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Review Article

ACE2: from protection of liver disease to propagation of COVID-19

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Twenty years ago, the discovery of angiotensin-converting enzyme 2 (ACE2) was an important breakthrough dramatically enhancing our understanding of the renin–angiotensin system (RAS). The classical RAS is driven by its key enzyme ACE and is pivotal in the regulation of blood pressure and fluid homeostasis. More recently, it has been recognised that the protective RAS regulated by ACE2 counterbalances many of the deleterious effects of the classical RAS. Studies in murine models demonstrated that manipulating the protective RAS can dramatically alter many diseases including liver disease. Liver-specific overexpression of ACE2 in mice with liver fibrosis has proved to be highly effective in antagonising liver injury and fibrosis progression. Importantly, despite its highly protective role in disease pathogenesis, ACE2 is hijacked by SARS-CoV-2 as a cellular receptor to gain entry to alveolar epithelial cells, causing COVID-19, a severe respiratory disease in humans. COVID-19 is frequently life-threatening especially in elderly or people with other medical conditions. As an unprecedented number of COVID-19 patients have been affected globally, there is an urgent need to discover novel therapeutics targeting the interaction between the SARS-CoV-2 spike protein and ACE2. Understanding the role of ACE2 in physiology, pathobiology and as a cellular receptor for SARS-CoV-2 infection provides insight into potential new therapeutic strategies aiming to prevent SARS-CoV-2 infection related tissue injury. This review outlines the role of the RAS with a strong focus on ACE2-driven protective RAS in liver disease and provides therapeutic approaches to develop strategies to prevent SARS-CoV-2 infection in humans.

Introduction

The renin–angiotensin system (RAS) is a well characterised essential hormone system with pivotal roles in vascular biology, blood pressure regulation, the nervous system, electrolyte homeostasis, tissue injury, neoplasia and lipid homeostasis [1,2]. The RAS has both classical and protective arms with the balance between them deciding the net effect on organ homeostasis and role in tissue injury [3]. Over decades widespread interest in the RAS has resulted from the development and availability of effective pharmacotherapies that target hypertension and cardiovascular disease. Therapeutics that act on the RAS including the angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are commonly used in current medical practice. Additional interest in the RAS has been driven by the recognition of the essential roles of this hormone system in tissue injury including pathologies as diverse as the remodelling of cardiac tissue after myocardial infarction, the development of the vasculature in malignancy and progression of tissue fibrosis such as liver fibrosis that can result in cirrhosis [4]. It is now recognised that the carboxypeptidase angiotensin-converting enzyme 2 (ACE2) determines the balance between the classical and protective RAS [4,5].

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Traditionally, the classical RAS has been viewed as a fundamental circulating hormonal cascade in which the profibrotic and potent vasoconstrictor peptide angiotensin II (Ang II) is the central physiological effector. It is established that the classical RAS mediates Ang II effects through the Ang II type 1 receptor (AT1R). However, the last two decades has seen the widespread recognition of the protective RAS (alternate counter-regulatory pathway) and consists of ACE2, angiotensin-(1-7) (Ang-(1-7)), the proto-oncogene Mas receptor (MasR) and the Mas related G protein-coupled receptor type D (MrgD) [6–8]. ACE2 has an essential role in determining the balance between the classical and protective RAS. It has been demonstrated that the components of the protective RAS, ACE2, Ang-(1-7) and MasR, are expressed and are active in the liver in which they mediate vascular tone and the development of progressive fibrosis and eventual cirrhosis [2,9–11].

Cirrhosis is the hallmark of end-stage chronic liver disease which affects >600 million people [12] and is responsible for over 2 million deaths annually. Current therapeutic options to treat chronic liver disease are limited [13–15], frequently ineffective and often chronic injury persists driving fibrosis. Recent studies, which in agreement with previous reports [16], have shown that liver fibrosis involves a coordinated response to chronic liver injury in which hepatic stellate cells (HSCs) and other cells of the myofibroblast lineage play a central role [2,11,17]. The pathways that lead to activation of these cells and perpetuation of the fibrogenic response in the liver are incompletely understood. It is clear that a range of cytokines, growth factors and vasoactive peptides are involved that may be potential targets for therapeutic intervention. Ang II, the main effector peptide of the classical RAS, has been shown to be a key mediator of tissue fibrosis in a number of diseases, including chronic heart and kidney diseases and diabetes. Although its role in liver disease is less well-established, we demonstrated that Ang II plays a central role in the pathogenesis of chronic liver disease and that the RAS is a promising potential target for antifibrotic therapies [2,10].

Moreover, ACE2 has received a great deal of interest as it has been hijacked by coronaviruses to gain entry to lung epithelial cells, causing severe acute respiratory syndrome (SARS) in humans [18,19] and now the highly infectious and lethal coronavirus disease 2019 (COVID-19) [20]. Apart from immediate and direct targets of SARS-CoV-2 such as the epithelial cells of the respiratory tract and lung alveolar epithelial cells, emerging evidence suggests that other organs that express high levels of ACE2 are potentially vulnerable for deadly infection by SARS-CoV-2 [21–28]. Despite its devastating global impact on human health and socioeconomic endpoints, a cure for this highly lethal virus has not been established. Therefore, there is a major need to develop novel therapeutics for the prevention and treatment of COVID-19.

In this review, we aim to provide an overview of the RAS with a strong focus on the hepatic RAS and how manipulation of the ACE2-driven protective RAS provides greater benefits over that of the ACE-driven classical RAS in liver disease. Moreover, we attempt to provide potential new therapeutic strategies aiming to prevent SARS-CoV-2 binding to ACE2 and host cell membrane fusion in COVID-19 patients. It is expected that this strategy helps prevent multiorgan failure caused by secondary viral invasion in such patients.

Overview of the renin–angiotensin system

The major components of the RAS are shown in Figure 1. The classical RAS cascade (Figure 1) is initiated with the hydrolysis of the precursor angiotensinogen, synthesised in hepatocytes, to angiotensin 1 (Ang I) by the aspartic protease, renin. Ang I is, in turn, converted to Ang II by the zinc metallopeptidase, ACE, which is present in abundance in the liver [9]. The actions of Ang II are mediated via specific G protein-coupled receptors (GPCRs), the AT1R and AT2R, which bind Ang II with differing affinities; although the AT1R is plentiful in the liver, AT2R gene expression is not detectable in normal or diseased liver. The discovery of other novel RAS components such as the carboxypeptidase ACE2 [29,30], a homologue of ACE, capable of degrading Ang II and forming Ang-(1-7), and MasR, the receptor for Ang-(1-7) [31], has emphasised the increasing complexity and multiplicity of biochemical pathways comprising the RAS. Recently, another vasodilator peptide named alamandine, formed from Ang-(1-7) or Ang A, and a vasodilatory GPCR, MrgD, have been identified [7,32]. Together these components form a novel protective arm of the RAS, a pathway that is important in counteracting the proliferative and profibrotic actions of Ang II [33–37]. ACE2 is pivotal in the regulation of the balance between the protective and classical arms but the inter-relationship is now clearly more complex. Ang I can be directly converted to Ang-(1-7) through the activity of neutral endopeptidase (NEP) [3]. Further, Ang-(1-9) is a precursor to Ang-(1-7) formed by ACE2 from Ang I. ACE is responsible for Ang-(1-9) conversion to Ang-(1-7). Ang-(1-7) is the major endogenous ligand for the MasR. Importantly, there are novel agonists CGEN-865S and AVE0091 which bind MasR such that this is now a potential new therapeutic approach [38].

Apart from its profibrotic and proliferative role, experimental studies have demonstrated that Ang II is also involved in key events of the inflammatory process [39]. RAS activity on leukocytes (T- and B-lymphocytes and macrophages) has been shown to contribute to end-organ injury in the heart [40], vasculature [41] and kidney [42,43]. Ang II acts via

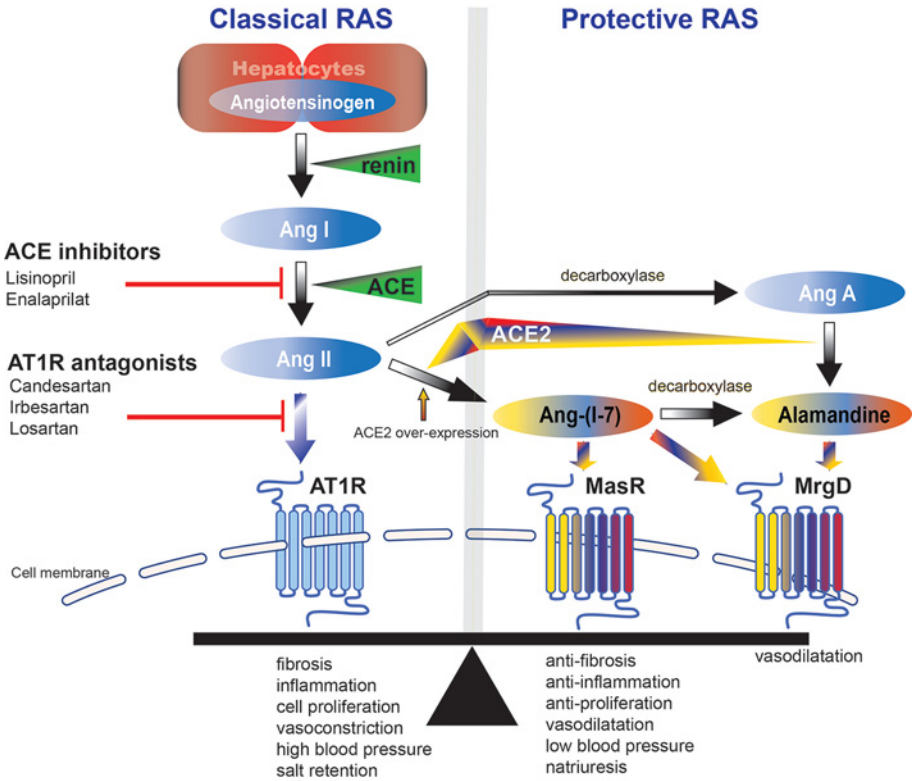

Figure 1. Overview of the renin–angiotensin system

The effects of the renin–angiotensin system (RAS) are determined by the balance between its classical arm and the protective counter-regulatory arm. Classical arm (classical RAS) consists angiotensin-converting enzyme (ACE), angiotensin II (Ang II), angiotensin II type 1 receptor (AT1R), which mediate vasoconstriction and blood pressure regulation, salt retention, cell proliferation and proinflammatory and profibrogenic pathways. The protective counter-regulatory arm (protective RAS) consists angiotensin–converting enzyme 2 (ACE2), angiotensin (1-7) (Ang-(1-7)) and the Mas receptor (MasR), directly opposing the deleterious effects of the classical RAS. Alamandine formed from angiotensin A (Ang A) or Ang-(1-7) mediates its vasodilatory action via the Mas-related G protein-coupled receptor type D (MrgD).

the AT1R to mediate recruitment of inflammatory cells into the tissue through the regulation of adhesion molecules [44] and chemokines by resident cells [45–47]. Moreover, Ang II directly activates infiltrating inflammatory cells and their many functions, including chemotaxis, proliferation and production of inflammatory mediators [43,48–50]. Most recently, studies have shown circulating leukocytes possess all the components of the RAS and can produce Ang II endogenously [43,48,51,52].

The hepatic renin–angiotensin system

Progressive liver injury is typically characterised by inflammatory cell infiltrate, progressive fibrosis and resultant regenerative/repair responses. The tissue repair response that ensues following liver injury involves inflammatory cells, that are integral to initiating the repair process and for secreting proinflammatory cytokines (e.g. interleukin-6 (IL-6), tumour necrosis factor-α (TNFα) and monocyte chemoattractant protein-1 (MCP-1)); and mesenchymal cells, phenotypically transformed interstitial fibroblasts (activated HSCs or myofibroblasts), which are responsible for collagen turnover and fibrous tissue formation. Each of these cellular actions in the microenvironment of repair is associated with molecular events that lead to the *de novo* generation of Ang II. It is well established that Ang II is pivotal to the progression of hepatic fibrosis, stimulating HSC production of pro-fibrotic cytokines, transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF), and increasing matrix formation [34,53,54].

While it has long been considered that the circulating RAS is important in disease pathogenesis, work reported from the beginning of this century has made the realisation that the local RAS or intra-organ RAS plays a key role in liver disease [9,54–56] and has now been described in a number of organs including the heart, kidney and pancreas [1,57–59]. We have provided strong evidence that both the classical and protective RAS pathways are integral to the

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function of the liver [4]. Upon liver injury, caused by experimental cholestasis in rats or hepatitis C viral infection in humans, these pathways are up-regulated contributing to the pathogenesis of hepatic fibrosis [4]. In a bile duct ligation (BDL) rat model of experimental cholestasis and hepatic fibrosis, there is a greater than 10-fold and 4-fold increase in ACE and ACE2 activity, respectively, and an up-regulation of the AT1R and MasR [9,56]. Further, we have shown that the protective RAS is functionally active in the liver and that ACE2, MasR and Ang-(1-7) peptide levels are altered in a concerted manner upon liver injury [4,9,56].

Development of liver fibrosis can be initiated from numerous insults to the liver. Of which, non-alcoholic fatty liver disease (NAFLD) is the most common liver disease characterised by accumulation of fats within hepatocytes [60]. Further NAFLD is the hepatic manifestation of the metabolic syndrome and is strongly linked to metabolic diseases such as diabetes and obesity [61–63]. It is increasingly clear however that the classical RAS is a central player in the development of NAFLD and hepatic insulin resistance [64] and that blockade of the classical RAS pathway with angiotensin receptor blockers is expected to shift the balance between the two RAS arms towards the protective RAS, leading to improvement in hepatic insulin sensitivity and steatosis in NAFLD [65–67]. It has also been shown that increased endogenous production of Ang-(1-7) in transgenic rats or administration of Ang-(1-7) to rats or mice improves insulin sensitivity, glucose and lipid metabolism and prevents hepatic steatosis [68–70]. Conversely, mice lacking ACE2, the main enzyme responsible for endogenous Ang-(1-7) production [3], had aggravated high fat diet-induced insulin resistance [71]. Collectively, these findings suggest that ACE2-driven protective RAS is a potential target in NAFLD, and provide an insight into novel therapeutic interventional strategies that can be adapted before it progresses to its severe inflammatory phenotype, non-alcoholic steatohepatitis (NASH), and to liver fibrosis. Indeed, unpublished observations from the author's laboratory suggest that liver-specific ACE2 gene therapy in diabetic NAFLD mice markedly inhibit NAFLD progression to liver fibrosis.

ACE inhibitors and angiotensin receptor blockers in the treatment of liver fibrosis

In liver disease, overactivation of the classical RAS leads to potent vasoconstriction, cell proliferation, proinflammatory and profibrotic cytokine secretion, tissue remodelling and liver fibrosis [9–11,34,54,55,72]. While circulating levels of Ang II is elevated in the systemic circulation of cirrhotic patients [73], both circulating and hepatic levels of the peptide is elevated in preclinical animal models [9–11]. The important role of the classical RAS and therapies targeting this system including ACE inhibitors and ARBs in cardiovascular disorders such as hypertension, heart failure and coronary artery disease, and chronic kidney disease has been well established [74,75] and reviewed recently in corroboration with ACE2 and COVID-19 [1,76,77].

