Angiotensin-converting enzyme 2 (ACE2), a first homolog of 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, renal and respiratory systems. For instance, in the acute respiratory distress syndrome (ARDS), ACE, AngII, and AT1R promote the disease pathogenesis, whereas ACE2 and the AT2R protect from ARDS. Importantly, ACE2 has been identified as a key SARS-coronavirus receptor and plays a protective role in SARS pathogenesis. Furthermore, the recent explosion of research into the ACE2 homolog, collectrin, has revealed a new physiological function of ACE2 as an amino acid transporter, which explains the pathogenic role of gene mutations in Hartnup disorder. This review summarizes and discusses the recently unveiled roles for ACE2 in disease pathogenesis.

Key Words: Acute respiratory distress syndrome (ARDS); Amino acid transporter; Angiotensin-converting enzyme 2 (ACE2); Cardiovascular disease; Severe acute respiratory syndrome (SARS)

The renin–angiotensin system (RAS) plays a central role in the control of cardiovascular and renal functions by maintaining homeostasis of blood pressure (BP) and electrolyte balance. Abnormal activation of the RAS is associated with the pathogenesis of cardiovascular and renal diseases such as hypertension, myocardial infarction and heart failure.1-6 The protease, renin, cleaves angiotensinogen to generate angiotensin (Ang) I. The angiotensin-converting enzyme (ACE) is a critical protease for regulating the RAS by cleaving AngI to generate AngII, which is a key regulator of the RAS and exerts biological functions through the specific receptors AngII receptor type 1 (AT1R) and AngII receptor type 2.7-9 Thus, for many years ACE has been known as a key enzyme in the regulation of the RAS (Figure 1).

In 2000, a homolog of ACE was cloned by 2 independent groups and termed ACE2.10,11 Whereas ACE cleaves AngI into AngII,7,8 ACE2 removes a single residue from AngI to yield Ang1–9,10,11 and cleaves a single residue from AngII to generate Ang1–711 (Figure 1). Several studies support a counter regulatory role for Ang1–7 by opposing many AT1R-mediated actions. Thus, ACE2 has emerged as a potent negative regulator of RAS, playing an opposing role to ACE in diverse organ systems including the heart, kidneys and lungs. In the respiratory system our group found that ACE, AngII, and AT1R function as lung-injury-promoting factors, whereas ACE2 protects from lung injury.12 In 2003, severe acute respiratory syndrome (SARS) caused by the SARS-coronavirus (SARS-CoV) spread quickly throughout the world causing more than 800 deaths from severe respiratory failure. ACE2 was identified as a receptor for SARS-CoV in vitro,13 and we confirmed that ACE2 was an essential receptor for SARS-CoV in vivo.14 We also demonstrated that down-regulation of ACE2 by SARS-CoV infection participates in the development of severe lung failure in SARS.14 Moreover, both ACE2 and its homolog, collectrin, bind amino acid transporter B0AT1, which is essential for neutral amino acid absorption in the intestine15 and kidney,16 and explains the pathogenesis of Hartnup disorder. In this review we shall discuss the recently unveiled roles for ACE2 in disease pathogenesis.

ACE2, a New Member of the RAS

The human ACE gene, located on chromosome 17, encodes a 180-kDa protein with 2 homologous domains.7 ACE is anchored to the plasma membrane through a single carboxy-terminal transmembrane domain and, at the cell surface, ACE functions as an ectoenzyme that hydrolyzes circulating peptides. ACE may be cleaved from the cell surface and act as a soluble enzyme, but the biological significance of soluble ACE remains unclear. In 2000, ACE2 was cloned as a first homolog of human ACE10,11 and mapped to the X chromosome. ACE2 is an 805 amino acid type I transmembrane glycoprotein with an extracellular catalytic domain with a molecular weight of approximately 120kDa. Tissue localization of ACE2 is predominantly observed in the heart, kidneys and testes, and at a lower level in a wide variety of tissues including the colon and lung.10 Early studies showed that
ACE2 is confined to the endothelium, but further studies showed ACE2 expression in cardiomyocytes, in the kidney on the luminal surface of tubular epithelial cells, and in testes on adult Leydig cells. Similar to ACE, ACE2 has 2 domains: the amino-terminal catalytic domain and the carboxy-terminal domain. The catalytic domain has one active site (the zinc metallopeptidase domain) and shows 41.8% sequence identity with the amino domain of ACE. Despite the sequence similarity of their catalytic domains, ACE and ACE2 appear to act on different peptide substrates. Whereas ACE cleaves AngI into AngII, ACE2 cleaves a single residue from AngII to generate Ang1–7, which has an opposing role to ACE by counterbalancing AT1R-mediated actions. Because ACE2 is a major Ang1–7-forming enzyme, its identification has added further support to the biological significance of Ang1–7. Of note, gene targeting of ACE resulted in increased expression in rat and humans to be determined, but myocardial infarction increases ACE2 expression in rat and humans and ACE2 overexpression inhibits hypoxia-induced collagen production in vitro. Interestingly, Ace and Ace2 double knockout mice rescue the cardiac contractility phenotype of Ace2 single knockout mice and also reverses the increased AngII peptide levels.

