

Review Article

The emerging role of ACE2 in physiology and disease

I Hamming,¹* ME Cooper,² BL Haagsmans,³ NM Hooper,⁴ R Korstanje,¹ ADME Osterhaus,³ W Timens,¹ AJ Turner,⁴ G Navis⁵ and H van Goor¹

¹Department of Pathology and Laboratory Medicine, University Medical Center Groningen and University of Groningen, The Netherlands

²Baker Medical Research Institute, St Kilda Central, Melbourne, Australia and Department of Medicine, University of Melbourne, Austin & Repatriation Medical Centre (Repatriation Campus), Heidelberg West, Victoria, Australia

³Department of Virology, Erasmus Medical Center, Dr Molewaterplein 50, 3015 GE Rotterdam, The Netherlands

⁴Proteolysis Research Group, Institute of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK

⁵Department of Nephrology, University Medical Center Groningen and University of Groningen, The Netherlands

*Correspondence to:

I Hamming, Department of Pathology and Laboratory Medicine, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands.
E-mail: i.hamming@path.umcg.nl

No conflicts of interest were declared.

Abstract

The renin–angiotensin–aldosterone system (RAAS) is a key regulator of systemic blood pressure and renal function and a key player in renal and cardiovascular disease. However, its (patho)physiological roles and its architecture are more complex than initially anticipated. Novel RAAS components that may add to our understanding have been discovered in recent years. In particular, the human homologue of ACE (ACE2) has added a higher level of complexity to the RAAS. In a short period of time, ACE2 has been cloned, purified, knocked-out, knocked-in; inhibitors have been developed; its 3D structure determined; and new functions have been identified. ACE2 is now implicated in cardiovascular and renal (patho)physiology, diabetes, pregnancy, lung disease and, remarkably, ACE2 serves as a receptor for SARS and NL63 coronaviruses. This review covers available information on the genetic, structural and functional properties of ACE2. Its role in a variety of (patho)physiological conditions and therapeutic options of modulation are discussed. Copyright © 2007 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.

Keywords: ACE2; RAAS; ACE; renin; angiotensin

Received: 3 November 2006

Revised: 8 January 2007

Accepted: 25 January 2007

Introduction

The renin–angiotensin–aldosterone system (RAAS) is cardinal in renal and cardiovascular physiology and pathophysiology. Its architecture and functions are more complex than previously assumed. In the classical RAAS, the protease renin, which is secreted from renal juxtaglomerular cells, acts on the circulating precursor angiotensinogen to generate angiotensin (Ang) I (Figure 1). Ang I is converted by the dipeptidyl carboxypeptidase angiotensin-converting enzyme (ACE) to Ang II, the main effector substance of the RAAS, with potent vasoconstrictive, pro-inflammatory, and pro-fibrotic properties. Consequently, ACE inhibitors (ACEi) and Ang II receptor blockers (ARBs) are effective in hypertension, heart failure, and progressive renal damage.

Recently, angiotensin fragments other than Ang II were also proposed to be relevant, in particular Ang(1–7), which mediates vasodilatation, anti-proliferation, and apoptosis, thereby opposing the effects of Ang II [1]. Further complexity was introduced by the discovery of an ACE homologue, ACE2. This enzyme cleaves Ang I into Ang(1–9), which can be converted to Ang(1–7) by ACE (Figure 1).

Furthermore, ACE2 degrades Ang II to Ang(1–7). It has therefore been suggested that ACE2 acts in a counter-regulatory manner to ACE by shifting the balance between Ang II and Ang(1–7), thus acting as a functional clearance mechanism for Ang II.

High ACE2 gene expression was initially reported in the testis, kidney, and heart [2,3]. Later studies showed widespread distribution of both rodent and human ACE2 in the lung, liver, small intestine, and brain, albeit much lower than in the kidneys [4–7]. Diverse roles have emerged for ACE2 since its identification in 2000 [2,3]. Some 200 papers have subsequently addressed its structure, functions, and role in cardiovascular and renal disease, diabetes, SARS coronavirus infection, and lung injury.

This review covers available information on the genetic, structural, and functional properties of ACE2. Its role in a variety of (patho)physiological conditions and therapeutic options of modulation will be discussed.

