

# ACE2: Angiotensin II/Angiotensin-(1–7) Balance in Cardiac and Renal Injury

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**Abstract** Our current recognition of the renin-angiotensin system is more convoluted than originally thought due to the discovery of multiple novel enzymes, peptides, and receptors inherent in this interactive biochemical cascade. Over the last decade, angiotensin-converting enzyme 2 (ACE2) has emerged as a key player in the pathophysiology of hypertension and cardiovascular and renal disease due to its pivotal role in metabolizing vasoconstrictive/hypertrophic/proliferative angiotensin II into favorable angiotensin-(1–7). This review addresses the considerable advancement in research on the role of tissue ACE2 in the development and progression of hypertension and cardiac and renal injury. We summarize the results from recent clinical and experimental studies suggesting that serum or urine soluble ACE2 may serve as a novel biomarker or independent risk factor relevant for diagnosis and prognosis of cardiorenal disease. We also review recent proceedings on novel therapeutic approaches to enhance ACE2/angiotensin-(1–7) axis.

**Keywords** Angiotensin-converting enzyme 2 · Angiotensin II · Angiotensin-(1–7) · Heart · Kidney · Hypertension · Left ventricular remodeling · Heart failure · Diabetes · Renal disease

## Introduction

An ever-emerging body of experimental and clinical evidence continues to support a key role of the renin-angiotensin system (RAS) in the pathogenesis of hypertension. In the last decade, our understanding of the convoluted RAS has expanded onto the existence of novel angiotensins that counteract the hypertensive, growth-promoting, and proliferative effects of angiotensin II (Ang II). Indeed, angiotensin-(1–7) [Ang-(1–7)], its forming enzyme angiotensin-converting enzyme 2 (ACE2), and receptor Mas have been a topic of interest not only in hypertension research but also across different research areas, reflecting pleiotropic effects of RAS effector hormones. This review addresses the considerable advancement of knowledge on these novel components of the RAS, with focus on ACE2. The significance of this progress is made apparent from a search of papers in PubMed over the last three years with terms such as ACE2, heart, and kidneys. These keywords yielded over 200 publications.

## The Conventional vs. Alternate RAS

The conventional RAS has been viewed as a classical hormonal system comprising the enzymatic cleavage of the decapeptide angiotensin I (Ang I) in the circulation by renal renin from liver-derived angiotensinogen. Further cleavage of two amino acids from the C-terminal part of Ang I by angiotensin-converting enzyme (ACE), primarily in the pulmonary circulation, leads to formation of Ang II, which contributes to the regulation of blood pressure by influencing

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vascular smooth muscle cells and sodium and volume homeostasis as well as aldosterone secretion. The Ang II effects are mediated through its two known plasma membrane receptors, angiotensin type 1 (AT1) and AT2 receptors. There is some controversy about the outcome of AT2 activation, but a majority of reports points toward opposing actions of AT2 on vascular tone and sodium homeostasis.

As opposed to this classical endocrine system, where the action of the hormone takes place quite remotely from its origin, the concept of the local-tissue RAS with paracrine, autocrine, and intracrine actions has become increasingly appreciated in the last two decades, underlining the role of the RAS in regulating cell growth and proliferation, inflammation, and cytokine production. Indeed, a growing body of evidence testifies that each and every component of the RAS is found throughout diverse tissues and organs, including the heart, vasculature, kidneys, brain, lung, and reproductive tissues. Importantly, recent studies identifying new enzymes (ACE2) or new substrates for known enzymes (chymase, ACE), peptides [Ang-(1–12), Ang-(1–9), Ang-(1–7)], and receptors (renin/prorenin receptor, Mas receptor) have brought novel insight into the role of the RAS in pathophysiology of hypertension and related cardiovascular and renal disease. In healthy and diseased human heart, for example, we recently showed a role for chymase in the formation of Ang II from Ang-(1–12), a new precursor for downstream angiotensin peptides [1•, 2, 3]. In this review, we will provide an update on ACE2 counteracting the majority of Ang II cardiovascular and renal effects, as well as its usefulness as a novel biomarker and therapeutic target for cardiovascular and renal disease.

### ACE2/Ang-(1–7)/mas axis

The heptapeptide Ang-(1–7) [Asp<sup>1</sup>-Arg<sup>2</sup>-Val<sup>3</sup>-Tyr<sup>4</sup>-Ile<sup>5</sup>-His<sup>6</sup>-Pro<sup>7</sup>-] is a truncated form of Ang II, lacking phenylalanine in the eighth position. Its functional role in counterbalancing Ang II actions was recognized long before the discovery of its forming enzyme ACE2 and related receptor Mas. As previously reviewed [4, 5], Ang-(1–7) induces systemic and regional vasodilation, diuresis and natriuresis, and exerts antiproliferative and antigrowth effects in vascular smooth muscle cells, cardiac myocytes, and fibroblasts as well as glomerular and proximal tubular cells. Cardiorenal protective effects of Ang-(1–7) are mediated by the Mas receptor through different signaling pathways, including several autocooids, mitogen-activated protein kinase (MAPK), AKT, NADPH oxidase, transforming growth factor (TGF)- $\beta$ 1, epidermal growth factor (EGF) receptor, and NF-kappaB activity.

ACE2 was discovered in 2000 as a homologue enzyme to the better-known ACE, sharing many features of the enzymes belonging to a family of zinc metalloproteinases. Similar to ACE, ACE2 is a plasma membrane-bound ectoenzyme, although

soluble forms in plasma and urine are also found [6, 7•]. Shedding of ACE2 has frequently been associated with the activity of tumor necrosis factor alpha-converting enzyme (TACE) [8]. In contrast to ACE, which has two active-site domains and acts as dicarboxypeptidase, ACE2 expresses only one catalytic site and acts as a monocarboxypeptidase, removing one amino acid from the C-terminus of its substrates. ACE2 metabolizes Ang I and Ang II into Ang-(1–9) and Ang-(1–7), respectively, with higher preference for Ang II degradation [9]. Other known ACE2 substrates belong to the apelin family (apelin 13, apelin 17, apelin 36) which, in addition to Ang-(1–7), exert important protective cardiovascular actions [10]. ACE2 is also insensitive to the actions of known ACE inhibitors [11, 12].

