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Angiotensin-Converting Enzyme 2: A new component of renin-angiotensin system (RAS) and a receptor for SARS-coronavirus-2

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Abstract

The classical renin–angiotensin system (RAS) regulates the physiological hemostasis and diseases of cardiovascular system. Recently, with the outbreak of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causing the disease COVID-19, scientists are struggling to understand how SARS-CoV-2 resembles and differs from SARS-CoV in the genomic and transcriptional levels. In short period, a growing body of research has been released and reported about new factors in RAS, such as angiotensin converting enzyme 2 (ACE2) and angiotensin (1-7) [Ang (1-7)]. The ACE2 has been shown to be protective factor in cardiovascular diseases through different mechanisms and also an important tool for entry and pathogenesis of SARS-CoVs. This review summarizes the current knowledge about ACE2 as a new factor that broadened the activity of the RAS and at the same time as a receptor for SARS-CoV and SARS-CoV-2 facilitating cell entry.

Conclusion: Grasping the cellular activity of ACE2 and Ang (1-7) is required for better therapies for cardiovascular diseases. In addition, strategies targeting ACE2 may offer beside vaccines a novel approach in the search for prevention and management of COVID 19.

Keywords: Severe acute respiratory syndrome (SARS), Coronavirus-2 (COV-2), Angiotensin converting enzyme 2 (ACE 2), Renin angiotensin system (RAS), Angiotensin (1-7) [Ang (1-7)].

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الانزيم المحول للأنجيوتنسين 2: عنصر جديد في نظام الرينين أنجيوتنسين (RAS) ومستقبل ل SARS-coronavirus-2

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ملخص الدراسة

يعمل نظام الرينين -أنجيوتنسين التقليدي (RAS) على تنظيم الوظائف الفسيولوجية وأمراض واضطرابات نظام القلب والأوعية الدموية. ومؤخراً، ومع تفشي متلازمة الالتهاب الرئوي الحاد للفيروس التاجي -2 (SARS-CoV-2) المسمى سارس والمسبب لمرض الكوفيد 19، شرع العلماء لفهم أوجه الشبه والاختلاف بين سارس- CoV-2 و SARS- CoV في المستويات الجينومية والنسخية. وفي الفترة الأخيرة تم إصدار أعداد ومجموعات متزايدة من البحوث التي تناولت العوامل الجديدة في نظام الرنين-انجيوتنسين، مثل الإنزيم المحول للأنجيوتنسين 2 والمسمى (ACE2) والأنجيوتنسين (1-7). وقد تم أثبات أن ACE2عامل وقائي في أمراض القلب والأوعية الدموية من خلال آليات مختلفة وأيضا أداة هامة ومستقبل لدخول الفيروسات التاجية وكعامل ممرض لها مسببا الالتهاب الرؤي. ويلخص هذا الاستعراض ما تناولته المراجع للمعرفة الحالية لانزيم ACE2 كعامل جديد وموسع لنشاط RASوفي نفس الوقت كمستقبل للفيروس SARS CoV & SARS-CoV-2 ومسهل لدخولهما الخلية.

الاستنتاج: إن استيعاب النشاط الخلوي لـ ACE2 و (1-7) Angمطلوب لعلاج أفضل لأمراض القلب والأوعية الدموية. وبالإضافة إلى ذلك، قد تقدم الاستراتيجيات التي تستهدف ACE2 بجانب اللقاحات نهجا جديدا في البحث عن الوقاية والعلاج لمرض COVID 19.

الكلمات مفتاحية: متلازمة الالتهاب الرئوي الحاد، الفيروس التاجي -2، الإنزيم المحول للانجيوتنسين-2، نظام الرنين الانجيوتنسين، انجيوتنسين (1-7).

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Introduction

The classical renin–angiotensin system (RAS) that has been evolved over 60 years influences multiple physiological pathways at both intracellular and endocrine levels. In addition, local RASs are present in various tissue throughout the body. RAS, both circulating and tissue RAS, regulates the cardiovascular function and also possesses a causative role in the development of hypertension [1]. Nowadays, understanding of RAS has substantially increased and seems to be more complex than previously thought. In a simplified pathway, the RAS exerts its action through the hormone angiotensin II (Agn II), a key molecule, resulting in multiple effects. In this system, two proteolytic enzymes are involved which are the aspartic protease renin and the angiotensin-converting enzyme (ACE) that was discovered by Leonard Skeggs in 1956 [2].