The ACE2 gene and protein

The 40 kb ACE2 gene is located on chromosome Xp22 and contains 18 exons, many of which closely

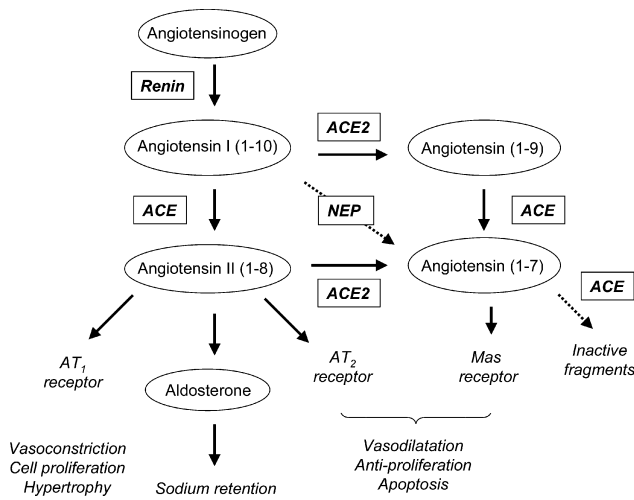


Figure 1. Schematic diagram of the renin–angiotensin–aldosterone system which shows the role of ACE and ACE2 in the metabolism of the various angiotensin peptides. Modified from Warner et al [101], with permission

resemble exons in the *ACE* gene [3]. Two alternative transcripts of the mouse *ACE2* gene have been identified which probably arise by alternative splicing [8]. Recently, an alternative 5'-untranslated exon of human *ACE2* and new polymorphisms have been reported [9].

The human *ACE2* protein is a typical zinc metallopeptidase, which comprises 805 amino acids and is 40% identical in sequence with ACE, although it only contains a single catalytic domain. Critical active site residues, including the His-Glu-Met-Gly-His zinc-binding motif, are highly conserved. *ACE2* is a type I integral membrane glycoprotein orientated with the N-terminus and the catalytic site facing the extracellular space (an ectoenzyme), where it can metabolize circulating peptides. The small C-terminal, cytoplasmic domain has a number of potential regulatory sites. The similarity with ACE relates only to its topology and much of the extracellular domain (Figure 2); the juxtamembrane, transmembrane, and cytoplasmic domains of *ACE2* share similarity with the renal, transmembrane protein collectrin [10] (Figure 2), which stimulates insulin exocytosis and pancreatic beta-cell proliferation [11,12]. Targeted deletion of the *collectrin* gene in mice suggests that it also plays a major role in regulating renal amino acid transport [13].

Substrate specificity of ACE2

ACE2, a strict carboxypeptidase, hydrolyses its substrates by removing a single amino acid from their respective C-termini, rather than a dipeptide, as does ACE. *ACE2* therefore has the ability to convert the decapeptide Ang I to Ang(1–9) and the octapeptide Ang II to Ang(1–7). A kinetic study evaluating the comparative roles of ACE and *ACE2* in angiotensin metabolism [14] established that Ang II is hydrolysed two orders of magnitude more efficiently by

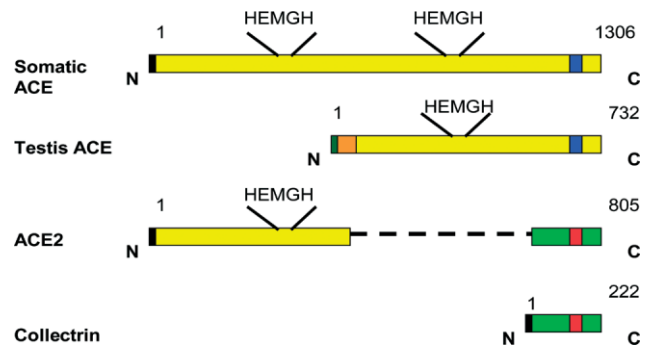


Figure 2. The domain structure of somatic and testis ACE, ACE2, and collectrin. Each protein is a type I integral membrane protein with a cleaved signal peptide (black), an N-terminal ectodomain, a transmembrane domain (blue in ACE; red in ACE2 and collectrin), and a C-terminal cytoplasmic domain. Somatic ACE contains two HEMGH zinc binding active sites, while testis ACE and ACE2 contain a single HEMGH motif. Collectrin lacks a HEMGH motif. The ectodomain of ACE2 (yellow) is more similar to the N-terminal domain of somatic ACE, while its juxtamembrane stalk, and transmembrane and cytoplasmic domains are more similar to collectrin. The numbers indicate the amino acid residues in each protein. Modified from Turner and Hooper [102], with permission

ACE2 than Ang I. Hence, the major role of *ACE2* in angiotensin metabolism seems to be the production of Ang(1–7), whose actions oppose those of Ang II. Different *in vivo* studies strongly support the concept that a major role of *ACE2* is indeed the generation of Ang(1–7) from Ang II and that its conversion of Ang I to Ang(1–9) is not normally of physiological importance [15–20], except possibly under conditions that raise Ang II levels, eg ACEi or ARB treatment [21].

Although most studies have focused on the role of *ACE2* in angiotensin metabolism, the enzyme has broad substrate specificity. In addition to Ang I and Ang II, *ACE2* hydrolyses apelin-13, neurotensin-(1–11), dynorphin A-(1–13), β -casomorphin-(1–7), and ghrelin [22]. Although the ACE substrate bradykinin is not hydrolysed by *ACE2*, its metabolite des-Arg [9]-[bradykinin], which is an agonist for the B1 bradykinin receptor, is hydrolysed and a role for *ACE2* in bradykinin metabolism cannot yet be dismissed. It is likely that other potential physiological substrates for *ACE2* will emerge.

