



Published in final edited form as:

Circ Res. 2016 April 15; 118(8): 1313–1326. doi:10.1161/CIRCRESAHA.116.307708.

Role of the ACE2/Angiotensin 1–7 axis of the Renin-Angiotensin System in Heart Failure

Vaibhav B. Patel^{1,2}, Jiu-Chang Zhong^{3,4}, Maria B. Grant⁵, and Gavin Y. Oudit^{1,2,6}

¹Division of Cardiology, Department of Medicine, University of Alberta, Edmonton, Canada

²Mazankowski Alberta Heart Institute, University of Alberta, Edmonton, Canada

³State Key Laboratory of Medical Genomics, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

⁴Shanghai Key Laboratory of Hypertension, Shanghai Institute of Hypertension, Shanghai, China

⁵Department of Ophthalmology, Indiana University School of Medicine, Indianapolis, IN, USA

⁶Department of Physiology, University of Alberta, Edmonton, Canada

Abstract

Heart failure remains the most common cause of death and disability, and a major economic burden, in industrialized nations. Physiological, pharmacological, and clinical studies have demonstrated that activation of the renin-angiotensin system is a key mediator of heart failure progression. Angiotensin converting enzyme 2 (ACE2), a homologue of ACE, is a monooxypeptidase that converts angiotensin II (Ang II) into angiotensin 1–7 (Ang 1–7) which, by virtue of its actions on the Mas receptor, opposes the molecular and cellular effects of Ang II. ACE2 is widely expressed in cardiomyocytes, cardiofibroblasts, and coronary endothelial cells. Recent preclinical translational studies confirmed a critical counter-regulatory role of ACE2/Ang 1–7 axis on the activated renin-angiotensin system that results in heart failure with preserved ejection fraction. While loss of ACE2 enhances susceptibility to heart failure, increasing ACE2 level prevents and reverses the heart failure phenotype. ACE2 and Ang 1–7 have emerged as a key protective pathway against heart failure with reduced and preserved ejection fraction. Recombinant human ACE2 has been tested in phase I and II clinical trials without adverse effects while lowering and increasing plasma Ang II and Ang 1–7 levels, respectively. This review discusses the transcriptional and post-transcriptional regulation of ACE2 and the role of the ACE2/Ang 1–7 axis in cardiac physiology and in the pathophysiology of heart failure. The pharmacological and therapeutic potential of enhancing ACE2/Ang 1–7 action as a novel therapy for heart failure is highlighted.

Address for Correspondence: Gavin Y. Oudit, MD, PhD, FRCPC, Division of Cardiology, Department of Medicine, Mazankowski Alberta Heart Institute, University of Alberta, Edmonton, T6G 2S2, Alberta, Canada, Phone: 780-407-6589, Fax: 780-407-6452, gavin.oudit@ualberta.ca.

Disclosures

NONE.

Keywords

Angiotensin II; Angiotensin 1–7; Angiotensin 1–9; Angiotensin converting enzyme 2; heart failure; recombinant human ACE2

The renin-angiotensin system (RAS) is a peptidergic system that functions in the homeostatic control of the cardiovascular and renal systems and in regulating extracellular fluid volume. Inhibition of the RAS plays a central role in alleviating the increased morbidity and mortality of patients with heart failure (HF).^{1, 2} The RAS consists of a series of enzymatic reactions that result in generation of angiotensin (Ang) II. In the first step, renin (an aspartyl proteinase secreted by kidney into the circulation) cleaves hepatic peptide angiotensinogen to produce Ang I in the blood. Ang I is then hydrolyzed by angiotensin-converting enzyme (ACE) in the second step, producing the octapeptide Ang II. This biologically active peptide acts on Ang II type 1 and type 2 receptors (AT₁R and AT₂R) (Figure 1A). Ang II promotes vasoconstriction, inflammation, salt and water reabsorption, and oxidative stress via the activation of AT₁R.³ These detrimental effects of Ang II/AT₁R have encouraged the quest for a counter-regulatory axis of the activated RAS. RAS was initially thought to function as a systemic entity not localized to any specific tissue. However, this notion of systemic RAS was challenged by observations that many tissues are capable of synthesizing the key components of RAS,^{4–6} including heart,^{4, 7, 8} kidney,⁹ vasculature,⁹ pancreas,^{6, 10} retina,^{11, 12} brain,^{6, 13} and others. The local RAS could produce peptides at the tissue level that show autocrine effects (on the cells where they are being produced), paracrine effects (on neighboring cells), or endocrine effects (on a distant organ or tissue; via systemic circulation).^{6, 14}

Our conception of the RAS family has seen substantial changes with the identification of angiotensin-converting enzyme 2 (ACE2), a homologue of ACE. ACE2 is a monocarboxypeptidase that degrades Ang I into a nonapeptide, Ang 1–9 and Ang II into a heptapeptide, Ang 1–7 (Figure 1A). The discovery of ACE2, Ang 1–9, and Ang 1–7 unravels a distinct enzymatic pathway for degradation of Ang I and Ang II as endogenous negative regulation of RAS activation. Moreover, ACE2 has been identified as an important RAS regulator capable of mitigating the deleterious actions mediated by Ang II and AT₁R. This is of particular importance in pathological conditions where the RAS is activated. Ang 1–7 is a biologically active peptide exerting a wide array of actions, many of which are opposite to those attributed to Ang II.^{15–18} In 2003, an endogenous orphan receptor, Mas (MasR), was identified as the Ang 1–7 receptor. A779, a MasR antagonist, has been shown to block the majority of Ang 1–7 effects.^{17, 19–22} Ang 1–9 has also shown beneficial biological effects via AT₂R that result in cardioprotection.^{23–26} Thus, while ACE/Ang II/AT₁R is a well-established axis of the RAS, the ACE2/Ang 1–7/MasR and ACE2/Ang 1–9/AT₂R axes have emerged as physiological antagonists that counter-regulate the activate RAS.^{16, 27–31} Taken together, the cardioprotective effects of ACE2 can be attributed to i) degradation of Ang I to Ang 1–9, limiting the availability of substrate for ACE action, ii) degradation of Ang II, limiting its detrimental effects, and iii) generation of Ang 1–7, exerting its cardioprotective effects. Several lines of evidence suggest that ACE2 level and/or activity balances two different arms. Decreased ACE2 activity results in activation of the

Ang II/AT₁R axis, contributing to increased progression of heart disease. Increased ACE2 level/activity leads to activation of ACE2/Ang 1–9 and ACE2/Ang 1–7 axes, leading to protection against heart disease (Figure 1B). In this review, we highlight the role of ACE2/Ang 1–7 in counter-regulation of Ang II actions, different approaches to manipulating ACE2/Ang 1–7 levels, and the potential of enhancing ACE2 action as a therapy for HF.

ACE2: Discovery, biochemistry, and regulation

a. Discovery of ACE2 and its differences from ACE

ACE2 or ACE homologue (ACEH) was discovered as a zinc metalloproteinase by two different groups in 2000. ACE2 was initially identified from human HF and lymphoma cDNA libraries^{32, 33} and was later shown to serve as a receptor for the SARS coronavirus.³⁴ It was found to possess an apparent signal peptide, a transmembrane domain, and a single metalloproteinase active site containing an HEXXH zinc-binding domain.^{32, 33} ACE2 is a type I transmembrane protein with an extracellular N-terminal domain containing the catalytic site and an intracellular C-terminal tail. Similar to ACE, the catalytic site of ACE2 is exposed (an ‘ectoenzyme’) to circulating vasoactive peptides.³⁵ Expression of a soluble truncated form of ACE2 in CHO cells produced a glycoprotein of 120 kDa that was able to cleave Ang I and II but not bradykinin.³³ Other critical residues typical of the ACE family are conserved in ACE2. Tipnis et al. discovered that the ACE2 gene contains 18 exons, with several having considerable size similarity to the first 17 exons of human ACE.³³ The metalloproteinase catalytic domains of ACE2 and ACE are 42% identical according to the findings of Donoghue et al.³² In spite of such similarity though, unlike ACE, ACE2 does not convert Ang I to Ang II. In fact, ACE2 activity is inhibited by EDTA but is unaffected by ACE inhibitors such as captopril and Lisinopril.^{32, 33, 36} Further research revealed a major difference in enzymatic actions of ACE and ACE2. ACE acts a dipeptidyl carboxypeptidase (removing a dipeptide from the C-terminus of substrate) whereas ACE2 acts as a mono-carboxypeptidase (removing a single amino acid) that degrades Ang I to generate the nonapeptide Ang 1–9 and Ang II to generate the heptapeptide Ang 1–7.^{32, 33} Later studies focused on ACE2 purification and characterization of its catalytic activity, showing a pH optimum of 6.5 and enhancement of ACE2 activity by monovalent anions, including Cl⁻ and F⁻, but not Br⁻.³⁷ This is consistent with the activity of ACE.³⁸ However, ACE2 was later shown to possess one Cl⁻ binding site compared to two Cl⁻ sites in ACE.³⁹ Out of 126 biological peptides tested with ACE2 using LC-MS, ACE2 hydrolyzed three peptides with high efficiency: Ang II, apelin-13, and dynorphin A 1–13. ACE2 also showed a preference for cleaving C-terminal amino acids with peptides ending in Pro-X, where X is a hydrophobic amino acid.^{38, 40} This cleavage preference of ACE2 was supported by a key experiment in which a dipeptide, Pro-Phe, completely inhibited ACE2 activity at 180 μM with Ang II as the substrate.⁴¹ In a search for the active site residues of ACE2, site-directed mutagenesis revealed that Arg273 is critical for substrate binding and its replacement causes complete loss of enzyme activity.³⁹

The difference in ACE and ACE2 enzymatic activity became more evident upon the discovery that human ACE2 catalytic efficiency is 400-fold higher with Ang II as a substrate than with Ang I.^{38, 42} To further unravel the biological role and importance of ACE2, several

ACE2 inhibitors were designed and synthesized via substrate-based⁴³ and structure-based⁴⁴ pharmacophore design and virtual screening. MLN-4760, a potent and selective inhibitor developed with substrate-based design, has been a key tool for *in vivo* and *in vitro* studies.⁴³ In the last 15 years, distinct roles of ACE2 have been discovered ranging from catalytic activities with various substrates, functional SARS coronavirus receptor, and an amino acid transporter.^{34, 40, 45, 46} ACE2 was initially thought to be expressed only in heart, kidney, and testes,³³ but was eventually found to be widely expressed in various organ systems including the cardiovascular system, kidneys, lungs, and brain, in which it exerts important actions to maintain cardiovascular homeostasis.^{47–52} In the heart, ACE2 is localized to cardiomyocytes (contracting cardiac muscle cells), cardiac fibroblasts, and the coronary vascular endothelium.^{53, 54} MasR is also present on cardiomyocytes, cardiac fibroblasts, and endothelial cells.^{19, 55–57}

b. Proteolytic processing, transcriptional, and post-transcriptional regulation of ACE2

Various molecules are shed from cell surfaces by the action of a disintegrin and metalloproteinase (ADAM) 17, also known as tumor necrosis factor- α converting enzyme (TACE).^{58–60} ADAM17-mediated proteolysis of ACE2 releases an enzymatically active ectodomain from the cell surface, generating a soluble, active form of the enzyme. Lambert et al. confirmed the ectodomain shedding of heterologously expressed ACE2 in HEK293 cells and endogenously expressed ACE2 in Huh7 cells. Small interfering RNA (siRNA) against ADAM17 reduced the shedding of ACE2 and ADAM17 overexpression increased it, providing direct evidence of ADAM17-mediated ectodomain shedding of ACE2. Lambert et al. later discovered that calmodulin, a ubiquitous calcium binding protein, associates with ACE2 and prevents its shedding, an action inhibited by calmodulin inhibitors.⁶¹ However, increased ACE2 shedding mediated by calmodulin inhibitors was only partially blocked by metalloproteinase inhibitor, suggesting the involvement of alternate proteolytic pathways not yet identified.⁶¹ The initial observation of ACE2 shedding was further confirmed and shown to be a constitutive and regulated phenomenon in various cell types including CHO cells, fibroblasts, 3T3-L1 adipocytes, neurons, cardiomyocytes, and proximal tubular cells.^{53, 62–64} In particular, we identified a positive feedback mechanism in the RAS whereby Ang II facilitates the loss of its negative regulator, ACE2.⁵³ Ang II action on AT₁R leads to phosphorylation (mediated by p38 mitogen-activated protein kinase [MAPK]) and activation of ADAM17, resulting in increased ACE2 shedding (Figure 2).^{53, 65} Shedding of membrane-bound ACE2 is likely responsible for the loss of myocardial ACE2^{66, 67} and elevation in plasma ACE2 activity in HF that correlates with worsened prognosis.^{68, 69} The biological and clinical significance of ACE2 ectodomain shedding is yet to be fully characterized. The inhibition of ectodomain shedding of ACE2 by manipulating the enzyme activity of ADAM17 could have therapeutic potential in HF.