### ACE2 and Cardiac Disease

ACE2 is widely distributed, and in the heart, its presence has been documented in the coronary vessels as well as in cardiac myocytes and fibroblasts. The first study using ACE2 knock-out (ACE2 KO) mice testified to the importance of this intracardiac enzyme in regulation of cardiac structure and function. ACE2 gene deletion resulted in major defect in cardiac contractility associated with increased plasma and heart Ang II [13]. Subsequent studies from our laboratory showed ACE2-dependent generation of Ang-(1–7) from Ang II in isolated hearts from mRen2 transgenic rats [14]. In the same animal model, we further showed that chronic ACE2 inhibition with MLN-4760 for 28 days worsened cardiac remodeling that was associated with increased cardiac Ang II levels [15]. Genetic ablation or pharmacological inhibition of ACE2 has been also associated with exacerbation of left ventricular remodeling and dysfunction in response to myocardial infarction [16, 17], diabetes [18•], and aging [19]. In agreement with these studies are the recent reports on significant reduction of ACE2 protein in response to pressure overload [20•] and the further worsening of cardiac remodeling and development of systolic dysfunction, and consequent heart failure, due to biomechanical stress imposed on the background of ACE2 gene deletion [21, 22]. Cardiac remodeling and dysfunction under these conditions were linked to protein kinase C-mediated activation of p47<sup>phox</sup> NADPH oxidase subunit, resulting in augmented oxidative stress, and extracellular signal-regulating protein kinases (ERK)1/2 activation, as well as increased matrix metalloproteinase 2 and 9 activities. Moreover, a critical role of increased action of Ang II and/or loss of Ang-(1–7) effects has been proven in a subsequent report, where blockade of AT1 receptors by irbesartan or infusion of Ang-(1–7) exerted a comparable level of cardioprotection in pressure-overloaded ACE2 null mice [23•]. Beneficial signaling pathways were confirmed in cultured cardiac myocytes and fibroblast isolated from pressure-

overload ACE2 KO mice. Similarly, irbesartan reduced systolic dysfunction in diabetic ACE2 KO mice, reducing the enhanced activation of NADPH oxidase and metalloproteinases [18•]. Thus blockade of Ang II action or Ang-(1–7) supplementation may be an effective treatment for heart failure associated with ACE2 downregulation.

On the other side, increased ACE2 mRNA, protein, and activity were reported in failing human hearts [24–27] and several experimental models of myocardial infarction [16, 25]. Moreover, increased ACE2 activity in the plasma of heart failure patients correlates with unfavorable clinical outcomes [6, 28•]. It seems that increased ACE2 levels and Ang-(1–7) formation are compensatory mechanisms to impede the progression of heart failure, keeping in mind that ACE2 overexpression provided cardioprotection in rats subjected to myocardial infarction [29]. Importantly, our laboratory documented the beneficial upregulation of ACE2 mRNA in infarcted rat hearts after 28 days of treatment with AT1 receptor antagonists [30]. In cardiac remodeling following prolonged nitric oxide inhibition [31] or heart failure due to experimental myocarditis [32], cardioprotective effects of AT1 receptor blockade were also mediated through ACE2/Ang-(1–7) axis. These data are in agreement with several *in vivo* and *in vitro* reports documenting negative ACE2 regulation by Ang II and endothelin through activation of MAPK pathways [33–37]. Moreover, Ang-(1–7)-mediated MAPK phosphatase activation counteracted inhibitory effects of Ang II on ACE2 in cardiomyocytes and vascular smooth muscle cells [36, 38], while lentivirus-mediated overexpression of Ang-(1–7) increased ACE2 gene expression in cardiac tissue of rats subjected to coronary artery ligation [39]. In agreement with the data, Ang-(1–7) increased ACE2 expression in neonatal cardiac myocytes under hypoxic conditions [39]. However, a recent report that both Ang II and Ang-(1–7) positively regulated ACE2 [40] in human cardiac fibroblasts warrants further studies in addressing ACE2 regulation and related signaling pathways.

An increasing body of evidence suggests that ACE2 gene polymorphisms are associated with left ventricular hypertrophy [41–44], coronary heart disease and myocardial infarction [45], cardiac remodeling, and urinary protein level in men with type 2 diabetes and coronary heart disease [46], as well as with hypertension [42, 47, 48], hypertension in women [49], and antihypertensive response to ACE inhibitors in women [50]. On the other hand, some reports found no association between ACE2 and hypertension [51, 52] and ACE2 and risk for sudden cardiac death in women or men [53], underscoring the necessity for further evaluation of the impact of ACE2 gene variants on cardiovascular disease in the human population.

### Renoprotective Role of ACE2

In human and rodent kidney, ACE2 was found in glomerular podocytes and mesangial cells and along different tubular

segments of the nephron. Several studies pointed to the lack of ACE2 expression in endothelium of glomerular capillaries and small renal arterioles [54–56], while ACE2 is highly expressed in vascular smooth muscle cells and endothelium of larger interlobular arteries [54]. Most of the studies stressed a much higher expression in tubules as compared to glomerulus [54, 56–58] and ACE2 expression in mouse kidney was found to be 20-fold higher than in mouse heart tissue [59].

Such a high and wide distribution throughout the nephron unit testifies to the importance of the ACE2 contribution to the maintenance of renal hemodynamics and tubulo-glomerular function. Indeed, genetic, pharmacological, and functional loss of ACE2 resulted in increased albuminuria and progression to glomerulosclerosis. ACE2 genetic ablation resulted in subtle fibrillar collagen accumulation in glomerular mesangium seen only under electron microscope in early age, while it led to severe glomerulosclerosis in aging mice [60]. Moreover, when ACE2 KO mice were crossed with Akita mice (a model of type 1 diabetes), more advanced glomerular damage was induced independently of blood pressure and glucose levels [61]. Similarly, ACE2 deletion in mice subjected to unilateral ureteral obstruction increased Ang II and decreased Ang-(1–7) in obstructed kidney, leading to exaggerated renal inflammation and fibrosis associated with enhanced TGF- $\beta$ /Smad2/3 and NF- $\kappa$ B signaling pathways [62]. Kidney Ang II was consistently many times higher, and hypertension, renal oxidative stress, and inflammation were more severe, in ACE2 KO than wild-type mice following Ang II infusion, confirming the critical role of ACE2 in the regulation of kidney Ang II metabolism, blood pressure, and tissue damage [63, 64]. In agreement, pharmacological inhibition of ACE2 by MLN-4760 worsened albuminuria in two mouse model of diabetes, db/db mice [56] and diabetes induced by streptozotocin (STZ) [65]. Although renal Ang II content and intrarenal Ang II signaling were not consistently evaluated in these studies, specific AT1 receptor antagonists ameliorated or prevented renal damage in some of the studies [56, 60, 61], demonstrating that development of renal pathology was, indeed, Ang II-mediated.

