Angiotensin-converting enzyme 2 (ACE2) is a negative regulator of the renin-angiotensin system, and functions as the key SARS coronavirus receptor and stabilizer of neutral amino acid transporters. ACE2 catalyzes the conversion of angiotensin II to angiotensin 1–7, thereby counterbalancing ACE activity. Accumulating evidence indicates that the enzymatic activity of ACE2 has a protective role in cardiovascular diseases. Loss of ACE2 can be detrimental, as it leads to functional deterioration of the heart and progression of cardiac, renal, and vascular pathologies. Recombinant soluble human ACE2 protein has been demonstrated to exhibit beneficial effects in various animal models, including cardiovascular diseases. ACE2 is a multifunctional enzyme and thus potentially acts on other vasoactive peptides, such as Apelin, a vital regulator of blood pressure and myocardium contractility. In addition, ACE2 is structurally a chimeric protein that has emerged from the duplication of 2 genes: homology with ACE at the carboxypeptidase domain and homology with Collectrin in the transmembrane C-terminal domain. ACE2 has been implicated in the pathology of Hartnup’s disease, a disorder of amino acid homeostasis, and, via its function in amino acid transport, it has been recently revealed that ACE2 controls intestinal inflammation and diarrhea, thus regulating the gut microbiome. This review summarizes and discusses the structure and multiple functions of ACE2 and the relevance of this key enzyme in disease pathogenesis. (Circ J 2013; 77: 301–308)

Key Words: Angiotensin-converting enzyme 2 (ACE2); Amino acid transporter; Acute respiratory distress syndrome (ARDS); Renin-angiotensin system (RAS); Severe acute respiratory syndrome (SARS)

Carboxypeptidase Domain of ACE2

ACE2 catalyzes enzymatic reactions by utilizing zinc ions, coordinated by conserved histidines within the active site (the HEXXH motif), to facilitate a nucleophilic attack on the carboxyl bond of the substrate by a water molecule, forming a non-covalently bound intermediate. Structural analyses of native ACE2 compared with ACE2 in the presence of an inhibitor have revealed a large “hinge-bending” motion, in which the catalytic subdomains I and II of the peptidase domain exhibit open-to-close transitions. This movement appears to be induced by binding of the inhibitor and repositions key resi-
Despite their similarities, ACE and ACE2 function differently; ACE releases a C-terminal dipeptide from its substrate (dipeptidylpeptidase), whereas ACE2 cleaves a single amino acid (monocarboxypeptidase).\textsuperscript{1,2} ACE2 catalyzes peptides, with a substrate preference for hydrolysis between proline and a hydrophobic or basic C-terminal residue.\textsuperscript{18} Whereas ACE converts AngI to the potent vasoconstrictor Ang II,\textsuperscript{19} ACE2 cleaves AngI to generate the presumably inactive Ang1–9 peptide,\textsuperscript{1,2} which then can be converted to the vasodilator peptide Ang1–7 by ACE or other peptidases.\textsuperscript{1} Importantly, ACE2 directly metabolizes Ang II to generate Ang1–7 with much higher efficiency than converting AngI to Ang1–9 (Figures 1, 3). The resolution of the ACE2 crystal structure revealed that these differences in substrate specificity are a result of the smaller binding pocket in ACE2 where arginine-273 makes a salt-bridge with the C-terminus of the substrate, whereas in ACE this residue is substituted by a smaller glutamine.\textsuperscript{17} Although enzymes were known to generate Ang1–7 (e.g., prolyl-endopeptidase 24.26,\textsuperscript{3} thimet oligopeptidase,\textsuperscript{5} and neprilysin\textsuperscript{6}), ACE2 is clearly the main enzyme that catalyzes this reaction in vitro and in vivo.\textsuperscript{1,2,18} The ACE2 product, Ang1–7 peptide, has been shown to interact with the G protein-coupled receptor Mas to mediate its vasoprotective effects.\textsuperscript{20,21} ACE2 also acts on the C-terminal amino acids of the peptides Apelin-13 and Apelin-36 with high catalytic efficiency in vitro\textsuperscript{18} (Figure 4). Apelin is synthesized as a 77 amino acid pre-pro-hormone, which is processed into the 36 amino acid peptide Apelin-36; further proteolytic cleavage generates Apelin-13.\textsuperscript{22,23} Systemic administration of Apelin-13 induced hypotension in rats and mice.\textsuperscript{24,25} Interestingly, modification of the C-terminal residue of Apelin-13 (F13A) resulted in a loss of its hypotensive function and antagonism of wild-type Apelin-13,\textsuperscript{26} implicating ACE2 in the metabolism of Apelin peptides.