Renin transforms angiotensinogen, an α-2 globulin produced by the liver and released into the circulation, into the decapeptide angiotensin I (Ang I). Then, ACE converts Ang I into the octapeptide Ang II [3], one of the major players in RAS, Figure 1. It is worth to mention that Ang II is also converted into Ang III [4,5] and then Ang IV [6] which are shorter peptide fragments of RAS with lower plasma levels and activity [7]. For details, RAS activities have been described by Castrop *et al* [8].

Most of the major known actions of RAS are related to the activity of Ang II. Moreover, it is well known that increase activity of RAS is associated with numerous cardiovascular disorders. Ang II acts on various receptors such as Ang II type 1 (AT1R) and type 2 receptors (AT2R) [9,10]. Following binding to AT1R, Ang II induces pleiotropic effects including vasoconstriction, cell proliferation (mitogenic effect), inflammation, generation of reactive oxygen species, fibrosis and tubular ion exchange [11] as well as induction of plasminogen activator inhibitor-1 [12]. Additionally, nitric oxide reduction is mediated through AT1R by inducing excessive release of vascular superoxide radicals produced by stimulation of NADH/NADPH oxidase which inactivates nitric oxide synthase [13]. Under pathological conditions, Ang II has been shown to promote cardiac hypertrophy remodeling by stimulation of matrix protein expression and PAI-1 [14], vascular remodeling and extracellular matrix deposition [15]. All these effects are implicated in the development of cardiovascular disorders including hypertension, atherosclerosis and heart failure. On the other hand, binding of Ang II to AT2R promotes vasodilatation and inhibition of cell growth. These effects seem to be minor to Ang II.

Angiotensin-Converting Enzyme 2 (ACE2): A new RAS component

The enzyme that hydrolyzes Ang II and lowers blood pressure is called angiotensin converting enzyme 2 (ACE2) [16]. ACE2 was discovered independently by two research groups in 2000. Tipnis *et al* [17] and Donoghue *et al* [16] have used differing genomic-based strategies and naming it ACE2. It is the human homologue of ACE but it seems to have different substrate specificity, which is why captopril and lisinopril are ineffective to inhibit and

subsequently reduce the proteolytical activity of ACE2 [17,18]. It has been found that ACE 2, as ACE, is a type I integral membrane protein, fixed on the cell surface to hydrolyze circulating peptides [8].

ACE2 has the ability to hydrolyze angiotensin 1 into Ang (1-9) [16] and Ang II into the heptapeptide angiotensin (1-7) [Ang (1-7)] by cleaving the carboxy-terminal amino acid from Ang II [19,20]. Ang (1-7) is then metabolized by ACE into inactive fragment Ang (1-5) and ACE-inhibitors prolong Ang (1-7) half-life [21]. Due to these actions ACE2 represents as a new component and broadens the activity of RAS. A study in China mentioned that the hydrolysis of Ang II by ACE 2 is with much higher efficiency than that for Ang 1 [22], where it is estimated to be approximately 90% active towards Ang II [23].
Ang (1-7) acts through its specific receptor, G-protein-coupled receptor, called Mas [24,25] which mediates vasodilatation, antiproliferation and apoptosis [26]. These actions place ACE2 as a counteractor of RAS with a protective effect. On other side, ACE2 is also able to hydrolyze opioid dynorphin A, apelin-13 and ghrelin [20,24].

Interestingly, there are two forms of the ACE2 enzyme; the membrane bound and a soluble form, which is separated from the membrane surface by proteolytic cleavage and needed further identification and characterization [17,27,28].
Although the establishment of the formation of soluble form came first from in vitro studies [16,17]. Recently, in vivo studies have detected the soluble form in certain human body fluid, including urine, plasma, and cell culture medium by different technique [29,30].