Despite the large number of studies providing convincing evidence in preclinical models of liver injury [2,78], randomised large clinical trials supporting the use of these drugs in human liver disease is relatively scarce. We and other investigators have demonstrated that RAS blockade using ACE inhibitors [79,80] or AT1R antagonists [55,81–83] significantly attenuate hepatic fibrosis. Majority of the studies using ACE inhibitors and ARBs has been conducted in patients with hepatitis C infection, showing an antifibrotic effect [84–89]. Small studies that have been conducted in patients with NASH also provided evidence that these treatment regimens improved liver fibrosis [90–92]. However, these trials were small prospective studies and lacked the consistency across the studies in the assessment of liver fibrosis. On the other hand, despite the positive outcome of small aforementioned trials on liver fibrosis where ACE inhibitors or ARBs were administered for a short period of time, a cohort of patients of the multicentre randomised controlled trial of antiviral treatment for chronic hepatitis C (HALT-C) receiving ACE inhibitors or ARBs for three and half years did not show any improvement in liver fibrosis [93]. These conflicting evidence for the use of ACE inhibitors or ARBs as an antifibrotic therapy may partly be attributable to a higher proportion of patients who also had diabetes, a well-recognised predictor that drives hepatitis C progression. It is also not known whether long-term ACE inhibition may activate alternative enzymatic pathways such as chymases that can contribute to the pool of Ang II by catalysing Ang I breakdown [94]. On the other hand, ACE inhibition may lead to the accumulation of bradykinin, a potent vasodilator in the circulation, causing systemic hypotension in cirrhotic patients [95]. It is also accepted that a major obstacle in using ACE inhibitors and/or ARBs is that these drugs, which are not specific to the liver, lower peripheral resistance, causing systemic hypotension and renal dysfunction [96]. Moreover, they are poorly tolerated in cirrhosis and apparently less effective in advanced cirrhosis such as in patients with Child Pugh B and C [96].

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ACE2 as a potential target in the treatment of liver fibrosis

ACE2-driven protective RAS in liver disease

In comparison with the classical RAS driven by its key enzyme ACE, discovered in 1956 [97], the discovery of ACE2 in the year 2000 [29,30] has fulfilled the missing component of the protective RAS, which is now well recognised for its pivotal role in counter-balancing the deleterious effects of the classical RAS [2,4]. Recent studies provide a number of lines of evidence that the ACE2-driven protective RAS offers an alternate and potentially more effective treatment target in liver fibrosis [2,4]. This is based on the fact that ACE2 can be targeted to produce dual effects; breakdown of the profibrotic peptide Ang II and the production of the antifibrotic peptide Ang-(1-7) [3]. Thus, ACE2 activation is expected to reduce the deleterious activity of the classical RAS comprising ACE/Ang II/AT1R while at the same time, to increase the beneficial activity of the protective RAS comprising ACE2/Ang-(1-7)/MasR [10,73]. Work from our laboratory and others demonstrated that circulating level of Ang-(1-7) peptide is elevated in cirrhotic patients and in preclinical animal models [9,73,98]. In line with this, we and others further demonstrated that oral (in a vehicle of cyclodextrin) as well as parenteral administration of Ang-(1-7) peptide inhibited liver inflammation, and fibrosis of the liver, heart and lungs, formation of atherosclerotic lesions, and improved glycaemic indices and Type 2 diabetes [11,68,69,73,99–105]. In support of these findings, it has been shown that non-peptide MasR agonist AVE0991 has renoprotective and cardiac protective actions [106,107]. Conversely, pharmacological blockade of MasR by A779 aggravated liver fibrosis induced by cholestatic liver injury in rats [37]. This supports the findings that mice lacking the MasR have impaired heart functions [108].

Compelling evidence that supports for an antifibrotic role of Ang-(1-7) has provided the impetus for developing novel therapeutic strategies for liver fibrosis by targeting the protective RAS [109]. Further, since ACE2 is the driving force in this pathway, therapeutic administration of recombinant human ACE2 (rhACE2) to rats with liver fibrosis induced by cholestatic or toxic injury reduces liver fibrosis, as reflected by reduced activation of HSCs [110]. In support of this, further studies in preclinical animal models have demonstrated that rhACE2 is beneficial for the prevention of myocardial fibrosis, cardiac dysfunction and hypertension [111,112], acute lung injury, pulmonary hypertension and lung fibrosis [113] and the improvement of kidney function in diabetic nephropathy as well as in Ang II-induced renal fibrosis [114,115]. These findings in preclinical models provided much needed evidence that ACE2 of the protective RAS is a potential target to improve vascular and tissue anomalies including fibrosis.

The successful outcomes from preclinical models of diseases of the liver, kidney, heart and lungs laid a solid foundation for human studies. A direct translatability of rhACE2 is highlighted by the finding that this form of ACE2 administered intravenously to healthy volunteers produced no adverse cardiovascular effects and well tolerated [116]. In an open label pilot study and a randomised, double-blind, placebo-controlled phase II trial in patients with pulmonary arterial hypertension (PAH), a single intravenous infusion of rhACE2 caused a significant improvement in cardiac output and pulmonary vascular resistance, reduced circulating inflammatory markers, and increased the ratio of circulating Ang-(1-7) to Ang II concentration without producing unwanted systemic side effects [117,118]. However, rhACE2 therapy in adequately powered randomised clinical trials in patients with lung, heart, kidney or liver disease is yet to be undertaken.

It could be argued that rhACE2 may be a better choice for diseases such as hypertension and PAH because the treatment should be directed to circulating Ang II and the breakdown of Ang II to Ang-(1-7) rescues hypertensive phenotype. However, the tolerability of parenterally delivered rhACE2 for liver, renal or cardiac fibrosis is yet to be seen in an adequately powered randomised, double-blind, placebo-controlled trial. Nevertheless, parenteral rhACE2 therapy is expected to alter circulating RAS but not the local or tissue RAS which appears to play a central role, as suggested by findings of preclinical animal models [2]. In this context, there can be several disadvantages with parenteral administration of rhACE2, including daily injection regimes, a procedure that is invasive in a clinical setting and an expensive approach [109]. Moreover, a treatment strategy utilising bioactive peptides such as Ang-(1-7) is an option; however, parenterally or orally administered peptide is not expected to deliver the peptide only to the target organ and therefore, it is highly likely to produce off-target effects, including an effect on blood pressure.

ACE2 gene therapy for liver fibrosis

To circumvent the potential off-target effects and to directly target the organ, an ideal approach would be to increase ACE2 expression/activity in a tissue- or organ-specific manner. Thus, the enhanced expression and activity of tissue-specific ACE2 would be expected to provide several benefits; decrease the local levels of Ang II, increase the local levels of Ang-(1-7) and absence or minimum off-target effects (Figure 2). This can be achieved by tissue-specific overexpression of ACE2 using a suitable and efficient gene delivery system [119]. The adeno-associated viral (AAV) vector has been demonstrated to be efficient in the delivery of a transgene, and provides many advantages over other

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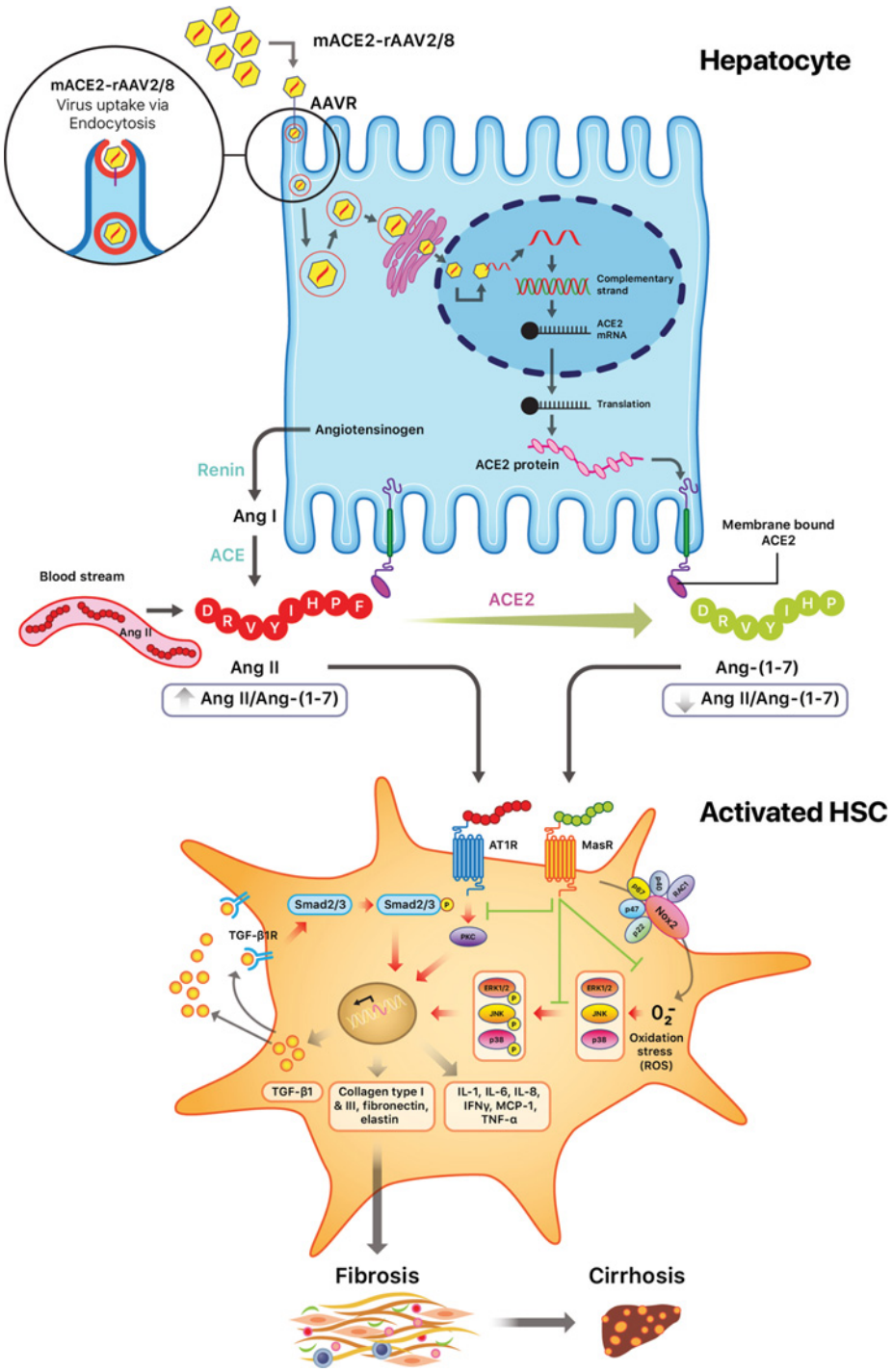

Figure 2. Improvement of liver fibrosis by ACE2 gene therapy

Angiotensin-converting enzyme 2 (ACE2) gene therapy for liver fibrosis using recombinant adeno-associated viral (AAV) vector pseudotyped with AAV-2 genome and liver-specific capsid-8 and carrying murine ACE2 (mACE2-rAAV2/8). Liver-specific overexpression of murine ACE2 in mice with liver disease shifts the increased ratio of hepatic angiotensin II (Ang II) to angiotensin-(1-7) (Ang-(1-7)) to a decreased hepatic ratio of Ang II to Ang-(1-7) by breaking down Ang II to Ang-(1-7). Thus, ACE2 gene therapy approach provides dual benefits; reduced hepatic Ang II levels resulting in a reduced liver fibrosis and increased hepatic Ang-(1-7) levels resulting in an increased anti-fibrotic activity. Abbreviations: mRNA, messenger RNA; Ang I, angiotensin I; ACE, angiotensin-converting enzyme; activated HSC, Activated hepatic stellate cells; ROS, reactive oxygen species; IL, interleukin; TNF-α, tumour necrosis factor-α; MCP-1, monocyte chemoattractant protein-1; IFNγ, interferon-γ; TGF-β1, transforming growth factor-β1; Smad2/3, Smad family members 2/3. Reproduced with permission [11].

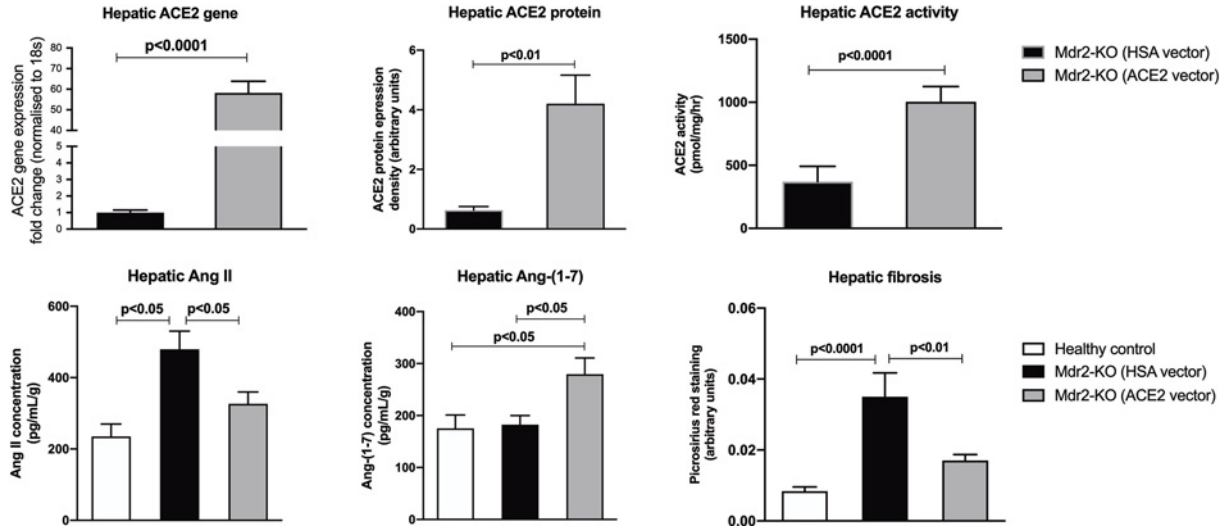

Figure 3. ACE2 gene therapy inhibits biliary fibrosis

A single intraperitoneal injection of mACE2-rAAV2/8 vector or the control HSA-rAAV2/8 vector was given to 3-month-old Mdr2 knockout mice with biliary disease. At 3 months post-treatment, animals were euthanised to collect liver samples to measure ACE2 gene and protein expression, and ACE2 protein activity, hepatic levels of Ang II and Ang-(1-7) peptides, and biliary fibrosis by quantification of picrosirius red staining. Reproduced with permission [11].

candidate viral vectors and display key features such as replicative defectiveness, non-pathogenicity, minimal immunogenicity and broad tissue tropism in both preclinical animal models and humans, and widely used in clinical trials targeting inherited metabolic diseases [119,120].

Prospects for gene therapy for a wide range of disorders have become a reality with the U.S. Food and Drug Administration (FDA), for the first time, approving a gene therapy protocol using an AAV vector in 2017, following a similar first-time approval by the European Commission in 2012 [121]. In line with this, we developed a safe and effective therapeutic approach using a pseudotyped AAV vector, which uses the recombinant AAV2 genome and liver-specific AAV8 capsid (rAAV2/8) to deliver murine ACE2 (mACE2-rAAV2/8). The specificity of ACE2 gene transcription was further enhanced by using strong liver-specific double promoters, ApoE/human α1-antitrypsin. We demonstrated that a single intraperitoneal injection of mACE2-rAAV2/8 produced sustained elevation of liver ACE2 expression for up to 6 months without affecting other major organs such as heart, lungs, brain, intestines and kidneys [10].

Treatment with mACE2-rAAV2/8 administered to short-term mouse models, representing human liver disease associated with biliary fibrosis, alcoholic liver fibrosis and non-alcoholic fatty liver disease (NAFLD) markedly reduced hepatic fibrosis in all three models (Figure 2). As expected, increased liver ACE2 expression and activity was accompanied by increased hepatic Ang-(1-7) levels with a concomitant decrease in hepatic Ang II levels [10]. ACE2 gene therapy thus provided a therapeutic strategy to shift the balance between the two arms of the RAS towards the protective pathway whereby the ratio of Ang II to Ang-(1-7) was decreased in ACE2-treated animals (Figure 2). Furthermore, work from our laboratory has confirmed the effectiveness of this therapeutic strategy in mice with genetic deletion of multidrug resistance protein-2 (Mdr2 knockout) [11], a long-term animal model with biliary lesions of progressive hepatobiliary fibrosis that resemble those of human primary sclerosing cholangitis [122–124]. Figure 3 shows ACE2 gene and protein expression, and ACE2 protein activity, hepatic levels of Ang II and Ang-(1-7), and biliary fibrosis as measured by picrosirius red staining, in 6-month-old Mdr2 knockout mice at 3 months post-treatment with a single intraperitoneal injection of mACE2-rAAV2/8 vector or the control HSA-rAAV2/8 vector. Thus, by extending our gene therapy application from 8-week short-term models of liver disease [10] up to a 9-month long-term model of biliary disease, we were able to confirm that liver-specific ACE2 gene delivery is a potential therapy for patients with liver disease. Work is currently in progress in the author's laboratory to show that this therapy can be translated into human trials by demonstrating the therapeutic effect of human ACE2 delivered to humanised mice with liver disease using human liver-specific AAV vectors.