**Figure 1.** Current view of angiotensin-converting enzyme 2 (ACE2) in the renin-angiotensin system. ACE2 was identified as a major angiotensin 1–7 (Ang1–7)-forming enzyme. Angiotensin I (Ang I) serves as a substrate for both ACE and ACE2. Ang II is known to act as a vasoconstrictor in vivo. Both ACE and ACE2 are involved in the production of Ang1–7, which binds the Mas receptor and induces vasodilation.

**Figure 2.** Angiotensin-converting enzyme 2 (ACE2) is a chimeric protein with homology to both ACE and Collectrin. Each protein is a type I integral protein with a signal peptide, depicted in gray, and a transmembrane domain shown in black. The zinc-binding motif (HEMGH) repeats twice in ACE and once in ACE2, and is located within the homology region denoted by the yellow box. Regions of homology between ACE2 and Collectrin are denoted in green, whereas homology of ACE and ACE2 is shown in blue. The numbers refer to the amino acids in each human protein.
Multiple Functions of ACE2

ACE2 as a SARS Receptor and an Integrin Substrate

Although ACE2 functions as a mono-carboxypeptidase to catalyze Ang II cleavage, further studies demonstrated that ACE2 has additional biological functions. In 2003, the epidemic of SARS threatened the world and ACE2 was identified as a functional receptor for the causative pathogen, SARS-CoV. Interaction of SARS-CoV with ACE2 is initiated via trimers of the SARS Spike protein, which extends into a hydrophobic pocket of the ACE2 catalytic domain. This ACE2-Spike interaction leads to endocytosis of virus particles through internalization with ACE2, induces the fusion of virus and host cells, and establishes SARS-CoV infection. However, this process does not require or affect the peptidase activity of ACE2, because cells expressing catalytic inactive mutants of ACE2 continue to be susceptible to SARS-CoV infection.

Figure 3. Multiple roles of angiotensin-converting enzyme 2 (ACE2) determined in genetic experiments. (A) ACE2 is a potent negative regulator of the renin-angiotensin system, catalyzing the conversion of Ang II to Ang1–7. ACE2 is an essential receptor for the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) (B) and also interacts with amino acid transporters and integrins (C).

Figure 4. Domain structure and molecular functions of angiotensin-converting enzyme 2 (ACE2). The N-terminus extracellular domain of ACE2 (blue) functions as a carboxypeptidase, SARS receptor, and integrin substrate, while the C-terminus Collectrin region (green) is crucial for binding to the neutral amino acid transporters B0AT1.

SARS, severe acute respiratory syndrome; TM, transmembrane region.
ACE2 are still permissive for SARS-CoV infection, and ACE2 substrates are still accessible to the catalytic pocket of ACE2 when the SARS Spike protein is bound. Furthermore, in the structure of SARS Spike-bound ACE2, the catalytically-active site of ACE2 was not blocked by the SARS Spike protein. Thus, ACE2 functions as a SARS receptor independently of its peptidase activity (Figure 3).

It has been reported that ACE2 is internalized together with SARS-CoV upon infection and that endocytosis is essential for virus entry. The internalization can take place even when recombinant SARS Spike protein, the SARS-CoV surface ligand for receptor binding, interacts with ACE2. In addition, it has been suggested that shedding of the ACE2 ectodomain is involved in the transmembrane domain internalization for further virus particle-host cell fusions. Clathrin-dependent and -independent entry of SARS-CoV into target cells have been proposed. However, the role of the cytoplasmic tail of ACE2 is controversial; for instance, deletion of the cytoplasmic tail of ACE2 did not affect SARS-CoV entry in I experiment setup, whereas it attenuated SARS-CoV entry in another study. Apart from being the SARS receptor, Lin et al biochemically purified the integrin β1 as an ACE2 interacting molecule from homogenates of failing human hearts, and a recent study has shown that the ectodomain of ACE2 binds to integrin β1 and integrin α5 (Figure 3), suggesting that ACE2 could be an integrin substrate. Thus, ACE2 might have a role in integrin-mediated signaling in cardiovascular diseases.