A reporter system using the 3'-UTR of an ACE2 transcript was used to determine the functionality of putative microRNA (miRNA) binding sites identified *in vitro*. In a luciferase reporter assay containing ACE2 3'-UTR, miR-421 strikingly decreased ACE2 protein levels while loss of miR-421 reversed these effects, implying that miR-421 modulates ACE2 expression via post-translational repression rather than degradation of mRNA transcripts. This identified miR-421 as a potential regulator of ACE2 and was the first demonstration of

post-transcriptional regulation of ACE2.⁷⁰ ACE2 mRNA expression is also regulated by Sirtuin 1 (SIRT1). Energy stress by hypoxia and adenosine monophosphate kinase (AMPK) activation by 5-amino-4-imidazolecarboxamide riboside (AICAR) increase the cellular ratio of NAD⁺ to NADH and increase ACE2 expression.⁷¹ SIRT1, in the presence of a possible but unknown cofactor, binds to the promoter region of *ACE2* and this binding is promoted by AICAR. AICAR-induced ACE2 expression is inhibited by an inhibitor of SIRT1, providing strong evidence for the SIRT1-mediated transcriptional regulation of ACE2 under conditions of energy stress (Figure 2).⁷¹ Similarly, apelin also increases ACE2 promoter activity *in vitro* and upregulates ACE2 expression in failing hearts *in vivo* (Figure 2).⁷² Therapeutically, agents that increase ACE2 expression (SIRT1 activators, apelin) or inhibitors of negative regulators of ACE2 (TACE or miR-421) could be utilized to enhance ACE2 activity and counteract cardiovascular diseases including HF.

Role of ACE2/Ang 1–7 in HF

Heart failure is a growing epidemic with high morbidity and mortality at an international scale. Acute and chronic HF is characterized by activation of several signaling pathways associated with pathological hypertrophy and maladaptive ventricular remodeling. HF is caused by damage to or loss of cardiomyocytes and contributes to diminished systolic performance and diastolic dysfunction in the failing heart.^{73, 74} HF involves changes in cardiac structure, myocardial composition, myocyte deformation, and multiple biochemical and molecular alterations, collectively referred to as adverse myocardial remodeling. Despite improvements in medical and surgical therapies, cardiac diseases remain the leading cause of death in North America, with ischemic and hypertensive heart disease as the leading cause of HF.^{75–77}

Diabetes mellitus and obesity are major causes of morbidity and mortality in all parts of the world including North America.⁷⁸ Diabetes mellitus is characterized by insulin insufficiency that is frequently associated with severe cardiovascular complications and increased risk for hypertension, HF, and myocardial infarction (MI).^{79–81} Obesity itself is an independent risk factor for development of HF with preserved ejection fraction (HF-pEF), independent of other comorbid conditions.^{82–84} The rising global tide of obesity and diabetes will likely contribute further to the increasing prevalence of systolic and diastolic HF.^{78, 80, 85–87} Although the mechanisms underlying the intertwined relationship among diabetes, obesity, hypertension, and cardiovascular events remain to be fully defined, major culprits that have been implicated are cardiovascular inflammation, oxidative stress, mitochondrial dysfunction, and insulin resistance, all closely linked with abnormalities in the RAS.^{88–91}

Neurohormonal changes such as activation of the RAS and increased Ang II levels play a pivotal role in adverse myocardial remodeling and progression to HF.^{2, 92, 93} Indeed, pharmacological antagonism of the RAS using ACE inhibitors (ACEi) or AT₁R blockers (ARB) is a cornerstone of current medical therapy for human HF, including diabetic cardiomyopathy.^{75, 94} While these pharmacotherapies for HF provide benefits, patients with HF continue to be plagued by clinical deterioration, high morbidity, and mortality.⁷⁷ Irrespective of the capacity of ACE inhibitors to inhibit ACE action, Ang II levels can remain elevated in optimally treated HF patients. About 50% of the patients using ongoing

ACEi therapy exhibit elevated levels of Ang II, the result of activation of mast cell chymase.^{95–98} Therefore, there is an urgent need to identify alternative strategies to minimize the detrimental effects of Ang II and treat HF.

ACE2, by virtue of its action on Ang I and Ang II, is nature's endogenous ACE inhibitor at the cellular level (Figure 3). Ang 1–9, the product of ACE2 degradation of Ang I, has recently shown promising anti-hypertrophic, anti-fibrotic, and anti-hypertensive effects. These beneficial effects result in cardioprotection against hypertension and MI.^{23–26} Adenoviral delivery of Ang 1–9 in H9c2 cardiomyocytes has shown anti-hypertrophic effects comparable to adenoviral Ang 1–7 delivery.²³ Moreover, RhoA/Rho kinase inhibition has shown potent anti-hypertensive effects that were mediated via the upregulation of vascular and plasma ACE2 and increased plasma Ang 1–9 levels, without an increase in Ang 1–7 levels.²⁶ This suggests a potential role for Ang 1–9 in the anti-hypertensive effects of RhoA/Rho-kinase inhibition.

Both Ang I and Ang II can function as the preferred substrate for ACE2. Studies using recombinant human ACE2 (rhACE2) and ACE2 purified from sheep tissues showed Ang II as a preferred substrate for ACE2.^{33, 38, 41, 67, 99, 100} In sheep, conversion from Ang I to Ang 1–9 was not detected while the proximal tubules contained robust ACE2 activity that converted Ang II to Ang 1–7.¹⁰¹ In contrast, changes in ACE2 correlated with plasma Ang 1–9 levels in rats.¹⁰² In a recent study Ye et al. demonstrated that rhACE2 generated Ang 1–7 and Ang 1–9 while recombinant murine ACE2 generated predominantly Ang 1–7.¹⁰³ In addition, the therapeutic effects of rhACE2 is highly dependent on Ang 1–7 action in rodents^{30, 67, 100} and in human studies rhACE2 clearly lowered plasma Ang II levels resulting in increased plasma Ang 1–7 levels.^{104–106} However, it remains possible that the contribution of Ang 1–9 in ACE2's beneficial effects may be underestimated and requires further investigation with a clear emphasis on human studies.

Ang 1–7 activates MasR and exerts various effects, the majority of which antagonize Ang II's effects.^{15, 20} These effects include i) activation of the phosphatidylinositol 3-kinase (PI3K)-Akt-endothelial nitric oxide synthase (eNOS) pathway; ii) inhibition of protein kinase C (PKC)-p38 MAPK pathways and iii) inhibition of collagen expression to limit cardiac fibrosis (Figure 3).^{19, 107, 108} To understand the relative contributions of inhibiting the Ang II/AT₁R axis and activating the Ang 1–7/MasR axis to cardioprotective effects, we studied the effects of irbesartan and Ang 1–7 supplementation in pressure-overload-induced HF in ACE2 knockout mice.³⁰ We found functional redundancy in the anti-fibrotic and anti-hypertrophic effects and suppression of pathological signaling. The cardioprotective effects of irbesartan and Ang 1–7 were equivalent, suggesting similar significance of both axes.

a. Role of ACE2/Ang 1–7 in hypertension

Activated RAS and Ang II are established key mediators of hypertension, therefore ACE2 is hypothesized to be a potent modulator of blood pressure and its deficiency leads to hypertension. In a preclinical model of hypertension, ACE2 gene maps to a defined quantitative trait locus on the X-chromosome previously identified as a quantitative locus for blood pressure.⁷ Recent studies suggest an association between ACE2 activity and blood pressure levels.^{109, 110} Serum ACE2 activity was higher in patients with hypertension

compared to healthy individuals. In hypertensive patients with type 1 diabetes, serum ACE2 activity was positively correlated with systolic blood pressure in both males and females.¹¹⁰ These studies suggest that elevated ACE2 may be a “compensatory response” to the hypertension. Indeed, the anti-hypertensive role of ACE2 has also been established in various preclinical models of hypertension.^{28, 111–113} Lentiviral overexpression of ACE2 results in increased expression of anti-hypertensive components of RAS (Ang 1–7, MasR and AT₂R) attenuating the elevated blood pressure.^{111, 112} Similarly, rhACE2 pretreatment alleviated hypertension induced by acute Ang II infusion and was associated with decreased plasma Ang II and increased plasma Ang 1–7 levels.⁹⁹ Cyclodextrin-encapsulated Ang 1–7, AVE0091, and CGEN856S (MasR agonists) have shown blood pressure-lowering effects in hypertensive animals.¹¹⁴ The anti-hypertensive effects of ACE2/Ang 1–7 generated interest in potential cardioprotective effects against hypertensive heart diseases, a group of disorders that includes HF, ischemic heart disease, hypertensive heart disease, and left ventricular hypertrophy.

b. Role of ACE2/Ang 1–7 in HF with reduced ejection fraction (HF-rEF)

ACE2 plays a critical role in the control of cardiac physiology and altered ACE2 expression or activity is linked to the progression of heart disease (Figure 1B). In heart, ACE2 is expressed in various cells including the cardiomyocytes, cardiac fibroblasts, and coronary endothelial cells,¹¹⁵ where it negates Ang II actions and also activates Ang 1–7/MasR signaling (Figure 3). ACE2 expression is highly affected by pathological disease conditions, suggesting its role in counter-regulating the development of cardiac diseases. In the human population, genetic variations in the *ACE2* gene correlate with susceptibility to cardiovascular disease.^{116–118} Single nucleotide polymorphisms of *ACE2* are associated with variation in septal wall thickness, ventricular hypertrophy,¹¹⁶ and coronary artery disease.¹¹⁷

The first report on the role of ACE2 as an essential regulator of cardiac function came soon after its discovery.⁷ In that study, ACE2 knockout mice showed reduced systolic function. The decrease in systolic function was both sex- and time-dependent, with more severe abnormalities in male than in female mice, and a more pronounced phenotype in older animals. ACE2 knockout mice also showed increased Ang II levels, which were rescued with genetic ablation of ACE.⁷ Consistently, we found age-dependent dilated cardiomyopathy in ACE2 knockout mice. This resulted in reduced systolic function along with increased cardiac inflammation and oxidative stress.²⁹ Myocardial ACE2 protein levels were decreased in pressure-overload-induced HF, suggesting an inverse relationship between myocardial ACE2 protein levels and disease progression.^{22, 67} In addition, loss of ACE2 resulted in worsened pathological remodeling in response to pressure-overload-induced biomechanical stress. This was associated with systolic dysfunction and ventricular dilation. Both were deemed due to activation of the myocardial NAPDH oxidase system, superoxide production, and matrix metalloproteinase (MMP) activation, which was attributed to increased local Ang II levels (Figure 3; Table).^{30, 119, 120} Post-MI remodeling and coronary artery disease is one of the most common causes of HF.¹²¹ MI increased ACE2 mRNA expression in humans, mice, and rats,^{122, 123} whereas loss of ACE2 or inhibition of ACE2 by C16, resulted in worsening of MI-induced cardiac dysfunction, increased infarct size, MMP

activation, cardiac extracellular matrix disruption, and inflammation (Table).^{123, 124} Lentiviral^{125, 126} or adenoviral¹²⁷ overexpression of ACE2 ameliorated MI-induced cardiac remodeling. In addition, lentiviral infection of cultured fibroblasts decreased the acute hypoxic exposure-induced production of collagen.¹²⁸

