneumonia characterized by high fever and the development of acute respiratory distress syndrome (ARDS) [19–21]. ARDS is the most severe form of acute lung injury, characterized by pulmonary edema, accumulation of inflammatory cells, and severe hypoxia [19–21]. ARDS has a very high mortality rate of 30–50% and affects nearly one million individuals worldwide/year. ARDS can be triggered by various diseases such as sepsis, trauma, aspiration, acute pancreatitis, or pneumonias including infections with SARS-CoV or avian and human influenza viruses [19, 20, 22, 23].

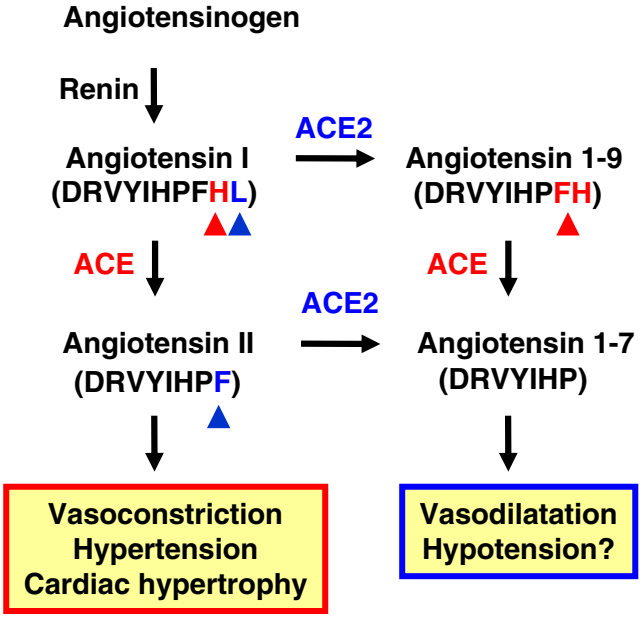

Fig. 1 Current view of ACE and ACE2 functions. ANG I (DRVYIHPFHL) serves as a substrate for both ACE and ACE2. ANG II (DRVYIHPF) is known to act as vasoconstrictor in vivo. The function of angiotensin 1–9 (DRVYIHPFH) is not well understood. Both ACE and ACE2 are involved in the production of the vasodilator peptide angiotensin 1–7 (DRVYIHPF). *Red arrowheads* ACE cleavage site; *blue arrowheads* ACE2 cleavage sites. It should be noted that ACE2 is an unspecific protease and can cleave multiple additional substrates, such as apelin

The SARS-CoV receptor ACE2 is expressed in the lungs of healthy and diseased humans [24], and our recent studies definitely showed that ACE2 is indeed an essential receptor for SARS infections in mice in vivo [13]. In the pathogenesis of ARDS, ANG II is upregulated by ACE and drives severe lung failure via the ANG II type 1 (AT1) receptor [25]. On the other hand, ACE2 and the ANG II type 2 receptor regulate opposing effects and have protective roles against lung injury. In addition, SARS-CoV-mediated down-regulation of ACE2 appears to play a causative role in severe acute lung injury in SARS through enhanced activation of AT1 receptors [13].

ACE2 in cardiovascular diseases

The beneficial effects of ACE inhibitors or AT1 receptor blockers on hypertension in the clinics have been functionally proven by in vivo models using mice deficient in ACE, angiotensinogen, or the ANG II type 1a receptor, all of which mice show lower blood pressure [9, 26, 27] (Fig. 1). The in vitro activity of ACE2 to cleave ANG II to angiotensin-(1–7) encouraged researchers to propose that ACE2 is also a major regulator of blood pressure homeostasis. After our initial study showing reduced ACE2 expression in different hypertensive rat strains [10], the upregulation of ACE2 expression is reported in the under-nutrition programmed hypertension rats [28] and AT1 receptor blocker-treated [29] or all-trans retinoic acid-treated [30] hypertensive rats, accompanied by a reduction in blood pressure. On the other hand, it was surprising to find that *ace2* knockout mice per se show apparently normal blood pressures [10]. However, it is likely that mouse-strain-specific background genes might influence the effects of ACE2 on blood pressure. Despite