Previously, some effects of the Ang (1-7) have been reported in laboratory animal studies and in vitro using isolated organ [31,32]. Ang (1-7) induces distinct actions including increase release of prostaglandin E2 from isolated rabbit vasa deferentia [32], counteract the detrimental action of Ang II, including vasoconstriction [26], vasopressin release in the brain [31], dilation of porcine coronary artery rings [33], diuretic actions and increase the glomerular filtration rate in rats [34] and natriuretic actions associated with prostaglandin I_2 release in isolated rat kidneys [35].

Recently, a growing body of evidence indicates that the described Ang (1-7), formed by the activity of ACE and ACE2 [8,20] see (Figure1), also showing these effects in human tissues, plays a role in metabolic pathways in human endothelial cells [36] and increases the functional spectrum of RAS through counteracting the detrimental vasoconstricting and mitogenic effects of Ang II [37]. Moreover, Ang (1-7) may contribute to cardiovascular regulation since it is devoid of vasoconstrictor and central pressor actions [38]. Furthermore, the signaling mechanisms stand behind these effects need further investigations and illustrations [36].

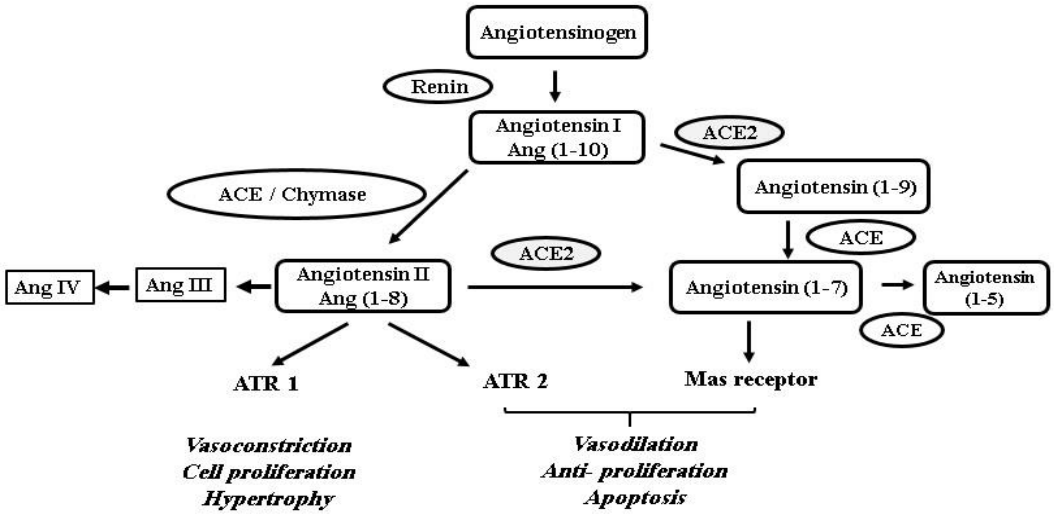

Figure 1: Angiotensin Converting Enzyme 2 in Renin Angiotensin System

Angiotensinogen is transformed by renin to octapeptide angiotensin I that is converted into decapeptide angiotensin II by angiotensin converting enzyme (ACE) or local by chymase. Angiotensin II acts on angiotensin receptor 1 (AT1R) and angiotensin receptor 2 (AT2R) where its major actions are mediated through AT1R. Angiotensin II can be converted into angiotensin III and angiotensin IV which have lower activity. ACE2 cleaves Angiotensin I into Angiotensin (1-9) that can be converted by ACE into the heptapeptide Angiotensin (1-7). ACE2 hydrolysis Angiotensin II into Angiotensin (1-7) with higher efficiency than that for Angiotensin I into Angiotensin (1-9). Angiotensin (1-7) acts on Mas receptors and oppose the effects of Angiotensin II.

Concerning the molecular mechanism of Ang (1-7), Mas receptors can be stimulated by Ang (1-7) or to a lesser extent by other angiotensin peptides [39]. This stimulation induces the release of arachidonic acid [25], nitric oxide [40,41] and to upregulate ACE2 expression [42] which represents a positive feedback mechanism as part of counterbalance pathway of RAS. In addition, the activation of Mas receptors by ang (1-7) can also alter other signaling pathways [43].