Cell biology and shedding of ACE2

Knowledge of the basic cell biology of *ACE2* still remains limited, partly because few cell models expressing *ACE2* at high levels are available [23]. *ACE2* is expressed as a cell-surface non-raft protein with little intracellular localization, and the protein does not readily internalize. However, binding of the SARS viral spike protein to *ACE2* does trigger enzyme internalization, down-regulating activity from the cell surface. In polarized cells, *ACE2* is exclusively targeted to the apical surface [23,24], in contrast to ACE

which distributes equally between apical and basolateral surfaces [23]. Another mechanism for down-regulating ACE2 at the cell-surface is by proteolytic shedding of its extracellular domain. This shedding, which is also undergone by ACE, is stimulated by phorbol esters and is blocked by inhibitors of the ADAMs family of zinc metalloproteinases. ADAM17 (TACE) is implicated as the primary enzyme involved in the regulated shedding of ACE2 [25], resulting in detectable levels of ACE2 in plasma and urine [14,23,26].

ACE2 and the heart

The importance of ACE2 in cardiac physiology and disease was initially suggested by two independent groups, based on cardiac ACE2 expression, particularly in endothelial cells [2,3]. Subsequent studies revealed ACE2 not only in endothelial cells and smooth muscle cells from intra-myocardial vessels, but also in cardiac myocytes [27] (Figure 3).

The importance of ACE2 in cardiac function was strengthened by Crackower *et al*, who described cardiac dysfunction in *Ace2* knock-out (KO) mice [28]. Specifically, there was a 40% decrease in fractional shortening with slight ventricular dilatation. Interestingly, there was thinning of the left ventricular (LV) wall rather than LV hypertrophy and/or cardiac fibrosis. These changes progressed with age and were more prominent in male mice [28]. The hearts of these *Ace2* KO mice showed increased Ang II levels and up-regulation of hypoxia-inducible genes. The authors suggest that cardiac function is modulated by the balance between ACE and ACE2, and that the increase in local cardiac Ang II was involved in these abnormalities. This is supported by the fact that the cardiac phenotype and increased Ang II levels were completely reversible by concomitant deletion of the *ACE* gene in *Ace2* KO mice. It remains unclear, however, why, despite elevated Ang II levels, the hearts of these *Ace2* KO mice did not show any cardiac hypertrophy or fibrosis. This may partly be related to Ang II-independent effects of ACE2, such as effects of the