**Collectrin** Domain of ACE2 Regulates Amino Acid Transporter Expression

Structural homology searches show that ACE2 is a chimeric protein that has emerged from the duplication of 2 genes: homology with ACE at the catalytic domain and homology with Collectrin (gene name: transmembrane protein 27 [Tmem27]) in the transmembrane C-terminal domain. Transcriptome analysis from partially nephrectomized rats identified Collectrin as an upregulated gene in regenerating collecting ducts. Collectrin shares 47.8% identity with the C-terminus of ACE2, but, unlike ACE2, lacks an active carboxypeptidase catalytic domain (Figure 2). An initial report indicated that Collectrin localizes to the cytoplasm of collecting duct epithelial cells. However, gene-targeting studies in mice showed that Collectrin is an essential regulator of neutral amino acid transporters and is predominantly localized in the brush borders of proximal tubular epithelial cells. Excessive amounts of neutral amino acids (tyrosine and phenylalanine) appear in the urine of Collectrin knockout mice. Biochemical studies reveal that Collectrin binds to the B4AT1 neutral amino acid transporter (and probably other neutral amino acid transporters) in the kidney where it controls expression of these transporters on the cell surface that are required for amino acid reabsorption in the proximal tubules. Despite its structural similarity, ACE2 does not bind to amino acid transporters in kidneys. Instead, it is highly expressed on the luminal surface of small intestinal epithelial cells, and in the gut, ACE2 binds to the B4AT1 amino acid transporter and contributes to the absorption of dietary neutral amino acids (Figure 3). Importantly, the peptidase activity of ACE2 is not necessary for pairing with the amino acid transporter (Figure 4).

ACE2 as a Negative Regulator of the RAS in the Cardiovascular System

Genetic analysis in rat models of hypertension have mapped Ace2 as a quantitative trait locus) onto the X chromosome. Those hypertensive rats show reduced ACE2 transcripts and protein expression in both heart and kidney. Transgenic ACE2 overexpression in the vessels of SHRSP rats reduces blood pressure (BP) and improves endothelial function, and neuronal overexpression of ACE2 also attenuates hypertension. In humans, several studies have shown a strong association of ACE2 polymorphisms to hypertension in female Chinese patients with metabolic syndrome, essential hypertension or diabetes-associated hypertension. Thus, together with biochemical data that ACE2 degrades Ang II to generate Ang1–7, it appears that ACE2 plays a profound role in controlling BP. However, genetic inactivation of ACE2 using homologous recombination results in no apparent alterations in BP in the basal state. In another line of ACE2 knockout mice, BP was significantly increased following Ang II infusions, in sharp contrast to the spontaneous hypotension observed in ACE knockout mice, suggesting that, in addition to the Ang II system, ACE2 might regulate BP through other peptide systems, such as bradykinin and/or Apelin. Nevertheless, exogenous supplementation of ACE2 by gene transfer decreased BP in SHR hypertensive rats, and recombinant ACE2 treatment attenuated Ang II-induced hypertension specifically. Thus, ACE2 clearly must function as a negative regulator of the RAS in BP control. In addition to BP regulation, ACE2 delivery has also shown beneficial effects on atherosclerosis in animal models, suggesting that ACE2 confers endothelial protection.

ACE2 is highly expressed in the heart and in multiple studies ACE2 has been reported to function as a regulator of heart failure. The key finding has been that ACE2 null mice display impaired cardiac contractility, which is associated with aging and/or cardiac pressure overload in several different lines of ACE2 knockout mice. Impaired cardiac contractility is correlated with elevated cardiac and plasma Ang II levels, and Ace and Ace2 (double-knockout) mice or treatment with AT1 receptor blockers reversed the cardiac phenotype of ACE2 knockout mice. Similarly, in myocardial infarction, loss of ACE2 accelerates maladaptive left ventricular remodeling. Mechanistically, the age-dependent cardiomyopathy in ACE2 knockout mice is likely mediated by Ang II-induced oxidative stress and induction of inflammation through AT1 receptor downstream PI3K (phosphatidylinositol-3-kinase) signaling.

In addition to the counterbalancing effects between ACE and ACE2, it has been proposed that ACE2 might control heart functions via Ang1–7 and Apelin. In particular, treatment with Ang1–7 peptide can improve myocardial performance, cardiac remodeling, and even survival in rodent heart failure models, including ischemia/reperfusion injury, myocardial infarction, and hypertension-induced cardiomyopathy. The selective Mas receptor ligand, AVE-0991, has actions similar to the cardioprotective effects of the Ang1–7 peptide, and Mas receptor knockout mice display reduced heart contractility, suggesting that Ang1–7 via its putative receptor Mas mediates these effects. Functional crosstalk between ACE2 and the Ang1–7-Mas system has been recently demonstrated experimentally; in pressure overload-induced heart failure, the Ang1–7 peptide can rescue the systolic dysfunction present in Ace2 null mice and attenuate the increase in NADPH oxidase activation.