Some studies have reported that at least some of the beneficial effects of using ACEI or ARBs could be related to increase expression and activity of ACE2. This has been investigated in in vitro where it has been shown that administration of ACEIs [44] or ARBs [45] in normal and post-MI rats increased cardiac ACE2 mRNA, protein and activity. Moreover, future research has to deal with the most addressed questions and problems that focus on the possible therapeutic potential of ACE2, Ang (1-7) and its Mas receptors representing the alternative pathway of RAS.

Angiotensin-converting enzyme 2: A receptor for SARS-Coronavirus-2

Since the identification of severe acute respiratory syndrome (SARS) as a new illness spread in 2002-2003, a huge body of investigations and experiments has been undergoing in order to identify the pathogen, its life cycle and possible therapeutic potentials [46], which culminated

with the identity proof of a new SARS-coronavirus (SARS-CoV) as the SARS pathogen [47].

In vitro studies, using different cell lines**,** demonstrated that ACE2 protein is a SARS-CoV receptor in vitro [48]. Later on, in 2005, Kuba *et al* [49]. Reported the first genetic proof that ACE2 is a vital SARS-CoV receptor in vivo. This receptor is the key for virus entry and is required to facilitate binding to the virus through its spike protein for entry into host cells and subsequently virus replication. In addition, Zhou *et al*. (2020**)** demonstrated through in vitro experiments using different cells with human ACE2 that SARS-CoV2 uses ACE2 as a cellular entry receptor, too [50].

In fact, coronaviruses contain main structural proteins, the envelop, membrane, nucleocapsid and the spike protein (termed S protein) which is a transmembrane spike glycoprotein positioned on the surface [51]. Through its two distinct functional domains (S1and S2 subunit), the spike plays a role in viral binding, fusion, entry and induction of neutralizing antibody and T-cell responses [47,52]. The S1/S2 cleavage site (called Furin cleave site) in the S protein distinguishes SARS-CoV-2 from SARS-CoV, that does not bear this cleavage site [47,53,54]. It has been reported that both SARS-CoV and SARS- CoV-2 share ACE2 as receptor to bind on cells and TMPRSS2 (transmembrane protease serine 2) as the main protease facilitating their entry into cells [50]. But, opposite to SARS-CoV, SARS-CoV-2 bears a Furin cleavage site, as mentioned before, and the cleavage at this site by Furin increases binding affinity of SARS-CoV-2 to ACE 2 [55,56]. The present of Furin with TMPRSS2 together might increase permissiveness of respiratory tract cells for SARS-CoV-2 and S protein priming after ACE2 receptor binding, (Figure 2). That is why SARS CoV-2 has a higher infection rate than SARS CoV. Furin is an enzyme cleaves paired amino acids and is highly expressed in lungs [56].

Interestingly the SARS-CoV infections and the Spike protein of the SARS-CoV reduce ACE2 expression. After binding the SARS-CoV Surface-Spike protein to ACE2 receptor, the expression of these receptors is downmodulated as shown in mice model [49]. Loss of ACE expression leads to increase activity of RAS resulting in acute lung failure [57]. So, the lethality of SARS-CoV-2 is partly mediated through over suppression of ACE2. Imai *et al.* have shown that RAS is implicated in severe acute lung injury and the SARS-CoV receptor ACE 2, as the counteractor of Ang II, plays a protective role in acute lung injury as shown in mice model [57]. Following virus binding, suppression of ACE 2 receptors results in deregulation of RAS promoting Ang II mediated disease pathogenesis inducing lung edemas and impairment of lung function. This damage to the lung has been proven by injecting SARS-CoV Spike into mice which has worsen lung injury that is reversed by ARBs (angiotensin receptor blockers) treatment [49,57].