In many models of genetic or induced hypertension, kidney ACE2 was decreased in adult animals [13, 66, 67]. Importantly, in the kidneys of spontaneously hypertensive rats (SHR), an excellent model of essential hypertension in humans, the developmental pattern of ACE2 expression was altered before the onset of hypertension, suggesting a causative role of altered ACE2 expression in the development of hypertension [68]. Increased salt intake and related kidney damage were associated with reduced cortical expression of ACE2 in obese Zucker rats [69] and increased ACE/ACE2 mRNA and protein ratio in glomeruli of uninephrectomized WKY [70], respectively. The similar ratio between ACE and ACE2 was also found in renal biopsies from patients with hypertension when compared to subjects with normal blood pressure [71], as well as in patients

with IgA nephropathy [72] and diabetes [57, 73]. No differences in ACE2 expression between patients with focal segmental glomerulosclerosis and control cohort has been reported [73]; these data suggest that decrease in ACE2 is not a general response to kidney injury. In contrast to clinical studies on diabetic patients, there are some discrepancies with regard to the expression and activity of ACE2 in experimental diabetes [56, 58, 59]. This may have stemmed from differences in animal models and nephron segment studied as well as the degree of the disease. Nevertheless, reduced ACE2 expression and activity in glomeruli was consistently found in experimental diabetes as well [56, 58]. Importantly, podocyte-specific overexpression of human ACE2 transiently attenuated the development of STZ-induced diabetic nephropathy [74•], providing support for the crucial role of glomerular ACE2 in the onset of diabetes-related kidney disease. Furthermore, in contrast to the association between ACE2 gene variants and hypertension or cardiac disease, no association was found between ACE2 gene polymorphisms and diabetic nephropathy or complications such as increased blood pressure or hemoglobin A1C [75, 76].

Reperfusion of ischemic kidney led to decrease in mRNA for ACE2 and consecutive changes in renal Ang II and Ang-(1–7), as did subtotal nephrectomy [25, 77, 78]. There were some discrepancies as to whether Ang-(1–7) supplementation was beneficial under the condition of nephron loss [79], but it was demonstrated that both Ang-(1–7) and losartan normalized cortical ACE2 expression [77]. Despite normalization of plasma Ang II by Ang-(1–7), there were no changes in blood pressure, albuminuria, and glomerular filtration rate consistent with increased mesangial area in glomeruli of rats treated with the heptapeptide [77]. Indeed, growth-stimulating effects of Ang-(1–7) associated with extracellular matrix protein production were demonstrated in human mesangial cells [80]. On the other hand, acute renal injury due to limb ischemia-reperfusion in mice is exacerbated with ACE2 deletion and rescued with its overexpression, which correlates with adequate changes in circulating but not tissue Ang II and Ang-(1–7) [81]. Nevertheless, these studies add to the evidence that targeting ACE2 in Ang II degradation and/or Ang-(1–7) formation might be a valuable therapeutic approach for renal disease of different etiologies.

Our laboratory was among the first to show that ACE inhibitors or AT1 receptor antagonist given for two weeks enhanced renal ACE2 activity that was associated with increased Ang-(1–7) in plasma and urine of normotensive animals [33]. The concomitant activation of unopposed AT2 receptors contributing to the ACE2 upregulation by AT1 receptor blockade cannot be ruled out. Indeed, a study published just a month ago revealed ACE2 and Ang-(1–7) upregulation in response to treatment with an AT2 agonist in obese Zucker rats that was associated with reduction of blood pressure and increased urinary sodium excretion [82•]. Other

studies in rodents [78] and humans with non-diabetic kidney disease [73] has suggested that upregulation of ACE2 may have abated the progression of renal disease. These studies are in accord with in vitro reports that Ang II, in AT1 fashion, downregulated ACE2 in human tubular cells via MAPK (ERK1/2 and p38) pathway [83]. However, our most recent study, in which we employed six-week treatment with olmesartan, showed that a marked reduction of blood pressure was associated with increased renal ACE2 protein, but not activity, in hypertensive mRen2.Lewis rats [84]. Posttranscriptional changes were suggested in studies in which a discrepancy was also found between ACE2 mRNA, protein, and/or activity in kidneys of diabetic animals [59, 85]. Together, these studies strongly suggest that a pathophysiological role of ACE2 should be assessed through comprehensive analysis, including not only its expression on the level of mRNA and protein but also enzymatic activity.

### **Circulating and Urinary ACE2: Novel Biomarker for Cardiac and Renal Disease?**

As it was previously mentioned, a cleavage of the catalytically active ectodomain of ACE2 results in a smaller protein fragment found in plasma, serum, and urine of humans and experimental animals. The very low ACE2 activities were detected in human plasma of healthy individuals and were frequently related to the presence of an endogenous inhibitor [86, 87]. In contrast, increased serum ACE2 activities were measured in patients with acute and chronic heart failure [6, 28, 88–90] and were correlated positively with plasma B-type natriuretic peptide (BNP), a potent predicting factor of mortality and morbidity in heart failure [6, 28, 88]. Higher ACE2 activity was associated with more severe disease and lower ejection fraction, regardless of the presence or absence of ischemic disease; in another study, however, increased ACE2 activity may have reflected coronary heart disease associated with diabetes [91]. In patients with chronic systolic heart failure, plasma ACE2 activity was predictive of unfavorable clinical outcome independently of ejection fraction and BNP. Moreover, it was suggested that measurements of both ACE2 activity and BNP may be of greater value in predicting the occurrence of adverse cardiac events [6, 28].

With regard to renal disease, augmented serum ACE2 activity was associated with microalbuminuria in patients with type 1 diabetes [91]. In addition, urinary ACE2 correlated positively with the degree of proteinuria [92] and albuminuria [93] in patients with type 2 diabetes, translating findings of increased ACE2 protein and activity in urine [7] and serum [94] of diabetic experimental models. While there are some differences in the expression of renal ACE2 between human and experimental diabetic nephropathy in the current literature, a study by Wysocki et al. clearly suggests that urinary ACE2

reflected renal rather than systemic source [7]. Few studies reported gender differences with respect to circulating ACE2; ACE2 was higher in males vs. females in healthy subjects and in patients with renal disease [87, 91, 95], mimicking gender disparity in the development of cardiovascular disease. Together, these studies suggest that measurement of soluble ACE2 may be a helpful diagnostic and prognostic marker for patients with cardiovascular and/or renal disease. While it is still unknown whether increased soluble ACE2 originated from increased tissue synthesis or augmented tissue shedding, it may reflect a compensatory, albeit still insufficient, response to adverse stimuli. Further increase in serum ACE2 after therapeutic intervention in patients with acute decompensated heart failure was associated with favorable clinical outcomes [90], providing additional support for therapeutic approaches to increase ACE2 activity in various diseases.