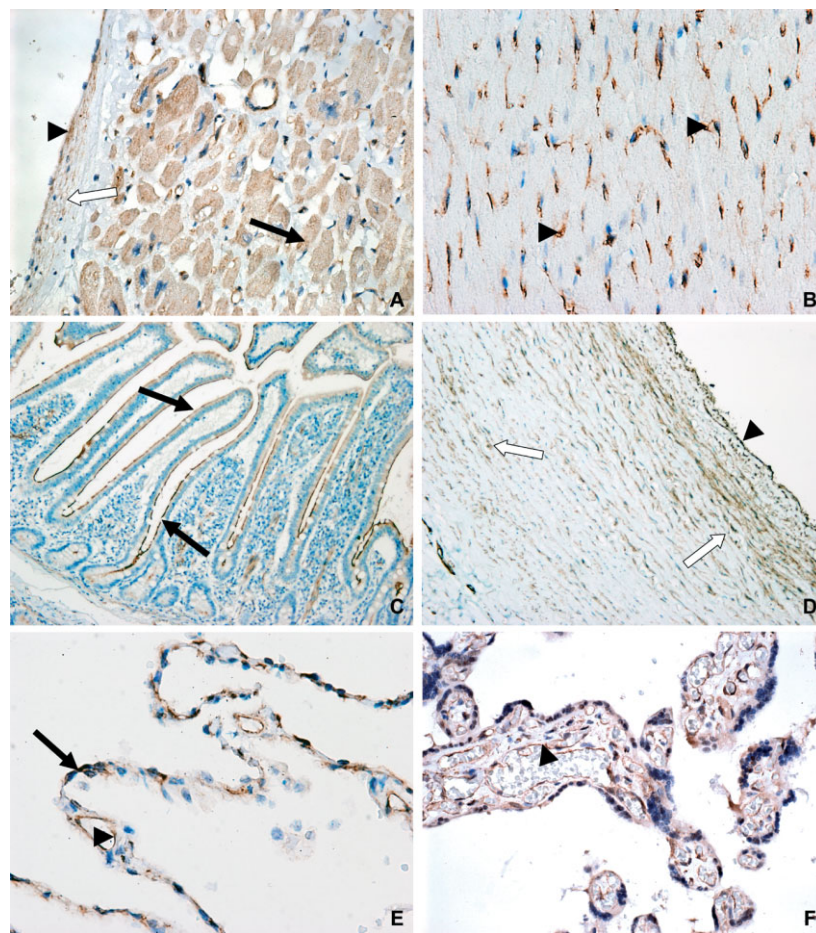


Figure 3. Immunohistochemical staining pattern of ACE2 in several organs. In the healthy human heart (A), ACE2 is expressed in cardiomyocytes (closed arrow), vascular endothelium (arrowhead), and smooth muscle cells (open arrow). In rat heart (B), ACE2 is predominantly expressed in vascular endothelium (arrowhead). (C) ACE2 expression in human small intestine (jejunum); abundant staining can be found in the brush border of enterocytes (arrow). In human aorta (D), ACE2 is expressed in the endothelium (arrowhead) and vascular smooth muscle cells (open arrow). In healthy human lung (E), ACE2 is present in alveolar epithelial cells (arrow) and in capillary endothelial cells (arrowhead). In human placenta (F), positive staining for ACE2 is found in the placental villi [syncytiotrophoblast, cytotrophoblast, vascular endothelium (arrowhead), and smooth muscle cells]

other substrates described above [22], which also can influence cardiac contractility.

Gurley *et al* generated an *Ace2* KO mouse [29], deleting the same exon as the Crackower group [28], and although demonstrating changes in blood pressure regulation, particularly in response to Ang II, these investigators could not detect a specific cardiac phenotype. In another study, Yamamoto *et al* [30] also failed to identify cardiac abnormalities in their *Ace2* KO mice. However, they did demonstrate reduced cardiac contractility after transverse aortic constriction (TAC), a model of pressure overload. TAC was associated, when compared with wild-type mice subjected to the same procedure, with a marked increase in cardiac Ang II levels and increased fibrosis, LV dilation, and myofibrillar disarray. Indeed, in *Ace2* KO mice followed for a longer period, this reduction in myocardial contractility led to pulmonary congestion and death [30].

Recent studies suggest that ACE2 possibly influences the electrical pathways of the heart. In *ACE2* transgenic mice, cardiac conduction disturbances were present and some animals developed lethal ventricular fibrillation [31]. The level of ACE2 up-regulation correlated with the severity of the conduction disturbance.

Accumulating evidence indicates that over-activity of cardiac RAAS and myocardial Ang II production contributes to the progression of heart failure. Several studies characterized ACE2 expression and activity in heart failure. In experimental myocardial infarction, increased cardiac ACE2 expression was found in the infarct zone and the surrounding ischaemic zone [27]. Moreover, local up-regulation of ACE2 was also found in explanted human hearts with ischaemic cardiomyopathy [20,27,32] and idiopathic dilated cardiomyopathy [20,32]. These findings may imply that the up-regulation of ACE2 is a compensatory response to the ischaemic insult and that the consequent increase in the vasodilatory Ang(1–7) may confer cardio-protective effects in an attempt to counterbalance the effects of Ang II. Other groups did not observe up-regulation of ACE2 [33] or demonstrated down-regulation of cardiac ACE2 in experimental heart failure [21,34], albeit in different rat strains.

Several studies noted a marked increase in cardiac ACE2 expression in response to RAAS blockade by ACEi [21,35], ARB [33–35], or an aldosterone antagonist [34,36] (a diuretic with specific benefits in heart failure [37]). This was interpreted as Ang II receptor blockade conferring cardio-protection not only by reducing harmful Ang II-mediated effects, but also by locally increasing Ang(1–7), which, through binding to a putative Ang(1–7) receptor, Mas 1 [38], is postulated to have beneficial effects on the heart [1].

The potential for ACE2 to modulate cardiac function and remodelling is additionally suggested by the finding that lenti-viral vector encoding mouse ACE2 injected intracardially in Sprague–Dawley rats significantly attenuated cardiac hypertrophy and myocardial

fibrosis induced by Ang II infusion [39]. Moreover, ACE2 overexpression after neonatal development provides protection from high blood pressure and cardiac pathophysiology in the SHR rat [40].

Altogether, these studies suggest that ACE2 is important in cardiac function and that ACE2-related effects contribute to cardio-protective effects of ACE inhibitors and ARBs. The increased ACE2 gene expression and activity during RAAS blockade suggest that at least part of their mode of action results from hydrolysis of the vasoconstrictor mitogenic Ang II to the vasodilator anti-proliferative Ang(1–7) through a feed-forward mechanism within the RAAS. Understanding the roles of ACE2 in heart failure may optimize current therapies and ultimately guide the development of new therapeutic strategies.

ACE2 and hypertension

It was initially hypothesized that disruption of the delicate balance between ACE and ACE2 would result in abnormal blood pressure control [41]; ACE2 might protect against increases in blood pressure and, conversely, ACE2 deficiency might lead to hypertension. The localization of ACE2 in vascular endothelial cells and smooth muscle cells [6] (Figure 3) supports this.