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Pathogenesis of COVID-19

Coronavirus pandemics in the twenty-first century

Emerging in December in 2019, in Wuhan, China, the novel coronavirus 'severe acute respiratory syndrome-coronavirus-2′ (SARS-CoV-2) has rapidly spread across 191 countries with over 56 million positive cases, and 1.3 million and 48,600 deaths, as of 18 November 2020 [125]. This novel disease was recognised earlier this year as a pandemic by the World Health Organisation (WHO), and continues to cause an enormous global health, social and economic impacts. Coronavirus is common and it makes up to 30% of common colds [126]. However, this is the third member of the coronavirus family to have caused a pandemic since the turn of this century [127]. The first coronavirus that crossed this barrier and infected humans in the year 2002 was SARS-CoV that was identified in Guangdong province of Southern China, spreading across 26 countries with more than 8000 infections and 774 deaths [128]. The second coronavirus, MERS-CoV (Middle East respiratory syndrome coronavirus), which emerged in 2012 in Saudi Arabia has spread across 27 countries with 2494 infections and 858 deaths [129]. It is thought that all these coronaviruses are closely related and came from a common origin in bats [130]. However, in contrast with the two other members of the family to have crossed the species barrier and caused a pandemic, SARS-CoV-2, which causes coronavirus disease-2019 (COVID-19), is highly virulent and contagious [131].

Role of ACE2 in COVID-19

SARS-CoV-2

SARS-CoV-2 is a single-stranded RNA virus containing ∼30 kb genome that consists of up to 14 open reading frames (ORFs) flanked by 5′- and 3′-untranslated regions (UTRs) [132,133]. The vital structural proteins of the viral envelope are mainly composed of highly glycosylated spike (S) protein, membrane/matrix protein (M) and envelope protein (E) that are embedded in a lipid bilayer [132]. While these structural proteins together with nucleocapsid (N) protein are important for virus assembly during viral replication, the subunit proteins of the spike protein which exit as a trimeric prefusion state play the leading role in receptor recognition, binding and entry into host cell. Thus, receptor-binding domain (RBD) of the subunit 1 (S1) protein binds to the receptor, ACE2, leading to destabilisation of the prefusion trimer which results in the cleavage of S1 subunit by host proteases including transmembrane protease serine 2 (TMPRSS2) [20]. This exposes the S2 subunit containing fusion peptide (FP) sequences that undergo a conformational change to acquire a fusion-able state, leading to host cell membrane fusion and viral entry [134,135].

ACE2 binding of SARS-CoV-2

Since viral S protein of both SARS-CoV-2 and SARS-CoV dictates the host cell infection by binding to cell surface receptor, ACE2, which is expressed at relatively high level in lung alveolar epithelial cells [136], the amino acid sequence of the S protein is key to develop drugs and vaccine. While the S subunits from both SARS-CoV-2 and SARS-CoV share a high degree of structural similarity, the binding affinity of the S1 subunit of SARS-CoV-2 to ACE2 is ∼6- to 22-fold higher compared to that of SARS-CoV S1 subunit [135,137]. The difference between the affinities of the two related viruses may be explained by the observation that although the N-terminal amino acid residues (SARS-CoV-2$^{331\text{-}429}$ / SARS-CoV$^{318\text{-}416}$) of the RBD is relatively well conserved, the C-terminal amino acid residues (SARS-CoV-2$^{430\text{-}527}$ / SARS-CoV$^{417\text{-}513}$) containing the residues of the RBD that interact with ACE2 is more variable [138]. In support of the sequence differences at the C-terminal residues of the RBD of S1 subunit that probably influences the binding affinities, atomic details of binding interface studies provide evidence that natural substitution of key amino acid residues of SARS-CoV-2 receptor binding motif (RBM) led to higher affinity for ACE2 binding with relatively higher van der Waals bonds than SARS-CoV [139]. A stronger interaction between ACE2 and the RBM of SARS-CoV-2 as compared with SARS-CoV is further reflected by the findings of structure-guided sequence alignment studies that showed that glutamine residue at 493 (Gln493) of the SARS-CoV-2 RBM not only interacts with 3 amino acid residues of ACE2 (Lys31, His34 and Glu35) which are considered as virus binding hotspots [140,141], but also forms a hydrogen bond with Glu35 whereas the comparable residue of the SARS-CoV (Asn479) interacts only with His34 of ACE2 [137]. This concept was further strengthened by studies in which the crystal structure of the RBD of SARS-CoV-2 in complex with ACE2 showed the formation of a hydrogen bond not only between Gln493 of SARS-CoV-2 RBM and Glu35 of ACE2 but also with Lys31 of ACE2 [142]. A detailed characterisation of the structure of the interface between the S1 RBM of SARS-CoV-2 and ACE2 residues provides numerous avenues to develop promising therapeutic strategies that may include *in silico*-screening of small molecule drugs targeting the interface between the viral spike protein and the receptor.

Host–cell membrane fusion of SARS-CoV-2

While the observed differences between the SARS-CoV and SARS-CoV-2 in binding to ACE2 receptor is relatively well characterised, host cell membrane fusion of S2 subunit shows some striking similarities between the SARS-CoV and SARS-CoV-2 [143,144]. Proteolytic cleavages at the S1/S2 subunits and S2' site upstream of fusion peptide (FP) domains by host proteases such as TMPRSS2 expose two FP sequences immediately downstream of S2′ site, allowing the insertion of the fusion sequences into the host cell membrane [20,145–147].

In this membrane fusion process, it has been proposed that Ca^{2+} ions play a dominant role in the ordering of FP domains of most enveloped viruses, thus facilitating the merge of FP domains with host cell membrane [144,148–150]. As in host cell membrane fusion of Rubella virus, a strong Ca^{2+} dependency of SARS-CoV FPs has been suggested to be prerequisite events for membrane-ordering effects of the two FP domains with lipid bilayer [144,148,149,151]. The requirement for two Ca^{2+} ions to make salt bridges by binding to conserved negatively charged hydrophobic residues in the FPs such as aspartic and glutamic acid residues, has been suggested to promote greater membrane ordering and host cell membrane fusion [143,144]. Straus and colleagues in their studies using mutant FP residues and electron spin resonance spectroscopy demonstrated that negatively charged E891 (Glu891) of MERS-CoV FP domain 1 is a critical residue for Ca^{2+} binding, membrane ordering and fusion [143]. They further provided evidence using pseudo viral particles decorated with MERS-CoV S subunit protein that the infectivity of Huh-7 cells by pseudo particles was attenuated by depletion of intracellular or extracellular Ca^{2+} concentration whereas intracellular Ca^{2+} depletion completely abrogated SARS-CoV infectivity [149]. This difference in the ability of membrane fusion and infectivity of the two SARS viruses is supported by isothermal titration calorimetry studies, which in agreement with previous reports [149], suggested that unlike MERS-CoV FP that requires one Ca^{2+} ion for membrane ordering, SARS-CoV binds two Ca^{2+} ions, forming a salt bridge with each of the two FP domains [143,149]. These findings reinforce the importance of targeting Ca^{2+} channels for the design and development of new drugs to treat patients with COVID-19 or repurposing drugs currently in clinical practice for other medical conditions such as ion channel blockers. Nevertheless, these drugs help prevent the virus from attacking other vital organs in those patients who are in early stages of disease progression.

Role of basigin in COVID-19

Like ACE2 a widely expressed protein, basigin, with multiple physiological and pathobiological functions is also implicated as a SARS-CoV-2 binding partner. Basigin, also known as CD147 or EMMPRIN and encoded by the BSG gene, is a highly multifunctional abundant 269aa type 1 integral glycosylated membrane protein. Basigin has now been implicated in the binding of SARS-CoV-2 spike protein and virus entry into host cells. However, the role of basigin in SARS-CoV-2 entry is contested. Irrespective of a direct role for basigin in SARS-CoV-2 cell entry, it may be of importance in COVID-19 disease pathogenesis through virus dependent functional activation of associated pathways.

Basigin is a highly multifunctional protein [152] with distinct binding partners and structural domains determining its functions at specific locations. Other than its widely accepted role as a regulator of matrix metalloproteinase (MMP) induction [153], basigin has multiple roles in inflammation including leukocyte recruitment and adhesion [154,155]. Cyclophilins (CyP)-A and B are the two basigin ligands known to mediate chemotaxis [156]. Basigin has been shown to be important for leukocyte recruitment in rheumatoid arthritis, multiple sclerosis and inflammatory lung disease, with mAb α-basigin interventions leading to reduced neutrophil, T-cell and monocytes/macrophage infiltration [154,155,157]. At sites of tissue injury basigin mediates leukocyte aggregation which appears responsible for the amplification of inflammatory responses [158].

Basigin complexes on the surface of erythrocytes bind with the Rh5-CyRPA-Ripr complex of malaria *P. falciparum* [159]. The basigin Rh5-CyRPA-Ripr interaction occurs through a complex multiprotein complex with the insertion of pathogen proteins into the host cell membrane. In this context, there are multiple interactions between the host and pathogen proteins to which basigin is a necessary and essential component [159]. Basigin has also been implicated in measles virus (MeV) entry to epithelial cells [160]. Functionally this appears to have importance as the basigin ligand CyPB incorporates in the MeV virion and the degree of cellular incorporation of the MeV viral particle directly affects cell entry [160].

Basigin's role as SARS-CoV-2 receptor for viral entry is being debated (Figure 4). However, *in silco* and molecular dynamic simulation studies provide evidence that T lymphocytes, which do not appear to express ACE2 [136], strongly interact through C-terminal domain of basigin with Arg403, Asn481 and Gly502 of the SARS-CoV-2 spike protein [161]. However, the tropism of SARS-CoV-2 to lung alveolar epithelium does not match the tissue expression

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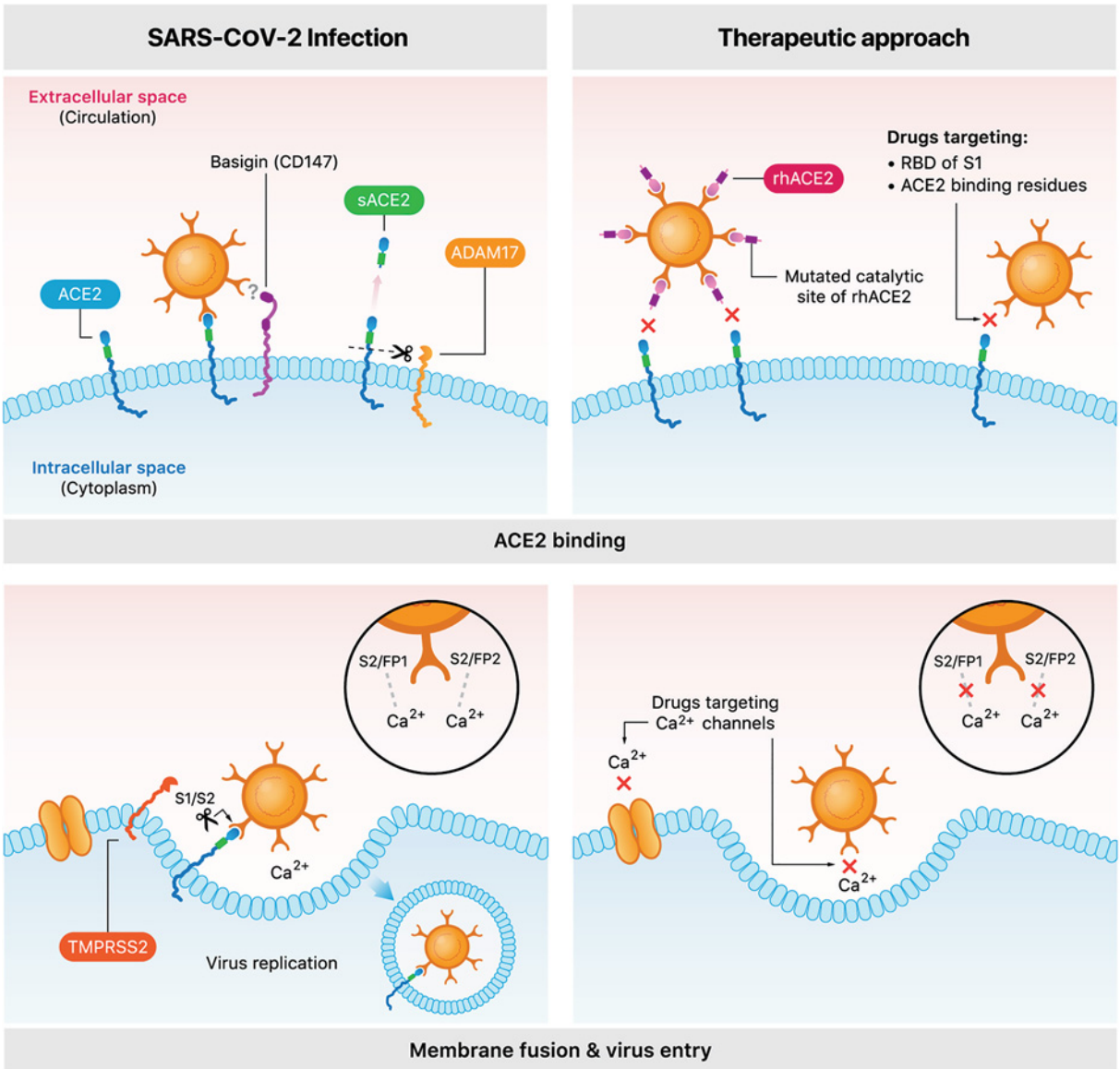

Figure 4. Role of ACE2 in SARS-CoV-2 infection and therapies targeting receptor binding and membrane fusion

Left: Spike (S) protein of SARS-CoV-2 (S1 subunit) binds to cellular ACE2 receptor (top), followed by host cell membrane fusion aided by fusion peptides (FPs) of S2 subunit (bottom), leading to endocytosis of the virus and loss of cell surface ACE2. Right: Treatment with recombinant human angiotensin-converting enzyme 2 (rhACE2) is expected to mop up SARS-CoV-2 in the circulation by competing with cellular ACE2 for binding to S1 protein (top). Drugs that can be used to target the interface between the ACE2 and the receptor-binding domain (RBD) of S1 protein to prevent viral entry to host cell (top). Ion channel blockers can be adopted to deplete intracellular and extracellular Ca^{2+} concentration to prevent the host cell membrane fusion with FP of S2 protein (bottom). Abbreviations: sACE2, soluble ACE2 - cellular ACE2 is shed into the circulation by the activity of metalloproteinase ADAM17; TMPRSS2, transmembrane protease serine 2 cleaves S1 subunit, leaving S2 subunit for host cell membrane fusion.

of basigin [162]. Further the recombinant full ectodomains and the S1 domain of the viral spike protein do not interact with the basigin expressed on HEK293 cell lines. Importantly, a basigin ligand CyPA is needed for SARS-CoV replication raising a functional interaction and suggests that CyPA may also participate in SARS-CoV-2 infection [163]. Can these apparent disparate results be explained? At a minimum, current data indicate that SARS-CoV-2 spike proteins bind to basigin. Variation in binding can be explained by the binding partners of basigin which determine the cellular actions as well as highly variable basigin glycosylation that favours binding with highly glycosylated

proteins such as the spike protein of SARS-CoV-2 [162,164]. Moreover, even in the absence of basigin directly mediating SAR-CoV-2 entry it is likely that in the respiratory and alveolar epithelium, the functional consequence of the basigin and SAR-CoV-2 interaction may be explained by some of the devastating inflammatory manifestations of COVID-19, such as the 'cytokine storm', through phenotypes such as leukocyte aggregation [158].