### Novel Therapeutic Advances to Enhance ACE2/Ang-(1–7) axis

An increasing body of evidence suggests that novel therapeutic approaches to augment ACE2, and consequently decrease Ang II while increasing Ang-(1–7) actions, may be particularly beneficial in multiple disease states associated with elevated Ang II/Ang-(1–7) ratio. Our laboratory was among the first to show that classical ACE inhibitors and AT1 receptor antagonists also augmented ACE2 activity in heart and kidneys of normotensive and hypertensive rats as well as in the heart of rats with myocardial infarction [30, 33, 34, 37]. As exercise is well accepted as one of the most powerful lifestyle interventions in the treatment of hypertension and cardiovascular disease, it is important to note that cardiac ACE decreased and ACE2 increased following exercise in both lean and obese rat strains [96]. Thus, the reciprocal changes in ACE/ACE2 were recognized as the underlying cardioprotective molecular mechanisms for development of non-pathological left ventricular hypertrophy in response to aerobic exercise, and the regulatory role of specific microRNAs was suggested [97].

In most recent studies, recombinant human ACE2 (rhACE2) concealed Ang II induced cardiac remodeling and dysfunction and related pathological signaling events in ACE2-deficient mice, decreasing plasma and tissue Ang II and increasing plasma Ang-(1–7) [20]. It also attenuated the development of dilated cardiomyopathy in pressure-overloaded wild-type mice. Moreover, Ang-(1–7) mediated the effects of rhACE2 in suppressing Ang II-induced oxidative stress, expression of profibrotic genes, and ERK1/2 signaling in cultured cardiomyocytes and fibroblasts [20]. Lower levels of Ang II and increased Ang-(1–7) were consistently associated with slower progression of diabetic nephropathy due to supplementation with rhACE2 in a murine

experimental model of type 1 diabetes [98]. In SHR, rhACE2 attenuated hypertension and cardiac, kidney, and vascular oxidative stress [99], while both human [100] and mouse rACE2 [101] attenuated Ang II-induced hypertension by decreasing plasma Ang II.

In keeping with the above-referenced findings, overexpression of ACE2 by adeno- or lentivirus transfection exerted cardiac protection in Ang II-infused rats [102], rats with myocardial infarction [29] and diabetic cardiomyopathy [103], as well as renoprotective action in diabetic nephropathy [104]. Moreover, podocyte-specific overexpression of hACE2 transiently attenuated the development of diabetic nephropathy [74], reflected in early protection from albuminuria, partial preservation of podocyte number and specific podocyte proteins nephrin and synaptopodin, as well as decreased profibrotic TGF- $\beta$ 1. Similarly, novel compounds with ACE2 activation ability reduced blood pressure in SHR [105] and improved cardiac function in diabetic rats [106], although further studies are necessary to confirm that the effects are, indeed, related to the relevant changes in Ang peptides.

Relative failure of classical RAS blockade in preventing development of end-stage heart and kidney disease could be explained, at least in part, by incomplete suppression of Ang II in response to the therapy with ACE inhibitors or AT1 receptor antagonists. Moreover, there are some conflicting reports as to the ability of the RAS blockade to upregulate ACE2 to facilitate Ang II metabolism [84, 107]. Therefore, more complete Ang II inhibition may be achieved by combining classical RAS blockade with novel approaches to enhance ACE2 activity, facilitating Ang II degradation. We recently reviewed alternative non-ACE-related pathways for Ang II production in human and experimental heart disease, including the activity of chymase on Ang-(1–12), a new precursor for downstream angiotensin peptides [1, 2, 3]. Other studies have also reported the importance of chymase derived from mast cells in development of left ventricular dysfunction [108]. In this context, in addition to classical blockade of Ang II synthesis and action by ACE inhibitors or AT1 receptor blockers, therapeutic approaches to augment ACE2 and consequently decrease Ang II while increasing Ang-(1–7) actions may be particularly beneficial under conditions when these alternative Ang II synthetic pathways are overactive.

### Conclusion

Experimental and clinical studies continue to provide novel evidence on the crucial role of ACE2/Ang-(1–7) in counterbalancing vasoconstrictor/hypertrophic/proliferative effects of Ang II determining the onset and progression of hypertension and cardiac and renal damage. Additional studies are needed to advance initial progress on the pharmacological and genetic therapeutic approaches to enhance ACE2 activity,

aiming to decrease Ang II while increasing Ang-(1-7) actions. This may be of particular interest when complete suppression of Ang II in response to therapy with ACE1 or AT1 receptor antagonist is not achieved or when alternative Ang II synthetic pathways are overactive. Further research is needed to confirm whether serum or urine soluble ACE2 may serve as a novel biomarker or independent risk factor relevant to diagnosis and prognosis of cardiorenal disease.

#### Compliance with Ethics Guidelines

**Conflict of Interest** Jasmina Varagic, Sarfaraz Ahmad, Sayaka Nagata, and Carlos M. Ferrario declare that they have no conflict of interest.

**Human and Animal Rights and Informed Consent** With regard to the authors' research cited in this paper, all procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000 and 2008, and all institutional and national guidelines for the care and use of laboratory animals were followed.

#### References

Papers of particular interest, published recently, have been highlighted as:

- Of importance

1. Ahmad S, Simmons T, Varagic J, Moniwa N, Chappell MC, Ferrario CM. Chymase-dependent generation of angiotensin II from angiotensin-(1-12) in human atrial tissue. *PLoS One*. 2011;6(12):e28501. *The first paper to show the chymase-dependent generation of Ang II from the novel intermediate precursor Ang-(1-12) in atrial tissue from patients undergoing cardiac surgery for primary control of atrial fibrillation.*
2. Ahmad S, Wei CC, Tallaj J, Dell'Italia LJ, Moniwa N, Varagic J, et al. Chymase mediates angiotensin-(1-12) metabolism in normal human hearts. *J Am Soc Hypertens*. 2013;7(2):128–36.
3. Ferrario CM, Ahmad S, Nagata S, Simington S, Varagic J, Kon N, et al. An evolving story of angiotensin II-forming pathways in rodents and humans. *Clin Sci*. 2013;156(7):461–9.
4. Ferrario CM, Varagic J. The ANG-(1-7)/ACE2/mas axis in the regulation of nephron function. *Am J Physiol Renal Physiol*. 2010;298(6):F1297–305.
5. Varagic J, Trask AJ, Jessup JA, Chappell MC, Ferrario CM. New angiotensins. *J Mol Med (Berl)*. 2008;86(6):663–71.
6. Epelman S, Shrestha K, Troughton RW, Francis GS, Sen S, Klein AL, et al. Soluble angiotensin-converting enzyme 2 in human heart failure: relation with myocardial function and clinical outcomes. *J Card Fail*. 2009;15(7):565–71.
7. Wysocki J, Garcia-Halpin L, Ye M, Maier C, Sowers K, Burns KD, et al. Regulation of urinary ACE2 in diabetic mice. *Am J Physiol Renal Physiol*. 2013;305(4):F600–11. *In db/db mice and STZ-induced diabetes serum, urinary, and renal cortex ACE2 were increased. This study suggests that urinary ACE2 reflected renal rather than systemic source.*
8. Lambert DW, Yarski M, Warner FJ, Thomhill P, Parkin ET, Smith AI, et al. Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute

- respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *J Biol Chem*. 2005;280(34):30113–9.
9. 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(Pt 1):45–51.
10. Kazemi-Bajestani SM, Patel VB, Wang W, Oudit GY. Targeting the ACE2 and Apelin Pathways Are Novel Therapies for Heart Failure: Opportunities and Challenges. *Cardiol Res Pract*. 2012;2012:823193.
11. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res*. 2000;87(5):E1–9.
12. 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(43):33238–43.
13. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*. 2002;417(6891):822–8.
14. Trask AJ, Averill DB, Ganten D, Chappell MC, Ferrario CM. Primary role of angiotensin-converting enzyme-2 in cardiac production of angiotensin-(1-7) in transgenic Ren-2 hypertensive rats. *Am J Physiol Heart Circ Physiol*. 2007;292(6):H3019–24.
15. Trask AJ, Groban L, Westwood BM, Varagic J, Ganten D, Gallagher PE, et al. Inhibition of angiotensin-converting enzyme 2 exacerbates cardiac hypertrophy and fibrosis in Ren-2 hypertensive rats. *Am J Hypertens*. 2010;23(6):687–93.
16. Kassiri Z, Zhong J, Guo D, Basu R, Wang X, Liu PP, et al. Loss of angiotensin-converting enzyme 2 accelerates maladaptive left ventricular remodeling in response to myocardial infarction. *Circ Heart Fail*. 2009;2(5):446–55.
17. Kim MA, Yang D, Kida K, Molotkova N, Yeo SJ, Varki N, et al. Effects of ACE2 inhibition in the post-myocardial infarction heart. *J Card Fail*. 2010;16(9):777–85.
18. Patel VB, Bodiga S, Basu R, Das SK, Wang W, Wang Z, et al. 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(10):1322–35. *This study pinpoints the crucial role of ACE2 in development of diabetic cardiomyopathy through Ang II-dependent mechanisms.*
19. Oudit GY, Kassiri Z, Patel MP, Chappell M, Butany J, Backx PH, et al. Angiotensin II-mediated oxidative stress and inflammation mediate the age-dependent cardiomyopathy in ACE2 null mice. *Cardiovasc Res*. 2007;75(1):29–39.
20. Zhong J, Basu R, Guo D, Chow FL, Byrns S, Schuster M, et al. Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. *Circulation*. 2010;122(7):717–28. *This comprehensive study provides critical evidence for beneficial effects of hrACE2 in Ang II- and pressure overload-induced cardiac remodeling and dysfunction. The cardioprotection was associated with attenuation of signaling pathways and molecules relevant for hypertrophy, fibrosis, and oxidative stress and correlated with reduction in Ang II and elevation in Ang-(1-7).*
21. Bodiga S, Zhong JC, Wang W, Basu R, Lo J, Liu GC, et al. Enhanced susceptibility to biomechanical stress in ACE2 null mice is prevented by loss of the p47(phox) NADPH oxidase subunit. *Cardiovasc Res*. 2011;91(1):151–61.
22. Yamamoto K, Ohishi M, Katsuya T, Ito N, Ikushima M, Kaibe M, et al. Deletion of angiotensin-converting enzyme 2 accelerates pressure overload-induced cardiac dysfunction by increasing local angiotensin II. *Hypertension*. 2006;47(4):718–26.