Importantly, Ang 1–7 treatment has shown noticeable cardioprotective effects in preclinical models of non-ischemic and ischemic cardiomyopathy.^{15, 21, 126, 129} Ang 1–7 suppressed cardiomyocyte growth *in vitro* and inhibited myocardial infarction-induced ventricular hypertrophy *in vivo*. Ang 1–7 also decreased myocardial levels of pro-inflammatory cytokines (TNF α and IL-6) leading to alleviation of cardiac inflammation.^{21, 126} These results confirm the important contribution of Ang 1–7 in the cardioprotective effects of ACE2 (Figure 4).

c. Role of ACE2/Ang 1–7 in HF with preserved ejection fraction (HF-pEF)

HF-pEF, also termed diastolic HF, is often associated with a normal or smaller heart size and diastolic filling abnormalities. It accounts for approximately 30% of all HF patients, with a similar mortality rate to patients with HF-rEF.^{84, 130} Ang II-induced diastolic dysfunction is a clinically relevant, widely accepted preclinical model of HF-pEF. We and others found that loss of ACE2 resulted in worsened cardiac dysfunction, cardiac hypertrophy, and fibrosis, leading to greater diastolic dysfunction in response to Ang II (Table).^{67, 131} Importantly, treatment with rhACE2 decreased plasma and myocardial Ang II levels and increased plasma Ang 1–7 levels, providing definitive evidence for a key role of ACE2 in the metabolism of Ang II.⁶⁷ Furthermore, rhACE2 attenuated pathological changes mediated by Ang II, reducing myocardial hypertrophy and fibrosis and correcting diastolic dysfunction. However, treatment with rhACE2 did not affect baseline plasma Ang II, Ang 1–7, or blood pressure in wild-type mice. This suggests that substrate availability is a limiting factor in ACE2 enzymatic activity.¹³² The pursuit of molecular mechanisms for these actions identified rhACE2's capacity to inhibit the Ang II effects on TGF- β 1 activation and collagen production.^{57, 67, 133} Loss of ACE2 also resulted in increased production of reactive oxygen species (ROS) via NADPH oxidase 2 activation, which is also suppressible by rhACE2.⁶⁷ Lentiviral overexpression of ACE2 protects the heart against myocardial injuries induced by Ang II in rats, confirming the role of ACE2 in counteracting HF-pEF.^{134,30} We assessed the contribution of Ang 1–7/MasR activation to the favorable effects shown by rhACE2 in the Ang II-induced murine HF model; inhibition of Ang 1–7/MasR signaling resulted in loss of rhACE2 mediated cardioprotective effects. However, this observation does not rule out the potential contribution of Ang 1–9 to the protective effects of rhACE2. An appropriate preclinical study is required to assess the relative contributions of Ang 1–9 and Ang 1–7.¹⁰⁰ ACE2 is an endogenous regulator of activated RAS-induced HF-pEF and enhancing ACE2 has a marked beneficial effect.

d. Role of ACE2/Ang 1–7 in diabetes and obesity-associated cardiomyopathy

Diabetes and obesity are major causes of morbidity and mortality in all parts of the world including Canada.⁷⁸ Studies of the ACE2/Ang 1–7 axis in diabetes and obesity-associated cardiac dysfunction have shed light on the critical role of this pathway in counter-regulation of the Ang II/AT₁R axis (Figure 4). In human type 1 diabetes, elevated plasma ACE2

activity correlated with microvascular and macrovascular complications, increased systolic blood pressure, and the duration of diabetes,¹¹⁰ strongly supporting a key clinical role for the ACE2 system in cardiovascular disease that is secondary to diabetes. The role of ACE2 in diabetic cardiovascular complications has been studied in various preclinical models of diabetes. Tools such as ACE2 knockout mice,^{123, 135} adenoviral ACE2 gene transfer,¹³⁶ rhACE2,¹³⁷ ACE2 activators and inhibitors,^{138–140} Ang 1–7 supplementation,¹⁴¹ Ang 1–7/MasR activator (AVE0991)¹⁴² and Ang 1–7/MasR receptor blockade (A779)¹⁴² have been utilized to assess the role of ACE2/Ang 1–7 in diabetic cardiovascular complications.

We studied the role of ACE2 in preventing progression of type 1 diabetic cardiovascular complications¹³⁵ using a clinically relevant animal model of diabetes, the Akita mouse. Akita type 1 diabetic hearts show diastolic dysfunction associated with reduced levels of the cardiac SERCA2a and increased myocardial lipotoxicity.¹⁴³ Loss of ACE2 in these hearts, in Akita/ACE2 knockout double mutants, resulted in systolic dysfunction.¹³⁵ Akita/ACE2 knockout hearts exhibited increased NADPH oxidase activity, ROS production, and protein kinase C and MMP activation, leading to increased degradation of the cardiac extracellular matrix. This study demonstrated a key role for ACE2 as a negative regulator of activated RAS in diabetic cardiomyopathy.¹³⁵ Further studies have validated our findings for this essential role of ACE2 in diabetic cardiomyopathy.^{136, 138–140, 144} We also identified beneficial effects of Ang 1–7 in type 2 diabetic cardiomyopathy. By reducing cardiac hypertrophy, lipotoxicity, and adipose inflammation, in combination with increased adipose triglyceride lipase, Ang 1–7 completely rescued diastolic dysfunction in the db/db type 2 diabetic murine model.^{141, 145}

Obesity is characterized by excessive fat accumulation in adipose tissues throughout the body and is the most common nutritional disorder in industrialized countries. Obesity is associated with increased morbidity and mortality and is a risk factor for development of HF-pEF, independent of other comorbid conditions.^{82–84, 146} We studied the role of ACE2 in obesity induced by high fat diet and associated cardiac dysfunction.¹⁴⁷ Loss of ACE2 was associated with worsened obesity-associated HF-pEF due to increased epicardial adipose tissue inflammation, myocardial lipotoxicity, and cardiac metabolic abnormalities (Table). These findings coupled with the protective effects of ACE2/Ang 1–7 in the vasculature supports a key role of adipose tissue inflammation and microvascular dysfunction in the pathogenesis of HF-pEF.^{69, 148} Importantly, Ang 1–7 prevented these changes and rescued HF-pEF in ACE2 knockout mice, validating its critical role in ACE2-mediated cardioprotection (Figure 4). As such, enhancing the ACE2/Ang 1–7 pathways represents a potential therapy for HF-pEF, which currently lacks effective therapies.

Therapeutic approaches and potential of enhancing ACE2/Ang 1–7 in HF

Irrespective of the capacity of ACEi to inhibit ACE action, Ang II levels can remain elevated in optimally treated HF patients; about 50% of patients using ongoing ACEi therapy exhibit elevated levels of Ang II.^{95–98} The generation of plasma and tissue Ang II by non-ACE related enzymes such as chymase suggests that enhancing ACE2 action may indeed have a unique therapeutic role.^{67, 96} In fact, ACEi and ARB have been shown to upregulate the expression of ACE2 or prevent the loss of ACE2.^{102, 149} ADAM17-mediated ACE2

shedding represents a mechanism by which Ang II induces a positive feedback mechanism in the tissue-localized RAS leading to its dysregulation. This results in the neurohumoral imbalance that is typical of HF.¹⁵⁰ Inhibiting TACE-mediated shedding of ACE2 from the surface of cardiac cells, leading to retention of ACE2 enzymatic activity within the cardiac microenvironment, might have therapeutic potential. ACE2 is post-transcriptionally regulated by miR-421, inhibition of which may result in increased ACE2 expression. As ACE2 is also subject to transcriptional regulation by SIRT1 and apelin, SIRT1 activators or apelin may have therapeutic benefits by enhancing the actions of ACE2.

A well-studied tool to enhance ACE2 action is rhACE2. A randomized, double-blinded, placebo-controlled study administered Intravenous rhACE2 to healthy human subjects and found that the rhACE2 was well-tolerated. Despite marked changes in angiotensin system peptide concentrations, hypotension was absent, suggesting the presence of effective compensatory mechanisms in healthy volunteers.¹⁰⁶ rhACE2 is primarily responsible for the conversion of Ang II into Ang 1–7 but can also convert Ang 1–10 into Ang 1–9.¹⁵¹ In healthy human volunteers treated with rhACE2, Ang II levels were reduced but Ang 1–7 levels were increased or remained unchanged.^{104, 106} Importantly, in a recently completed phase II trial in patients with acute lung injury, rhACE2 resulted in sustained reduction in plasma Ang II levels and elevation in Ang 1–7 levels.¹⁰⁵ We propose that assessment of plasma RAS peptide levels can allow the tailoring of rhACE2 therapy for human HF. rhACE2 provided beneficial effects against Ang II-induced HF-pEF and pressure-overload-induced HF-rEF in murine models of HF (Table).⁶⁷ Thus, using rhACE2 as a therapy is very much a viable option and the advancement of rhACE2 in clinical trials provides the translational impact of rhACE2 findings in murine models.^{104, 105} Several ACE2 activators and Ang 1–7/MasR agonists have been developed. In addition, novel approaches, including oral ACE2 and Ang 1–7 biencapsulated in plant cells, have been designed and used in preclinical studies, showing promising cardioprotective effects.^{152–156} Lastly, gene therapy approaches could be utilized to achieve the tissue-specific delivery of ACE2/Ang 1–7.

Autologous cell-based therapy using putative progenitor cells such as CD34⁺ cells could be an attractive therapeutic approach for diabetic vascular complications. However, these cells are dysfunctional in diabetic individuals. Peripheral CD34⁺ cells isolated from patients with diabetes exhibit reduced proliferative potential and migratory function, which could be attributed to decreased eNOS activity, increased ROS levels, and advanced glycation end-products.^{157, 158} As ACE2 and Ang 1–7 are potent activators of eNOS¹⁹ and anti-oxidants,^{100, 135} the ACE2/Ang 1–7/MasR axis should improve CD34⁺ cell function and result in increased reparative efficacy. Indeed, Ang 1–7 increased the vascular reparative function of CD34⁺ cells isolated from patients with diabetes.¹⁵⁹

Conclusions

ACE2 has emerged as the dominant mechanism for negative regulation of the RAS, by metabolizing Ang II into the beneficial peptide Ang 1–7. This important biochemical and physiological property is being harnessed as potential therapy for HF. Since the discovery of ACE2 in 2000, tremendous progress has been made in elucidating its biochemical actions and its key role in heart disease and HF. ACE2 is widely expressed and regulates the

fundamental cellular biology of cardiomyocytes, cardiofibroblasts, and coronary endothelial cells in both HF-rEF and HF-pEF models. Ang 1–7 has also emerged in HF models as a physiologically active peptide with protective effects. Enhancing Ang 1–7 action may also provide marked therapeutic effects in HF. Clinical and experimental studies clearly support a physiological and pathophysiological role for ACE2/Ang 1–7 in HF, and studies indicate that increasing/activating ACE2/Ang 1–7 results in beneficial effects to prevent heart disease and HF. Further experimental studies are required that combine rhACE2/ACE2 activators with RAS blockers (such as ACE inhibitors or AT₁R blockers) to determine if this combined approach offers additional benefits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding

VBP received support from Alberta Innovates-Health Solutions (AI-HS) and Heart & Stroke Foundation post-doctoral fellowships. JCZ was supported by the National Basic Research Program of China (2014CB542300), the National Major Research Plan Training Program (91339108), the National Natural Science Foundation of China (81170246 & 81370362), the Shanghai Pujiang Talents Program of the Shanghai Science & Technology Committee (11PJ1408300), and a Shanghai Municipal Education Commission-Gaofeng Clinical Medicine Grant (20152509). We acknowledge funding support from the Canadian Institutes of Health Research, Heart & Stroke Foundation, and AI-HS to GYO and the National Institutes of Health (HL110170) to MBG and GYO.