Novel therapeutics for COVID-19

Patients with COVID-19

Among diverse physiological roles of ACE2 in many tissues and vascular beds, the protective role of ACE2 against lung injury is paramount since this multifunctional protein lies in the interface between exterior air and alveolar cells [165,166]. Intriguingly, despite its protective role in the lung, ACE2 has been hijacked by SARS-CoV and SARS-CoV-2 to gain entry to alveolar epithelia cells, causing severe respiratory disease in humans [20,137,167–169]. What is remarkable in this process is that not only the virus gain entry into alveolar epithelial cells, but at the same, it destroys the protective machinery driven by ACE2, thus causing dual negative impact in the lungs.

It is likely that the driving force in many gravely ill patients' secondary complications following the appearance of respiratory symptoms is a disastrous overreaction of the immune system known as a 'cytokine storm' where immune cells start to attack healthy tissues [170]. This phenomenon may lead to blood vessels leak, dropping blood pressure, clot formation, possibly leading to catastrophic multiorgan failure such as stroke, heart and kidney failure [28,171–173]. While the lung is the primary battle zone disrupting healthy oxygen transfer with subsequent damage to lung vasculature triggered by cytokine storm, it is highly likely that lung alveolar epithelial cells release a large fraction of the virus into the circulation, leading to viral-mediated direct effects on other major organs such as the kidneys, heart, brain and intestines [174]. Indeed, autopsy of dead patients showed viral inclusion bodies and particles in the kidneys, and inflammatory cells and apoptotic bodies in the heart and small intestine, implying that the virus could directly invade other organs [21,23,26–28]. This is not surprising given that along with vascular endothelium across the body, these are the organs that highly express ACE2 receptor in both humans and rodents and thus potentially vulnerable to infection [28,136,175]. Moreover, the possibility that the virus can directly infect nerve cells, particularly neurons in the medulla oblongata of the brain stem that controls the functioning of the heart and the lungs, and the reported loss of sense of smell and taste in COVID-19 patients, suggests neurological damage and rapid deterioration of patients' condition [22,24,25,28].

A study found that 318 of 417 (over 50%) patients admitted to the hospital with confirmed COVID-19 had abnormal liver test results [176,177] with over 20% of patients showing ALT levels more than three times the upper limit of normal values [178]. Additionally, over 20% of patients who had liver injury with abnormal liver function test had a higher risk of progressing to severe disease [178,179]. It is yet to be seen whether liver abnormalities in COVID-19 patients are a direct consequence of SARS-CoV-2 infection and replication in hepatocytes [180]. It should be noted however that abundant ACE2 expression that occurs in the normal liver [136] is further elevated in cirrhotic patients and in preclinical animal models with liver disease [9,56]. Given the high ACE2 expression, it could be argued that the liver is a potential target for direct viral invasion in patients with respiratory symptoms, with or without liver disease [180].

Moreover, a significant percentage of COVID-19 patients are transferred to the intensive care unit (ICU) around median day 8 (day 5–14) from first admission to the hospital [171,181] and the median duration from admission to the ICU to death is 7 days [182]. It could be argued that the latency period between hospitalisation and ICU admission may be enough for the virus to enter and destroy other vital organs including the kidney, heart, liver, gut and neuronal system. Therefore, it appears that secondary invasion of the virus targeting other major organs may be expected to cause catastrophic multiorgan failure [28,172,173].

Therapeutic strategies for COVID-19

Despite many clinical trials undertaken in COVID-19 patients worldwide, an effective treatment for this highly contagious disease is yet to be identified. In addition, there is no accepted *in vitro* model or animal model to screen approved drugs that could be repurposed to treat COVID-19 patients. In our search for novel drugs for COVID-19, it is imperative that potential drug candidates need to be selected by screening large databases of small molecules that will be expected to undergo a rigorous *in vitro* and/or *in vivo* testing for their effectiveness against SARS-CoV-2 infection. The *in vitro* model adopted by Monteil and colleagues used native SARS-CoV-2 on Vero-E6 cells, and organoids derived from human embryonic stem cells (kidney organoid) and induced pluripotent stem cells (capillary organoid) to investigate the effectiveness of recombinant ACE2 proteins on infectivity of SARS-CoV-2 [183]. While this model provides an ideal flatform to investigate the therapeutic potential of drug candidates, the use of native

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SARS-CoV-2 is apparently not possible in many laboratory settings. Therefore, a simple and robust in vitro-based platform utilising SARS-CoV-2 viral like particles (VLPs) will enable fast, reliable and rapid screening of existing as well as potential new therapeutics in a physical containment 2 (PC2) laboratory setting [184]. In addition, the re-current spill over of coronaviruses into humans from their reservoir in bats strongly suggests that future zoonotic transmission events are inevitable and supports the need for ongoing and robust methods of antiviral drug screening and development.

Repurposed drugs or novel drug candidates can be tested to block entry of SARS-CoV-2 to host cells, particularly in those patients who are in the early stage of disease progression such as those patients who only show respiratory illness. It is now clear that therapies targeting the SARS-CoV-2 infection can be implemented at three levels. This includes an early stage of prevention of the virus binding to cellular ACE2 receptor and host cell membrane fusion, and late stage of virus replication within the cell. However, as discussed above, strategies that target the prevention of ACE2 binding and host cell membrane fusion is of paramount importance since these approaches essentially eliminate the viral invasion of vital organs other than the lungs in infected patients. In fact, a combined treatment targeting both receptor binding and membrane fusion is expected to block a higher proportion of the virus from entering target cells. Combined treatments have been successfully adopted for viral diseases; for example, the hepatitis C virus is now being treated using a combined treatment with two antiviral drugs directed at viral replication [185].

Impact of loss of cellular ACE2

While circulating RAS plays a pivotal role in blood pressure regulation and fluid homeostasis in normal physiology and as discussed above [186,187], it is now well recognised that the local tissue RAS plays a predominant role in disease pathogenesis in many organs including liver disease [10,11,188,189]. It has been argued that loss of cellular ACE2 due to the endocytosis associated with SARS-CoV-2 infection poses a great risk to patients with COVID-19 as the loss of ACE2 is expected to shift the balance between the two arms of the RAS towards the classical RAS, thus exacerbating the condition in which Ang II-driven cytokine release can be enhanced [2,10,11]. Moreover, the loss of ACE2 also expected have adverse effects on RAS-independent functions of ACE2 such as the regulation of neutral amino acid transport in the gut epithelial cells [190]. The possible impact of tissue ACE2 loses in patients with COVID-19 including the impact on those COVID-19 patients who have secondary complications such as Type 2 diabetes, heart and kidney diseases, and gut dysbiosis has been reviewed recently [1].

Recombinant ACE2

The first approach to inhibit the interaction between the host cell membrane ACE2 and the S1-RBM of SARS-CoV-2 can be accomplished by utilising recombinant human soluble ACE2 (rhsACE2). Thus, the approach adopted by Monteil and colleagues using parenterally administered rhsACE2 that competes with SARS-CoV-2 for binding to cellular ACE2 (Figure 4) has a therapeutic potential in patients with COVID-19 [183]. Direct translatability of rhsACE2 is highlighted by the finding that this form of ACE2 administered intravenously to healthy volunteers produced no adverse effects and well tolerated [116] Supporting this, work from our laboratory reported that up to 6000-fold overexpression of cellular ACE2 in the liver of healthy mice had no adverse effect [10]. Although rhsACE2 has been reported to reduce the cellular uptake of the SARS-CoV-2 by up to 5000-fold [183], this has not been tested in *in vivo* model due to a lack of an appropriate *in vivo* model.

Approaches that utilise exogenously administered rhsACE2 may compromise blood pressure since circulating rhsACE2 is expected to breakdown potent vasoconstrictor peptide Ang II, likely causing systemic hypotension and renal failure [191]. The effect on blood pressure can be further impacted by the production of vasodilator peptide, Ang-(1-7), the product of Ang II breakdown [2,10,11]. This potential problem on blood pressure can be circumvented by introducing mutations into the catalytic domain of rhsACE2 (Figure 4) [192,193]. The canonical function of ACE2 is to function as a carboxypeptidase [194]. On the basis of the ability of ACE2 to cleave biological peptides, a consensus sequence of Pro-X-Pro-hydrophobic has been derived as the target for the ACE2 catalytic activity [194]. Furthermore, this catalytic activity has been narrowed down to a number of residues located within the catalytic domain of ACE2 [192]. Particularly, Arg273 has been identified as a critical residue for substrate binding [192]. Site-directed mutagenesis to replace the arginine with a glutamine residue (R273Q) that represents a positive to neutral change in the side chain while maintaining most of the hydrophobic surface area has demonstrated that the loss of positive charge on the side chain at residue 273 has a profound effect on the substrate binding [192]. Furthermore, mutations on H345A/L and H505A/L also results in enzyme activity being dramatically reduced. Further investigations have revealed that His345 as the hydrogen bond donor/acceptor during the formation of the tetrahedral peptide intermediate [192]. Thus, these residues of ACE2 are highly critical for the catalytic activity. Therefore, if the choice is to use rhsACE2 as

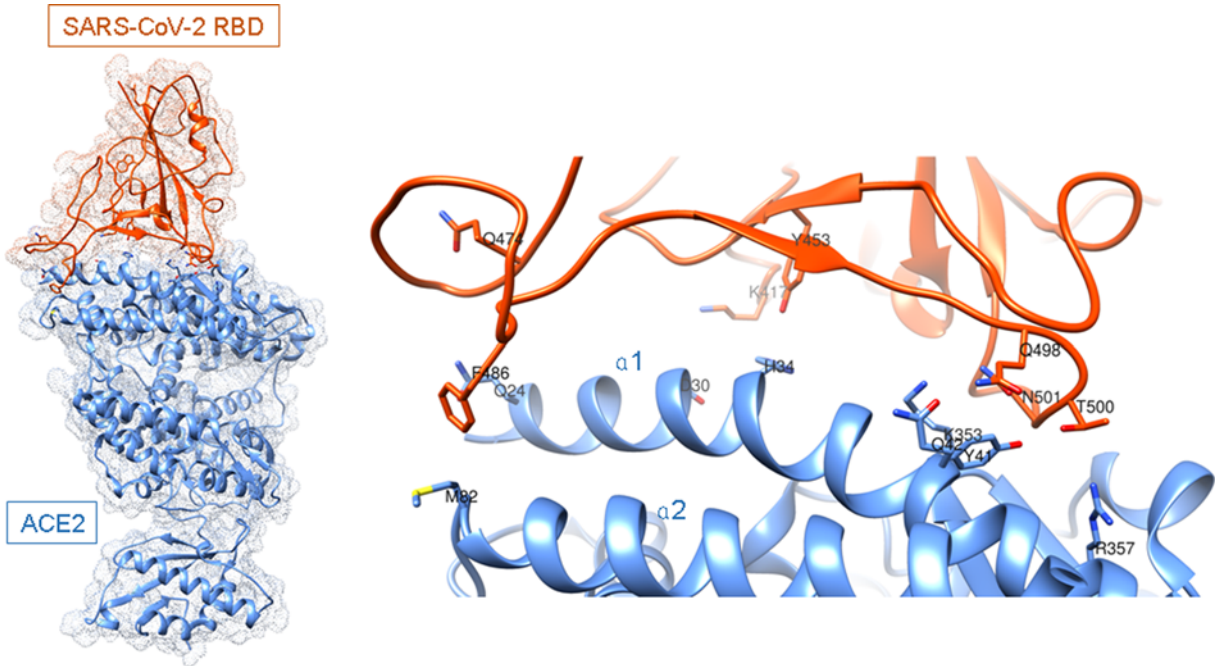

Figure 5. ACE2 interaction with the RBD of the SARS-CoV-2 spike protein

The SARS-CoV-2 S1-receptor binding domain (RBD) interaction with the ACE2 is shown as a ribbon diagram using the crystal structure PDB: 6m17 [196]. The right panel shows the residues on ACE2 (Q24(Glu), H34(His), Y41(Tyr), Q42(Glu), M82(Met), K353(Lys), R357(Arg)) interacting with the residues on S1-RBM (K417(Lys), Y453(Tyr), Q474(Glu), F486(Phe), Q498(Glu), T500(Thr), N501(Asp)) [196].

a therapy in COVID-19 patients, it is important that these critical residues will need to be mutated to make rhsACE2 catalytically inactive to prevent an increased breakdown of circulating Ang II and subsequent effect on blood pressure [191]. Catalytically inactive rhsACE2 could compete with the host ACE2 for binding to the S1-RBM of SARS-CoV-2, thus preventing or reducing the 'effective viral load' in the circulation that is available for host receptor engagement. Because rhsACE2 administration should be performed as an intravenous treatment, this therapy could only be applicable to those patients who are currently admitted to a hospital. Furthermore, since the treatment is a short-term treatment, the risk of developing anti-rhsACE2 antibodies is also minimum.

Targeting ACE2 binding interface

Coronaviruses have been known to use membrane bound ACE2 as a point of entry to the host cell [195]. The arch shaped -1 helix on the ACE2 peptidase domain (PD) mainly interacts with the RBM of the S1 protein while -2 helix and the β3-4 loop of the ACE2 make limited contacts [196]. The crystal structure of this interaction [196] provides an excellent opportunity to inhibit the viral entry by inhibiting the ACE2-S1 interaction. Binding of the S1 RBM on ACE2 is mainly hinged on the arch shaped -1 helix of the ACE2 [196]. Therefore, the surface of the RBM interacts over a significant surface area on the ACE2. This provides two options for the inhibition of SARS-CoV-2 binding to the ACE2: (a) designing small molecule inhibitors that bind to the S1-RBM and inhibit the interaction with the ACE2 [197,198], and (b) use the ACE2 residues that interact with the S1-RBM as the target for the drug binding and inhibit the interaction with the SARS-CoV-2 (Figure 4).

While repurposing the approved drugs provides fairly rapid solutions, *in silico* drug screening with molecular dynamics simulations could also be employed to screen and identify novel drug candidates that can be used to inhibit the receptor binding and/or the host cell membrane fusion of not only SARS-CoV-2 [199] but also coronaviruses of future pandemics. Based on the co-crystal structure of the RBD and the ACE2, the S1-RBD forms a pocket around the residues R403(Arg), D405(Asp), Q409(Glu), K417(Lys), I418(Ile), L455(Leu), Y453(Tyr), Y495(Tyr), F497(Phe), N501(Asp) and Y505(Tyr). This pocket is ∼17 Å long and ∼9 Å in width and lined with hydrophobic residues. This could be utilised as a drug binding pocket and screen for molecules that bind to this region at a high affinity (Figure 5). Binding a molecule to this pocket could significantly reduce the interactions between the S1-RBD and the ACE2

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resulting in lowering the affinity between the virus and the receptor.

Similarly, the same approach can be undertaken to screen for molecules binding to the ACE2 surface and prevent the RBD interaction with the host receptor. However, the RBD interacting surface of ACE2 does not appear to possess any druggable pockets. Therefore, screening for small molecules that could bind to the ACE2 surface to inhibit the interaction with RBD could be far-fetched. However, if a peptide can be derived from ACE2 and S1-RBD interacting surface, this peptide could be used to inhibit the interaction between the ACE2 and S1-RBD. In situations where the protein surfaces do not facilitate small molecule inhibitors, peptide-based inhibitors have become an emerging technique. A recent review has provided a comprehensive review of peptide inhibitors and their application and draw backs [200]. Given the ACE2 surface characteristics, developing a peptide inhibitor to mimic the RBD-binding surface on ACE2 could be equally valuable as developing small molecule inhibitors that bind to the S1-RBD. Furthermore, a similar approach could be adopted by using a peptide that binds to the RBD and interfere with the ACE2 binding. One of the disadvantages of using peptide-based inhibitors is their susceptibility to host proteases.