Since hypertension was linked to loci on the X chromosome [42,43] and *ACE2* was mapped to the X chromosome [3], *ACE2* became a candidate gene underlying the loci linked to hypertension. Crackower *et al* [28] were the first to test *ACE2* as the gene underlying the blood pressure locus on the X chromosome. They showed reduced expression of renal *ACE2* in the salt-sensitive Sabra hypertensive rat compared with the normotensive rat. Both hypertensive SHR and SHRSP rats showed reduced renal ACE2 protein levels compared with the normotensive Sabra and WKY strains. Two other groups confirmed some of these findings by showing that SHR rats have lower renal ACE2 mRNA, protein, and activity compared with WKY rats [44,45]. However, others were unable to detect any differences in renal ACE2 mRNA, protein, and activity between adult hypertensive rats and their normotensive controls [46]. On close scrutiny, in SHRSP rats the allele of the previously identified blood pressure locus on rat chromosome X contributes to a blood pressure-lowering effect [42], while in the salt-sensitive Sabra hypertensive rat this allele contributes to a blood pressure-increasing effect [43], suggesting that it is not the same gene that underlies the blood pressure locus. Surprisingly, while the allelic effects of the blood pressure locus are discordant between these hypertensive strains, a similar, ie concordant, reduction in renal ACE2 expression was reported [28], which reduces the possibility that *ACE2* is the candidate gene underlying the blood pressure locus.

Additional arguments came from studies in *Ace2* KO mice. *Ace2* KO mice on a mixed B6/129 background had normal blood pressure compared with the

wild type [28]. However, a recent study which also studied *Ace2* KO animals on homozygous B6 and 129 backgrounds showed that *Ace2* KO animals with a B6 background have a significantly lower blood pressure compared with wild-type animals [29], suggesting that the genetic background with modifier genes present in the 129 strain may counteract the absence of *Ace2*, or that the 129 allele of another gene involved in blood pressure regulation moved with the KO into the B6 background and caused the difference in blood pressure in these animals.

So far, three human association studies of *ACE2* polymorphisms with hypertension have been performed [47–49]. Two of these studies, using Chinese cohorts, reported an association between a SNP in intron 3 of *ACE2* and blood pressure in females with metabolic syndrome [49] and females with essential hypertension [48]. However, the use of only one SNP cannot rule out the possibility of this SNP being in linkage disequilibrium with SNPs in neighbouring genes that could cause the difference in blood pressure.

Altogether, the role of *ACE2* in hypertension is not conclusive. Functional studies that show blood pressure effects after the administration of *ACE2* inhibitors or stimulators are needed to further elucidate its significance in hypertension.

ACE2 and the kidney

ACE2 is highly expressed in the kidney [7]; however, its role in the kidney has not been fully elucidated.

In the human kidney, *ACE2* is predominantly found in the proximal tubular brush border [50,51], where it co-localizes with *ACE* [23,24]. Moreover, *ACE2* is found in endothelial and smooth muscle cells of renal vessels [50,51] and in glomerular visceral (in podocyte/slit diaphragm complex) and parietal epithelial cells [51,52] (Figure 4). The distribution of *ACE2* is species-specific. In human kidneys, the *ACE2* expression pattern is comparable to that of mouse kidneys [5,53], whereas in rat kidneys, *ACE2* is predominantly found in glomeruli and to a lesser extent in tubules (Figure 4).

Several lines of evidence support a role for renal *ACE* in renal damage [54]. Individual differences in renal *ACE* activity predict the susceptibility for proteinuria-associated renal damage in experimental conditions [55,56]. Furthermore, Ang II is increased in damaged tubules as a possible mediator of further renal damage in experimental and human renal disorders [57,58]. A disrupted balance between intrarenal *ACE* and *ACE2* with consequent high levels of Ang II might therefore contribute to progressive renal damage. Indeed, in experimental hypertension and diabetes, renal *ACE2* expression is decreased [28,59]. Moreover, Tikellis *et al* showed that in the kidneys of SHR rats *ACE2* expression follows a developmental pattern with declining expression during development and onset of hypertension [45]. In line with the beneficial effects of RAAS blockade on cardiac *ACE2* as mentioned earlier, renal *ACE2* activity is also increased in response to *ACEi* and ARB [59,60]. In