23. Patel VB, Bodiga S, Fan D, Das SK, Wang Z, Wang W, et al. 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(6):1195–203. *When ACE2 is absent, a blockade of Ang II action or Ang-(1-7) supplementation provides comparable cardioprotection in an experimental model of heart failure due to pressure overload revealing striking redundancy of these two counterregulatory mechanisms.*
24. Zisman LS, Keller RS, Weaver B, Lin Q, Speth R, Bristow MR, et al. Increased angiotensin-(1-7)-forming activity in failing human heart ventricles: evidence for upregulation of the angiotensin-converting enzyme Homologue ACE2. *Circulation*. 2003;108(14):1707–12.
25. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, et al. Myocardial infarction increases ACE2 expression in rat and humans. *Eur Heart J*. 2005;26(4):369–75.
26. Goulter AB, Goddard MJ, Allen JC, Clark KL. ACE2 gene expression is up-regulated in the human failing heart. *BMC Med*. 2004;2:19.
27. Ohtsuki M, Morimoto S, Izawa H, Ismail TF, Ishibashi-Ueda H, Kato Y, et al. Angiotensin converting enzyme 2 gene expression increased compensatory for left ventricular remodeling in patients with end-stage heart failure. *Int J Cardiol*. 2010;145(2):333–4.
28. Wang Y, Moreira MC, Heringer-Walther S, Ebermann L, Schultheiss HP, Wessel N, et al. Plasma ACE2 activity is an independent prognostic marker in Chagas' disease and equally potent as BNP. *J Card Fail*. 2010;16(2):157–63. *This study suggests that measurements of both ACE2 activity and BNP may be of greater value in prediction morbidity and mortality in patients with heart failure.*
29. Zhao YX, Yin HQ, Yu QT, Qiao Y, Dai HY, Zhang MX, et al. ACE2 overexpression ameliorates left ventricular remodeling and dysfunction in a rat model of myocardial infarction. *Hum Gene Ther*. 2010;21(11):1545–54.
30. Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension*. 2004;43(5):970–6.
31. Inaba S, Iwai M, Furuno M, Kanno H, Senba I, Okayama H, et al. Role of angiotensin-converting enzyme 2 in cardiac hypertrophy induced by nitric oxide synthase inhibition. *J Hypertens*. 2011;29(11):2236–45.
32. Sukumaran V, Veeraveedu PT, Gurusamy N, Yamaguchi K, Lakshmanan AP, Ma M, et al. Cardioprotective effects of telmisartan against heart failure in rats induced by experimental autoimmune myocarditis through the modulation of angiotensin-converting enzyme-2/angiotensin 1-7/mas receptor axis. *Int J Biol Sci*. 2011;7(8):1077–92.
33. Ferrario CM, Jessup J, Gallagher PE, Averill DB, Brosnihan KB, Ann TE, et al. Effects of renin-angiotensin system blockade on renal angiotensin-(1-7) forming enzymes and receptors. *Kidney Int*. 2005;68(5):2189–96.
34. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation*. 2005;111(20):2605–10.
35. Gallagher PE, Chappell MC, Ferrario CM, Tallant EA. Distinct roles for ANG II and ANG-(1-7) in the regulation of angiotensin-converting enzyme 2 in rat astrocytes. *Am J Physiol Cell Physiol*. 2006;290(2):C420–6.
36. Gallagher PE, Ferrario CM, Tallant EA. MAP kinase/phosphatase pathway mediates the regulation of ACE2 by angiotensin peptides. *Am J Physiol Cell Physiol*. 2008;295(5):C1169–74.
37. Jessup JA, Gallagher PE, Averill DB, Brosnihan KB, Tallant EA, Chappell MC, et al. Effect of angiotensin II blockade on a new congenic model of hypertension derived from transgenic Ren-2 rats. *Am J Physiol Heart Circ Physiol*. 2006;291(5):H2166–72.
38. Gallagher PE, Ferrario CM, Tallant EA. Regulation of ACE2 in cardiac myocytes and fibroblasts. *Am J Physiol Heart Circ Physiol*. 2008;295(6):H2373–9.
39. Qi Y, Shenoy V, Wong F, Li H, Afzal A, Mocco J, et al. Lentivirus-mediated overexpression of angiotensin-(1-7) attenuated ischaemia-induced cardiac pathophysiology. *Exp Physiol*. 2011;96(9):863–74.
40. Lin CS, Pan CH, Wen CH, Yang TH, Kuan TC. Regulation of angiotensin converting enzyme II by angiotensin peptides in human cardiofibroblasts. *Peptides*. 2010;31(7):1334–40.
41. Lieb W, Graf J, Gotz A, Konig IR, Mayer B, Fischer M, et al. 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(1):88–96.
42. Patel SK, Wai B, Ord M, MacIsaac RJ, Grant S, Velkoska E, et al. Association of ACE2 genetic variants with blood pressure, left ventricular mass, and cardiac function in Caucasians with type 2 diabetes. *Am J Hypertens*. 2012;25(2):216–22.
43. van der Merwe L, Cloete R, Revera M, Heradien M, Goosen A, Corfield VA, et al. Genetic variation in angiotensin-converting enzyme 2 gene is associated with extent of left ventricular hypertrophy in hypertrophic cardiomyopathy. *Hum Genet*. 2008;124(1):57–61.
44. Wang SX, Fu CY, Zou YB, Wang H, Shi Y, Xu XQ, et al. Polymorphisms of angiotensin-converting enzyme 2 gene associated with magnitude of left ventricular hypertrophy in male patients with hypertrophic cardiomyopathy. *Chin Med J (Engl)*. 2008;121(1):27–31.
45. Yang W, Huang W, Su S, Li B, Zhao W, Chen S, et al. 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(5):333–40.
46. Chaoxin J, Daili S, Yanxin H, Ruwei G, Chenlong W, Yaobin T. The influence of angiotensin-converting enzyme 2 gene polymorphisms on type 2 diabetes mellitus and coronary heart disease. *Eur Rev Med Pharmacol Sci*. 2013;17(19):2654–9.
47. Huang W, Yang W, Wang Y, Zhao Q, Gu D, Chen R. Association study of angiotensin-converting enzyme 2 gene (ACE2) polymorphisms and essential hypertension in northern Han Chinese. *J Hum Hypertens*. 2006;20(12):968–71.
48. Zhong J, Yan Z, Liu D, Ni Y, Zhao Z, Zhu S, et al. Association of angiotensin-converting enzyme 2 gene A/G polymorphism and elevated blood pressure in Chinese patients with metabolic syndrome. *J Lab Clin Med*. 2006;147(2):91–5.
49. Lu N, Yang Y, Wang Y, Liu Y, Fu G, Chen D, et al. ACE2 gene polymorphism and essential hypertension: an updated meta-analysis involving 11,051 subjects. *Mol Biol Rep*. 2012;39(6):6581–9.
50. Fan X, Wang Y, Sun K, Zhang W, Yang X, Wang S, et al. Polymorphisms of ACE2 gene are associated with essential hypertension and antihypertensive effects of Captopril in women. *Clin Pharmacol Ther*. 2007;82(2):187–96.
51. Benjafield AV, Wang WY, Morris BJ. No association of angiotensin-converting enzyme 2 gene (ACE2) polymorphisms with essential hypertension. *Am J Hypertens*. 2004;17(7):624–8.
52. Zhou JB, Yang JK. Meta-analysis of association of ACE2 G8790A polymorphism with Chinese Han essential hypertension. *J Renin Angiotensin Aldosterone Syst*. 2009;10(1):31–4.
53. Sotoodehnia N, Li G, Johnson CO, Lemaitre RN, Rice KM, Rea TD, et al. Genetic variation in angiotensin-converting enzyme-related pathways associated with sudden cardiac arrest risk. *Heart Rhythm*. 2009;6(9):1306–14.
54. Lely AT, Hamming I, van GH, Navis GJ. Renal ACE2 expression in human kidney disease. *J Pathol*. 2004;204(5):587–93.