In addition to Ang II, ACE2 also acts on the peptides Apelin-13 and Apelin-36 with high catalytic efficacy in vitro. Apelin-13 and Apelin-36 are generated by proteolytic processing of a 77 amino acid Apelin pre-prohormone. Although systemic administration of Apelin peptides triggers hypotension in rats and mice, Apelin and API (Apelin receptor)
knockout mice both showed little alterations in BP at baseline. Interestingly, Apelin knockout mice showed aging- or stress-associated cardiac contractility defects, similar to the heart phenotype of ACE2 knockout mice, suggesting that ACE2 and Apelin may share the same signaling pathway to control heart functions. Although further studies are required to validate the proposed crosstalk, in humans both ACE2 and Apelin polymorphisms are associated with parameters of left ventricular hypertrophy and BP responses to potassium supplementation, and soluble ACE2 activity is increased in patients with myocardial dysfunction and correlates with disease severity, implicating physiological roles of ACE2 and Apelin in patients.

**ACE2 in the Kidney**

In 2002, it was reported that ACE2 is abundantly expressed in the kidney, and the activity of ACE2 is even higher in the kidney cortex than in heart tissue. ACE2 has emerged as a protective molecule against kidney diseases in the context of negative regulation of the RAS. For instance, deletion of Ace2 leads to spontaneous late-onset nephritic glomerulosclerosis and accelerates diabetic kidney injury in both Akita diabetic mice and streptozocin-induced diabetic mice. In line with these results, pharmacological ablation of ACE2 by MLN-4760 has resulted in increased albuminuria and glomerular pathology in the kidneys of db/db diabetic mice and streptozocin-induced diabetic mice. These phenotypes are, at least in part, dependent on Ang II signaling and can be rescued by AT1 receptor blockade. Thus, these data definitively indicate that ACE2 is renoprotective. In hypertensive SHR rats, with the onset of hypertension, the tubular expression of ACE2 in the kidney is downregulated compared with control rats. Diabetic nephropathy in experimental models has also been shown to alter both the glomerular and tubular expression of ACE2, and recently, the involvement of ACE2 in glucose transport was implicated. There is an early increase in ACE2 expression and activity in the kidneys of diabetic db/db and Akita mice. The highest expression of ACE2 mRNA found in renal proximal tubules was, however, significantly reduced in the tubules from diabetic rats. Thus, reduced ACE2 expression might contribute to the pathogenesis and progression of kidney diseases.

**ACE2 in the Lung**

Acute respiratory distress syndrome (ARDS) is the most severe form of acute lung injury, which affects approximately 1 million individuals worldwide/year and has a mortality rate of at least 30–50%. ARDS can be triggered by multiple diseases, such as sepsis, aspiration, trauma, acute pancreatitis, or pneumonias following infections with SARS-CoV or the avian and human influenza viruses. Previous studies showed a correlation between an ACE insertion/deletion polymorphism and the severity of ARDS in humans, and ACE inhibitor treatments in rodent ARDS models suggested that the RAS could have a role in ARDS/acute lung injury. Intriguingly, despite having a normal lung structure and function, Ace2 knockout mice exhibit very severe pathology of ARDS/acute lung injury compared with wild-type control mice, defined by enhanced vascular permeability, increased lung edema, neutrophil accumulation, and markedly worsened lung function. AT1 blocker treatment or additional Ace deficiency on an Ace2 knockout background rescues the severe acute lung injury phenotype of Ace2 single mutant mice. Importantly, treatment with catalytically-active, but not enzymatically-inactive, recombinant ACE2 protein improved the symptoms of acute lung injury in wild-type mice, as well as in Ace2 knockout mice. Furthermore, in a recent study of large animals in a sepsis ARDS model, recombinant ACE2 protein significantly improved the respiratory failure and increased the oxygen levels of ARDS-affected pigs. Thus, in acute lung injury, ACE, Ang II, and the AT1 receptor function as lung injury-promoting factors, while the negative regulation of Ang II levels by ACE2 protects against lung injury. Moreover, in other lung injury models, such as bleomycin-induced lung fibrosis and monocrotaline-induced pulmonary hypertension, ACE2 has been recently shown to protect against chronic lung injury, fibrosis, and pulmonary vasoconstriction. These results indicate that ACE2 may serve as an entirely novel therapeutic for chronic lung diseases as well as acute lung injury. The efficacy of recombinant soluble human ACE2 protein on acute lung failure is currently being tested in phase II human clinical trials.