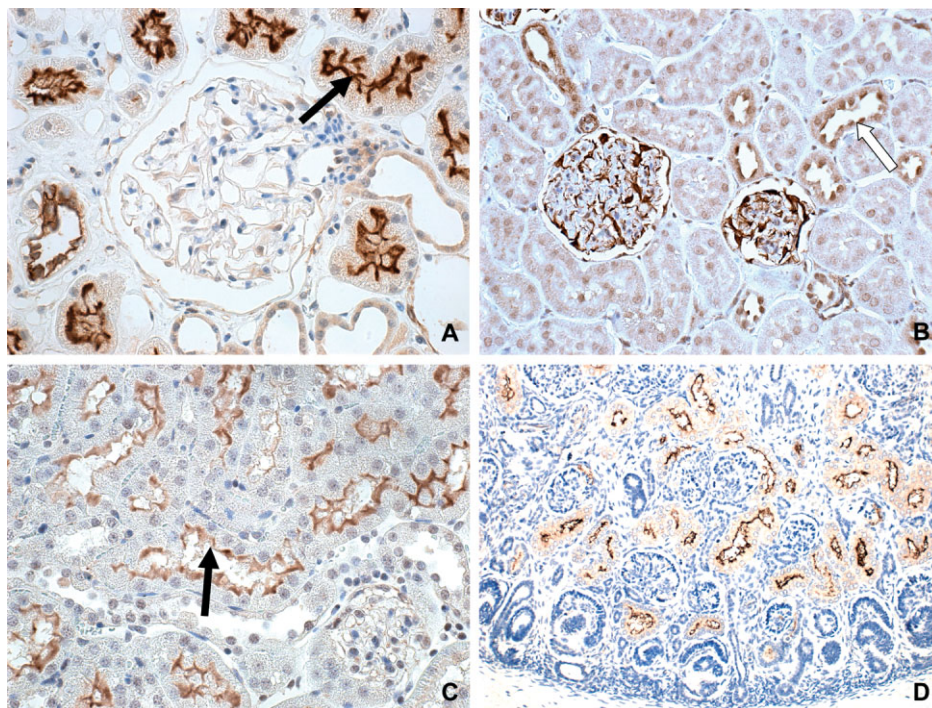


Figure 4. Immunohistochemical staining pattern of *ACE2* in the kidney. In the healthy human kidney (A), *ACE2* is predominantly present in the brush border of proximal tubular cells (arrow) and to a lesser extent in the glomerular visceral and parietal epithelium. In rat kidney (B), *ACE2* is predominantly found in glomeruli and to a lesser extent in distal tubules (open arrow). In mouse kidney (C), the distribution pattern of *ACE2* is comparable to human kidney with predominant expression in proximal tubules (arrow). *ACE2* is also expressed during human nephrogenesis (gestational age 16 weeks) (D)

contrast, further enhancement of the therapeutic efficacy of ACEi resulted in an unexpected reduction in renal ACE2 expression compared with ACEi alone [61]. It looks like despite extensive research, the mechanisms of the effects of ACEi are still not completely understood [62]. Further studies on the regulation of renal ACE2 during RAAS blockade might help to elucidate this.

Initial reports on *Ace2* KO mice did not show any renal structural and functional abnormalities [28,29], but recent studies showed that male *Ace2*^{-/-} mice develop age-dependent glomerulosclerosis and albuminuria, whereas the renal vasculature and interstitium were relatively protected [63]. The authors hypothesized that the glomerular abnormalities were caused by chronic exposure to increased circulating and tissue Ang II, as these abnormalities were abolished by ARB treatment. The glomerulo-protective role of ACE2 is supported by studies in which chronic infusion of the ACE2 inhibitor MLN4760 increased albuminuria in db/db mice, resulting in increased deposition of glomerular fibronectin [52]. Albuminuria could be prevented by ARB, indicating Ang II dependency. Moreover, these diabetic female db/db mice have decreased glomerular ACE2 staining compared with db/m heterozygous littermates [52]. The authors suggest that glomerular ACE2, present in the podocyte/slit diaphragm complex, could normally be reno-protective by favouring rapid degradation of Ang peptides and thereby preventing exposure to high levels of Ang II.

Other studies support the assumption that increased ACE2 activity tied with decreased ACE activity may reflect a protective mechanism by limiting the renal accumulation of Ang II and favouring Ang(1–7) formation. Increased ACE2 expression is indeed coupled with profound reduction of ACE expression in renal tubules of young db/db mice [53]. The authors speculate that this might be an early reno-protective response. The kidneys of streptozotocin-induced diabetic mice also show increased ACE2 expression at the post-transcriptional level [64]. In humans, *de novo* expression of ACE2 is found in glomerular and peritubular endothelium in biopsies in patients with primary and secondary renal disease, as well as in renal transplants [51]. In renal biopsies of non-diabetic and diabetic patients, significant up-regulation of the *ACE* gene, and not the *ACE2* gene, was found in diabetic nephropathy [65]. Moreover, no associations between polymorphisms in the *ACE2* gene and diabetic nephropathy could be established [66].

Altogether, ACE2 appears to be involved in the pathogenesis of renal damage, but its precise role is unclear and further studies are needed, in particular during renal disease.

ACE2 and pregnancy

The placenta is an organ with major Ang(1–7) and ACE2 expression [67] (Figure 3), suggesting that

ACE2 may be involved in mother–fetus interactions, which is interesting regarding a potential role for ACE2 in fetal programming and pregnancy. In pregnant rats, renal expression of ACE2 is increased compared with virgin controls [15]. A recent study showed no differences in ACE2 expression between normotensive and pre-eclamptic placentas in the third trimester [67]. However, pre-eclampsia is determined early in pregnancy; thus, the role of ACE2 in pre-eclampsia should be further studied, for example, in animal models.