Targeting host cell membrane fusion

As described above, Ca^{2+} has been shown to play an important role in the process of fusing SARS-CoV-2 with the host membrane. Viral membrane fusion is the process by which the virus envelop merge with the host cell membrane to deliver the viral genetic materials. SARS-CoV-2 virus membrane fusion occurs after ACE2 binding and once both viral membrane and the host membrane are proximal to each other. During the process of membrane fusion Ca^{2+} ions are believed to be used by the fusion peptides for orienting themselves on the host cell membrane. This provides a unique opportunity for inhibiting the process of virus genetic material delivery to the host cell by inhibiting the peptide orienting process. If a small molecule can be used to compete with the residues that binds Ca^{2+}, it would be possible to inhibit the fusion peptide orientation. However, given the small size of the binding site it would be unwise to take such approach. Therefore, as an alternative if the amount of available Ca^{2+} for the virus can be depleted one should be able to achieve a similar effect.

Calcium transporters located on the cell surface act as regulators of Ca^{2+} concentration in the extracellular space. Therefore, if an inhibitor could be used to inhibit Ca^{2+} channels then the amount of Ca^{2+} available can be effectively regulated. Consequently, this would provide a control over the membrane fusion step. Currently there are a number of Ca^{2+} channel blockers available that are used to treat patients with hypertension and heart arrhythmia. Thus, it would probably be the right time to revisit those drugs and repurpose them as a potential treatment for SARS-CoV-2 (Figures 4 and 5).

While repurposing the approved drugs such as ion channel blockers provides fairly rapid solutions as in the identification of drug candidates to inhibit receptor binding, *in silico* drug screening can be effectively employed to identify such drugs that have the potential to inhibit host cell membrane fusion of the virus by depleting extracellular Ca^{2+} concentration. A number of open source and proprietary molecular docking software packages are available to be used in the process but Autodock [201] and Autodock Vina [202] have shown to be the most popular with most of the drug screening campaigns. This software is relying on the availability of docking ready compound databases. One of the most commonly used such database is the ZINC database [203] that contains over 230 million purchasable small molecule compounds in ready-to-dock, 3D formats. Docking algorithms can provide an estimation of the binding affinity and based on the calculated value, top hits can be selected, and further validation could be performed with molecular dynamics simulations. Finally, those promising compounds can be tested in *in vitro* and *in vivo* studies.

Conclusion

Since its discovery two decades ago, ACE2 has been the central focus of a large number of studies that investigated the protective role of the alternate RAS in many pathological conditions including cardiovascular, renal, lung and liver disease. The fact that ACE2 breaks down potent profibrotic peptide Ang II to antifibrotic peptide Ang-(1-7) and that Ang-(1-7) administration to preclinical animal models with liver disease dramatically improves liver fibrosis provided a great opportunity to use ACE2 as a potential therapy for liver fibrosis. Thus, we have reported a successful application of ACE2 gene therapy to improve liver fibrosis in an organ-specific manner in mice with acute as well as chronic liver disease. Our findings provided much needed impetus to translate experimental ACE2 gene therapy work into future clinical trials in patients with liver disease. Incidentally, of the three coronavirus pandemics of this century, both SARS-CoV and SARS-CoV-2 have hijacked ACE2 to gain entry into alveolar epithelial cells of the lungs. However, compared with SARS-CoV infection, COVID-19 caused by SARS-CoV-2 is a devastating respiratory illness in humans and frequently life-threatening especially in elderly or people with other medical conditions. A massive amount of data that were generated in response to SARS-CoV-2 pandemic as well as the findings from studies

conducted in response to SARS-CoV pandemic, have provided avenues to develop novel therapeutics or to repurpose approved drugs to treat COVID-19. This effort of developing drugs to target virus binding and cellular entry, and replication is important for treating COVID-19 patients until such time an effective vaccine becomes available for this disease and for similar coronavirus infections in the future.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang I, angiotensin I; Ang II, angiotensin II; Ang-(1-7), angiotensin-(1-7); ARB, angiotensin receptor blocker; AT1R, angiotensin II type 1 receptor; AT2R, angiotensin II type 2 receptor; MasR, mas receptor; COVID-19, coronavirus disease 2019; CTGF, connective tissue growth factor; FP, fusion peptide; GPCR, G protein-coupled receptor; HEK293, human embryonic kidney 293; HSA-rAAV2, human serum albumin-recombinant adeno-associated virus; HSC, hepatic stellate cell; IL-6, interleukin-6; mACE2-rAAV2, murine ACE2-recombinant adeno-associated virus; MCP-1, monocyte chemoattractant protein-1; Mdr2, multidrug resistance protein-2; MERS-CoV, Middle East respiratory syndrome coronavirus; MMP, matrix metalloproteinase; MrgD, mas related G protein-coupled receptor type D; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NEP, neutral endopeptidase; ORF, open reading frame; PAH, pulmonary arterial hypertension; PD, peptidase domain; RAS, renin–angiotensin system; RBD, receptor-binding domain; RBM, receptor-binding motif; rhACE2, recombinant human ACE2; rhsACE2, recombinant human soluble ACE2; S protein, spike protein; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TGF-β1, transforming growth factor-β1; TMPRSS2, transmembrane protease serine 2; TNFα, tumour necrosis factor-α; VLP, virus-like particle.

References

1 Gheblawi, M., Wang, K., Viveiros, A., Nguyen, Q., Zhong, J.C., Turner, A.J. et al. (2020) Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System: Celebrating the 20th Anniversary of the Discovery of ACE2. *Circ. Res.* **126**, 1456–1474, https://doi.org/10.1161/CIRCRESAHA.120.317015

2 Grace, J.A., Herath, C.B., Mak, K.Y., Burrell, L.M. and Angus, P.W. (2012) Update on new aspects of the renin-angiotensin system in liver disease: clinical implications and new therapeutic options. *Clin. Sci. (Lond.)* **123**, 225–239, https://doi.org/10.1042/CS20120030

3 Herath, C.B., Lubel, J.S., Jia, Z., Velkoska, E., Casley, D., Brown, L. et al. (2009) Portal pressure responses and angiotensin peptide production in rat liver are determined by relative activity of ACE and ACE2. *Am. J. Physiol. Gastrointest. Liver Physiol.* **297**, G98–G106, https://doi.org/10.1152/ajpgi.00045.2009

4 Warner, F.J., Lubel, J.S., McCaughan, G.W. and Angus, P.W. (2007) Liver fibrosis: a balance of ACEs? *Clin. Sci. (Lond.)* **113**, 109–118, https://doi.org/10.1042/CS20070026

5 Santos, R.A.S., Sampaio, W.O., Alzamora, A.C., Motta-Santos, D., Alenina, N., Bader, M. et al. (2018) The ACE2/Angiotensin-(1-7)/MAS Axis of the Renin-Angiotensin System: Focus on Angiotensin-(1-7). *Physiol. Rev.* **98**, 505–553, https://doi.org/10.1152/physrev.00023.2016

6 Paz Ocaranza, M., Riquelme, J.A., Garcia, L., Jalil, J.E., Chiong, M., Santos, R.A.S. et al. (2020) Counter-regulatory renin-angiotensin system in cardiovascular disease. *Nat. Rev. Cardiol.* **17**, 116–129, https://doi.org/10.1038/s41569-019-0244-8

7 Tetzner, A., Gebolys, K., Meinert, C., Klein, S., Uhlich, A., Trebicka, J. et al. (2016) G-Protein-Coupled Receptor MrgD Is a Receptor for Angiotensin-(1-7) Involving Adenylyl Cyclase, cAMP, and Phosphokinase A. *Hypertension* **68**, 185–194, https://doi.org/10.1161/HYPERTENSIONAHA.116.07572

8 Gunarathne, L.S., Angus, P.W. and Herath, C.B. (2019) Blockade of Mas Receptor or Mas-Related G-Protein Coupled Receptor Type D Reduces Portal Pressure in Cirrhotic but Not in Non-cirrhotic Portal Hypertensive Rats. *Front. Physiol.* **10**, 1169, https://doi.org/10.3389/fphys.2019.01169

9 Herath, C.B., Warner, F.J., Lubel, J.S., Dean, R.G., Jia, Z., Lew, R.A. et al. (2007) Upregulation of hepatic angiotensin-converting enzyme 2 (ACE2) and angiotensin-(1-7) levels in experimental biliary fibrosis. *J. Hepatol.* **47**, 387–395, https://doi.org/10.1016/j.jhep.2007.03.008

10 Mak, K.Y., Chin, R., Cunningham, S.C., Habib, M.R., Torresi, J., Sharland, A.F. et al. (2015) ACE2 Therapy Using Adeno-associated Viral Vector Inhibits Liver Fibrosis in Mice. *Mol. Ther.* **23**, 1434–1443, https://doi.org/10.1038/mt.2015.92

11 Rajapaksha, I.G., Gunarathne, L.S., Asadi, K., Cunningham, S.C., Sharland, A., Alexander, I.E. et al. (2019) Liver-Targeted Angiotensin Converting Enzyme 2 Therapy Inhibits Chronic Biliary Fibrosis in Multiple Drug-Resistant Gene 2-Knockout Mice. *Hepatol. Commun.* **3**, 1656–1673, https://doi.org/10.1002/hep4.1434
12 Poynard, T., McHutchison, J., Manns, M., Myers, R.P. and Albrecht, J. (2003) Biochemical surrogate markers of liver fibrosis and activity in a randomized trial of peginterferon alfa-2b and ribavirin. *Hepatology* **38**, 481–492, https://doi.org/10.1053/jhep.2003.50319
13 Patel, K. and Shackel, N.A. (2014) Current status of fibrosis markers. *Curr. Opin. Gastroenterol.* **30**, 253–259, https://doi.org/10.1097/MOG.0000000000000059
14 El Bialy, S.A., El Kader, K.F. and El-Ashmawy, M.B. (2011) Current progress in antifibrotics. *Curr. Med. Chem.* **18**, 3082–3092, https://doi.org/10.2174/092986711796391679
15 Poelstra, K., Beljaars, L. and Melgert, B.N. (2013) Cell-specific delivery of biologicals: problems, pitfalls and possibilities of antifibrotic compounds in the liver. *Drug Discov. Today* **18**, 1237–1242, https://doi.org/10.1016/j.drudis.2013.05.013
16 Bataller, R. and Brenner, D.A. (2005) Liver fibrosis. *J. Clin. Invest.* **115**, 209–218, https://doi.org/10.1172/JCI24282
17 Friedman, S.L. (2008) Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol. Rev.* **88**, 125–172, https://doi.org/10.1152/physrev.00013.2007
18 Acharya, K.R., Sturrock, E.D., Riordan, J.F. and Ehlers, M.R. (2003) Ace revisited: a new target for structure-based drug design. *Nat. Rev. Drug Discov.* **2**, 891–902, https://doi.org/10.1038/nrd1227
19 Turner, A.J., Hiscox, J.A. and Hooper, N.M. (2004) ACE2: from vasopeptidase to SARS virus receptor. *Trends Pharmacol. Sci.* **25**, 291–294, https://doi.org/10.1016/j.tips.2004.04.001
20 Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S. et al. (2020) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **181**, 271e278–280e278, https://doi.org/10.1016/j.cell.2020.02.052
21 Farkash, E.A., Wilson, A.M. and Jentzen, J.M. (2020) Ultrastructural Evidence for Direct Renal Infection with SARS-CoV-2. *J. Am. Soc. Nephrol.* **31**, 1683–1687, https://doi.org/10.1681/ASN.2020040432
22 Iadecola, C., Anrather, J. and Kamel, H. (2020) Effects of COVID-19 on the Nervous System. *Cell* **83**, 16–27, https://doi.org/10.1016/j.cell.2020.08.028
23 Lamers, M.M., Beumer, J., van der Vaart, J., Knoops, K., Puschhof, J., Breugem, T.I. et al. (2020) SARS-CoV-2 productively infects human gut enterocytes. *Science* **369**, 50–54, https://doi.org/10.1126/science.abc1669
24 Lechien, J.R., Hopkins, C. and Saussez, S. (2020) Sniffing out the evidence; It's now time for public health bodies recognize the link between COVID-19 and smell and taste disturbance. *Rhinology* **58**, 402–403
25 Li, Y.C., Bai, W.Z. and Hashikawa, T. (2020) The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. *J. Med. Virol.* **92**, 552–555, https://doi.org/10.1002/jmv.25728
26 Puelles, V.G., Lutgehetmann, M., Lindenmeyer, M.T., Sperhake, J.P., Wong, M.N., Allweiss, L. et al. (2020) Multiorgan and Renal Tropism of SARS-CoV-2. *N. Engl. J. Med.* **383**, 590–592, https://doi.org/10.1056/NEJMc2011400
27 Varga, Z., Flammer, A.J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A.S. et al. (2020) Endothelial cell infection and endotheliitis in COVID-19. *Lancet* **395**, 1417–1418, https://doi.org/10.1016/S0140-6736(20)30937-5
28 Zou, X., Chen, K., Zou, J., Han, P., Hao, J. and Han, Z. (2020) Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front. Med.* **14**, 185–192, https://doi.org/10.1007/s11684-020-0754-0
29 Tipnis, S.R., Hooper, N.M., Hyde, R., Karran, E., Christie, G. and Turner, A.J. (2000) A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* **275**, 33238–33243, https://doi.org/10.1074/jbc.M002615200
30 Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N. et al. (2000) A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ. Res.* **87**, E1–E9, https://doi.org/10.1161/01.RES.87.5.e1
31 Santos, R.A., Simoes e Silva, A.C., Maric, C., Silva, D.M., Machado, R.P., de Buhr, I. et al. (2003) Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 8258–8263, https://doi.org/10.1073/pnas.1432869100
32 Lautner, R.Q., Villela, D.C., Fraga-Silva, R.A., Silva, N., Verano-Braga, T., Costa-Fraga, F. et al. (2013) Discovery and characterization of alamandine: a novel component of the renin-angiotensin system. *Circ. Res.* **112**, 1104–1111, https://doi.org/10.1161/CIRCRESAHA.113.301077
33 Bataller, R., Gabele, E., Parsons, C.J., Morris, T., Yang, L., Schoonhoven, R. et al. (2005) Systemic infusion of angiotensin II exacerbates liver fibrosis in bile duct-ligated rats. *Hepatology* **41**, 1046–1055, https://doi.org/10.1002/hep.20665
34 Bataller, R., Gabele, E., Schoonhoven, R., Morris, T., Lehnert, M., Yang, L. et al. (2003) Prolonged infusion of angiotensin II into normal rats induces stellate cell activation and proinflammatory events in liver. *Am. J. Physiol. Gastrointest. Liver Physiol.* **285**, G642–G651, https://doi.org/10.1152/ajpgi.00037.2003
35 Bataller, R., Gines, P., Nicolas, J.M., Gorbig, M.N., Garcia-Ramallo, E., Gasull, X. et al. (2000) Angiotensin II induces contraction and proliferation of human hepatic stellate cells. *Gastroenterology* **118**, 1149–1156, https://doi.org/10.1016/S0016-5085(00)70368-4
36 Gallagher, P.E. and Tallant, E.A. (2004) Inhibition of human lung cancer cell growth by angiotensin-(1-7). *Carcinogenesis* **25**, 2045–2052, https://doi.org/10.1093/carcin/bgh236
37 Pereira, R.M., Dos Santos, R.A., Teixeira, M.M., Leite, V.H., Costa, L.P., da Costa Dias, F.L. et al. (2007) The renin-angiotensin system in a rat model of hepatic fibrosis: evidence for a protective role of Angiotensin-(1-7). *J. Hepatol.* **46**, 674–681, https://doi.org/10.1016/j.jhep.2006.10.018
38 Karnik, S.S., Singh, K.D., Tirupula, K. and Unal, H. (2017) Significance of angiotensin 1-7 coupling with MAS1 receptor and other GPCRs to the renin-angiotensin system: IUPHAR Review 22. *Br. J. Pharmacol.* **174**, 737–753, https://doi.org/10.1111/bph.13742
39 Suzuki, Y., Ruiz-Ortega, M., Lorenzo, O., Ruperez, M., Esteban, V. and Egido, J. (2003) Inflammation and angiotensin II. *Int. J. Biochem. Cell Biol.* **35**, 881–900, https://doi.org/10.1016/S1357-2725(02)00271-6