During 2003, a newly identified illness termed “severe acute respiratory syndrome (SARS)” spread rapidly worldwide. International cooperative teams swiftly isolated a novel coronavirus as the SARS pathogen (SARS-CoV) and determined the SARS-CoV genome sequence. Surprisingly, ACE2 was identified by co-immunoprecipitation techniques as a functional receptor in vitro. Subsequently, in a SARS infection model in Ace2 knockout mice, ACE2 was indeed identified as the essential receptor for SARS infections in vivo. One mystery of SARS-CoV is why, in contrast to other coronaviruses, such infections trigger severe lung disease with such high mortality. Accumulating evidence indicates that severe SARS infections are dependent on the burden of viral replication, as well as on the immunopathological consequences of the host response. In addition to aberrant activation of the immune system, our own studies have implicated direct involvement of ACE2 in SARS pathogenesis. Intriguingly, both SARS-CoV infection and challenge with recombinant SARS Spike protein triggered a marked downregulation of ACE2 expression in both lungs and cell culture. Thus, SARS-CoV-infected or Spike protein-treated mice resemble Ace2 knockout mice. Similar to Ace2 mutant mice, Spike-treated wild-type mice show markedly more severe pathology in acute lung injury. Therefore, downregulation of the SARS receptor, ACE2, by SARS-CoV infection activates the RAS and contributes to the pathogenesis of severe ARDS/acute lung injury in SARS.

**ACE2 in Hartnup’s Disorder and Intestinal Epithelial Immunity**

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. Collectrin is expressed in β-cells of the pancreas under the transcriptional control of hepatocyte nuclear factor 1, where it was implicated in insulin exocytosis or β-cell proliferation. Surprisingly, using genetargeting studies of Collectrin in vivo, Collectrin turned out to be a critical binding partner for neutral amino acid transporters. Collectrin physically associates with numerous apical amino acid transporters in the kidney and controls their expression and transport functions. As a consequence, Collectrin mutant mice exhibit a marked defect in the reabsorption of amino acids in the proximal tubules of the kidneys. Biochemically,
Collectrin binds to BAT1-family amino acid transporters and controls polarized expression of these transporters on the cell surface.

Hartnup’s disorder is a hereditary familial disease characterized by a pellagra-like light-sensitive rash, cerebellar ataxia, emotional instability, and amino aciduria. Gene mutations in the BAT1 (Slc6a19) neutral amino acid transporter have been identified as a cause of Hartnup’s disorder. Despite the lack of mutations of ACE2 and Collectrin in Hartnup’s disease, among the known human Hartnup mutations in BAT1, the A69T and R240Q missense mutants were shown to be functionally linked to ACE2 and Collectrin.

In addition to Hartnup’s disorder, a recent study by our group revealed that ACE2 regulates intestinal epithelial immunity by controlling amino acid homeostasis, expression of antimicrobial peptides, and the ecology of the gut microbiome. The finding can molecularly explain why amino acid malnutrition can cause intestinal inflammation and diarrhea. Because such RAS-independent functions of ACE2 have not yet been observed in cardiovascular systems, such as hearts, kidneys, and blood vessels, further studies are warranted.

Conclusions

ACE2 has been established as an in vivo negative regulator of systemic and local RAS function through its peptidase activity. The carboxypeptidase activity of ACE2 limits the availability of Ang II, generates counter-regulatory vasodilator peptides such as Ang1–7, and may also act on other peptide systems such as Apelin or dynorphin. Importantly, ACE2 has also been identified as the key SARS receptor and plays a protective role in SARS-mediated ARDS. These findings led to the development of recombinant human ACE2 protein as a potential novel therapeutic agent, which is now being tested in a clinical trial. During the course of study of ACE2 function, we also made an entirely unexpected find: ACE2 and Collectrin are essential for the expression of ACE2 function, we also made an entirely unexpected finding: ACE2 and Collectrin are essential for the expression of amino acid transporters, which added another level of complexity to this key regulator of the RAS system. Understanding the complexity and molecular crosstalk of the RAS system will not only broaden our knowledge of the evolution and biology of the cardiovascular system, but also lead to the development of new and refined therapeutic strategies for various diseases.

Acknowledgments

K.K. is supported by the Funding Program for Next Generation World-Leading Researchers from the Japan Society for the Promotion of Science (NEXT Program), and the KAKEN (23390155) from the Japanese Ministry of Science. Y.L. is supported by the NEXT Program, KAKEN, Takeda Foundation and Uehara Foundation. J.M.P. is supported by IMBA, and the LeDucq Foundation.

References


Ware LB. Pathophysiology of acute lung injury and the acute respiratory distress syndrome. Semin Respir Crit Care Med 2006; 27: 337–349.