ACE2 and lung disease

There is abundant expression of RAS components in the lung, including ACE and ACE2. Activation of the intrapulmonary RAS could influence the pathogenesis of lung injury [68]. Indeed, increased levels of ACE have been associated with pulmonary hypertension [69,70], sarcoidosis [71,72], idiopathic pulmonary fibrosis [73], and the acute respiratory distress syndrome [74,75]. The alleged role for ACE2 as a counter-regulatory mechanism of ACE may therefore be crucial in the lung. ACE2 is present in type I and type II alveolar epithelial cells and to a lesser extent in bronchiolar epithelial cells (Figure 3) [6,76]. Furthermore, as in other organs, ACE2 is present in endothelial cells and in arterial smooth muscle cells. With respect to the role of ACE2 in pulmonary hypertension, it can be envisaged that in particular, the presence and function in smooth muscle cells of small arterioles are of relevance, although the actual role in this disease is unknown [77].

Ace2 KO mice do not have lung abnormalities when compared with their wild-type littermates [28]. However, it was recently shown that loss of ACE2 expression precipitates severe acute lung failure [76]. Moreover, injection of recombinant human ACE2 attenuates acute lung failure in *Ace2* KO as well as in wild-type mice [76]. With respect to the possible role of ACE2 in the lung in relation to acute lung injury in particular, the relationship to the ACE2 expression at the alveolar capillary interface is of interest. It is tempting to speculate that increased ACE2 may play a role in reducing the initial leakage over the alveolar capillary interface. This would then slow down the vicious circle that often occurs after a damaging effect to this interface and leads to the clinical pathological picture of diffuse alveolar damage with intra-alveolar oedema and fibrin deposits. These data support a critical role for the intrapulmonary RAS in the pathogenesis of acute lung injury and show that ACE2 is a key molecule involved in the development and progression of acute lung failure.

ACE2 and human coronaviruses

ACE2 acts as a receptor for two coronaviruses (CoV): severe acute respiratory syndrome (SARS)-CoV and

human CoV-NL63 [78,79], positive stranded RNA viruses with a 'corona'-like appearance [80]. Their genome is packaged together with several membrane proteins, the RNA binding nucleoprotein and the receptor binding spike protein that protrudes through the virion membrane. HCoV-NL63 infection causes clinical respiratory symptoms resembling those observed in children infected with common cold viruses. SARS-CoV causes severe lower respiratory tract disease including fever, non-productive cough, myalgia, and dyspnoea [81]. A specific region within the SARS-CoV spike protein (S1) interacts with ACE2 [79,82,83]. The crystal structure at 2.9 angstrom resolution of this receptor binding domain bound with the peptidase domain of human ACE2 shows that it presents a gently concave surface, which cradles the N-terminal lobe of the peptidase [82]. After engagement with ACE2, SARS-CoV fuses with host cell membranes, by which the conformational changes of the two heptad regions located in the S2 region, HR-1 and HR-2, cause the formation of an oligomeric structure, leading to fusion between the viral and target-cell membranes.

Using soluble ACE2 molecules, peptides derived thereof, and antibodies directed against ACE2, the SARS-CoV infection can be blocked [79,84]. Conversely, expression of ACE2 in refractory cell lines resulted in SARS-CoV replication [85]. Experiments in *Ace2* KO mice revealed the importance of ACE2 as a receptor for SARS-CoV [86]. Moreover, autopsy specimens of patients who died of SARS revealed that ACE2-expressing cells are a direct target of SARS-CoV [87].

Besides ACE2, lysosomal proteases such as cathepsin-L are required for productive SARS-CoV [88,89], explaining discrepancies observed between ACE2 expression and absence of SARS-CoV replication in endothelial cells [6,85]. Members of the DC-SIGN family of proteins may enhance SARS-CoV infection but are not sufficient to infect cells [90].

Murine and rat ACE2 less efficiently bound the S1 domain of late-phase SARS-CoV isolates and supported less efficient S-protein-mediated infection [91]. In addition, spike proteins from isolates of palm civets utilized civet ACE2 more efficiently than human ACE2, whereas late-phase isolates such as TOR2 utilized both receptors with equal efficiency [92]. Subsequent studies demonstrated that sequence variation in the spike protein binding sites of ACE2 hindered efficient binding [92] (Figure 5). The lower affinity of some of these S proteins could be complemented by altering specific residues within the S-protein-binding site of human ACE2 to those of civet ACE2, or by altering S-protein residues 479 and 487 to residues conserved during the 2002–2003 outbreak. This indicates that specific molecular interactions are important in the adaptation process of SARS-CoV to human cells. Animal precursors of SARS-CoV are thus likely to be less pathogenic to humans, and exposure to such viruses may have led to limited clinical symptoms

but antigenic stimulation that results in a serological response. This was observed when SARS-CoV re-emerged in Guangdong in 2003, causing milder clinical disease [93]. Similarly, animal traders had high seroprevalence for human and animal SARS coronavirus, without having a history of SARS.