40 Nicoletti, A., Heudes, D., Mandet, C., Hinglais, N., Bariety, J. and Michel, J.B. (1996) Inflammatory cells and myocardial fibrosis: spatial and temporal distribution in renovascular hypertensive rats. *Cardiovasc. Res.* **32**, 1096–1107, https://doi.org/10.1016/S0008-6363(96)00158-7
41 Guzik, T.J., Hoch, N.E., Brown, K.A., McCann, L.A., Rahman, A., Dikalov, S. et al. (2007) Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J. Exp. Med.* **204**, 2449–2460, https://doi.org/10.1084/jem.20070657
42 Suzuki, Y., Ruiz-Ortega, M., Gomez-Guerrero, C., Tomino, Y. and Egido, J. (2003) Angiotensin II, the immune system and renal diseases: Another road for RAS? *Nephrol. Dialysis Transplant.* **18**, 1423–1426, https://doi.org/10.1093/ndt/gfg223
43 Jurewicz, M., McDermott, D.H., Sechler, J.M., Tinckam, K., Takakura, A., Carpenter, C.B. et al. (2007) Human T and natural killer cells possess a functional renin-angiotensin system: further mechanisms of angiotensin II-induced inflammation. *J. Am. Soc. Nephrol.* **18**, 1093–1102, https://doi.org/10.1681/ASN.2006070707
44 Krejcy, K., Eichler, H.G., Jilma, B., Kapiotis, S., Wolzt, M., Zanaschka, G. et al. (1996) Influence of angiotensin II on circulating adhesion molecules and blood leukocyte count in vivo. *Can. J. Physiol. Pharmacol.* **74**, 9–14, https://doi.org/10.1139/y95-218
45 Kanno, K., Tazuma, S., Nishioka, T., Hyogo, H. and Chayama, K. (2005) Angiotensin II participates in hepatic inflammation and fibrosis through MCP-1 expression. *Dig. Dis. Sci.* **50**, 942–948, https://doi.org/10.1007/s10620-005-2669-7
46 Marra, F., DeFranco, R., Grappone, C., Milani, S., Pastacaldi, S., Pinzani, M. et al. (1998) Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: correlation with monocyte infiltration. *Am. J. Pathol.* **152**, 423–430
47 Marra, F., Romanelli, R.G., Giannini, C., Failli, P., Pastacaldi, S., Arrighi, M.C. et al. (1999) Monocyte chemotactic protein-1 as a chemoattractant for human hepatic stellate cells. *Hepatology* **29**, 140–148, https://doi.org/10.1002/hep.510290107
48 Jankowski, V., Vanholder, R., Van Der Giet, M., Henning, L., Tlle, M., Schnfelder, G. et al. (2005) Detection of angiotensin II in supernatants of stimulated mononuclear leukocytes by matrix-assisted laser desorption ionization time-of-flight/time- of-flight mass analysis. *Hypertension* **46**, 591–597, https://doi.org/10.1161/01.HYP.0000177436.09733.d4
49 Weidanz, J.A., Jacobson, L.M., Muehrer, R.J., Djamali, A., Hullett, D.A., Sprague, J. et al. (2005) ATR blockade reduces IFN-γ production in lymphocytes in vivo and in vitro. *Kidney Int.* **67**, 2134–2142, https://doi.org/10.1111/j.1523-1755.2005.00318.x
50 Weinstock, J.V. and Kassab, J. (1986) Chemotactic response of splenic mononuclear cells to angiotensin II in murine schistosomiasis. *J. Immunol.* **137**, 2020–2024
51 Hoch, N.E., Guzik, T.J., Chen, W., Deans, T., Maalouf, S.A., Gratze, P. et al. (2009) Regulation of T-cell function by endogenously produced angiotensin II. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **296**, R208–R216, https://doi.org/10.1152/ajpregu.90521.2008
52 Coppo, M., Boddi, M., Bandinelli, M., Degl'Innocenti, D., Ramazzotti, M., Marra, F. et al. (2008) Angiotensin II upregulates renin-angiotensin system in human isolated T lymphocytes. *Regul. Pept.* **151**, 1–6, https://doi.org/10.1016/j.regpep.2008.07.010
53 Bataller, R., Sancho-Bru, P., Gines, P., Lora, J.M., Al-Garawi, A., Sole, M. et al. (2003) Activated human hepatic stellate cells express the renin-angiotensin system and synthesize angiotensin II. *Gastroenterology* **125**, 117–125, https://doi.org/10.1016/S0016-5085(03)00695-4
54 Paizis, G., Cooper, M.E., Schembri, J.M., Tikellis, C., Burrell, L.M. and Angus, P.W. (2002) Up-regulation of components of the renin-angiotensin system in the bile duct-ligated rat liver. *Gastroenterology* **123**, 1667–1676, https://doi.org/10.1053/gast.2002.36561
55 Paizis, G., Gilbert, R.E., Cooper, M.E., Murthi, P., Schembri, J.M., Wu, L.L. et al. (2001) Effect of angiotensin II type 1 receptor blockade on experimental hepatic fibrogenesis. *J. Hepatol.* **35**, 376–385, https://doi.org/10.1016/S0168-8278(01)00146-5
56 Paizis, G., Tikellis, C., Cooper, M.E., Schembri, J.M., Lew, R.A., Smith, A.I. et al. (2005) Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. *Gut* **54**, 1790–1796, https://doi.org/10.1136/gut.2004.062398
57 Leung, P.S. (2004) The peptide hormone angiotensin II: its new functions in tissues and organs. *Curr. Protein Pept. Sci.* **5**, 267–273, https://doi.org/10.2174/1389203043379693
58 Leung, P.S. (2007) The physiology of a local renin-angiotensin system in the pancreas. *J. Physiol.* **580**, 31–37, https://doi.org/10.1113/jphysiol.2006.126193
59 Ferrario, C.M. (2006) Angiotensin-converting enzyme 2 and angiotensin-(1-7): an evolving story in cardiovascular regulation. *Hypertension* **47**, 515–521, https://doi.org/10.1161/01.HYP.0000196268.08909.fb
60 Leung, C., Rivera, L., Furness, J.B. and Angus, P.W. (2016) The role of the gut microbiota in NAFLD. *Nat. Rev. Gastroenterol. Hepatol.* **13**, 412–425, https://doi.org/10.1038/nrgastro.2016.85
61 Loomba, R. and Sanyal, A.J. (2013) The global NAFLD epidemic. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 686–690, https://doi.org/10.1038/nrgastro.2013.171
62 Friedman, S.L., Neuschwander-Tetri, B.A., Rinella, M. and Sanyal, A.J. (2018) Mechanisms of NAFLD development and therapeutic strategies. *Nat. Med.* **24**, 908–922, https://doi.org/10.1038/s41591-018-0104-9
63 Watt, M.J., Miotto, P.M., De Nardo, W. and Montgomery, M.K. (2019) The Liver as an Endocrine Organ-Linking NAFLD and Insulin Resistance. *Endocr. Rev.* **40**, 1367–1393, https://doi.org/10.1210/er.2019-00034
64 Paschos, P. and Tziomalos, K. (2012) Nonalcoholic fatty liver disease and the renin-angiotensin system: Implications for treatment. *World J. Hepatol.* **4**, 327–331, https://doi.org/10.4254/wjh.v4.i12.327
65 Cao, X., Yang, F.Y., Xin, Z., Xie, R.R. and Yang, J.K. (2014) The ACE2/Ang-(1-7)/Mas axis can inhibit hepatic insulin resistance. *Mol. Cell. Endocrinol.* **393**, 30–38, https://doi.org/10.1016/j.mce.2014.05.024
66 Souza-Mello, V. (2017) Hepatic structural enhancement and insulin resistance amelioration due to AT1 receptor blockade. *World J Hepatol* **9**, 74–79, https://doi.org/10.4254/wjh.v9.i2.74
67 Borem, L.M.A., Neto, J.F.R., Brandi, I.V., Lelis, D.F. and Santos, S.H.S. (2018) The role of the angiotensin II type I receptor blocker telmisartan in the treatment of non-alcoholic fatty liver disease: a brief review. *Hypertens. Res.* **41**, 394–405, https://doi.org/10.1038/s41440-018-0040-6

68 Feltenberger, J.D., Andrade, J.M., Paraiso, A., Barros, L.O., Filho, A.B., Sinisterra, R.D. et al. (2013) Oral formulation of angiotensin-(1-7) improves lipid metabolism and prevents high-fat diet-induced hepatic steatosis and inflammation in mice. *Hypertension* **62**, 324–330, https://doi.org/10.1161/HYPERTENSIONAHA.111.00919

69 Santos, S.H., Andrade, J.M., Fernandes, L.R., Sinisterra, R.D., Sousa, F.B., Feltenberger, J.D. et al. (2013) Oral Angiotensin-(1-7) prevented obesity and hepatic inflammation by inhibition of resistin/TLR4/MAPK/NF-kappaB in rats fed with high-fat diet. *Peptides* **46**, 47–52, https://doi.org/10.1016/j.peptides.2013.05.010

70 Santos, S.H., Braga, J.F., Mario, E.G., Porto, L.C., Rodrigues-Machado Mda, G., Murari, A. et al. (2010) Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1-7). *Arterioscler. Thromb. Vasc. Biol.* **30**, 953–961, https://doi.org/10.1161/ATVBAHA.109.200493

71 Takeda, M., Yamamoto, K., Takemura, Y., Takeshita, H., Hongyo, K., Kawai, T. et al. (2013) Loss of ACE2 exaggerates high-calorie diet-induced insulin resistance by reduction of GLUT4 in mice. *Diabetes* **62**, 223–233, https://doi.org/10.2337/db12-0177

72 Bataller, R., Schwabe, R.F., Choi, Y.H., Yang, L., Paik, Y.H., Lindquist, J. et al. (2003) NADPH oxidase signal transduces angiotensin II in hepatic stellate cells and is critical in hepatic fibrosis. *J. Clin. Invest.* **112**, 1383–1394, https://doi.org/10.1172/JCI18212

73 Lubel, J.S., Herath, C.B., Tchongue, J., Grace, J., Jia, Z., Spencer, K. et al. (2009) Angiotensin-(1-7), an alternative metabolite of the renin-angiotensin system, is up-regulated in human liver disease and has antifibrotic activity in the bile-duct-ligated rat. *Clin. Sci. (Lond.)* **117**, 375–386, https://doi.org/10.1042/CS20080647

74 Lang, C.C. and Struthers, A.D. (2013) Targeting the renin-angiotensin-aldosterone system in heart failure. *Nat. Rev. Cardiol.* **10**, 125–134, https://doi.org/10.1038/nrcardio.2012.196

75 Xie, X., Liu, Y., Perkovic, V., Li, X., Ninomiya, T., Hou, W. et al. (2016) Renin-Angiotensin System Inhibitors and Kidney and Cardiovascular Outcomes in Patients With CKD: A Bayesian Network Meta-analysis of Randomized Clinical Trials. *Am. J. Kidney Dis.* **67**, 728–741, https://doi.org/10.1053/j.ajkd.2015.10.011

76 Clerkin, K.J., Fried, J.A., Raikhelkar, J., Sayer, G., Griffin, J.M., Masoumi, A. et al. (2020) COVID-19 and Cardiovascular Disease. *Circulation* **141**, 1648–1655, https://doi.org/10.1161/CIRCULATIONAHA.120.046941

77 Wang, K., Gheblawi, M. and Oudit, G.Y. (2020) Angiotensin Converting Enzyme 2: A Double-Edged Sword. *Circulation* **142**, 426–428, https://doi.org/10.1161/CIRCULATIONAHA.120.047049

78 Simoes, E.S.A.C., Miranda, A.S., Rocha, N.P. and Teixeira, A.L. (2017) Renin angiotensin system in liver diseases: Friend or foe? *World J. Gastroenterol.* **23**, 3396–3406, https://doi.org/10.3748/wjg.v23.i19.3396

79 Ohishi, T., Saito, H., Tsusaka, K., Toda, K., Inagaki, H., Hamada, Y. et al. (2001) Anti-fibrogenic effect of an angiotensin converting enzyme inhibitor on chronic carbon tetrachloride-induced hepatic fibrosis in rats. *Hepatol. Res.* **21**, 147–158, https://doi.org/10.1016/S1386-6346(01)00102-4

80 Yoshiji, H., Kuriyama, S. and Fukui, H. (2002) Angiotensin-I-converting enzyme inhibitors may be an alternative anti-angiogenic strategy in the treatment of liver fibrosis and hepatocellular carcinoma. Possible role of vascular endothelial growth factor. *Tumour Biol.* **23**, 348–356, https://doi.org/10.1159/000069792

81 Yoshiji, H., Kuriyama, S., Yoshii, J., Ikenaka, Y., Noguchi, R., Nakatani, T. et al. (2001) Angiotensin-II type 1 receptor interaction is a major regulator for liver fibrosis development in rats. *Hepatology* **34**, 745–750, https://doi.org/10.1053/jhep.2001.28231

82 Kanno, K., Tazuma, S. and Chayama, K. (2003) AT1A-deficient mice show less severe progression of liver fibrosis induced by CCl(4). *Biochem. Biophys. Res. Commun.* **308**, 177–183, https://doi.org/10.1016/S0006-291X(03)01357-3

83 Wei, H.S., Lu, H.M., Li, D.G., Zhan, Y.T., Wang, Z.R., Huang, X. et al. (2000) The regulatory role of AT 1 receptor on activated HSCs in hepatic fibrogenesis:effects of RAS inhibitors on hepatic fibrosis induced by CCl(4). *World J. Gastroenterol.* **6**, 824–828, https://doi.org/10.3748/wjg.v6.i6.824

84 Corey, K.E., Shah, N., Misdraji, J., Abu Dayyeh, B.K., Zheng, H., Bhan, A.K. et al. (2009) The effect of angiotensin-blocking agents on liver fibrosis in patients with hepatitis C. *Liver Int.* **29**, 748–753, https://doi.org/10.1111/j.1478-3231.2009.01973.x

85 Yoshiji, H., Noguchi, R. and Fukui, H. (2005) Combined effect of an ACE inhibitor, perindopril, and interferon on liver fibrosis markers in patients with chronic hepatitis C. *J. Gastroenterol.* **40**, 215–216, https://doi.org/10.1007/s00535-004-1523-6

86 Yoshiji, H., Noguchi, R., Kojima, H., Ikenaka, Y., Kitade, M., Kaji, K. et al. (2006) Interferon augments the anti-fibrotic activity of an angiotensin-converting enzyme inhibitor in patients with refractory chronic hepatitis C. *World J. Gastroenterol.* **12**, 6786–6791, https://doi.org/10.3748/wjg.v12.i42.6786

87 Debernardi-Venon, W., Martini, S., Biasi, F., Vizio, B., Termine, A., Poli, G. et al. (2007) AT1 receptor antagonist Candesartan in selected cirrhotic patients: effect on portal pressure and liver fibrosis markers. *J. Hepatol.* **46**, 1026–1033, https://doi.org/10.1016/j.jhep.2007.01.017

88 Rimola, A., Londono, M.C., Guevara, G., Bruguera, M., Navasa, M., Forns, X. et al. (2004) Beneficial effect of angiotensin-blocking agents on graft fibrosis in hepatitis C recurrence after liver transplantation. *Transplantation* **78**, 686–691, https://doi.org/10.1097/01.TP.0000128913.09774.CE

89 Terui, Y., Saito, T., Watanabe, H., Togashi, H., Kawata, S., Kamada, Y. et al. (2002) Effect of angiotensin receptor antagonist on liver fibrosis in early stages of chronic hepatitis C. *Hepatology* **36**, 1022, https://doi.org/10.1053/jhep.2002.32679

90 Georgescu, E.F., Ionescu, R., Niculescu, M., Mogoanta, L. and Vancica, L. (2009) Angiotensin-receptor blockers as therapy for mild-to-moderate hypertension-associated non-alcoholic steatohepatitis. *World J. Gastroenterol.* **15**, 942–954, https://doi.org/10.3748/wjg.15.942