no direct correlation between essential hypertension and *ACE2* polymorphisms in an earlier clinical study [31], two recent Chinese population studies indicated that the *ACE2* G8790A polymorphism is associated with cardiac incompetence in essential hypertension patients or with hypertension in metabolic syndrome patients [32, 33]. These findings implicated the significance of the *ACE2* G8790A polymorphism in cardiovascular diseases. Of note, all of these studies investigated single nucleotide polymorphisms (SNPs) located in the introns of the *ACE2* gene; the effects of these SNPs on the activity and/or expression of ACE2 are still unknown. Nevertheless, the data of hypertensive rats and human ACE2 SNPs implicate the possible significance of ACE2 in blood pressure regulation, and further studies are awaited.

Initially, ACE2 was identified and cloned from human failing heart tissue [7]. In line with this, increased ACE2 expression in the failing heart has been reported independently [34–37], and the protective role of ACE2 in pressure-overload-induced heart failure was recently shown in a transverse aortic constriction model using *ace2* knockout mice [38]. Furthermore, the function of ACE2 in basal condition was defined by the echocardiography measurements showing a progressive impairment of heart contractility in aged *ace2*-gene-deficient mice without histological changes in the heart [10]. The observed phenotype of *ace2* mutant mice resembles the defective heart found in patients with cardiac stunning/hibernation [39]. Cardiac stunning and hibernation reflect adaptive responses to prolonged states of tissue hypoxia that can occur in coronary artery disease or following bypass surgery [39, 40]. Pathologically, chronic hypoxic conditions can lead to compensatory changes in myocyte metabolism [41], upregulation of hypoxia-induced genes [42, 43], and possibly resembling myocyte-specific vascular endothelial growth factor mutant mice [44]. The link between cardiac stunning/hibernation and the heart defect observed in *ace2* knockout mice has to be further investigated.

Interestingly, additional deletion of *ace* gene on an *ace2* mutant background rescued the cardiac contractility phenotype of *ace2* single knockout mice and also reversed the increased ANG II peptide levels [10]. The normal cardiac functions of *ace/ace2* double-mutant mice suggest that ACE's catalytic products account, at least in part, for the observed contractile impairment of old *ace2* single-mutant mice [10]. These observations for the first time demonstrated, at the genetic level, that ACE2 counterbalances the enzymatic actions of ACE. It seems that increased local cardiac ANG II might have been the cause for the cardiac abnormalities in *ace2*-deficient mice. Nevertheless, it remains unclear why the elevated heart ANG II levels do not induce cardiac hypertrophy in the *ace2*-deficient mice. For instance, in experimental models, it is well established that cardiomyocytes express ANG II receptors and undergo hypertrophy and fibrosis in response to ANG II stimulation [45]. Thus, it is important to note that ANG II-independent pathways could also play an important role in ACE/ACE2-regulated heart functions.

ACE2 in lung diseases

The major sites of ACE expression in our body are vascular endothelial cells in the lungs, and thus, it has been proposed that systemic ANG II is mainly generated in the lung [4, 5, 46]. Experimentally, a possible role of ANG II in lung disease has been implied from animal models of pulmonary fibrosis elicited by bleomycin or irradiation-mediated lung injury. In bleomycin-induced pulmonary fibrosis in rats or mice, ACE inhibitors or AT1 receptor blockers can attenuate epithelial apoptosis, interstitial fibrosis, and collagen deposition [47–50]. Those reports implicated ACE inhibitors or AT1 receptor blockers as possible therapy for pulmonary fibrosis seen in idiopathic pulmonary fibrosis [51], sarcoidosis [52], irradiation-induced pneumonitis [47], or fibrosis that develops as a side effect of chemotherapy in cancer patients [53]. However, there are not enough published clinical studies to make any firm conclusion whether these inhibitors indeed show beneficial effects. A recent cohort study of ARDS showed the significant association between ACE insertion/deletion (D) polymorphism and the susceptibility and outcome of ARDS [54]. The D allele of the human *ACE* gene confers increased ACE activity in plasma, and the DD genotype frequency was increased in the patients with ARDS compared with the control groups [54]. Moreover, the DD allele was significantly associated with mortality in the ARDS group. Thus, based on inhibitor experiments in rodents and *ACE* allelic correlation studies in humans, it has been suggested that the RAS could have a role in acute lung failure and pulmonary fibrosis.