55. Soler MJ, Ye M, Wysocki J, William J, Lloveras J, Batlle D. Localization of ACE2 in the renal vasculature: amplification by angiotensin II type 1 receptor blockade using telmisartan. *Am J Physiol Renal Physiol.* 2009;296(2):F398–405.
56. Ye M, Wysocki J, William J, Soler MJ, Cokic I, Batlle D. Glomerular localization and expression of Angiotensin-converting enzyme 2 and Angiotensin-converting enzyme: implications for albuminuria in diabetes. *J Am Soc Nephrol.* 2006;17(11):3067–75.
57. Mizuiri S, Hemmi H, Arita M, Ohashi Y, Tanaka Y, Miyagi M, et al. Expression of ACE and ACE2 in individuals with diabetic kidney disease and healthy controls. *Am J Kidney Dis.* 2008;51(4):613–23.
58. Tikellis C, Johnston CI, Forbes JM, Burns WC, Burrell LM, Risvanis J, et al. Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. *Hypertension.* 2003;41(3):392–7.
59. Wysocki J, Ye M, Soler MJ, Gurley SB, Xiao HD, Bernstein KE, et al. ACE and ACE2 activity in diabetic mice. *Diabetes.* 2006;55(7):2132–9.
60. Oudit GY, Herzenberg AM, Kassiri Z, Wong D, Reich H, Khokha R, et al. Loss of angiotensin-converting enzyme-2 leads to the late development of angiotensin II-dependent glomerulosclerosis. *Am J Pathol.* 2006;168(6):1808–20.
61. Wong DW, Oudit GY, Reich H, Kassiri Z, Zhou J, Liu QC, et al. Loss of angiotensin-converting enzyme-2 (Ace2) accelerates diabetic kidney injury. *Am J Pathol.* 2007;171(2):438–51.
62. Liu Z, Huang XR, Chen HY, Penninger JM, Lan HY. Loss of angiotensin-converting enzyme 2 enhances TGF-beta/Smad-mediated renal fibrosis and NF-kappaB-driven renal inflammation in a mouse model of obstructive nephropathy. *Lab Invest.* 2012;92(5):650–61.
63. Gurley SB, Allred A, Le TH, Griffiths R, Mao L, Philip N, et al. Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice. *J Clin Invest.* 2006;116(8):2218–25.
64. Zhong J, Guo D, Chen CB, Wang W, Schuster M, Loibner H, et al. Prevention of angiotensin II-mediated renal oxidative stress, inflammation, and fibrosis by angiotensin-converting enzyme 2. *Hypertension.* 2011;57(2):314–22.
65. Soler MJ, Wysocki J, Ye M, Lloveras J, Kanwar Y, Batlle D. ACE2 inhibition worsens glomerular injury in association with increased ACE expression in streptozotocin-induced diabetic mice. *Kidney Int.* 2007;72(5):614–23.
66. Prieto MC, Gonzalez-Villalobos RA, Botros FT, Martin VL, Pagan J, Satou R, et al. Reciprocal changes in renal ACE/ANG II and ACE2/ANG 1-7 are associated with enhanced collecting duct renin in Goldblatt hypertensive rats. *Am J Physiol Renal Physiol.* 2011;300(3):F749–55.
67. Zhong JC, Huang DY, Yang YM, Li YF, Liu GF, Song XH, et al. Upregulation of angiotensin-converting enzyme 2 by all-trans retinoic acid in spontaneously hypertensive rats. *Hypertension.* 2004;44(6):907–12.
68. Tikellis C, Cooper ME, Bialkowski K, Johnston CI, Burns WC, Lew RA, et al. Developmental expression of ACE2 in the SHR kidney: a role in hypertension? *Kidney Int.* 2006;70(1):34–41.
69. Samuel P, Ali Q, Sabuhi R, Wu Y, Hussain T. High Na intake increases renal angiotensin II levels and reduces expression of the ACE2-AT(2)R-MasR axis in obese Zucker rats. *Am J Physiol Renal Physiol.* 2012;303(3):F412–9.
70. Bernardi S, Toffoli B, Zennaro C, Tikellis C, Monticone S, Losurdo P, et al. High-salt diet increases glomerular ACE/ACE2 ratio leading to oxidative stress and kidney damage. *Nephrol Dial Transplant.* 2012;27(5):1793–800.
71. Wakahara S, Konoshita T, Mizuno S, Motomura M, Aoyama C, Makino Y, et al. Synergistic expression of angiotensin-converting enzyme (ACE) and ACE2 in human renal tissue and confounding effects of hypertension on the ACE to ACE2 ratio. *Endocrinology.* 2007;148(5):2453–7.
72. Mizuiri S, Hemmi H, Arita M, Aoki T, Ohashi Y, Miyagi M, et al. Increased ACE and decreased ACE2 expression in kidneys from patients with IgA nephropathy. *Nephron Clin Pract.* 2011;117(1):c57–66.
73. Reich HN, Oudit GY, Penninger JM, Scholey JW, Herzenberg AM. Decreased glomerular and tubular expression of ACE2 in patients with type 2 diabetes and kidney disease. *Kidney Int.* 2008;74(12):1610–6.
74. Nadarajah R, Milagres R, Dilauro M, Gutsol A, Xiao F, Zimpelmann J, et al. Podocyte-specific overexpression of human angiotensin-converting enzyme 2 attenuates diabetic nephropathy in mice. *Kidney Int.* 2012;82(3):292–303. *This study provides critical evidence that podocyte-specific overexpression of hACE2 transiently attenuated the development of diabetic nephropathy.*
75. Currie D, McKnight AJ, Patterson CC, Sadlier DM, Maxwell AP. Investigation of ACE, ACE2 and AGTR1 genes for association with nephropathy in Type 1 diabetes mellitus. *Diabet Med.* 2010;27(10):1188–94.
76. Frojdo S, Sjolind L, Parkkonen M, Makinen VP, Kilpikari R, Pettersson-Fernholm K, et al. Polymorphisms in the gene encoding angiotensin I converting enzyme 2 and diabetic nephropathy. *Diabetologia.* 2005;48(11):2278–81.
77. Dilauro M, Zimpelmann J, Robertson SJ, Genest D, Burns KD. Effect of ACE2 and angiotensin-(1-7) in a mouse model of early chronic kidney disease. *Am J Physiol Renal Physiol.* 2010;298(6):F1523–32.
78. Velkoska E, Dean RG, Burchill L, Levidiotis V, Burrell LM. Reduction in renal ACE2 expression in subtotal nephrectomy in rats is ameliorated with ACE inhibition. *Clin Sci (Lond).* 2010;118(4):269–79.
79. Velkoska E, Dean RG, Griggs K, Burchill L, Burrell LM. Angiotensin-(1-7) infusion is associated with increased blood pressure and adverse cardiac remodelling in rats with subtotal nephrectomy. *Clin Sci (Lond).* 2011;120(8):335–45.
80. Zimpelmann J, Burns KD. Angiotensin-(1-7) activates growth-stimulatory pathways in human mesangial cells. *Am J Physiol Renal Physiol.* 2009;296(2):F337–46.
81. Yang XH, Wang YH, Wang JJ, Liu YC, Deng W, Qin C, et al. Role of angiotensin-converting enzyme (ACE and ACE2) imbalance on tourniquet-induced remote kidney injury in a mouse hindlimb ischemia-reperfusion model. *Peptides.* 2012;36(1):60–70.
82. Ali Q, Wu Y, Hussain T. Chronic AT2 receptor activation increases renal ACE2 activity, attenuates AT1 receptor function and blood pressure in obese Zucker rats. *Kidney Int.* 2013;84(5):931–9. *This is the first study to show a contribution of AT2 receptor in the regulation of renal ACE2 of obese Zucker rats. ACE2 and related Ang-(1-7) upregulation was associated with reduction of blood pressure and increased urinary sodium excretion. AT2 receptor agonist increased ACE2 activity in HK-2 cells confirming a direct effect of AT2 activation in enhancing ACE2/Ang-(1-7) axis.*
83. Koka V, Huang XR, Chung AC, Wang W, Truong LD, Lan HY. Angiotensin II up-regulates angiotensin I-converting enzyme (ACE), but down-regulates ACE2 via the AT1-ERK/p38 MAP kinase pathway. *Am J Pathol.* 2008;172(5):1174–83.
84. Varagic J, Ahmad S, VonCannon JL, Moniwa N, Brosnihan KB, Wysocki J, et al. Predominance of AT(1) blockade over mas-mediated angiotensin-(1-7) mechanisms in the regulation of blood pressure and renin-angiotensin system in mRen2.Lewis rats. *Am J Hypertens.* 2013;26(5):583–90.
85. Tikellis C, Bialkowski K, Pete J, Sheehy K, Su Q, Johnston C, et al. ACE2 deficiency modifies renoprotection afforded by ACE inhibition in experimental diabetes. *Diabetes.* 2008;57(4):1018–25.
86. Lew RA, Warner FJ, Hanchapola I, Yarski MA, Manohar J, Burrell LM, et al. Angiotensin-converting enzyme 2 catalytic