**ACE2 in the Cardiovascular System**

In a genetic approach to isolating rat hypertension genes, the Ace2 gene was found to be mapped to a quantitative trait locus of the X chromosome for salt-sensitive hypertension in 3 different rat models. The hypertensive rats show reduced ACE2 transcript and protein expression in both heart and kidney. Consistently, transgenic ACE2 overexpression in the vessels of SHRSP rats reduces BP and improves endothelial function, and neuronal overexpression of ACE2 also attenuates hypertension. In humans, several studies have shown a strong association of ACE2 polymorphism to hypertension in female Chinese patients with metabolic syndrome or essential hypertension. Thus, together with the biochemical data that has identified ACE2 as a negative component of the RAS (ie, degrading AngII to generate Ang1–7), ACE2 can be thought to play a profound role in controlling BP. However, mouse knockout of Ace2 results in no apparent alterations in BP at basal levels, despite enhanced susceptibility for AngII-induced hypertension. This is in sharp contrast to a phenotype of spontaneous hypotension in ACE knockout mice and implies that ACE2 might regulate BP through other peptide systems, such as bradykinin and apelin, in addition to the AngII system. Initially, ACE2 was identified and cloned from human failing heart tissue. In line with this, increased ACE2 expression in disease hearts has been reported independently. The cardiac function of ACE2 in the basal condition was defined by echocardiography showing progressive impairment of heart contractility with age in Ace2-deficient mice, and histologically, the hearts of these mice show thinning of the left ventricular wall without cardiac fibrosis or hypertrophy. Thus, the observed phenotype of Ace2 mutant mice resembles the defective heart found in patients with cardiac stunning/hibernation, which are adaptive responses to the prolonged state of tissue hypoxia that can occur in coronary artery disease or following bypass surgery. Indeed, the hearts of Ace2 mutant mice show upregulation of mRNA expression of hypoxia-inducible genes such as BNIP3 and PAI-1. Whether ACE2 deficiency leads to hypoxia remains to be determined, but myocardial infarction increases ACE2 expression in rat and humans and ACE2 overexpression inhibits hypoxia-induced collagen production in vitro. Interestingly, Ace and Ace2 double knockout mice rescue the cardiac contractility phenotype of Ace2 single knockout mice and also reverses the increased AngII peptide levels. The
normal cardiac function of Ace/Ace2 double mutant mice suggest that catalytic products of ACE account for the observed contractile impairment of aged Ace2 single mutant mice. Indeed, AT1R blocker treatment rescues the impaired contractility of Ace2 knockout mice related to aging and other stress conditions. Furthermore, cardiac overexpression of ACE2 and Ang1–7 protects the heart from ischemia-induced pathophysiology and hypertensive cardiac remodeling, respectively. Therefore, ACE2 counterbalances the enzymatic actions of ACE through degrading of AngII to generate Ang1–7.

This counterbalancing action of ACE2 on ACE is also observed in other organs of the cardiovascular system. In atherosclerosis, ACE2 protects endothelial cells from AngII-mediated oxidative stress in an Ang1–7-dependent manner and attenuates atherosclerosis and stabilizes the atherosclerotic plaques. In the kidney, ACE2 is abundantly expressed, and deletion of Ace2 leads to spontaneous late-onset nephrotic glomerulosclerosis and accelerates diabetic kidney injury in Akita diabetic mice. These phenotypes are dependent on AngII signaling and are indeed rescued by AT1R blockade. These findings provide the genetic evidence that ACE2 counterbalances ACE function, negatively regulates AngII levels and thereby controls cardiac and renal homeostasis.