Our group investigated the role of ACE2 in ARDS by using *ace2* knockout mice. In three different ARDS models, acid-aspiration-induced ARDS, endotoxin-induced ARDS, and peritoneal sepsis-induced ARDS, *ace2* knockout mice show very severe disease compared with wild-type mice [25]. Loss of ACE2 expression in mutant mice resulted in enhanced vascular permeability, increased lung edema, neutrophil accumulation, and worsened lung function. Importantly, treatment with catalytically active recombinant ACE2 protein improved the symptoms of acute lung injury in wild-type mice, as well as in *ace2* knockout mice [25]. Thus, ACE2 plays a protective role in acute lung injury. Mechanistically, the negative regulation of ANG II levels by ACE2 accounts, in part, for the protective function of ACE2 in ARDS. For example, AT1 inhibitor treatment or

additional *ace* gene deficiency on an *ace2* knockout background rescues the severe phenotype of *ace2* single-mutant mice in acute lung injury [25]. In addition, *ace* knockout mice and *at1a receptor* knockout mice showed improved symptoms of acute lung injury [25]. Therefore, in acute lung injury, ACE, ANG II, and AT1 receptor function as lung-injury-promoting factors, while ACE2 protects from lung injury [25]. However, similar to the heart, we also have evidence that ACE2-regulated, but ANG II-independent, pathways [8, 55] might play a critical role in controlling ARDS following acute lung injury (Fig. 2).

ACE2 in SARS pathology

Within months of publication of the SARS-CoV genome [56, 57], ACE2 was identified as a potential receptor using in vitro cell line studies [11]. ACE2 has been demonstrated to bind SARS-CoV spike and to support "syncytia formation," the fusion of spike-protein-expressing cells into large multinucleated cells that can also be seen in "real" SARS infections [11]. After the identification of ACE2 as a SARS receptor in vitro, liver-specific ICAM3-grabbing nonintegrin (L-SIGN) (also known as CD209L) was reported as a second receptor for SARS-CoV infection [58] (Fig. 2). Certainly, both receptors seem functional for in vitro viral entry, but it was unclear whether those receptors are required for in vivo SARS infections.

Using a SARS infection model in *ace2* knockout mice, our group was able to show that ACE2 is indeed essential for SARS infections in vivo [13]. When *ace2* knockout mice are infected with SARS-CoV, they were resistant to virus infection [13]. No lung histology from *ace2* knockout mice challenged with SARS-CoV showed signs of inflammation [13], whereas some (but not all) SARS-infected wild-type mice displayed mild inflammation with leukocyte infiltration [13, 59, 60]. Thus, ACE2 is an essential receptor for SARS infections in vivo. Moreover, the importance of L-SIGN in SARS infection has been recently highlighted by the genetic analyses of a polymorphism that determines different numbers (3–9) of tandem repeat domains in exon 4 of the human L-SIGN protein [61]. Individuals homozygous for these tandem repeats (i.e., the same number of tandem repeats in both alleles) are less susceptible to SARS infections than ones heterozygous for the repeats (i.e., the different number of tandem repeats in each allele) [62]. It would be interesting to further investigate the functional importance of L-SIGN in vivo SARS infections using