Abbreviations

3'-UTR	3' untranslated region
ACE	Angiotensin converting enzyme
ACE2	Angiotensin converting enzyme 2
ACEi	ACE inhibitor
ADAM17	A disintegrin and metalloproteinase 17
ADH	Antidiuretic hormone
AICAR	5-amino-4-imidazolecarboxamide riboside
AMPK	Adenosine monophosphate kinase
Ang	Angiotensin
APA	Aminopeptidase A
ARB	AT ₁ R blocker
AT₁R	Angiotensin II type 1 receptor
AT₂R	Angiotensin II type 2 receptor

CPA	Carboxypeptidase A
DAG	Diacyl glycerol
eNOS	Endothelial nitric oxide synthase
ERK1/2	Extracellular signal-regulated kinase 1/2
IP3	Inositol triphosphate
HF	Heart failure
HF-pEF	Heart failure with preserved ejection fraction
HF-rEF	Heart failure with reduced ejection fraction
MAPK	Mitogen activated protein kinase
MasR	Mas receptor
MI	Myocardial infarction
miRNA	MicroRNA
MMP	Matrix metalloproteinases
NAD+	Nicotinamide adenine dinucleotide – oxidized form
NADH	Nicotinamide adenine dinucleotide – reduced form
NEP	Neutral endopeptidase
Nox2	NADPH oxidase 2
PCP	Prolyl carboxypeptidase
PEP	Prolyl endopeptidase
NADPH	Nicotinamide adenine dinucleotide phosphate
PKC	Protein kinase C
PI3K	Phosphatidylinositol 3-kinase
PLC	Phospholipase C
RAS	Renin-angiotensin system
ROS	Reactive oxygen species
rhACE2	Recombinant human ACE2
siRNA	Small interfering RNA
SIRT1	Sirtuin 1
TACE	Tumor necrosis factor- α converting enzyme

References

1. Givertz MM. Manipulation of the renin-angiotensin system. *Circulation*. 2001; 104:E14–18. [PubMed: 11479264]
2. Zaman MA, Oparil S, Calhoun DA. Drugs targeting the renin-angiotensin-aldosterone system. *Nat Rev Drug Discov*. 2002; 1:621–636. [PubMed: 12402502]
3. Bader M, Ganten D. Update on tissue renin-angiotensin systems. *J Mol Med (Berl)*. 2008; 86:615–621. [PubMed: 18414822]
4. Dzau VJ, Re R. Tissue angiotensin system in cardiovascular medicine. A paradigm shift? *Circulation*. 1994; 89:493–498. [PubMed: 8281685]
5. Lavoie JL, Sigmund CD. Minireview: Overview of the renin-angiotensin system--an endocrine and paracrine system. *Endocrinology*. 2003; 144:2179–2183. [PubMed: 12746271]
6. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. *Physiol Rev*. 2006; 86:747–803. [PubMed: 16816138]
7. 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, Penninger JM. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*. 2002; 417:822–828. [PubMed: 12075344]
8. Danser AH, Schalekamp MA. Is there an internal cardiac renin-angiotensin system? *Heart*. 1996; 76:28–32. [PubMed: 8983664]
9. Paul M, Wagner J, Dzau VJ. Gene expression of the renin-angiotensin system in human tissues. Quantitative analysis by the polymerase chain reaction. *J Clin Invest*. 1993; 91:2058–2064. [PubMed: 8387539]
10. Tahmasebi M, Puddefoot JR, Inwang ER, Vinson GP. The tissue renin-angiotensin system in human pancreas. *J Endocrinol*. 1999; 161:317–322. [PubMed: 10320830]
11. Wagner J, Jan Danser AH, Derkx FH, de Jong TV, Paul M, Mullins JJ, Schalekamp MA, Ganten D. Demonstration of renin mRNA, angiotensinogen mRNA, and angiotensin converting enzyme mRNA expression in the human eye: Evidence for an intraocular renin-angiotensin system. *Br J Ophthalmol*. 1996; 80:159–163. [PubMed: 8814748]
12. Tikellis C, Johnston CI, Forbes JM, Burns WC, Thomas MC, Lew RA, Yarski M, Smith AI, Cooper ME. Identification of angiotensin converting enzyme 2 in the rodent retina. *Curr Eye Res*. 2004; 29:419–427. [PubMed: 15764086]
13. Baltatu O, Silva JA Jr, Ganten D, Bader M. The brain renin-angiotensin system modulates angiotensin II-induced hypertension and cardiac hypertrophy. *Hypertension*. 2000; 35:409–412. [PubMed: 10642333]
14. Ribeiro-Oliveira A Jr, Nogueira AI, Pereira RM, Boas WW, Dos Santos RA, Simoes e Silva AC. The renin-angiotensin system and diabetes: An update. *Vasc Health Risk Manag*. 2008; 4:787–803. [PubMed: 19065996]
15. Mercure C, Yogi A, Callera GE, Aranha AB, Bader M, Ferreira AJ, Santos RA, Walther T, Touyz RM, Reudelhuber TL. Angiotensin(1–7) blunts hypertensive cardiac remodeling by a direct effect on the heart. *Circ Res*. 2008; 103:1319–1326. [PubMed: 18845809]
16. Oudit GY, Penninger JM. Recombinant human angiotensin-converting enzyme 2 as a new renin-angiotensin system peptidase for heart failure therapy. *Curr Heart Fail Rep*. 2011; 8:176–183. [PubMed: 21611889]
17. Alenina N, Xu P, Rentzsch B, Patkin EL, Bader M. Genetically altered animal models for mas and angiotensin-(1–7). *Exp Physiol*. 2008; 93:528–537. [PubMed: 18156169]
18. Bader M. Ace2, angiotensin-(1–7), and mas: The other side of the coin. *Pflügers Arch Eur J Physiol*. 2012; 465:79–85. [PubMed: 23463883]
19. Sampaio WO, Souza dos Santos RA, Faria-Silva R, da Mata Machado LT, Schifffrin EL, Touyz RM. Angiotensin-(1–7) through receptor mas mediates endothelial nitric oxide synthase activation via akt-dependent pathways. *Hypertension*. 2007; 49:185–192. [PubMed: 17116756]
20. Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SV, Lopes MT, Bader M, Mendes EP, Lemos VS, Campagnole-Santos MJ, Schultheiss

- HP, Speth R, Walther T. Angiotensin-(1-7) is an endogenous ligand for the g protein-coupled receptor mas. *Proc Natl Acad Sci U S A*. 2003; 100:8258–8263. [PubMed: 12829792]
21. Tallant EA, Ferrario CM, Gallagher PE. Angiotensin-(1-7) inhibits growth of cardiac myocytes through activation of the mas receptor. *Am J Physiol Heart Circ Physiol*. 2005; 289:H1560–1566. [PubMed: 15951342]
22. Zhang Y, Li B, Wang B, Zhang J, Wu J, Morgan T. Alteration of cardiac ace2/mas expression and cardiac remodelling in rats with aortic constriction. *Chin J Physiol*. 2014; 57:335–342. [PubMed: 25575522]
23. Flores-Munoz M, Godinho BM, Almalik A, Nicklin SA. Adenoviral delivery of angiotensin-(1-7) or angiotensin-(1-9) inhibits cardiomyocyte hypertrophy via the mas or angiotensin type 2 receptor. *PLoS One*. 2012; 7:e45564. [PubMed: 23029101]
24. Flores-Munoz M, Work LM, Douglas K, Denby L, Dominiczak AF, Graham D, Nicklin SA. Angiotensin-(1-9) attenuates cardiac fibrosis in the stroke-prone spontaneously hypertensive rat via the angiotensin type 2 receptor. *Hypertension*. 2012; 59:300–307. [PubMed: 22184331]
25. Ocaranza MP, Lavandero S, Jalil JE, Moya J, Pinto M, Novoa U, Apablaza F, Gonzalez L, Hernandez C, Varas M, Lopez R, Godoy I, Verdejo H, Chiong M. Angiotensin-(1-9) regulates cardiac hypertrophy in vivo and in vitro. *J Hypertens*. 2010; 28:1054–1064. [PubMed: 20411619]
26. Ocaranza MP, Rivera P, Novoa U, Pinto M, Gonzalez L, Chiong M, Lavandero S, Jalil JE. Rho kinase inhibition activates the homologous angiotensin-converting enzyme-angiotensin-(1-9) axis in experimental hypertension. *J Hypertens*. 2011; 29:706–715. [PubMed: 21330937]
27. Oudit GY, Crackower MA, Backx PH, Penninger JM. The role of ace2 in cardiovascular physiology. *Trends Cardiovasc Med*. 2003; 13:93–101. [PubMed: 12691672]
28. Lo J, Patel VB, Wang Z, Levasseur J, Kaufman S, Penninger JM, Oudit GY. Angiotensin-converting enzyme 2 antagonizes angiotensin ii-induced pressor response and nadph oxidase activation in wistar-kyoto rats and spontaneously hypertensive rats. *Exp Physiol*. 2013; 98:109–122. [PubMed: 22750422]
29. Oudit GY, Kassiri Z, Patel MP, Chappell M, Butany J, Backx PH, Tsushima RG, Scholey JW, Khokha R, Penninger JM. Angiotensin ii-mediated oxidative stress and inflammation mediate the age-dependent cardiomyopathy in ace2 null mice. *Cardiovasc Res*. 2007; 75:29–39. [PubMed: 17499227]
30. Patel VB, Bodiga S, Fan D, Das SK, Wang Z, Wang W, Basu R, Zhong J, Kassiri Z, Oudit GY. Cardioprotective effects mediated by angiotensin ii type 1 receptor blockade and enhancing angiotensin 1-7 in experimental heart failure in angiotensin-converting enzyme 2-null mice. *Hypertension*. 2012; 59:1195–1203. [PubMed: 22508831]
31. Danilczyk U, Penninger JM. Angiotensin-converting enzyme ii in the heart and the kidney. *Circ Res*. 2006; 98:463–471. [PubMed: 16514079]
32. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S. A novel angiotensin-converting enzyme-related carboxypeptidase (ace2) converts angiotensin i to angiotensin 1-9. *Circ Res*. 2000; 87:E1–9. [PubMed: 10969042]
33. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem*. 2000; 275:33238–33243. [PubMed: 10924499]
34. Li W, Moore MJ, Vasileva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. Angiotensin-converting enzyme 2 is a functional receptor for the sars coronavirus. *Nature*. 2003; 426:450–454. [PubMed: 14647384]
35. Warner FJ, Lew RA, Smith AI, Lambert DW, Hooper NM, Turner AJ. Angiotensin-converting enzyme 2 (ace2), but not ace, is preferentially localized to the apical surface of polarized kidney cells. *J Biol Chem*. 2005; 280:39353–39362. [PubMed: 16166094]
36. Turner AJ, Tipnis SR, Guy JL, Rice G, Hooper NM. Aceh/ace2 is a novel mammalian metallo-carboxypeptidase and a homologue of angiotensin-converting enzyme insensitive to ace inhibitors. *Can J Physiol Pharmacol*. 2002; 80:346–353. [PubMed: 12025971]