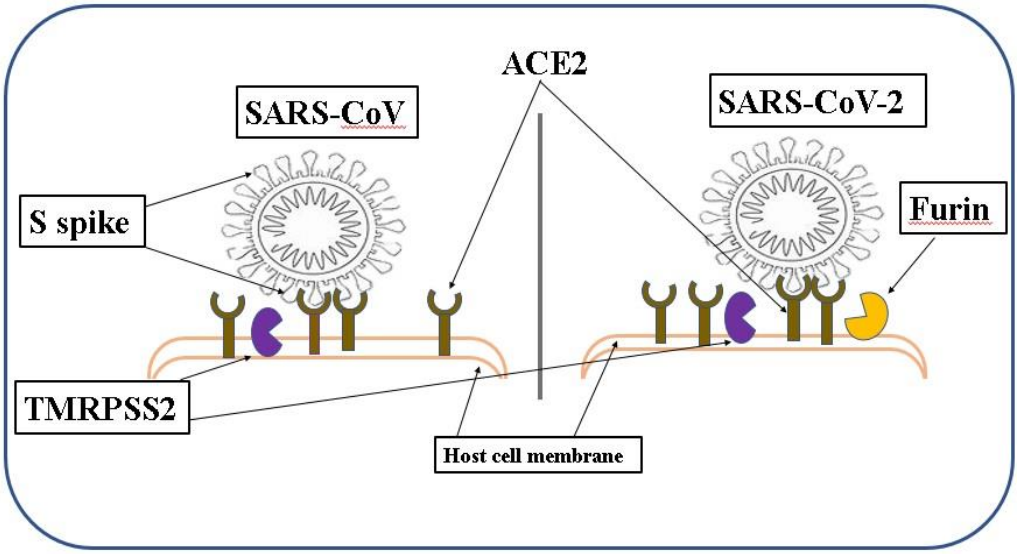

Figure 2: Binding of SARS-CoV-2 to Its Receptor on the Host Cell Membrane

The SARS-CoV S spike attaches to angiotensin converting enzyme 2 (ACE 2), then the transmembrane protease serine 2 (TMRPSS 2) cleaves the S protein this facilitates completely the entry of SARS-CoV but not SARS-CoV-2. SARS-CoV-2 is additionally and efficiently cleaved by the protease priming Furin at the cleavage site in S spike that facilitates its entry after receptor binding.

After the evolution of the positive-sense RNA virus termed the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019 (and temporary called 2019-nCoV) and by using computer modeling, many similarities have been found between SARS-CoV and SARS-CoV-2 including the receptor-binding domain in the spike proteins of both viruses Xu *et al* [58]. Also, crystal structure analysis revealed the strong binding of the spike protein to human ACE2 [59]. Further analysis demonstrated higher affinity of SARS-CoV-2 than SARS-CoV to human ACE2 which suggests its increasing ability to transmit from individual to other [22]. A part of interpretation, it seems to be due to the recognition of amino acid residue 394 (glutamine) in SARS-CoV-2 receptor by lysine 31 on the human ACE2 receptor [22,60]. All together suggest that ACE2 is a critical receptor for SARS-CoV-2.

Accumulating evidence supports that the lung is the most vulnerable target for SARS- CoV-2 than other organs and tissues such as the heart, kidney, intestine and endothelium where ACE2 are also expressed [27,61]. The likely reasons stand behind it can be summarized in the vast surface area of the lung [62], enrichment of the surface of the lung cells with ACE2 receptors, notably alveolar epithelial type II cells that represent as reservoir for viral invasion constituting almost 80% of ACE2-expressing cells as reported by Zhao *et al* in bioRvix, recently [63]**,** and the existence of multiple viral process-related genes in ACE2-expressing alveolar epithelial type II cells [63]. These genes include regulatory genes for viral processes, life cycle and assembly suggesting facilitation of coronaviruses replication in the lung by ACE2-expressing alveolar epithelial type II cells [62]. In fact, the multiorgan dysfunctions observed in patients

severely infected could be explained by ACE2 tissue distribution [64]. Furthermore, ACE2 proteins are expressed over cortical neurons and glial cells making them a potential target for the SARS-CoV-2 attack and becoming the possible bases of anosmia (loss of taste) seen in patients with COVID-19 [65]. Initially, anosmia and dysgeusia have been early seen in many patients with COVID-19, later on they were reported as significant symptoms for COVID-19 [66].