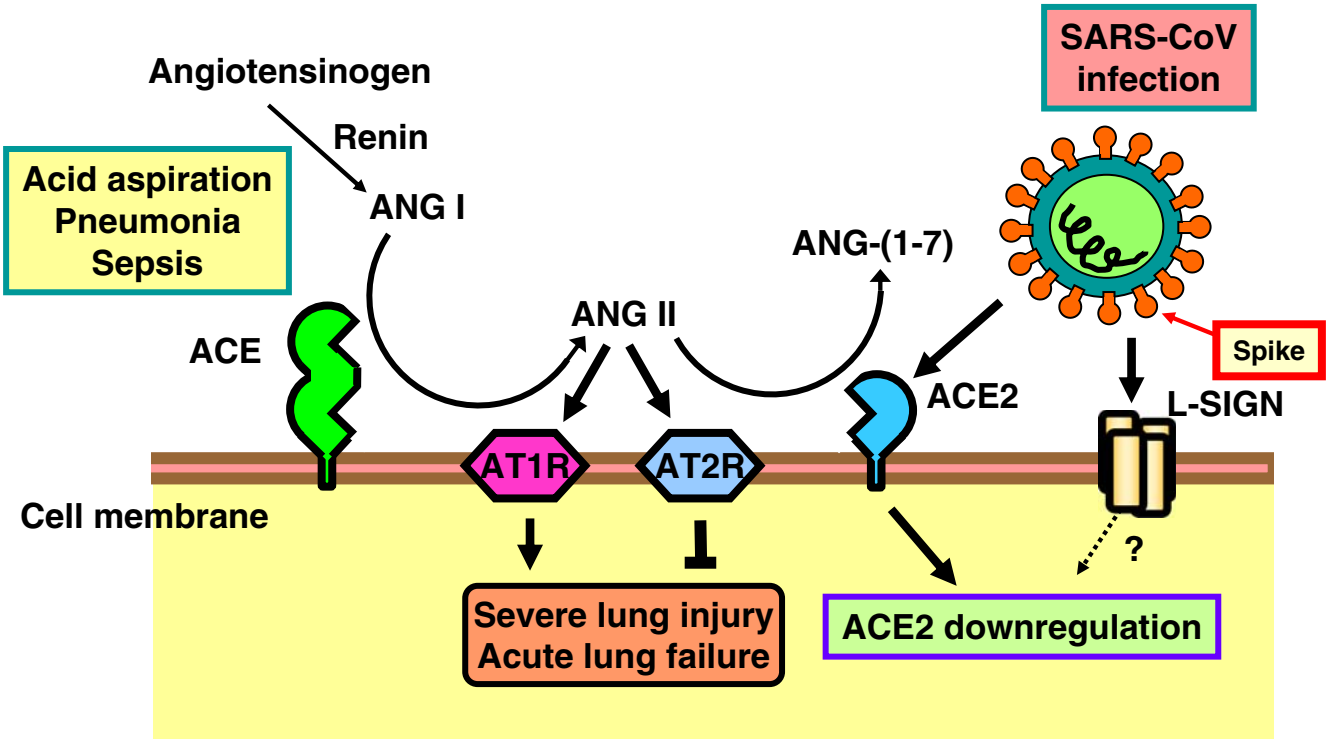

Fig. 2 Schematic diagram of the role of the RAS in acute lung failure and proposed SARS-CoV action. In acute lung injury, such as acid aspiration, pneumonia, or sepsis, the generation of ANG II from ANG I is enhanced by ACE, and ANG II induces acute lung failure through stimulation of the AT1 receptor, while ACE2 and ANG II type 2 receptor negatively regulate this pathway and protect from acute lung failure. On the other hand, SARS-CoV infection is mediated through binding of the SARS-Spike protein to ACE2 or L-SIGN and down-regulates the protective molecule ACE2, and thus leads to severe lung injury and acute lung failure

model systems such as L-SIGN transgenic mice, and to genetically compare the role of L-SIGN to that of ACE2 in *ace2*-gene-deficient mice.