91 Yokohama, S., Tokusashi, Y., Nakamura, K., Tamaki, Y., Okamoto, S., Okada, M. et al. (2006) Inhibitory effect of angiotensin II receptor antagonist on hepatic stellate cell activation in non-alcoholic steatohepatitis. *World J. Gastroenterol.* **12**, 322–326, https://doi.org/10.3748/wjg.v12.i2.322

92 Yokohama, S., Yoneda, M., Haneda, M., Okamoto, S., Okada, M., Aso, K. et al. (2004) Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. *Hepatology* **40**, 1222–1225, https://doi.org/10.1002/hep.20420

93 Abu Dayyeh, B.K., Yang, M., Dienstag, J.L. and Chung, R.T. (2011) The effects of angiotensin blocking agents on the progression of liver fibrosis in the HALT-C Trial cohort. *Dig. Dis. Sci.* **56**, 564–568, https://doi.org/10.1007/s10620-010-1507-8

94 Burnier, M. and Brunner, H.R. (2000) Angiotensin II receptor antagonists. *Lancet* **355**, 637–645, https://doi.org/10.1016/S0140-6736(99)10365-9

95 Vlachogiannakos, J., Tang, A.K., Patch, D. and Burroughs, A.K. (2001) Angiotensin converting enzyme inhibitors and angiotensin II antagonists as therapy in chronic liver disease. *Gut* **49**, 303–308, https://doi.org/10.1136/gut.49.2.303
96 Tandon, P., Abraldes, J.G., Berzigotti, A., Garcia-Pagan, J.C. and Bosch, J. (2010) Renin-angiotensin-aldosterone inhibitors in the reduction of portal pressure: a systematic review and meta-analysis. *J. Hepatol.* **53**, 273–282, https://doi.org/10.1016/j.jhep.2010.03.013
97 Skeggs, Jr, L.T., Kahn, J.R. and Shumway, N.P. (1956) The preparation and function of the hypertensin-converting enzyme. *J. Exp. Med.* **103**, 295–299, https://doi.org/10.1084/jem.103.3.295
98 Vilas-Boas, W.W., Ribeiro-Oliveira, Jr, A., Pereira, R.M., Ribeiro, R.C., Almeida, J., Nadu, A.P. et al. (2009) Relationship between angiotensin-(1-7) and angiotensin II correlates with hemodynamic changes in human liver cirrhosis. *World J. Gastroenterol.* **15**, 2512–2519, https://doi.org/10.3748/wjg.15.2512
99 Lula, I., Denadai, A.L., Resende, J.M., de Sousa, F.B., de Lima, G.F., Pilo-Veloso, D. et al. (2007) Study of angiotensin-(1-7) vasoactive peptide and its beta-cyclodextrin inclusion complexes: complete sequence-specific NMR assignments and structural studies. *Peptides* **28**, 2199–2210, https://doi.org/10.1016/j.peptides.2007.08.011
100 Grobe, J.L., Mecca, A.P., Mao, H. and Katovich, M.J. (2006) Chronic angiotensin-(1-7) prevents cardiac fibrosis in DOCA-salt model of hypertension. *Am. J. Physiol. Heart Circ. Physiol.* **290**, H2417–H2423, https://doi.org/10.1152/ajpheart.01170.2005
101 Benter, I.F., Yousif, M.H., Anim, J.T., Cojocel, C. and Diz, D.I. (2006) Angiotensin-(1-7) prevents development of severe hypertension and end-organ damage in spontaneously hypertensive rats treated with L-NAME. *Am. J. Physiol. Heart Circ. Physiol.* **290**, H684–H691, https://doi.org/10.1152/ajpheart.00632.2005
102 Loot, A.E., Roks, A.J., Henning, R.H., Tio, R.A., Suurmeijer, A.J., Boomsma, F. et al. (2002) Angiotensin-(1-7) attenuates the development of heart failure after myocardial infarction in rats. *Circulation* **105**, 1548–1550, https://doi.org/10.1161/01.CIR.0000013847.07035.B9
103 Santos, S.H., Giani, J.F., Burghi, V., Miquet, J.G., Qadri, F., Braga, J.F. et al. (2014) Oral administration of angiotensin-(1-7) ameliorates type 2 diabetes in rats. *J. Mol. Med. (Berl.)* **92**, 255–265, https://doi.org/10.1007/s00109-013-1087-0
104 Yang, J.M., Dong, M., Meng, X., Zhao, Y.X., Yang, X.Y., Liu, X.L. et al. (2013) Angiotensin-(1-7) dose-dependently inhibits atherosclerotic lesion formation and enhances plaque stability by targeting vascular cells. *Arterioscler. Thromb. Vasc. Biol.* **33**, 1978–1985, https://doi.org/10.1161/ATVBAHA.113.301320
105 Marques, F.D., Ferreira, A.J., Sinisterra, R.D., Jacoby, B.A., Sousa, F.B., Caliari, M.V. et al. (2011) An oral formulation of angiotensin-(1-7) produces cardioprotective effects in infarcted and isoproterenol-treated rats. *Hypertension* **57**, 477–483, https://doi.org/10.1161/HYPERTENSIONAHA.110.167346
106 Silveira, K.D., Barroso, L.C., Vieira, A.T., Cisalpino, D., Lima, C.X., Bader, M. et al. (2013) Beneficial effects of the activation of the angiotensin-(1-7) MAS receptor in a murine model of adriamycin-induced nephropathy. *PLoS One* **8**, e66082, https://doi.org/10.1371/journal.pone.0066082
107 Carvalho, M.B., Duarte, F.V., Faria-Silva, R., Fauler, B., da Mata Machado, L.T., de Paula, R.D. et al. (2007) Evidence for Mas-mediated bradykinin potentiation by the angiotensin-(1-7) nonpeptide mimic AVE 0991 in normotensive rats. *Hypertension* **50**, 762–767, https://doi.org/10.1161/HYPERTENSIONAHA.107.094987
108 Xu, P., Costa-Goncalves, A.C., Todiras, M., Rabelo, L.A., Sampaio, W.O., Moura, M.M. et al. (2008) Endothelial dysfunction and elevated blood pressure in MAS gene-deleted mice. *Hypertension* **51**, 574–580, https://doi.org/10.1161/HYPERTENSIONAHA.107.102764
109 Herath, C.B., Mak, K. and Angus, P.W. (2015) Role of the alternate RAS in liver disease and GI tract. In *The protective arm of the renin angiotensin system: Functional aspects therapeutic implications*, First ed., (Unger, T., Steckelings, U.M. and Santos, R.A.S., eds), pp. 239–245, Elsevier, London
110 Osterreicher, C.H., Taura, K., De Minicis, S., Seki, E., Penz-Osterreicher, M., Kodama, Y. et al. (2009) Angiotensin-converting-enzyme 2 inhibits liver fibrosis in mice. *Hepatology* **50**, 929–938, https://doi.org/10.1002/hep.23104
111 Zhong, J., Basu, R., Guo, D., Chow, F.L., Byrns, S., Schuster, M. et al. (2010) Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. *Circulation* **122**, 717–728, 718 p following 728, https://doi.org/10.1161/CIRCULATIONAHA.110.955369
112 Wysocki, J., Ye, M., Rodriguez, E., Gonzalez-Pacheco, F.R., Barrios, C., Evora, K. et al. (2010) Targeting the degradation of angiotensin II with recombinant angiotensin-converting enzyme 2: prevention of angiotensin II-dependent hypertension. *Hypertension* **55**, 90–98, https://doi.org/10.1161/HYPERTENSIONAHA.109.138420
113 Shenoy, V., Kwon, K.C., Rathinasabapathy, A., Lin, S., Jin, G., Song, C. et al. (2014) Oral delivery of Angiotensin-converting enzyme 2 and Angiotensin-(1-7) bioencapsulated in plant cells attenuates pulmonary hypertension. *Hypertension* **64**, 1248–1259, https://doi.org/10.1161/HYPERTENSIONAHA.114.03871
114 Zhong, J., Guo, D., Chen, C.B., Wang, W., Schuster, M., Loibner, H. et al. (2011) Prevention of angiotensin II-mediated renal oxidative stress, inflammation, and fibrosis by angiotensin-converting enzyme 2. *Hypertension* **57**, 314–322, https://doi.org/10.1161/HYPERTENSIONAHA.110.164244
115 Oudit, G.Y., Liu, G.C., Zhong, J., Basu, R., Chow, F.L., Zhou, J. et al. (2010) Human recombinant ACE2 reduces the progression of diabetic nephropathy. *Diabetes* **59**, 529–538, https://doi.org/10.2337/db09-1218
116 Haschke, M., Schuster, M., Poglitsch, M., Loibner, H., Salzberg, M., Bruggisser, M. et al. (2013) Pharmacokinetics and pharmacodynamics of recombinant human angiotensin-converting enzyme 2 in healthy human subjects. *Clin. Pharmacokinet.* **52**, 783–792, https://doi.org/10.1007/s40262-013-0072-7
117 Khan, A., Benthin, C., Zeno, B., Albertson, T.E., Boyd, J., Christie, J.D. et al. (2017) A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit. Care* **21**, 234, https://doi.org/10.1186/s13054-017-1823-x
118 Hemnes, A.R., Rathinasabapathy, A., Austin, E.A., Brittain, E.L., Carrier, E.J., Chen, X. et al. (2018) A potential therapeutic role for angiotensin-converting enzyme 2 in human pulmonary arterial hypertension. *Eur. Respir. J.* **51**, 1702638, https://doi.org/10.1183/13993003.02638-2017
119 Rajapaksha, I., Angus, P. and Herath, C.B. (2018) Adeno-associated virus (AAV)-mediated gene therapy for disorders of inherited and non-inherited origin. In *Gene therapy* (Bachtarzi, H., ed.), IntechOpen, London, United Kingdom

Clinical Science (2020) **134** 3137–3158
https://doi.org/10.1042/CS20201268

120 Alexander, I.E., Cunningham, S.C., Logan, G.J. and Christodoulou, J. (2008) Potential of AAV vectors in the treatment of metabolic disease. *Gene Ther.* **15**, 831–839, https://doi.org/10.1038/gt.2008.64
121 Ferreira, V., Petry, H. and Salmon, F. (2014) Immune Responses to AAV-Vectors, the Glybera Example from Bench to Bedside. *Front. Immunol.* **5**, 82, https://doi.org/10.3389/fimmu.2014.00082
122 Fickert, P., Fuchsbichler, A., Wagner, M., Zollner, G., Kaser, A., Tilg, H. et al. (2004) Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. *Gastroenterology* **127**, 261–274, https://doi.org/10.1053/j.gastro.2004.04.009
123 Fickert, P., Zollner, G., Fuchsbichler, A., Stumptner, C., Weiglein, A.H., Lammert, F. et al. (2002) Ursodeoxycholic acid aggravates bile infarcts in bile duct-ligated and Mdr2 knockout mice via disruption of cholangioles. *Gastroenterology* **123**, 1238–1251, https://doi.org/10.1053/gast.2002.35948
124 Van Nieuwkerk, C.M., Elferink, R.P., Groen, A.K., Ottenhoff, R., Tytgat, G.N., Dingemans, K.P. et al. (1996) Effects of Ursodeoxycholate and cholate feeding on liver disease in FVB mice with a disrupted mdr2 P-glycoprotein gene. *Gastroenterology* **111**, 165–171, https://doi.org/10.1053/gast.1996.v111.pm8698195
125 Johns Hopkins Coronavirus Resource Centre (2020) COVID-19 dashboard.
126 Mesel-Lemoine, M., Millet, J., Vidalain, P.O., Law, H., Vabret, A., Lorin, V. et al. (2012) A human coronavirus responsible for the common cold massively kills dendritic cells but not monocytes. *J. Virol.* **86**, 7577–7587, https://doi.org/10.1128/JVI.00269-12
127 Pitlik, S.D. (2020) COVID-19 Compared to Other Pandemic Diseases. *Rambam Maimonides Med. J.* **11**, https://doi.org/10.5041/RMMJ.10418
128 World Health Organization (WHO) (2004) Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003.
129 World Health Organization (WHO) (2019) Middle East respiratory syndrome coronavirus (MERS-CoV).
130 Andersen, K.G., Rambaut, A., Lipkin, W.I., Holmes, E.C. and Garry, R.F. (2020) The proximal origin of SARS-CoV-2. *Nat. Med.* **26**, 450–452, https://doi.org/10.1038/s41591-020-0820-9
131 Zhu, Z., Lian, X., Su, X., Wu, W., Marraro, G.A. and Zeng, Y. (2020) From SARS and MERS to COVID-19: a brief summary and comparison of severe acute respiratory infections caused by three highly pathogenic human coronaviruses. *Respir. Res.* **21**, 224, https://doi.org/10.1186/s12931-020-01479-w
132 Wu, F., Zhao, S., Yu, B., Chen, Y.M., Wang, W., Song, Z.G. et al. (2020) A new coronavirus associated with human respiratory disease in China. *Nature* **579**, 265–269, https://doi.org/10.1038/s41586-020-2008-3
133 Kim, D., Lee, J.Y., Yang, J.S., Kim, J.W., Kim, V.N. and Chang, H. (2020) The Architecture of SARS-CoV-2 Transcriptome. *Cell* **181**, 914e910–921e910, https://doi.org/10.1016/j.cell.2020.04.011
134 Walls, A.C., Tortorici, M.A., Snijder, J., Xiong, X., Bosch, B.J., Rey, F.A. et al. (2017) Tectonic conformational changes of a coronavirus spike glycoprotein promote membrane fusion. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 11157–11162, https://doi.org/10.1073/pnas.1708727114
135 Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.L., Abiona, O. et al. (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **367**, 1260–1263, https://doi.org/10.1126/science.abb2507
136 Hamming, I., Timens, W., Bulthuis, M.L., Lely, A.T., Navis, G. and van Goor, H. (2004) Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* **203**, 631–637, https://doi.org/10.1002/path.1570
137 Lan, J.H., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S. et al. (2020) Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* **581**, 215–220, https://doi.org/10.1038/s41586-020-2180-5
138 Jaimes, J.A., Andre, N.M., Chappie, J.S., Millet, J.K. and Whittaker, G.R. (2020) Phylogenetic Analysis and Structural Modeling of SARS-CoV-2 Spike Protein Reveals an Evolutionary Distinct and Proteolytically Sensitive Activation Loop. *J. Mol. Biol.* **432**, 3309–3325, https://doi.org/10.1016/j.jmb.2020.04.009
139 Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z. et al. (2020) Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell* **181**, 894e899–904e899, https://doi.org/10.1016/j.cell.2020.03.045
140 Li, F. (2008) Structural analysis of major species barriers between humans and palm civets for severe acute respiratory syndrome coronavirus infections. *J. Virol.* **82**, 6984–6991, https://doi.org/10.1128/JVI.00442-08
141 Wu, K., Peng, G., Wilken, M., Geraghty, R.J. and Li, F. (2012) Mechanisms of host receptor adaptation by severe acute respiratory syndrome coronavirus. *J. Biol. Chem.* **287**, 8904–8911, https://doi.org/10.1074/jbc.M111.325803
142 Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H. et al. (2020) Structural basis of receptor recognition by SARS-CoV-2. *Nature* **581**, 221–224, https://doi.org/10.1038/s41586-020-2179-y
143 Straus, M.R., Tang, T., Lai, A.L., Flegel, A., Bidon, M., Freed, J.H. et al. (2020) Ca(2+) Ions Promote Fusion of Middle East Respiratory Syndrome Coronavirus with Host Cells and Increase Infectivity. *J. Virol.* **94**, e00426–20, https://doi.org/10.1128/JVI.00426-20
144 Millet, J.K. and Whittaker, G.R. (2018) Physiological and molecular triggers for SARS-CoV membrane fusion and entry into host cells. *Virology* **517**, 3–8, https://doi.org/10.1016/j.virol.2017.12.015
145 Cannalire, R., Stefanelli, I., Cerchia, C., Beccari, A.R., Pelliccia, S. and Summa, V. (2020) SARS-CoV-2 Entry Inhibitors: Small Molecules and Peptides Targeting Virus or Host Cells. *Int. J. Mol. Sci.* **21**, https://doi.org/10.3390/ijms21165707
146 Gierer, S., Bertram, S., Kaup, F., Wrensch, F., Heurich, A., Kramer-Kuhl, A. et al. (2013) The spike protein of the emerging betacoronavirus EMC uses a novel coronavirus receptor for entry, can be activated by TMPRSS2, and is targeted by neutralizing antibodies. *J. Virol.* **87**, 5502–5511, https://doi.org/10.1128/JVI.00128-13
147 Shirato, K., Kawase, M. and Matsuyama, S. (2013) Middle East respiratory syndrome coronavirus infection mediated by the transmembrane serine protease TMPRSS2. *J. Virol.* **87**, 12552–12561, https://doi.org/10.1128/JVI.01890-13
148 Dube, M., Rey, F.A. and Kielian, M. (2014) Rubella virus: first calcium-requiring viral fusion protein. *PLoS Pathog.* **10**, e1004530, https://doi.org/10.1371/journal.ppat.1004530
149 Lai, A.L., Millet, J.K., Daniel, S., Freed, J.H. and Whittaker, G.R. (2017) The SARS-CoV Fusion Peptide Forms an Extended Bipartite Fusion Platform that Perturbs Membrane Order in a Calcium-Dependent Manner. *J. Mol. Biol.* **429**, 3875–3892, https://doi.org/10.1016/j.jmb.2017.10.017