ACE2 is down-regulated in the lungs of mice after acute lung injury, including SARS-CoV infection [86]. The cytokines IL-4 and IFN- γ down-regulated cell-surface expression of ACE2 (Figure 6), decreased ACE2 mRNA levels, and also inhibited SARS-CoV replication in Vero E6 cells [94]. Furthermore, experiments *in vitro* indicated that ACE2 expression is dependent on the differentiation state of epithelia [95], and a role for the GATA family of transcription factors in regulating the expression of ACE2 has been suggested [96]. Down-regulation of ACE2 expression may not only affect SARS-CoV entry, but also hamper angiotensin II cleavage, causing pathological changes due to angiotensin II type 1a receptor activation [86]. Therefore, intervention strategies using soluble recombinant ACE2 proteins may neutralize SARS-CoV and dampen lung pathology. However, studies analysing the role of ACE2 gene polymorphisms in the progression of SARS have not found evidence that these affect outcome [9,97].

Conclusions and future perspectives

ACE2 is now implicated in a variety of (patho) physiological processes. Further understanding of its role in disease will hopefully lead to the exploration of novel therapeutic options. Functional studies using ACE2 inhibitors will be essential to elucidate the regulatory mechanisms of ACE2. Moreover, relatively little progress has been made on the development of

Human:	30	DKFNHEAEDLFY	41
Mf:		-----	
Felis:		E-----E-S-	
Rn:		N---Q-----S-	
Mumu:		NN--Q-----S-	
Human:	82	MYPLQEIQNLTV	93
Mf:		-----	
Felis:		T---A--H-T--	
Rn:		NFS-----A-I	
Mumu:		SFS-----TPII	
Human:	353	KGDFR	357
Mf:		-----	
Felis:		-----	
Rn:		H----	
Mumu:		H----	

Figure 5. Alignment of amino acid sequences of ACE2 from human, macaque (Mf), cat (Felis), rat (Rn), and mouse (Mumu) critical to SARS-CoV spike protein interactions

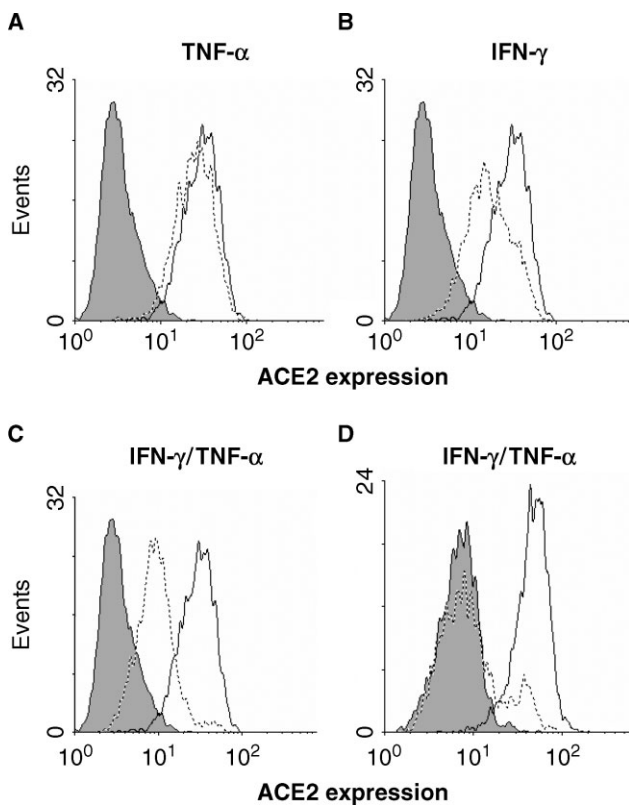


Figure 6. IFN-gamma down-regulates ACE2 expression in Vero E6 cells. ACE2 expression was determined by FACS analysis after treatment with TNF- α (A), IFN- γ (B), and IFN- γ combined with TNF- α (C), all at 48 h, or Vero E6 cells incubated for 96 h in the presence of IFN- γ combined with TNF- α (D). Dotted lines represent cytokine-treated cells, while thick lines represent mock-treated control cells. The shaded areas represent background staining. Modified from de Lang *et al* [94], with permission

such specific ACE2 inhibitors, largely because of lack of a clear therapeutic target. MLN4760 is the most potent and selective ACE2 inhibitor currently available [98]. Structure-based screening programmes have been applied to the identification of novel and selective ACE2 inhibitors with some success [99,100]. Since ACE2 opposes the vasoactive and proliferative actions of Ang II, up-regulation of ACE2 expression or activity is a much more desirable characteristic. Modification of ACE2 levels by stimulating its expression or exogenous administration of recombinant ACE2 may have beneficial effects in several disease conditions and should be the scope of future studies.

Acknowledgement

We thank Mirjan van Timmeren for critically reading the manuscript.

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