ACE2 in the Respiratory System

Acute respiratory distress syndrome (ARDS) is the most severe form of acute lung injury (ALI). Many pathogenic conditions can trigger ARDS, including sepsis, gastric juice aspiration, pancreatitis, trauma, and pathogens such as Bacillus anthracis (anthrax), Spanish flu virus, SARS-coronavirus (SARS-CoV), and H5N1 avian influenza virus. To date, no pharmacological therapies have been developed to improve the clinical outcome of ARDS in large-scale clinical trials, and the associated mortality remains very high despite modern intensive care medicine. Recent study has shown that genetic factors of ACE are involved in the onset or severity of ARDS. Large individual differences in plasma ACE concentrations have been reported among individuals, while the ACE plasma levels tend to be similar within families. The human ACE gene (dcp1) is located on chromosome 17q23 and contains a restriction fragment length polymorphism within the coding sequence of intron 16 defined by the presence (insertion, I) or absence (deletion, D) of a 287-bp repeat. This polymorphism determines the function of ACE and the human ACE D allele confers increased ACE activity. Importantly, recent cohort studies of ARDS patients showed a marked correlation between ACE I/D polymorphism and the susceptibility and mortality of ARDS. The D/D genotype was significantly increased in patients with ARDS compared with control patients without ARDS respiratory failure being ventilated in the intensive care unit. In addition, the ACE D/D allele correlated with mortality in the ARDS group, and patients carrying the ACE I/I genotype have shown a significantly increased survival rate. These findings implicate genetic factors of the RAS in the susceptibility, progression, and lethality of ARDS in human patients.

Our group investigated the role of the RAS in ALI/ARDS by using RAS-related knockout mice. In 3 different models, acid-aspiration-induced ARDS, endotoxin-induced ARDS, and peritoneal sepsis-induced ARDS, Ace2 knockout mice showed very severe disease compared with wild-type (WT) mice. 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 ALI in both the WT and Ace2 knockout mice. Thus, ACE2 plays a protective role in ALI. Mechanistically, the negative regulation of AngII levels by ACE2 accounts, in part, for the protective function of ACE2 in ARDS; for example, AT1R blocker treatment or additional Ace deficiency in an Ace2 knockout background rescues the severe phenotype of Ace2 single-mutant mice in ALI. In addition, Ace knockout mice and AT1Ra knockout mice show improved symptoms of ALI. Thus, in ALI, ACE, AngII, and AT1R all function as lung-injury-promoting...
factors, whereas ACE2 protects from lung injury (Figure 2). Furthermore, in models of other lung diseases, such as bleomycin-induced lung fibrosis and monocrotaline-induced pulmonary hypertension, ACE2 has recently been shown to protect from pathophysiology, implicating ACE2 proteins/gens and small compounds of ACE2 activator as possible therapeutics for pulmonary fibrosis as well as pulmonary hypertension. 57–59

ACE2 as a SARS Receptor

Within months after the identification of the SARS-CoV genome, 60,61 ACE2 was identified in in-vitro cell line studies as a potential receptor for SARS-CoV. 13 In those experiments, ACE2 protein was immunoprecipitated from cell lysates susceptible to SARS-CoV infection using the recombinant spike protein of SARS-CoV. 13 ACE2 can bind to the SARS-CoV spike and this binding supports ‘syncytia formation’, the fusion of spike-protein-expressing cells into the large multinucleated cells that are also observed in “real” SARS infections. Using a SARS infection model in Ace2 knockout mice, our group was able to show that ACE2 is essential for SARS infections in vivo. 14 When Ace2 knockout mice are infected with the SARS-CoV, they were resistant to virus infection: virus titers from the lung tissues of infected Ace2 knockout mice were 105-fold lower than that from the lungs of WT mice. 14 Moreover, none of the lung histology samples from Ace2 knockout mice challenged with SARS-CoV showed signs of inflammation, whereas some (but not all) SARS-infected WT mice displayed mild inflammation as defined by leukocyte infiltration. 14,62 One important question in SARS pathology is why infections with the SARS-CoV trigger severe ARDS with high mortality whereas infections with other coronaviruses result in quite mild disease. Severe SARS infections are determined by the burden of viral replication, as well as the host’s immune response to the virus. Our own studies have implicated 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 ARDS once the disease process has been initiated. 12,14 (Figure 2). Upon SARS-CoV infection, ACE2 expression in lungs is markedly downregulated in WT mice. Similarly, treatment with the recombinant SARS spike protein downregulates ACE2 expression in cell lines in vitro and in lungs in vivo. 14 Thus, SARS-CoV-infected or spike-protein-treated WT mice resemble Ace2 mutant mice, spike-protein-treated WT mice show markedly more severe pathology in ALI. 14 As a consequence, AngII peptide levels are increased in spike-protein-treated mice and these mice show worsened ARDS symptoms, both of which are partially reversed by pharmacological inhibition of the AT1R. In contrast, SARS spike protein challenge does not affect the ARDS symptoms in Ace2 knockout mice. 14 Thus, down-regulation of ACE2 expression in SARS-CoV infections may play a causal role in SARS pathogenesis, especially disease progression to ARDS. These findings provide a rational explanation for the severe disease pathology in SARS patients (Figure 3).