- activity in human plasma is masked by an endogenous inhibitor. *Exp Physiol*. 2008;93(5):685–93.
87. Roberts MA, Velkoska E, Ierino FL, Burrell LM. Angiotensin-converting enzyme 2 activity in patients with chronic kidney disease. *Nephrol Dial Transplant*. 2013;28(9):2287–94.
  88. Epelman S, Tang WH, Chen SY, Van LF, 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(9):750–4.
  89. Ortiz-Perez JT, Riera M, Bosch X, De Caralt TM, Perea RJ, Pascual J, et al. Role of circulating angiotensin converting enzyme 2 in left ventricular remodeling following myocardial infarction: a prospective controlled study. *PLoS One*. 2013;8(4):e61695.
  90. Shao Z, Shrestha K, Borowski AG, Kennedy DJ, Epelman S, Thomas JD, et al. Increasing serum soluble angiotensin-converting enzyme 2 activity after intensive medical therapy is associated with better prognosis in acute decompensated heart failure. *J Card Fail*. 2013;19(9):605–10.
  91. Soro-Paavonen A, Gordin D, Forsblom C, Rosengard-Barlund M, Waden J, Thorn L, et al. Circulating ACE2 activity is increased in patients with type 1 diabetes and vascular complications. *J Hypertens*. 2012;30(2):375–83.
  92. Wang G, Lai FM, Lai KB, Chow KM, Kwan CH, Li KT, et al. Urinary mRNA expression of ACE and ACE2 in human type 2 diabetic nephropathy. *Diabetologia*. 2008;51(6):1062–7.
  93. Park SE, Kim WJ, Park SW, Park JW, Lee N, Park CY, et al. High urinary ACE2 concentrations are associated with severity of glucose intolerance and microalbuminuria. *Eur J Endocrinol*. 2013;168(2):203–10.
  94. Yamaleyeva LM, Gilliam-Davis S, Almeida I, Brosnihan KB, Lindsey SH, Chappell MC. Differential regulation of circulating and renal ACE2 and ACE in hypertensive mRen2.Lewis rats with early-onset diabetes. *Am J Physiol Renal Physiol*. 2012;302(11):F1374–84.
  95. Soler MJ, Riera M, Crespo M, Mir M, Marquez E, Pascual MJ, et al. Circulating angiotensin-converting enzyme 2 activity in kidney transplantation: a longitudinal pilot study. *Nephron Clin Pract*. 2012;121(3–4):c144–50.
  96. Barretti DL, Magalhaes FC, Fernandes T, do Carmo EC, Rosa KT, Irigoyen MC, et al. Effects of aerobic exercise training on cardiac renin-angiotensin system in an obese Zucker rat strain. *PLoS One*. 2012;7(10):e46114.
  97. Fernandes T, Hashimoto NY, Magalhaes FC, Fernandes FB, Casarini DE, Carmona AK, et al. Aerobic exercise training-induced left ventricular hypertrophy involves regulatory MicroRNAs, decreased angiotensin-converting enzyme-angiotensin ii, and synergistic regulation of angiotensin-converting enzyme 2-angiotensin (1-7). *Hypertension*. 2011;58(2):182–9. *This study suggests a regulatory role for specific microRNAs in reciprocal regulation of cardiac ACE and ACE2 as an underlying cardioprotective molecular mechanism for the development of non-pathological left ventricular hypertrophy in response to aerobic exercise.*
  98. Oudit GY, Liu GC, Zhong J, Basu R, Chow FL, Zhou J, et al. Human recombinant ACE2 reduces the progression of diabetic nephropathy. *Diabetes*. 2010;59(2):529–38.
  99. Lo J, Patel VB, Wang Z, Levasseur J, Kaufman S, Penninger JM, et al. 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(1):109–22.
  100. Wysocki J, Ye M, Rodriguez E, Gonzalez-Pacheco FR, Barrios C, Evora K, et al. Targeting the degradation of angiotensin II with recombinant angiotensin-converting enzyme 2: prevention of angiotensin II-dependent hypertension. *Hypertension*. 2010;55(1):90–8.
  101. Ye M, Wysocki J, Gonzalez-Pacheco FR, Salem M, Evora K, Garcia-Halpin L, et al. 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(3):730–40.
  102. Huentelman MJ, Grobe JL, Vazquez J, Stewart JM, Mecca AP, Katovich MJ, et al. Protection from angiotensin II-induced cardiac hypertrophy and fibrosis by systemic lentiviral delivery of ACE2 in rats. *Exp Physiol*. 2005;90(5):783–90.
  103. Dong B, Yu QT, Dai HY, Gao YY, Zhou ZL, Zhang L, et al. Angiotensin-converting enzyme-2 overexpression improves left ventricular remodeling and function in a rat model of diabetic cardiomyopathy. *J Am Coll Cardiol*. 2012;59(8):739–47.
  104. Liu CX, Hu Q, Wang Y, Zhang W, Ma ZY, Feng JB, et al. Angiotensin-converting enzyme (ACE) 2 overexpression ameliorates glomerular injury in a rat model of diabetic nephropathy: a comparison with ACE inhibition. *Mol Med*. 2011;17(1–2):59–69.
  105. Hernandez Prada JA, Ferreira AJ, Katovich MJ, Shenoy V, Qi Y, Santos RA, et al. Structure-based identification of small-molecule angiotensin-converting enzyme 2 activators as novel antihypertensive agents. *Hypertension*. 2008;51(5):1312–7.
  106. Murca TM, Moraes PL, Capurro CA, Santos SH, Melo MB, Santos RA, et al. Oral administration of an angiotensin-converting enzyme 2 activator ameliorates diabetes-induced cardiac dysfunction. *Regul Pept*. 2012;177(1–3):107–15.
  107. Burchill LJ, Velkoska E, Dean RG, Griggs K, Patel SK, Burrell LM. Combination renin-angiotensin system blockade and angiotensin-converting enzyme 2 in experimental myocardial infarction: implications for future therapeutic directions. *Clin Sci (Lond)*. 2012;123(11):649–58.
  108. Wei CC, Hase N, Inoue Y, Bradley EW, Yahiro E, Li M, et al. Mast cell chymase limits the cardiac efficacy of Ang I-converting enzyme inhibitor therapy in rodents. *J Clin Invest*. 2010;120(4):1229–39.