Taken all together, it has been shown that ACE2 is not only the receptor for SARS-CoVs but also a pathogenic tool. The question is how susceptible the human host to the virus is. This issue with the rapid spread of the infection attract attention of the researchers and scientists to study the human genetic risk factors influencing disease progression. Some studies have reported the complex genetic contribution to the outcomes of infections, and the question is why some individuals develop life-threatening immune-mediated pathologies [67]. Future gene studies might contribute to understand key mediators of immune responses. In line with this and in search for molecules with therapeutic potential, human recombinant soluble ACE2 (hrsACE2) is being studied to block early stages of SARS-CoV-2 infections by inhibiting cellular binding and protecting the membrane bound ACE2 receptors.

Conclusion

Grasping the cellular activity of ACE2 and Ang (1-7) are required for better therapies for cardiovascular diseases. In addition, strategies targeting ACE2 may offer beside vaccines a novel approach in the search for prevention and management of COVID 19.

References

1. Sparks MA, Crowley SD, Gurley SB, Mirotsou M, Coffman TM. Classical renin-angiotensin system in kidney physiology. Compr Physiol 2014; 4(3): 1201-28. doi:10.1002/cphy.c130040.
2. Skeggs LT, Kahn JR, Shumway NP. The preparation and function of the hypertension converting enzyme. J Experimental Med 1956;103 (3): 295-9. doi:10.1084/jem.103.3.295.
3. Coates D. The angiotensin converting enzyme (ACE). International J Biochemistry Cell Biology 2003; 35 (6): 769-73. doi:10.1016/S13572725(02)00309-6.
4. Vauquelin G, Michotte Y, Smolders I, Sarre S, Ebinger G, Dupont A, *et al*. Cellular targets for angiotensin II fragments: pharmacological and molecular evidence. J Renin Angiotensin Aldosterone Syst 2002; 3:195-204.
5. Wright JW, Harding JW. Important role for angiotensin III and IV in the brain renin-angiotensin system. Brain Res Rev 1997; 25:96-124.
6. Chai SY, Fernando R, Peck G, Ye SY, Mendelsohn FA, Jenkins TA, *et al*. The angiotensin IV/AT4

receptor. Cell Mol Life Sci 2004; 61:2728-37.
7. Haulica I, Bild W, Serban DN. Angiotensin peptides and their pleiotropic actions. J Renin Angiotensin Aldosterone Syst 2005; 6: 121-31.
8. Castrop H, Höcherl K, Kurtz A, Schweda F, Todorov V. Physiology of kidney renin. Physiol Rev 2010; 90: 607-73.
9. Jeppesen PL, Christensen GL, Schneider M, Nossent AY, Jensen HB, Andersen DC, *et al*. Angiotensin II type 1 receptor signaling regulates microRNA differentially in cardiac fibroblasts and myocytes. British J Pharmacol 2011; 164 394-404.
10. Asano K, Zisman LS, Bristow MR. Angiotensin II receptors in the normal and failing heart. Heart Failure Reviews 1999; 3: 199-208.
11. Prabhakar SS. Regulatory and functional interaction of vasoactive factors in the kidney and extracellular PH. Kidney Int 2004; 66: 1742-54.
12. Kerins DM, Hao Q, Vaughan DE. Angiotensin induction of PAI-1 expression in endothelial cells is mediated by the hexapeptide angiotensin IV. J Clin Invest 1995; 96:2515-20.
13. Cheng WH, Lu PJ, Ho WY, Tung CS, Cheng PW, Hsiao M, *et al*. Angiotensin II inhibits neuronal nitric oxide synthase activation through the ERK1/2-RSK signaling pathway to modulate central control of blood pressure. Circ Res 2010; 106:788-95. doi: 10.1161/CIRCRESAHA.109.208439.
14. Kawano H, Do YS, Kawano Y, Starnes V, Barr M, Law RE, *et al*. Angiotensin II has multiple profibrotic effects in human cardiac fibroblasts. Circulation 2000; 101:1130-7.
15. Dzau VJ. Theodore cooper lecture: Tissue angiotensin and pathobiology of vascular disease: A unifying hypothesis. Hypertension 2001; 37: 1047-52.
16. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, *et al*. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res 2000; 87: E1-E9.
17. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril insensitive carboxypeptidase. J Biol Chem 2000; 275: 33238-43.
18. Cushman DW, Cheung HS, Sabo EF, Ondetti MA. Design of potent competitive inhibitors of angiotensin converting enzyme. Carboxyalkanoyl and mercaptoalkanoyl amino acids. Biochemistry 1977;16: 5484-91.
19. Ferrario CM, Chappell MC, Dean RH, Iyer SN. Novel angiotensin peptides regulate blood pressure, endothelial function, and natriuresis. J Am Soc Nephrol 1998; 9:1716-22.
20. Warner FJ, Smith AI, Hooper NM, Turner AJ. Angiotensin-converting enzyme-2: A molecular and cellular perspective. Cell Mol Life Sci 2004; 61: 2704-13. doi 10.1007/s00018-004-4240-7.
21. Allred AJ, Diz DI, Ferrario CM, Chappell MC. Pathways for angiotensin-(1–7) metabolism in pulmonary and renal tissues. Am J Physiol Renal Physiol 2000; 279: F841-F50.

22. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. J Virol 2020; 94: e00127-20. https ://doi.org/10.1128/jvi.00127 -20.
23. Guy JL, Jackson RM, Acharya KR, Sturrock ED, Hooper NM, Turner AJ. Angiotensin-converting enzyme-2 (ACE2): Comparative modelling of the active site, substrate specificity and chloride dependence. Biochemistry 2003; 42: 13185-92.
24. Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, *et al*. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. J Biol Chem 2002, 277:14838-43.
25. Santos RA, Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, *et al*. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci USA 2003; 100:8258-63.
26. Ferrario CM, Trask AJ, Jessup JA. Advances in the biochemical and functional roles of angiotensin converting enzyme 2 and angiotensin-(1-7) in the regulation of cardiovascular function. Am J Physiol Heart Circ Physiol 2005; 289:H2281-H2290.
27. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, *et al*. Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature 2002; 417:822-8.
28. Parvathy S, Oppong SY, Karran EH, Turner AJ, Hooper NM. Angiotensin converting enzyme secretase is inhibited by metalloprotease inhibitors and requires its substrate to be inserted in a lipid bilayer. Biochem J 1997; 292: 597-603.
29. Mizuiri S, Aoki T, Hemmi H, Arita M, Sakail K, Aikawa A. Urinary angiotensin-converting enzyme 2 in patients with CKD. Nephrology (Carlton) 2011; 16:567-72.
30. Xiao F, Hiremath S, Knoll G, Zimpelmann J, Srivaratharajah K, Jadhav D, *et al*. Increased urinary angiotensin-converting enzyme 2 in renal transplant patients with diabetes. PLoS One 2012; 7(5): e37649. doi: 10.1371/journal.pone.0037649
31. Ferrario CM, Santos RAS, Brosnihan KB, Block CH, Schiavone MT, Khosla MC, *et al*. A hypothesis regarding the function of angiotensin peptides in the brain. Clin Exp Hypertens 1988; 10(1):107-21.
32. Trachte GJ, Meixner K, Ferrario CM, Khosla MC. Prostaglandin production in response to angiotensin-(1–7) in rabbit isolated vasa deferentia. Prostaglandins 1990; 39:385-94.
33. Poersti I, Bara AT, Busse R, Hecker M. Release of nitric oxide by angiotensin-(1–7) from porcine coronary endothelium: implications for a novel angiotensin receptor. Br J Pharmacol 1994; 111:652-4.
34. Heyne N, Beer W, Muehlbauer B, Osswald H. Renal response to angiotensin-(1–7) in anaesthetized rats. Kidney Int 1995; 47:975-6.
35. Hilchey SD, Bell-Quilley CP. Association between the natriuretic action of Ang-(1–7) and selective stimulation of renal prostaglandin I2 release. Hypertension 1995; 25:1238-44.