37. Rushworth CA, Guy JL, Turner AJ. Residues affecting the chloride regulation and substrate selectivity of the angiotensin-converting enzymes (ace and ace2) identified by site-directed mutagenesis. *FEBS J.* 2008; 275:6033–6042. [PubMed: 19021774]
38. Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout K, Parsons T, Baronas E, Hsieh F, Acton S, Patane M, Nichols A, Tummino P. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J Biol Chem.* 2002; 277:14838–14843. [PubMed: 11815627]
39. Guy JL, Jackson RM, Jensen HA, Hooper NM, Turner AJ. Identification of critical active-site residues in angiotensin-converting enzyme-2 (ace2) by site-directed mutagenesis. *FEBS J.* 2005; 272:3512–3520. [PubMed: 16008552]
40. Clarke NE, Turner AJ. Angiotensin-converting enzyme 2: The first decade. *Int J Hypertens.* 2012; 2012:307315. [PubMed: 22121476]
41. Guy JL, Jackson RM, Acharya KR, Sturrock ED, Hooper NM, Turner AJ. Angiotensin-converting enzyme-2 (ace2): Comparative modeling of the active site, specificity requirements, and chloride dependence. *Biochemistry (Mosc).* 2003; 42:13185–13192.
42. Rice GI, Thomas DA, Grant PJ, Turner AJ, Hooper NM. Evaluation of angiotensin-converting enzyme (ace), its homologue ace2 and neprilysin in angiotensin peptide metabolism. *Biochem J.* 2004; 383:45–51. [PubMed: 15283675]
43. Dales NA, Gould AE, Brown JA, Calderwood EF, Guan B, Minor CA, Gavin JM, Hales P, Kaushik VK, Stewart M, Tummino PJ, Vickers CS, Ocain TD, Patane MA. Substrate-based design of the first class of angiotensin-converting enzyme-related carboxypeptidase (ace2) inhibitors. *J Am Chem Soc.* 2002; 124:11852–11853. [PubMed: 12358520]
44. Rella M, Rushworth CA, Guy JL, Turner AJ, Langer T, Jackson RM. Structure-based pharmacophore design and virtual screening for novel angiotensin converting enzyme 2 inhibitors. *J Chem Inf Model.* 2006; 46:708–716. [PubMed: 16563001]
45. Turner AJ, Hiscox JA, Hooper NM. Ace2: From vaso-peptidase to sars virus receptor. *Trends Pharmacol Sci.* 2004; 25:291–294. [PubMed: 15165741]
46. Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, Wild B, Camargo SM, Singer D, Richter A, Kuba K, Fukamizu A, Schreiber S, Clevers H, Verrey F, Rosenstiel P, Penninger JM. Ace2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature.* 2012; 487:477–481. [PubMed: 22837003]
47. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ace2 protein, the functional receptor for sars coronavirus. A first step in understanding sars pathogenesis. *J Pathol.* 2004; 203:631–637. [PubMed: 15141377]
48. Paizis G, Tikellis C, Cooper ME, Schembri JM, Lew RA, Smith AI, Shaw T, Warner FJ, Zuilli A, Burrell LM, Angus PW. Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. *Gut.* 2005; 54:1790–1796. [PubMed: 16166274]
49. Doobay MF, Talman LS, Obr TD, Tian X, Davisson RL, Lazartigues E. Differential expression of neuronal ace2 in transgenic mice with overexpression of the brain renin-angiotensin system. *Am J Physiol Regul Integr Comp Physiol.* 2007; 292:R373–381. [PubMed: 16946085]
50. Wong DW, Oudit GY, Reich H, Kassiri Z, Zhou J, Liu QC, Backx PH, Penninger JM, Herzenberg AM, Scholey JW. Loss of angiotensin-converting enzyme-2 (ace2) accelerates diabetic kidney injury. *Am J Pathol.* 2007; 171:438–451. [PubMed: 17600118]
51. Kuba K, Imai Y, Rao S, Jiang C, Penninger JM. Lessons from sars: Control of acute lung failure by the sars receptor ace2. *J Mol Med (Berl).* 2006; 84:814–820. [PubMed: 16988814]
52. Gembardt F, Sterner-Kock A, Imboden H, Spalteholz M, Reibitz F, Schultheiss HP, Siems WE, Walther T. Organ-specific distribution of ace2 mrna and correlating peptidase activity in rodents. *Peptides.* 2005; 26:1270–1277. [PubMed: 15949646]
53. Patel VB, Clarke N, Wang Z, Fan D, Parajuli N, Basu R, Putko B, Kassiri Z, Turner AJ, Oudit GY. Angiotensin ii induced proteolytic cleavage of myocardial ace2 is mediated by tace/adam-17: A positive feedback mechanism in the ras. *J Mol Cell Cardiol.* 2014; 66:167–176. [PubMed: 24332999]
54. Patel VB, Zhong JC, Fan D, Basu R, Morton JS, Parajuli N, McMurtry MS, Davidge ST, Kassiri Z, Oudit GY. Angiotensin-converting enzyme 2 is a critical determinant of angiotensin ii-induced loss

- of vascular smooth muscle cells and adverse vascular remodeling. *Hypertension*. 2014; 64:157–164. [PubMed: 24799609]
55. Santos RA, Castro CH, Gava E, Pinheiro SV, Almeida AP, Paula RD, Cruz JS, Ramos AS, Rosa KT, Irigoyen MC, Bader M, Alenina N, Kitten GT, Ferreira AJ. Impairment of in vitro and in vivo heart function in angiotensin-(1–7) receptor mas knockout mice. *Hypertension*. 2006; 47:996–1002. [PubMed: 16567589]
 56. Sampaio WO, Henrique de Castro C, Santos RA, Schiffrin EL, Touyz RM. Angiotensin-(1–7) counterregulates angiotensin ii signaling in human endothelial cells. *Hypertension*. 2007; 50:1093–1098. [PubMed: 17984366]
 57. Iwata M, Cowling RT, Gurantz D, Moore C, Zhang S, Yuan JX, Greenberg BH. Angiotensin-(1–7) binds to specific receptors on cardiac fibroblasts to initiate antifibrotic and antitrophic effects. *Am J Physiol Heart Circ Physiol*. 2005; 289:H2356–2363. [PubMed: 16024575]
 58. Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature*. 1997; 385:729–733. [PubMed: 9034190]
 59. Moss ML, Jin SL, Milla ME, Bickett DM, Burkhart W, Carter HL, Chen WJ, Clay WC, Didsbury JR, Hassler D, Hoffman CR, Kost TA, Lambert MH, Leesnitzer MA, McCauley P, McGeehan G, Mitchell J, Moyer M, Pahel G, Rocque W, Overton LK, Schoenen F, Seaton T, Su JL, Becherer JD, et al. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. *Nature*. 1997; 385:733–736. [PubMed: 9034191]
 60. Gooz M. Adam-17: The enzyme that does it all. *Crit Rev Biochem Mol Biol*. 2010; 45:146–169. [PubMed: 20184396]
 61. Lambert DW, Clarke NE, Hooper NM, Turner AJ. Calmodulin interacts with angiotensin-converting enzyme-2 (ace2) and inhibits shedding of its ectodomain. *FEBS Lett*. 2008; 582:385–390. [PubMed: 18070603]
 62. Iwata M, Silva Enciso JE, Greenberg BH. Selective and specific regulation of ectodomain shedding of angiotensin-converting enzyme 2 by tumor necrosis factor alpha-converting enzyme. *Am J Physiol Cell Physiol*. 2009; 297:C1318–1329. [PubMed: 19759332]
 63. Xia H, Sriramula S, Chhabra KH, Lazartigues E. Brain angiotensin-converting enzyme type 2 shedding contributes to the development of neurogenic hypertension. *Circ Res*. 2013; 113:1087–1096. [PubMed: 24014829]
 64. Salem ES, Grobe N, Elased KM. Insulin treatment attenuates renal adam17 and ace2 shedding in diabetic akita mice. *Am J Physiol Renal Physiol*. 2014; 306:F629–639. [PubMed: 24452639]
 65. Xu P, Derynck R. Direct activation of tace-mediated ectodomain shedding by p38 map kinase regulates egf receptor-dependent cell proliferation. *Mol Cell*. 2010; 37:551–566. [PubMed: 20188673]
 66. Wang W, Patel VB, Parajuli N, Fan D, Basu R, Wang Z, Ramprasath T, Kassiri Z, Penninger JM, Oudit GY. Heterozygote loss of ace2 is sufficient to increase the susceptibility to heart disease. *J Mol Med (Berl)*. 2014; 92:847–858. [PubMed: 24728465]
 67. Zhong J, Basu R, Guo D, Chow FL, Byrns S, Schuster M, Loibner H, Wang XH, Penninger JM, Kassiri Z, Oudit GY. Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. *Circulation*. 2010; 122:717–728. [PubMed: 20679547]
 68. Epelman S, Tang WH, Chen SY, Van Lente F, Francis GS, Sen S. Detection of soluble angiotensin-converting enzyme 2 in heart failure: Insights into the endogenous counter-regulatory pathway of the renin-angiotensin-aldosterone system. *J Am Coll Cardiol*. 2008; 52:750–754. [PubMed: 18718423]
 69. Putko BN, Wang Z, Lo J, Anderson T, Becher H, Dyck JR, Kassiri Z, Oudit GY. Alberta Heart Investigators. Circulating levels of tumor necrosis factor-alpha receptor 2 are increased in heart failure with preserved ejection fraction relative to heart failure with reduced ejection fraction: Evidence for a divergence in pathophysiology. *PLoS One*. 2014; 9:e99495. [PubMed: 24923671]

70. Lambert DW, Lambert LA, Clarke NE, Hooper NM, Porter KE, Turner AJ. Angiotensin-converting enzyme 2 is subject to post-transcriptional regulation by mir-421. *Clin Sci (Lond)*. 2014; 127:243–249. [PubMed: 24564768]
71. Clarke NE, Belyaev ND, Lambert DW, Turner AJ. Epigenetic regulation of angiotensin-converting enzyme 2 (*ace2*) by *sirt1* under conditions of cell energy stress. *Clin Sci (Lond)*. 2014; 126:507–516. [PubMed: 24147777]
72. Sato T, Suzuki T, Watanabe H, Kadowaki A, Fukamizu A, Liu PP, Kimura A, Ito H, Penninger JM, Imai Y, Kuba K. Apelin is a positive regulator of *ace2* in failing hearts. *J Clin Invest*. 2013; 123:5203–5211. [PubMed: 24177423]
73. Hunter JJ, Chien KR. Signaling pathways for cardiac hypertrophy and failure. *N Engl J Med*. 1999; 341:1276–1283. [PubMed: 10528039]
74. Braunwald E, Bristow MR. Congestive heart failure: Fifty years of progress. *Circulation*. 2000; 102:IV14–23. [PubMed: 11080127]
75. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, Jessup M, Konstam MA, Mancini DM, Michl K, Oates JA, Rahko PS, Silver MA, Stevenson LW, Yancy CW. 2009 focused update incorporated into the acc/aha 2005 guidelines for the diagnosis and management of heart failure in adults: A report of the american college of cardiology foundation/american heart association task force on practice guidelines: Developed in collaboration with the international society for heart and lung transplantation. *Circulation*. 2009; 119:e391–479. [PubMed: 19324966]
76. Arnold JM, Liu P, Demers C, Dorian P, Giannetti N, Haddad H, Heckman GA, Howlett JG, Ignaszewski A, Johnstone DE, Jong P, McKelvie RS, Moe GW, Parker JD, Rao V, Ross HJ, Sequeira EJ, Svendsen AM, Teo K, Tsuyuki RT, White M. Canadian Cardiovascular S. Canadian cardiovascular society consensus conference recommendations on heart failure 2006: Diagnosis and management. *Can J Cardiol*. 2006; 22:23–45. [PubMed: 16450016]
77. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. American Heart Association Statistics C, Stroke Statistics S. Heart disease and stroke statistics--2012 update: A report from the american heart association. *Circulation*. 2012; 125:e2–e220. [PubMed: 22179539]
78. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature*. 2001; 414:782–787. [PubMed: 11742409]
79. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: The framingham study. *Am J Cardiol*. 1974; 34:29–34. [PubMed: 4835750]
80. Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV, Mitch W, Smith SC Jr, Sowers JR. Diabetes and cardiovascular disease: A statement for healthcare professionals from the american heart association. *Circulation*. 1999; 100:1134–1146. [PubMed: 10477542]
81. Boudina S, Abel ED. Diabetic cardiomyopathy revisited. *Circulation*. 2007; 115:3213–3223. [PubMed: 17592090]
82. Kenchaiah S, Evans JC, Levy D, Wilson PW, Benjamin EJ, Larson MG, Kannel WB, Vasan RS. Obesity and the risk of heart failure. *N Engl J Med*. 2002; 347:305–313. [PubMed: 12151467]
83. Kenchaiah S, Sesso HD, Gaziano JM. Body mass index and vigorous physical activity and the risk of heart failure among men. *Circulation*. 2009; 119:44–52. [PubMed: 19103991]
84. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med*. 2006; 355:251–259. [PubMed: 16855265]
85. Krauss RM, Winston M, Fletcher BJ, Grundy SM. Obesity : Impact on cardiovascular disease. *Circulation*. 1998; 98:1472–1476.
86. Kopelman PG. Obesity as a medical problem. *Nature*. 2000; 404:635–643. [PubMed: 10766250]
87. From AM, Scott CG, Chen HH. The development of heart failure in patients with diabetes mellitus and pre-clinical diastolic dysfunction a population-based study. *J Am Coll Cardiol*. 2010; 55:300–305. [PubMed: 20117433]