ACE2 as an Amino Acid Transporter

Hartnup disorder is an autosomal recessive disorder characterized by a pellagra-like light-sensitive rash, cerebellar ataxia, emotional instability, and amino aciduria. 63 Gene mutations in B0AT1 (SLC6A19) neutral amino acid transporter have

---

**Figure 3.** Schematic diagram of the role of the renin–angiotensin system in acute lung injury (ALI) and the proposed action of the SARS-coronavirus (SARS-CoV). In ALI, such as acid aspiration, pneumonia or sepsis, the generation of angiotensin II (AngII) from angiotensin I (AngI) is enhanced by angiotensin-converting enzyme (ACE), and AngII induces ALI through stimulation of the AngII type 1 receptor (AT1R), whereas ACE2 and AngII type 2 receptor (AT2R) negatively regulate this pathway and are protective. On the other hand, SARS-CoV infection is mediated through binding of the SARS–spike protein to ACE2 or L-SIGN, which downregulates the protective molecule ACE2, and thus leads to severe lung injury and acute lung failure.
been identified as a cause of Hartnup disorder. The expression of B^AT1 in the kidney was recently shown to depend on its association with collectrin (Tmem27), which shares 47.8% identity with the C-terminal end of ACE2. The collectrin gene is located in immediate proximity to the ACE2 locus on the X chromosome and both genes share similar transcription factor binding sites. Surprisingly, in gene-targeting studies, the physiological in vivo function of collectrin was be a crucial binding partner for neutral amino acid transporters. In the absence of collectrin, B^AT1, XT2, and XT3 amino acid transporter proteins are not expressed on the apical surface of proximal tubular epithelial cells, and thus neutral amino acids are not reabsorbed but leaked into the urine. Therefore, collectrin physically associates with numerous apical amino acid transporters in the kidney and thereby controls their expression and transport functions.

Because of high homology with collectrin, it can be anticipated that ACE2 will be also important for amino acid reabsorption in the kidneys, but that is not the case. In the kidney, ACE2 does not colocalize with B^AT1, and Ace2 knockout mice do not show any aminoaciduria. Rather, ACE2 functions more significantly in the small intestine, where it is highly expressed. Immunofluorescence, coimmunoprecipitation, and functional experiments using WT and Ace2-null mice show that ACE2 is necessary for the expression of the Hartnup transporter B^AT1 in the intestines, suggesting differential functional association of mutant B^AT1 transporters with ACE2 and collectrin in the intestine and kidney, respectively. Importantly, among the human Hartnup gene mutations of B^AT1, the A69T and R240Q B^AT1 mutants have been shown to function as WT B^AT1 when expressed alone in vitro, though the patients suffer from the symptoms and the cause of the disease manifestations remain unknown. These mutations, however, are critical for the association with ACE2 and collectrin and cannot be physiologically activated by either of these proteins. Therefore, the interaction of ACE2 and B^AT1 amino acid transporter explains the previously unknown mechanism of the pathogenesis of Hartnup disorder.

**Perspectives**

ACE2, a first homolog of ACE, regulates the RAS by countering its activity. Accumulating evidence supports a critical role for ACE2 in disease pathogenesis, including cardiovascular, renal and respiratory diseases. In particular, ACE, AngII, and the AT1R promote ARDS, whereas ACE2 and the AT2R protect from ARDS. Importantly, ACE2 has been identified as a key SARS-CoV receptor and plays a protective role in SARS-CoV-mediated ARDS. These findings provide an opportunity to develop recombinant ACE2 as a novel drug in ARDS, together with an AT1R inhibitor or ACE inhibitor to possibly treat emerging infectious lung diseases such as avian influenza (H5N1) and novel 2009 influenza (H1N1). In addition, because ACE2 is an unspecific protease, such as avian influenza (H5N1) and novel 2009 influenza virus (H1N1), it would be also interesting to investigate its role, and that of its metabolites, including AngI–7, des-Arg(9)-bradykinin, apelin and dynorphin, in disease pathogenesis. Furthermore, the recent explosion of research into the ACE2 homolog, collectrin, has revealed a new physiological function for ACE2 and collectrin as amino acid transporters, independent of their catalytic activity. Although further studies are required, recent findings have revealed intriguing possibilities for the use of RAS-modulating agents/molecules, in particular ACE2, as novel therapeutic agents.

**References**


23. Crackower MA, Sarao R, Oudit GY, Yagal C, Kozieradzki I, Scanga SE, et al. Angiotensin-converting enzyme 2 is an essential regulat...