One mystery of SARS-CoV is why, in contrast to the other coronaviruses infecting humans, infections with the SARS-CoV trigger severe lung disease with such high mortality. Accumulating evidence further indicates that severe SARS infections are dependent on the burden of viral replication, as well as on the immunopathologic consequences of the host response in SARS pathogenesis (see reviews [63, 64]). Moreover, our own studies have implicated the involvement of the RAS in SARS pathogenesis: first, ACE2 is a critical SARS receptor in vivo and, second, ACE2 and other components of the RAS play a central role in controlling the severity of acute lung failure once the disease process has started [13]. Intriguingly, before the identification of ACE2 as a SARS receptor, it has been reported that some SARS patients develop impaired heart contractility using echocardiograhic measurements [65]. Certainly, the observed decrease in left ventricular performance during the acute infection phase may be related to a systemic inflammatory condition [66], which is not unique to SARS. Nevertheless, it is interesting to contemplate a possible link of the impaired heart function of SARS patients and the reduced heart contractility in *ace2* mutant mice. In line with this idea, wild-type mice infected with SARS-CoV showed markedly down-regulated ACE2 expression in lungs [13], as well as in hearts (unpublished). Moreover, treatment with recombinant SARS-Spike protein, without any other virus components, down-regulates ACE2 expression in vitro and in vivo [13]. Thus, Spike-treated wild-type mice resemble *ace2* knockout mice, and, similar to *ace2* mutant mice, Spike-treated wild-type mice show enhanced RAS signaling, leading to markedly more severe pathology in acute lung injury. Therefore, the down-regulation of ACE2 expression by Spike in SARS-CoV infections might be a possible explanation for SARS pathogenesis, especially in disease progression to ARDS. Although the enhanced RAS signaling is unique in SARS-CoV infection, the down-regulation of virus receptor by interaction with virus ligand is also seen in other viruses. For instance, CD4, a receptor for HIV, internalizes with HIV gp120, resulting in the disruption of immune cell functions [67], and CD46, a measles receptor, is down-regulated by measles hemagglutinin, leading to the impairment of complement pathways and immune systems [68]. On the other hand, any consequences of the down-regulation of sialo-glycoconjugate receptor by influenza A virus [69] are yet unknown. The finding of enhanced RAS signaling and severe acute lung injury as consequences of ACE2 down-regulation by SARS-CoV might possibly implicate that other emerging infectious lung diseases, like H5N1 avian flu, may also utilize the receptor down-regulation system to impair host immune/inflammatory systems and induce severe lung diseases. Thus, it is interesting to investigate the consequences of down-regulation of other virus receptors upon virus infection.

Concluding remarks

ACE2 has now been identified as a key factor for protection from ARDS/acute lung injury, and ACE2 functions as a critical SARS receptor in vivo. Since SARS Spike-protein-mediated ACE2 down-regulation appears to contribute to the severity of lung failure, these findings may explain how the SARS-CoV has turned into a lethal virus. In addition, in an acid aspiration ARDS mouse model, strong down-regulation of ACE2 protein in the injured lung was observed, while ACE expression remained unchanged. Thus, recombinant ACE2 protein could not only be a treatment to block the spreading of SARS but also to protect SARS patients from developing lung failure. Furthermore, those findings could apply to investigating the therapeutic efficacy of ACE2 in ARDS that develops in other emerging lung infectious diseases, like avian influenza A (H5N1) [70] or other diseases that affect lung function [71]. In addition to recombinant ACE2 protein therapy, ACE2 gene therapy would be another candidate. Recently, lentivirus-mediated gene delivery of ACE2 into rat hearts was shown to successfully attenuate ANG II-induced cardiac hypertrophy [72]. However, especially for the acute phase of ARDS, tissue-specific delivery of exogenous recombinant ACE2 protein might be the first line of choice while avoiding systemic adverse effects. We look forward to the further elucidation of the pathophysiological role of ACE2 and to the use of ACE2 as a therapeutic target.

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