150 Nathan, L., Lai, A.L., Millet, J.K., Straus, M.R., Freed, J.H., Whittaker, G.R. et al. (2020) Calcium Ions Directly Interact with the Ebola Virus Fusion Peptide To Promote Structure-Function Changes That Enhance Infection. *ACS Infect. Dis.* **6**, 250–260, https://doi.org/10.1021/acsinfecdis.9b00296
151 Dube, M., Etienne, L., Fels, M. and Kielian, M. (2016) Calcium-Dependent Rubella Virus Fusion Occurs in Early Endosomes. *J. Virol.* **90**, 6303–6313, https://doi.org/10.1128/JVI.00634-16
152 Chapple, C.E., Robisson, B., Spinelli, L., Guien, C., Becker, E. and Brun, C. (2015) Extreme multifunctional proteins identified from a human protein interaction network. *Nat. Commun.* **6**, 7412, https://doi.org/10.1038/ncomms8412
153 Calabro, S.R., Maczurek, A.E., Morgan, A.J., Tu, T., Wen, V.W., Yee, C. et al. (2014) Hepatocyte produced matrix metalloproteinases are regulated by CD147 in liver fibrogenesis. *PLoS One* **9**, e90571, https://doi.org/10.1371/journal.pone.0090571
154 Yurchenko, V., Constant, S., Eisenmesser, E. and Bukrinsky, M. (2010) Cyclophilin-CD147 interactions: a new target for anti-inflammatory therapeutics. *Clin. Exp. Immunol.* **160**, 305–317, https://doi.org/10.1111/j.1365-2249.2010.04115.x
155 Agrawal, S.M., Silva, C., Tourtellotte, W.W. and Yong, V.W. (2011) EMMPRIN: a novel regulator of leukocyte transmigration into the CNS in multiple sclerosis and experimental autoimmune encephalomyelitis. *J. Neurosci.* **31**, 669–677, https://doi.org/10.1523/JNEUROSCI.3659-10.2011
156 Yurchenko, V., O'Connor, M., Dai, W.W., Guo, H., Toole, B., Sherry, B. et al. (2001) CD147 is a signaling receptor for cyclophilin B. *Biochem. Biophys. Res. Commun.* **288**, 786–788, https://doi.org/10.1006/bbrc.2001.5847
157 Zhu, P., Lu, N., Shi, Z.G., Zhou, J., Wu, Z.B., Yang, Y. et al. (2006) CD147 overexpression on synoviocytes in rheumatoid arthritis enhances matrix metalloproteinase production and invasiveness of synoviocytes. *Arthritis Res. Ther.* **8**, R44, https://doi.org/10.1186/ar1899
158 Yee, C., Main, N.M., Terry, A., Stevanovski, I., Maczurek, A., Morgan, A.J. et al. (2019) CD147 mediates intrahepatic leukocyte aggregation and determines the extent of liver injury. *PLoS One* **14**, e0215557, https://doi.org/10.1371/journal.pone.0215557
159 Berzsenyi, M.D., Roberts, S.K., Preiss, S., Woollard, D.J., Beard, M.R., Skinner, N.A. et al. (2011) Hepatic TLR2 & TLR4 expression correlates with hepatic inflammation and TNF-alpha in HCV & HCV/HIV infection. *J. Viral Hepat.* **18**, 852–860
160 Watanabe, A., Yoneda, M., Ikeda, F., Terao-Muto, Y., Sato, H. and Kai, C. (2010) CD147/EMMPRIN acts as a functional entry receptor for measles virus on epithelial cells. *J. Virol.* **84**, 4183–4193, https://doi.org/10.1128/JVI.02168-09
161 Helal, M.A., Shouman, S., Abdelwaly, A., Elmehrath, A.O., Essawy, M., Sayed, S.M. et al. (2020) Molecular basis of the potential interaction of SARS-CoV-2 spike protein to CD147 in COVID-19 associated-lymphopenia. *J. Biomol. Struct. Dyn.*, https://doi.org/10.1080/07391102.2020.1822208
162 Radzikowska, U., Ding, M., Tan, G., Zhakparov, D., Peng, Y., Wawrzyniak, P. et al. (2020) Distribution of ACE2, CD147, CD26, and other SARS-CoV-2 associated molecules in tissues and immune cells in health and in asthma, COPD, obesity, hypertension, and COVID-19 risk factors. *Allergy* **75**, 2829–2845, https://doi.org/10.1111/all.14429
163 Liu, C., von Brunn, A. and Zhu, D. (2020) Cyclophilin A and CD147: novel therapeutic targets for the treatment of COVID-19. *Med. Drug Discov.* **7**, 100056, https://doi.org/10.1016/j.medidd.2020.100056
164 Huang, W., Luo, W.J., Zhu, P., Tang, J., Yu, X.L., Cui, H.Y. et al. (2013) Modulation of CD147-induced matrix metalloproteinase activity: role of CD147 N-glycosylation. *Biochem. J.* **449**, 437–448, https://doi.org/10.1042/BJ20120343
165 Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B. et al. (2005) Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **436**, 112–116, https://doi.org/10.1038/nature03712
166 Samavati, L. and Uhal, B.D. (2020) ACE2, Much More Than Just a Receptor for SARS-COV-2. *Front. Cell Infect. Microbiol.* **10**, 317, https://doi.org/10.3389/fcimb.2020.00317
167 Li, W., Moore, M.J., Vasilieva, N., Sui, J., Wong, S.K., Berne, M.A. et al. (2003) Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450–454, https://doi.org/10.1038/nature02145
168 Li, F., Li, W., Farzan, M. and Harrison, S.C. (2005) Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* **309**, 1864–1868, https://doi.org/10.1126/science.1116480
169 Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B. et al. (2005) A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.* **11**, 875–879, https://doi.org/10.1038/nm1267
170 Moore, J.B. and June, C.H. (2020) Cytokine release syndrome in severe COVID-19. *Science* **368**, 473–474, https://doi.org/10.1126/science.abb8925
171 Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y. et al. (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497–506, https://doi.org/10.1016/S0140-6736(20)30183-5
172 Shi, S., Qin, M., Shen, B., Cai, Y., Liu, T., Yang, F. et al. (2020) Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China. *JAMA Cardiol.* **5**, 802–810, https://doi.org/10.1001/jamacardio.2020.0950
173 Klok, F.A., Kruip, M., van der Meer, N.J.M., Arbous, M.S., Gommers, D., Kant, K.M. et al. (2020) Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: An updated analysis. *Thromb. Res.* **191**, 148–150, https://doi.org/10.1016/j.thromres.2020.04.041
174 Farcas, G.A., Poutanen, S.M., Mazzulli, T., Willey, B.M., Butany, J., Asa, S.L. et al. (2005) Fatal severe acute respiratory syndrome is associated with multiorgan involvement by coronavirus. *J. Infect. Dis.* **191**, 193–197, https://doi.org/10.1086/426870
175 Gembardt, F., Sterner-Kock, A., Imboden, H., Spalteholz, M., Reibitz, F., Schultheiss, H.P. et al. (2005) Organ-specific distribution of ACE2 mRNA and correlating peptidase activity in rodents. *Peptides* **26**, 1270–1277, https://doi.org/10.1016/j.peptides.2005.01.009
176 Sun, J., Aghemo, A., Forner, A. and Valenti, L. (2020) COVID-19 and liver disease. *Liver Int.* **40**, 1278–1281, https://doi.org/10.1111/liv.14470
177 Liu, F., Long, X., Zhang, B., Zhang, W., Chen, X. and Zhang, Z. (2020) ACE2 Expression in Pancreas May Cause Pancreatic Damage After SARS-CoV-2 Infection. *Clin. Gastroenterol. Hepatol.* **18**, 2128e2122–2130e2122, https://doi.org/10.1016/j.cgh.2020.04.040
178 Cai, Q., Huang, D., Yu, H., Zhu, Z., Xia, Z., Su, Y. et al. (2020) COVID-19: Abnormal liver function tests. *J. Hepatol.* **73**, 566–574, https://doi.org/10.1016/j.jhep.2020.04.006
179 Ji, D., Qin, E., Xu, J., Zhang, D., Cheng, G., Wang, Y. et al. (2020) Non-alcoholic fatty liver diseases in patients with COVID-19: A retrospective study. *J. Hepatol.* **73**, 451–453, https://doi.org/10.1016/j.jhep.2020.03.044

Clinical Science (2020) **134** 3137–3158
https://doi.org/10.1042/CS20201268

180 Zhao, B., Ni, C., Gao, R., Wang, Y., Yang, L., Wei, J. et al. (2020) Recapitulation of SARS-CoV-2 infection and cholangiocyte damage with human liver ductal organoids. *Protein Cell* **11**, 771–775, https://doi.org/10.1007/s13238-020-00718-6
181 Guan, W.J., Ni, Z.Y., Hu, Y., Liang, W.H., Ou, C.Q., He, J.X. et al. (2020) Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* **382**, 1708–1720, https://doi.org/10.1056/NEJMoa2002032
182 Yang, X., Yu, Y., Xu, J., Shu, H., Xia, J., Liu, H. et al. (2020) Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir. Med.* **8**, 475–481, https://doi.org/10.1016/S2213-2600(20)30079-5
183 Monteil, V., Kwon, H., Prado, P., Hagelkruys, A., Wimmer, R.A., Stahl, M. et al. (2020) Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. *Cell* **181**, 905e907–913e907, https://doi.org/10.1016/j.cell.2020.04.004
184 Xu, R., Shi, M., Li, J., Song, P. and Li, N. (2020) Construction of SARS-CoV-2 Virus-Like Particles by Mammalian Expression System. *Front. Bioeng. Biotechnol.* **8**, 862, https://doi.org/10.3389/fbioe.2020.00862
185 Falade-Nwulia, O., Suarez-Cuervo, C., Nelson, D.R., Fried, M.W., Segal, J.B. and Sulkowski, M.S. (2017) Oral Direct-Acting Agent Therapy for Hepatitis C Virus Infection: A Systematic Review. *Ann. Intern. Med.* **166**, 637–648, https://doi.org/10.7326/M16-2575
186 Wu, C.H., Mohammadmoradi, S., Chen, J.Z., Sawada, H., Daugherty, A. and Lu, H.S. (2018) Renin-Angiotensin System and Cardiovascular Functions. *Arterioscler. Thromb. Vasc. Biol.* **38**, e108–e116, https://doi.org/10.1161/ATVBAHA.118.311282
187 Ferrario, C.M., Trask, A.J. and Jessup, J.A. (2005) Advances in biochemical and functional roles of angiotensin-converting enzyme 2 and angiotensin-(1-7) in regulation of cardiovascular function. *Am. J. Physiol. Heart Circ. Physiol.* **289**, H2281–H2290, https://doi.org/10.1152/ajpheart.00618.2005
188 Crackower, M.A., Sarao, R., Oudit, G.Y., Yagil, C., Kozieradzki, I., Scanga, S.E. et al. (2002) Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* **417**, 822–828, https://doi.org/10.1038/nature00786
189 Yang, T. and Xu, C. (2017) Physiology and Pathophysiology of the Intrarenal Renin-Angiotensin System: An Update. *J. Am. Soc. Nephrol.* **28**, 1040–1049, https://doi.org/10.1681/ASN.2016070734
190 Hashimoto, T., Perlot, T., Rehman, A., Trichereau, J., Ishiguro, H., Paolino, M. et al. (2012) ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* **487**, 477–481, https://doi.org/10.1038/nature11228
191 Ferreira, A.J. and Raizada, M.K. (2008) Are we poised to target ACE2 for the next generation of antihypertensives? *J. Mol. Med. (Berl.)* **86**, 685–690, https://doi.org/10.1007/s00109-008-0339-x
192 Guy, J.L., Jackson, R.M., Jensen, H.A., Hooper, N.M. and Turner, A.J. (2005) Identification of critical active-site residues in angiotensin-converting enzyme-2 (ACE2) by site-directed mutagenesis. *FEBS J.* **272**, 3512–3520, https://doi.org/10.1111/j.1742-4658.2005.04756.x
193 Turner, A.J., Tipnis, S.R., Guy, J.L., Rice, G. and Hooper, N.M. (2002) ACEH/ACE2 is a novel mammalian metallocarboxypeptidase and a homologue of angiotensin-converting enzyme insensitive to ACE inhibitors. *Can. J. Physiol. Pharmacol.* **80**, 346–353, https://doi.org/10.1139/y02-021
194 Guy, J.L., Jackson, R.M., Acharya, K.R., Sturrock, E.D., Hooper, N.M. and Turner, A.J. (2003) Angiotensin-converting enzyme-2 (ACE2): comparative modeling of the active site, specificity requirements, and chloride dependence. *Biochemistry* **42**, 13185–13192, https://doi.org/10.1021/bi035268s
195 Bourgonje, A.R., Abdulle, A.E., Timens, W., Hillebrands, J.L., Navis, G.J., Gordijn, S.J. et al. (2020) Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *J. Pathol.* **251**, 228–248, https://doi.org/10.1002/path.5471
196 Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y. and Zhou, Q. (2020) Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* **367**, 1444–1448, https://doi.org/10.1126/science.abb2762
197 Benítez-Cardoza, C.G. and Vique-Sánchez, J.L. (2020) Potential inhibitors of the interaction between ACE2 and SARS-CoV-2 (RBD), to develop a drug. *Life Sci.* **256**, 117970, https://doi.org/10.1016/j.lfs.2020.117970
198 Hanson, Q.M., Wilson, K.M., Shen, M., Itkin, Z., Eastman, R.T., Shinn, P. et al. (2020) Targeting ACE2-RBD interaction as a platform for COVID19 therapeutics: Development and drug repurposing screen of an AlphaLISA proximity assay. *ACS Pharmacol. Transl. Sci.* , https://doi.org/10.1101/2020.06.16.154708
199 Hu ang, Y., Yang, C., Xu, X.F., Xu, W. and Liu, S.W. (2020) Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. *Acta Pharmacol. Sin.* **41**, 1141–1149, https://doi.org/10.1038/s41401-020-0485-4
200 Wójcik, P. and Berlicki, L (2016) Peptide-based inhibitors of protein-protein interactions. *Bioorg. Med. Chem. Lett.* **26**, 707–713, https://doi.org/10.1016/j.bmcl.2015.12.084
201 Morris, G.M., Huey, R. and Olson, A.J. (2008) Using AutoDock for ligand-receptor docking. *Curr. Protoc. Bioinformatics* **24**, Chapter 8, Unit 8.14, https://doi.org/10.1002/0471250953.bi0814s24
202 Trott, O. and Olson, A.J. (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **31**, 455–461
203 Irwin, J.J. and Shoichet, B.K. (2005) ZINC–a free database of commercially available compounds for virtual screening. *J. Chem. Inf. Model.* **45**, 177–182, https://doi.org/10.1021/ci049714+