88. Hayashi T, Takai S, Yamashita C. Impact of the renin-angiotensin-aldosterone-system on cardiovascular and renal complications in diabetes mellitus. *Curr Vasc Pharmacol*. 2010; 8:189–197. [PubMed: 19485896]
89. Nakao YM, Teramukai S, Tanaka S, Yasuno S, Fujimoto A, Kasahara M, Ueshima K, Nakao K, Hinotsu S, Nakao K, Kawakami K. Effects of renin-angiotensin system blockades on cardiovascular outcomes in patients with diabetes mellitus: A systematic review and meta-analysis. *Diabetes Res Clin Pract*. 2012; 96:68–75. [PubMed: 22197527]
90. Boudina S, Han YH, Pei S, Tidwell TJ, Henrie B, Tuinei J, Olsen C, Sena S, Abel ED. Ucp3 regulates cardiac efficiency and mitochondrial coupling in high fat-fed mice but not in leptin-deficient mice. *Diabetes*. 2012; 61:3260–3269. [PubMed: 22912419]
91. Boudina S, Sena S, Theobald H, Sheng X, Wright JJ, Hu XX, Aziz S, Johnson JI, Bugger H, Zaha VG, Abel ED. Mitochondrial energetics in the heart in obesity-related diabetes: Direct evidence for increased uncoupled respiration and activation of uncoupling proteins. *Diabetes*. 2007; 56:2457–2466. [PubMed: 17623815]
92. McMurray JJ. Consensus to emphasis: The overwhelming evidence which makes blockade of the renin-angiotensin-aldosterone system the cornerstone of therapy for systolic heart failure. *Eur J Heart Fail*. 2011; 13:929–936. [PubMed: 21816763]
93. Mehta PK, Griendling KK. Angiotensin ii cell signaling: Physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol*. 2007; 292:C82–97. [PubMed: 16870827]
94. Latini R, Tognoni G, Maggioni AP, Baigent C, Braunwald E, Chen ZM, Collins R, Flather M, Franzosi MG, Kjekshus J, Kober L, Liu LS, Peto R, Pfeffer M, Pizzetti F, Santoro E, Sleight P, Swedberg K, Tavazzi L, Wang W, Yusuf S. Clinical effects of early angiotensin-converting enzyme inhibitor treatment for acute myocardial infarction are similar in the presence and absence of aspirin: Systematic overview of individual data from 96,712 randomized patients. Angiotensin-converting enzyme inhibitor myocardial infarction collaborative group. *J Am Coll Cardiol*. 2000; 35:1801–1807. [PubMed: 10841227]
95. Li M, Liu K, Michalick J, Angus JA, Hunt JE, Dell'Italia LJ, Feneley MP, Graham RM, Husain A. Involvement of chymase-mediated angiotensin ii generation in blood pressure regulation. *J Clin Invest*. 2004; 114:112–120. [PubMed: 15232618]
96. Wei CC, Hase N, Inoue Y, Bradley EW, Yahiro E, Li M, Naqvi N, Powell PC, Shi K, Takahashi Y, Saku K, Urata H, Dell'Italia LJ, Husain A. Mast cell chymase limits the cardiac efficacy of angiotensin-converting enzyme inhibitor therapy in rodents. *J Clin Invest*. 2010; 120:1229–1239. [PubMed: 20335663]
97. Jorde UP, Ennezat PV, Lisker J, Suryadevara V, Infeld J, Cukon S, Hammer A, Sonnenblick EH, Le Jemtel TH. Maximally recommended doses of angiotensin-converting enzyme (ace) inhibitors do not completely prevent ace-mediated formation of angiotensin ii in chronic heart failure. *Circulation*. 2000; 101:844–846. [PubMed: 10694521]
98. Petrie MC, Padmanabhan N, McDonald JE, Hillier C, Connell JM, McMurray JJ. Angiotensin converting enzyme (ace) and non-ace dependent angiotensin ii generation in resistance arteries from patients with heart failure and coronary heart disease. *J Am Coll Cardiol*. 2001; 37:1056–1061. [PubMed: 11263608]
99. Wysocki J, Ye M, Rodriguez E, Gonzalez-Pacheco FR, Barrios C, Evora K, Schuster M, Loibner H, Brosnihan KB, Ferrario CM, Penninger JM, Batlle D. Targeting the degradation of angiotensin ii with recombinant angiotensin-converting enzyme 2: Prevention of angiotensin ii-dependent hypertension. *Hypertension*. 2010; 55:90–98. [PubMed: 19948988]
100. Patel VB, Takawale A, Ramprasath T, Das SK, Basu R, Grant MB, Hall DA, Kassiri Z, Oudit GY. Antagonism of angiotensin 1–7 prevents the therapeutic effects of recombinant human ace2. *J Mol Med (Berl)*. 2015; 93:1003–1013. [PubMed: 25874965]
101. Shaltout HA, Westwood BM, Averill DB, Ferrario CM, Figueroa JP, Diz DI, Rose JC, Chappell MC. Angiotensin metabolism in renal proximal tubules, urine, and serum of sheep: Evidence for ace2-dependent processing of angiotensin ii. *Am J Physiol Renal Physiol*. 2007; 292:F82–91. [PubMed: 16896185]
102. Ocaranza MP, Godoy I, Jalil JE, Varas M, Collantes P, Pinto M, Roman M, Ramirez C, Copaja M, Diaz-Araya G, Castro P, Lavandero S. Enalapril attenuates downregulation of angiotensin-

- converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. *Hypertension*. 2006; 48:572–578. [PubMed: 16908757]
103. Ye M, Wysocki J, Gonzalez-Pacheco FR, Salem M, Evora K, Garcia-Halpin L, Poglitsch M, Schuster M, Battle D. Murine recombinant angiotensin-converting enzyme 2: Effect on angiotensin ii-dependent hypertension and distinctive angiotensin-converting enzyme 2 inhibitor characteristics on rodent and human angiotensin-converting enzyme 2. *Hypertension*. 2012; 60:730–740. [PubMed: 22777933]
 104. ClinicalTrials.gov. [accessed january 2016] Safety and tolerability study of apn01 (recombinant human angiotensin converting enzyme 2). <https://clinicaltrials.gov/ct2/show/nct00886353>. Nlm identifier: Nct00886353
 105. ClinicalTrials.gov. [accessed january 2016] The safety, tolerability, pk and pd of gsk2586881 in patients with acute lung injury. <https://clinicaltrials.gov/ct2/show/nct01597635>. Nlm identifier: Nct00886353
 106. Haschke M, Schuster M, Poglitsch M, Loibner H, Salzberg M, Bruggisser M, Penninger J, Krahenbuhl S. Pharmacokinetics and pharmacodynamics of recombinant human angiotensin-converting enzyme 2 in healthy human subjects. *Clin Pharmacokinet*. 2013; 52:783–792. [PubMed: 23681967]
 107. Giani JF, Munoz MC, Mayer MA, Veiras LC, Arranz C, Taira CA, Turyn D, Toblli JE, Dominici FP. Angiotensin-(1–7) improves cardiac remodeling and inhibits growth-promoting pathways in the heart of fructose-fed rats. *Am J Physiol Heart Circ Physiol*. 2010; 298:H1003–1013. [PubMed: 20061544]
 108. Dias-Peixoto MF, Santos RA, Gomes ER, Alves MN, Almeida PW, Greco L, Rosa M, Fauler B, Bader M, Alenina N, Guatimosim S. Molecular mechanisms involved in the angiotensin-(1–7)/mas signaling pathway in cardiomyocytes. *Hypertension*. 2008; 52:542–548. [PubMed: 18695148]
 109. Patel SK, Velkoska E, Freeman M, Wai B, Lancefield TF, Burrell LM. From gene to protein-experimental and clinical studies of ace2 in blood pressure control and arterial hypertension. *Front Physiol*. 2014; 5:227. [PubMed: 25009501]
 110. Soro-Paavonen A, Gordin D, Forsblom C, Rosengard-Barlund M, Waden J, Thorn L, Sandholm N, Thomas MC, Groop PH. FinnDiane Study G. Circulating ace2 activity is increased in patients with type 1 diabetes and vascular complications. *J Hypertens*. 2012; 30:375–383. [PubMed: 22179088]
 111. Diez-Freire C, Vazquez J, Correa de Adjounian MF, Ferrari MF, Yuan L, Silver X, Torres R, Raizada MK. Ace2 gene transfer attenuates hypertension-linked pathophysiological changes in the shr. *Physiol Genomics*. 2006; 27:12–19. [PubMed: 16788004]
 112. Rentzsch B, Todiras M, Iliescu R, Popova E, Campos LA, Oliveira ML, Baltatu OC, Santos RA, Bader M. Transgenic angiotensin-converting enzyme 2 overexpression in vessels of shrsp rats reduces blood pressure and improves endothelial function. *Hypertension*. 2008; 52:967–973. [PubMed: 18809792]
 113. Yamazato M, Yamazato Y, Sun C, Diez-Freire C, Raizada MK. Overexpression of angiotensin-converting enzyme 2 in the rostral ventrolateral medulla causes long-term decrease in blood pressure in the spontaneously hypertensive rats. *Hypertension*. 2007; 49:926–931. [PubMed: 17325232]
 114. Te Riet L, van Esch JH, Roks AJ, van den Meiracker AH, Danser AH. Hypertension: Renin-angiotensin-aldosterone system alterations. *Circ Res*. 2015; 116:960–975. [PubMed: 25767283]
 115. Gallagher PE, Ferrario CM, Tallant EA. Regulation of ace2 in cardiac myocytes and fibroblasts. *Am J Physiol Heart Circ Physiol*. 2008; 295:H2373–2379. [PubMed: 18849338]
 116. Lieb W, Graf J, Gotz A, Konig IR, Mayer B, Fischer M, Stritzke J, Hengstenberg C, Holmer SR, Doring A, Lowel H, Schunkert H, Erdmann J. Association of angiotensin-converting enzyme 2 (ace2) gene polymorphisms with parameters of left ventricular hypertrophy in men. Results of the monica augsburg echocardiographic substudy. *J Mol Med (Berl)*. 2006; 84:88–96. [PubMed: 16283142]
 117. Yang W, Huang W, Su S, Li B, Zhao W, Chen S, Gu D. Association study of ace2 (angiotensin i-converting enzyme 2) gene polymorphisms with coronary heart disease and myocardial infarction in a chinese han population. *Clin Sci (Lond)*. 2006; 111:333–340. [PubMed: 16822235]

118. Keidar S, Strizevsky A, Raz A, Gamliel-Lazarovich A. Ace2 activity is increased in monocyte-derived macrophages from prehypertensive subjects. *Nephrol Dial Transplant*. 2007; 22:597–601. [PubMed: 17095582]
119. Bodiga S, Zhong JC, Wang W, Basu R, Lo J, Liu GC, Guo D, Holland SM, Scholey JW, Penninger JM, Kassiri Z, Oudit GY. Enhanced susceptibility to biomechanical stress in ace2 null mice is prevented by loss of the p47(phox) nadph oxidase subunit. *Cardiovasc Res*. 2011; 91:151–161. [PubMed: 21285291]
120. Yamamoto K, Ohishi M, Katsuya T, Ito N, Ikushima M, Kaibe M, Tatara Y, Shiota A, Sugano S, Takeda S, Rakugi H, Ogihara T. Deletion of angiotensin-converting enzyme 2 accelerates pressure overload-induced cardiac dysfunction by increasing local angiotensin ii. *Hypertension*. 2006; 47:718–726. [PubMed: 16505206]
121. Tendera M. The epidemiology of heart failure. *J Renin Angiotensin Aldosterone Syst*. 2004; 5(Suppl 1):S2–6. [PubMed: 15526238]
122. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, Tikellis C, Grant SL, Lew RA, Smith AI, Cooper ME, Johnston CI. Myocardial infarction increases ace2 expression in rat and humans. *Eur Heart J*. 2005; 26:369–375. discussion 322–364. [PubMed: 15671045]
123. Kassiri Z, Zhong J, Guo D, Basu R, Wang X, Liu PP, Scholey JW, Penninger JM, Oudit GY. Loss of angiotensin-converting enzyme 2 accelerates maladaptive left ventricular remodeling in response to myocardial infarction. *Circ Heart Fail*. 2009; 2:446–455. [PubMed: 19808375]
124. Kim MA, Yang D, Kida K, Molotkova N, Yeo SJ, Varki N, Iwata M, Dalton ND, Peterson KL, Siems WE, Walther T, Cowling RT, Kjekshus J, Greenberg B. Effects of ace2 inhibition in the post-myocardial infarction heart. *J Card Fail*. 2010; 16:777–785. [PubMed: 20797602]
125. Der Sarkissian S, Grobe JL, Yuan L, Narielwala DR, Walter GA, Katovich MJ, Raizada MK. Cardiac overexpression of angiotensin converting enzyme 2 protects the heart from ischemia-induced pathophysiology. *Hypertension*. 2008; 51:712–718. [PubMed: 18250366]
126. Qi Y, Shenoy V, Wong F, Li H, Afzal A, Mocco J, Sumners C, Raizada MK, Katovich MJ. Lentivirus-mediated overexpression of angiotensin-(1–7) attenuated ischaemia-induced cardiac pathophysiology. *Exp Physiol*. 2011; 96:863–874. [PubMed: 21685447]
127. Zhao YX, Yin HQ, Yu QT, Qiao Y, Dai HY, Zhang MX, Zhang L, Liu YF, Wang LC, Liu de S, Deng BP, Zhang YH, Pan CM, Song HD, Qu X, Jiang H, Liu CX, Lu XT, Liu B, Gao F, Dong B. Ace2 overexpression ameliorates left ventricular remodeling and dysfunction in a rat model of myocardial infarction. *Hum Gene Ther*. 2010; 21:1545–1554. [PubMed: 20507236]
128. Grobe JL, Der Sarkissian S, Stewart JM, Meszaros JG, Raizada MK, Katovich MJ. Ace2 overexpression inhibits hypoxia-induced collagen production by cardiac fibroblasts. *Clin Sci (Lond)*. 2007; 113:357–364. [PubMed: 17600530]
129. Loot AE, Roks AJ, Henning RH, Tio RA, Suurmeijer AJ, Boomsma F, van Gilst WH. Angiotensin-(1–7) attenuates the development of heart failure after myocardial infarction in rats. *Circulation*. 2002; 105:1548–1550. [PubMed: 11927520]
130. Henkel DM, Redfield MM, Weston SA, Gerber Y, Roger VL. Death in heart failure: A community perspective. *Circ Heart Fail*. 2008; 1:91–97. [PubMed: 19300532]
131. Alghamri MS, Weir NM, Anstadt MP, Elased KM, Gurley SB, Morris M. Enhanced angiotensin ii-induced cardiac and aortic remodeling in ace2 knockout mice. *J Cardiovasc Pharmacol Ther*. 2013; 18:138–151. [PubMed: 23043153]
132. Garabelli PJ, Modrall JG, Penninger JM, Ferrario CM, Chappell MC. Distinct roles for angiotensin-converting enzyme 2 and carboxypeptidase a in the processing of angiotensins within the murine heart. *Exp Physiol*. 2008; 93:613–621. [PubMed: 18356559]
133. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. *Circulation*. 1991; 83:1849–1865. [PubMed: 1828192]
134. Huentelman MJ, Grobe JL, Vazquez J, Stewart JM, Mecca AP, Katovich MJ, Ferrario CM, Raizada MK. Protection from angiotensin ii-induced cardiac hypertrophy and fibrosis by systemic lentiviral delivery of ace2 in rats. *Exp Physiol*. 2005; 90:783–790. [PubMed: 16049057]
135. Patel VB, Bodiga S, Basu R, Das SK, Wang W, Wang Z, Lo J, Grant MB, Zhong J, Kassiri Z, Oudit GY. Loss of angiotensin-converting enzyme-2 exacerbates diabetic cardiovascular

- complications and leads to systolic and vascular dysfunction: A critical role of the angiotensin ii/at1 receptor axis. *Circ Res.* 2012; 110:1322–1335. [PubMed: 22474255]
136. Dong B, Yu QT, Dai HY, Gao YY, Zhou ZL, Zhang L, Jiang H, Gao F, Li SY, Zhang YH, Bian HJ, Liu CX, Wang N, Xu H, Pan CM, Song HD, Zhang C, Zhang Y. Angiotensin-converting enzyme-2 overexpression improves left ventricular remodeling and function in a rat model of diabetic cardiomyopathy. *J Am Coll Cardiol.* 2012; 59:739–747. [PubMed: 22340266]
 137. Oudit GY, Liu GC, Zhong J, Basu R, Chow FL, Zhou J, Loibner H, Janzek E, Schuster M, Penninger JM, Herzenberg AM, Kassiri Z, Scholey JW. Human recombinant ace2 reduces the progression of diabetic nephropathy. *Diabetes.* 2010; 59:529–538. [PubMed: 19934006]
 138. Murca TM, Moraes PL, Capurco CA, Santos SH, Melo MB, Santos RA, Shenoy V, Katovich MJ, Raizada MK, Ferreira AJ. Oral administration of an angiotensin-converting enzyme 2 activator ameliorates diabetes-induced cardiac dysfunction. *Regul Pept.* 2012; 177:107–115. [PubMed: 22595130]
 139. Murca TM, Almeida TC, Raizada MK, Ferreira AJ. Chronic activation of endogenous angiotensin-converting enzyme 2 protects diabetic rats from cardiovascular autonomic dysfunction. *Exp Physiol.* 2012; 97:699–709. [PubMed: 22286369]
 140. Yousif MH, Dhaunsi GS, Makki BM, Qabazard BA, Akhtar S, Benter IF. Characterization of angiotensin-(1–7) effects on the cardiovascular system in an experimental model of type-1 diabetes. *Pharmacol Res.* 2012; 66:269–275. [PubMed: 22580236]
 141. Mori J, Patel VB, Abo Alrob O, Basu R, Altamimi T, Desaulniers J, Wagg CS, Kassiri Z, Lopaschuk GD, Oudit GY. Angiotensin 1–7 ameliorates diabetic cardiomyopathy and diastolic dysfunction in db/db mice by reducing lipotoxicity and inflammation. *Circ Heart Fail.* 2014; 7:327–339. [PubMed: 24389129]
 142. Al-Maghrebi M, Benter IF, Diz DI. Endogenous angiotensin-(1–7) reduces cardiac ischemia-induced dysfunction in diabetic hypertensive rats. *Pharmacol Res.* 2009; 59:263–268. [PubMed: 19166939]
 143. Basu R, Oudit GY, Wang X, Zhang L, Ussher JR, Lopaschuk GD, Kassiri Z. Type 1 diabetic cardiomyopathy in the akita (ins2wt/c96y) mouse model is characterized by lipotoxicity and diastolic dysfunction with preserved systolic function. *Am J Physiol Heart Circ Physiol.* 2009; 297:H2096–2108. [PubMed: 19801494]
 144. Tikellis C, Pickering R, Tsorotes D, Du XJ, Kiriazis H, Nguyen-Huu TP, Head GA, Cooper ME, Thomas MC. Interaction of diabetes and ace2 in the pathogenesis of cardiovascular disease in experimental diabetes. *Clin Sci (Lond).* 2012; 123:519–529. [PubMed: 22616805]
 145. Patel VB, Parajuli N, Oudit GY. Role of angiotensin-converting enzyme 2 (ace2) in diabetic cardiovascular complications. *Clin Sci (Lond).* 2014; 126:471–482. [PubMed: 24329564]
 146. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJ, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WH, Tsai EJ, Wilkoff BL. 2013 accf/aha guideline for the management of heart failure: Executive summary: A report of the american college of cardiology foundation/american heart association task force on practice guidelines. *Circulation.* 2013; 128:1810–1852. [PubMed: 23741057]
 147. Patel VB, Mori J, McLean BA, Basu R, Das SK, Ramprasath T, Parajuli N, Penninger JM, Grant MB, Lopaschuk GD, Oudit GY. Ace2 deficiency worsens epicardial adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity. *Diabetes.* 2016; 65:85–95. [PubMed: 26224885]
 148. Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: Comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol.* 2013; 62:263–271. [PubMed: 23684677]
 149. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, Diz DI, Gallagher PE. Effect of angiotensin-converting enzyme inhibition and angiotensin ii receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation.* 2005; 111:2605–2610. [PubMed: 15897343]
 150. McMurray JJ, Pfeffer MA. Heart failure. *Lancet.* 2005; 365:1877–1889. [PubMed: 15924986]

151. Poglitsch M, Domenig O, Schwager C, Stranner S, Peball B, Janzek E, Wagner B, Jungwirth H, Loibner H, Schuster M. Recombinant expression and characterization of human and murine ace2: Species-specific activation of the alternative renin-angiotensin-system. *Int J Hypertens*. 2012; 2012:428950. [PubMed: 22518284]
152. Coutinho DC, Monnerat-Cahli G, Ferreira AJ, Medei E. Activation of angiotensin-converting enzyme 2 improves cardiac electrical changes in ventricular repolarization in streptozotocin-induced hyperglycaemic rats. *Europace*. 2014; 16:1689–1696. [PubMed: 24741027]
153. Haga S, Tsuchiya H, Hirai T, Hamano T, Mimori A, Ishizaka Y. A novel ace2 activator reduces monocrotaline-induced pulmonary hypertension by suppressing the jak/stat and tgf-beta cascades with restored caveolin-1 expression. *Exp Lung Res*. 2015; 41:21–31. [PubMed: 25275723]
154. Qi Y, Zhang J, Cole-Jeffrey CT, Shenoy V, Espejo A, Hanna M, Song C, Pepine CJ, Katovich MJ, Raizada MK. Diminazene aceturate enhances angiotensin-converting enzyme 2 activity and attenuates ischemia-induced cardiac pathophysiology. *Hypertension*. 2013; 62:746–752. [PubMed: 23959549]
155. Shenoy V, Gjymishka A, Jarajapu YP, Qi Y, Afzal A, Rigatto K, Ferreira AJ, Fraga-Silva RA, Kearns P, Douglas JY, Agarwal D, Mubarak KK, Bradford C, Kennedy WR, Jun JY, Rathinasabapathy A, Bruce E, Gupta D, Cardounel AJ, Mocco J, Patel JM, Francis J, Grant MB, Katovich MJ, Raizada MK. Diminazene attenuates pulmonary hypertension and improves angiogenic progenitor cell functions in experimental models. *Am J Respir Crit Care Med*. 2013; 187:648–657. [PubMed: 23370913]
156. Shenoy V, Kwon KC, Rathinasabapathy A, Lin S, Jin G, Song C, Shil P, Nair A, Qi Y, Li Q, Francis J, Katovich MJ, Daniell H, Raizada MK. Oral delivery of angiotensin-converting enzyme 2 and angiotensin-(1–7) bioencapsulated in plant cells attenuates pulmonary hypertension. *Hypertension*. 2014; 64:1248–1259. [PubMed: 25225206]
157. Caballero S, Sengupta N, Afzal A, Chang KH, Li Calzi S, Guberski DL, Kern TS, Grant MB. Ischemic vascular damage can be repaired by healthy, but not diabetic, endothelial progenitor cells. *Diabetes*. 2007; 56:960–967. [PubMed: 17395742]
158. Jarajapu YP, Grant MB. The promise of cell-based therapies for diabetic complications: Challenges and solutions. *Circ Res*. 2010; 106:854–869. [PubMed: 20299675]
159. Jarajapu YP, Bhatwadekar AD, Caballero S, Hazra S, Shenoy V, Medina R, Kent D, Stitt AW, Thut C, Finney EM, Raizada MK, Grant MB. Activation of the ace2/angiotensin-(1–7)/mas receptor axis enhances the reparative function of dysfunctional diabetic endothelial progenitors. *Diabetes*. 2013; 62:1258–1269. [PubMed: 23230080]

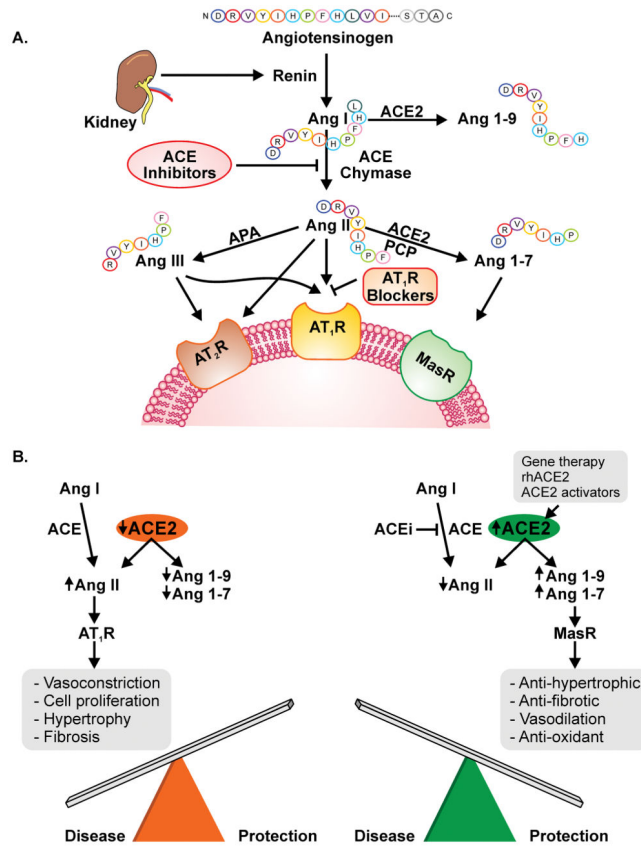


Figure 1. The enzymatic cascade of the RAS, key receptor systems, and the biological effects mediated by Ang II and Ang 1-7

(A) The RAS cascade showing the angiotensin peptide metabolic pathway.

Angiotensinogen, as the starting substrate, is cleaved by renin to Ang I. Ang I is cleaved by ACE to Ang II, which is cleaved by ACE2 to Ang 1-7. Ang II acts on AT₁ and AT₂ receptors. Ang 1-7 acts on Mas receptors and counterbalances the Ang II/AT₁R actions. (B) Decreased ACE2 shifts the balance in the RAS to the Ang II/AT₁R axis, resulting in disease progression. Increased ACE2 (by rhACE2, gene delivery, or ACE2 activators) shifts the balance to the Ang 1-7/MasR axis, leading to protection from disease.

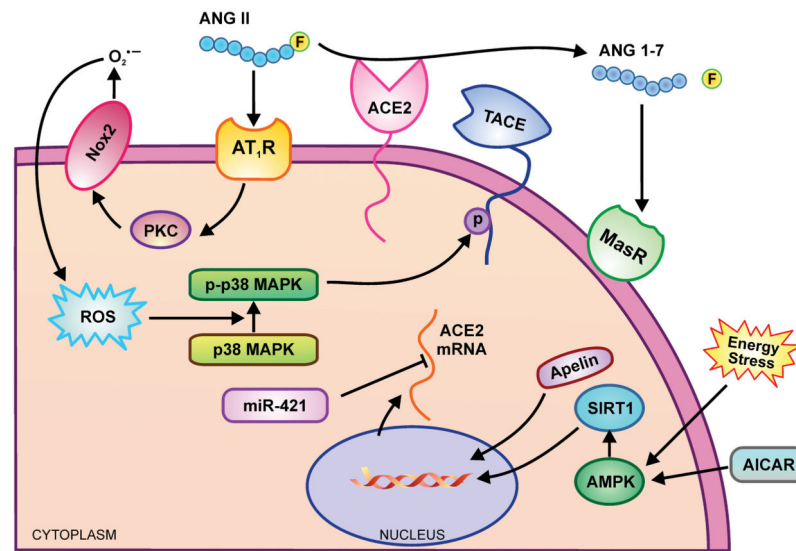


Figure 2. Transcriptional, post-transcriptional, and post-translational regulation of ACE2
 ACE2 expression is transcriptionally regulated by energy stress and activation of AMPK via SIRT1, which binds to the promoter region and facilitates ACE2 mRNA expression. Similarly, apelin binds to the promoter region of *ACE2* and enhances its expression. ACE2 mRNA is subject to post-transcriptional regulation by miR-421, which regulates protein expression. Ang II, the main effector peptide of the RAS, is produced by ACE and chymase in the heart and other tissues. ACE2, a monocarboxypeptidase, degrades Ang II into a heptapeptide, Ang 1–7. Ang II, via its action on AT₁R, promotes NOX2-dependent ROS formation. This leads to phosphorylation and activation of p38-MAPK and ultimately results in TACE phosphorylation (Thr735) and activation. Activated TACE proteolytically cleaves ACE2 and releases the active ACE2 ectodomain.

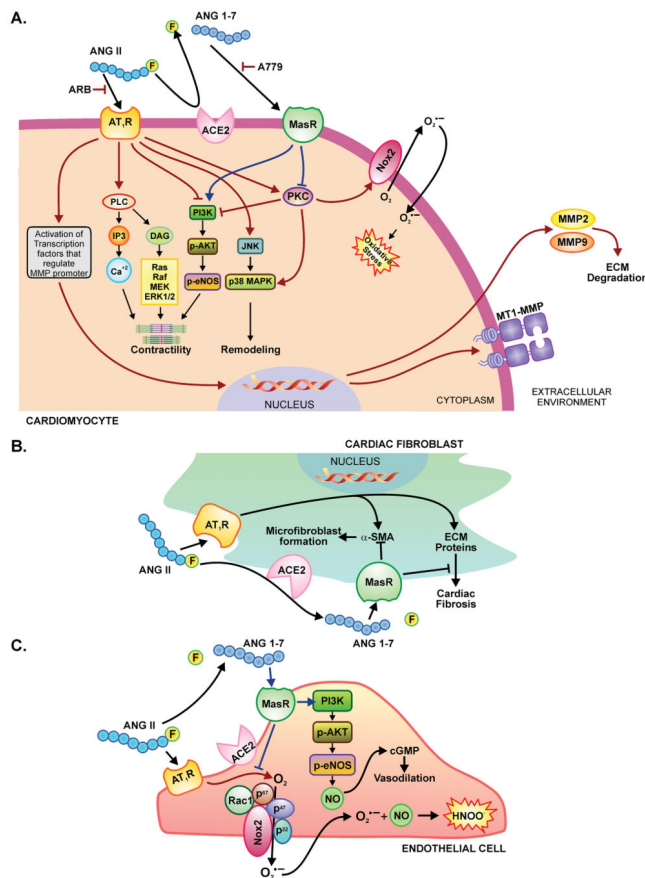


Figure 3. Cardiac effects of the Ang II/AT₁R axis and counter-regulation by the ACE2/Ang 1-7/MasR axis
 ACE-mediated generation of Ang II results in activation of various signaling pathways in cardiomyocytes, cardiac fibroblasts, and endothelial cells, resulting in adverse cardiac remodeling and cardiac dysfunction. Activation of the ACE2/Ang 1-7/MasR axis counter-regulates Ang II/AT₁R mediated effects and also stimulates cardiac contractility mediated by the PI3K-Akt-eNOS pathway.

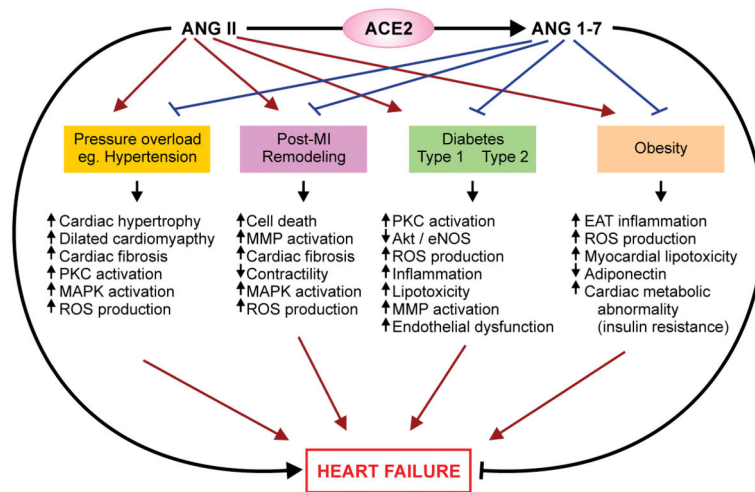


Figure 4. Central role of the ACE2/Ang 1–7 axis in HF: non-ischemic cardiomyopathy, MI, diabetic cardiomyopathy, and obesity-associated cardiac dysfunction
 Ang II/AT₁R is critically involved in the disease progression leading to non-ischemic, ischemic, and diabetic cardiomyopathy and to obesity-associated cardiac dysfunction. By converting Ang II to Ang 1–7, ACE2 shifts the balance to the cardioprotective ACE2/Ang 1–7/MasR axis. EAT: epicardial adipose tissue.

Table

Interventions to modulate ACE2 levels or activity and their effects in experimental models of heart failure.

Experimental intervention	Experimental model	Observation
Gain of Function		
Lentiviral overexpression	LAD * coronary artery ligation	6 weeks post-surgery: complete rescue of cardiac output, a 41% rescue of ejection fraction, a 44% rescue in contractility, and a 53% rescue in LV anterior (infarcted) wall thinning compared to control rats ¹¹⁷
Lentiviral overexpression	SHR	Attenuation of high blood pressure in the SHR, 18% reduction in left ventricular wall thickness, 12% increase in left ventricular end diastolic, and a 21% increase in end systolic diameters in lenti-ACE2-treated SHR; attenuation of peri-vascular fibrosis ¹⁰³
Lentiviral overexpression	Ang II infusion	Attenuation of the increased heart weight/body weight and myocardial fibrosis induced by Ang II infusion ¹²⁶
Lentiviral overexpression	Cardiac fibroblasts – hypoxia/re-oxygenation	Attenuation of both basal and hypoxia/re-oxygenation- induced collagen production by fibroblasts ¹²⁰
Adenoviral overexpression	LAD coronary artery ligation	4 weeks after ACE2 gene transfer: reduced LV volume and extent of myocardial fibrosis, increased LV ejection fraction and levels of ACE2 activity ¹¹⁹
rhACE2	Ang II infusion	Blunted the hypertrophic response and expression of hypertrophy markers; decreased ROS production; inhibited pathological signaling ⁶⁸ ; rhACE2 administration to WKY rats reduced Ang II infusion-induced pressor response, myocardial hypertrophy, pathological signaling, and superoxide production ²⁴
rhACE2	SHR	14-day administration of rhACE2 partly corrected hypertension, ROS production, and pathological signaling in the heart ²⁴
rhACE2	Transverse aortic constriction	rhACE2 partially prevented the pressure-overload- induced dilated cardiomyopathy and mRNA expression of disease markers and pro-fibrotic genes ⁶⁸
ACE2 activator (DIZE)	LAD coronary artery ligation	DIZE attenuated the MI-induced decrease in fractional shortening by 89%, improved dp/dtmax by 92%, and reversed ventricular hypertrophy by 18% ¹⁵¹
Loss of Function		
ACE2KO	Ang II infusion	Worsened cardiac fibrosis and pathological hypertrophy in ACE2KO mice ⁶⁸
ACE2KO	Transverse aortic constriction	Eccentric cardiac remodeling, increased pathological hypertrophy, and worsening of systolic performance; increased ROS production ^{67, 111, 112}
ACE2KO	LAD coronary artery ligation	Enhanced susceptibility to MI, with increased mortality, infarct expansion, and adverse ventricular remodeling ¹¹⁵
ACE2KO	Type 1 diabetes; Akita	Loss of ACE2 in type 1 diabetic mice resulted in HF-rEF with background HF-pEF in Akita mice ¹²⁹
ACE2KO	High fat diet- induced obesity	Loss of ACE2 worsens epicardial adipose tissue inflammation, myocardial metabolic abnormalities, and lipotoxicity, resulting in HF-pEF ¹⁴¹
ACE2 inhibitor (MLN4760)	(mRen2)27 hypertensive rats	Increased cardiac Ang II levels; increases in LV anterior, posterior, and relative wall thicknesses; increased interstitial collagen fraction area and cardiomyocyte hypertrophy ¹⁵⁷
ACE2 inhibitor (DX600)	Ang II stimulation of cultured cardiac fibroblasts	DX600 increased superoxide production and expression of CTGF, FKN, and phosphorylated ERK1/2; rhACE2 reduced these effects of Ang II ¹⁵⁸
ACE2 Inhibitor (C16)	Coronary artery ligation	Increase in MI size and reduction in LV % fractional shortening ¹¹⁶

* ACE2KO: ACE2 knockout; CTGF, connective tissue growth factor; DIZE, diminazene aceturate; ERK1/2, extracellular signal-regulated kinase 1/2; FKN, fractalkine; LAD, left anterior descending; LV, left ventricle; SHR, spontaneously hypertensive rats.