36. Meinert C, Gembardt f, Böhme I, Tetzner A, Wieland T, Greenberg B, *et al*. Identification of intracellular proteins and signaling pathways in human endothelial cells regulated by angiotensin-(1-7). J Proteomics 2016; 130: 129-39. doi: 10.1016/j.jprot.2015.09.020.
37. Tallant EA, Ferrario CM, Gallagher PE. Angiotensin-(1-7) inhibits growth of cardiac myocytes through activation of the Mas receptor. Am J Physiol Heart Circ Physiol 2005; 289:H1560-6.
38. Chappell MC, Iyer SN, Diz DI, Ferrario CM. Antihypertensive effects of angiotensin-(1-7). Braz J Med Biol Res 1998 ;31(9):1205-12. doi: 10.1590/s0100-879x1998000900014.
39. Gembardt F, Grajewski S, Vahl M, Schultheiss HP, Walther T. Angiotensin metabolites can stimulate receptors of the Mas-related genes family. Mol Cell Biochem 2008; 319:115-23.
40. Dias-Peixoto MF, Santos RA, Gomes ER, Alves MN, Almeida PW, Greco L, *et al*. Molecular mechanisms involved in the angiotensin-(1–7) Mas signaling pathway in cardiomyocytes. Hypertension 2008; 52:542-8.
41. Sampaio WO, Souza dos Santos RA, Faria-Silva R, da Mata Machado LT, Schiffrin EL, Touyz RM. Angiotensin-(1–7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. Hypertension 2007; 49:185-92.
42. Lin CS, Pan CH, Wen CH, Yang TH, Kuan TC. Regulation of angiotensin converting enzyme II by angiotensin peptides in human cardio-fibroblasts. Peptides 2010; 31:1334-40.
43. Gava E, Samad-Zadeh A, Zimpelmann J, Bahramifarid N, Kitten GT, Santos RA, *et al*. Angiotensin-(1–7) activates a tyrosine phosphatase and inhibits glucose-induced signaling in proximal tubular cells. Nephrol Dial Transplant 2009; 24:1766-73.
44. Ocaranza MP, Godoy I, Jalil JE, Varas M, Collantes P, Pinto M, *et al*. Enalapril attenuates downregulation of angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. Hypertension 2006; 48:572-8.
45. Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. Hypertension 2004; 43:970-6.
46. Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM, *et al*. A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med 2003; 348: 1986-94.
47. Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, *et al*. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 2003; 300: 1394-9.
48. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, *et al*. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003; 426:450-4.
49. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, *et al*. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS

coronavirus–induced lung injury. Nature Medicine 2005; 11(8):875-9. doi:10.1038/nm1267.
50. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, *et al*. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579:270-90. https: //doi.org/10.1038/ s4158 6-020-2012-7.
51. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, *et al*. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 2020; 581:215-27.
52. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV–a target for vaccine and therapeutic development. Nat Rev Microbiol 2009; 7(3): 226-36
53. Xu Y, Lou Z, Liu Y, Pang H, Tien P, Gao GF, *et al*. Crystal structure of severe acute respiratory syndrome coronavirus spike protein fusion core. J Biological Chemistry 2004; 279(47): 49414-9.
54. Lukassen S, Chua RL, Trefzer T, Kahn NC, Schneider MA, Muley T, *et al*. SARS-CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. EMBO J 2020; 39: e105114
55. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, *et al*. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020; 367: 1260 -3.
56. Coutard B, Valle C, de lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Research 2020; 176:104742. https://doi.org/10.1016/j.antiviral.2020.104742.
57. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, *et al*. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 2005; 436:112–6.
58. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, *et al*. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its Spike protein for risk of human transmission. Sci China Life Sci 2020; 63(3):457-60. https://doi.org/10.1007/s11427-020-1637-5.
59. Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science 2005; 309:1864-8.
60. Wu KL, Peng GQ, Wilken M, Geraghty RJ, Li F. Mechanisms of host receptor adaptation by severe acute respiratory syndrome coronavirus. J Biol Chem 2012; 287:8904-11.
61. Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus: A first step in understanding SARS pathogenesis. J Pathol 2004; 203:631-63.
62. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: Molecular mechanisms and potential therapeutic target. Intensive Care Med 2020; 46:586–90. https://doi.org/10.1007/s00134-020-05985-9.

63. Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. Bio Rvix 2020. https ://doi.org/10.1101/2020.01.26.919 98. (accessed on 2020-04-28).
64. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan China. Lancet 2020; 395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5.
65. Baig AM. Neurological manifestations in COVID-19 caused by SARS-CoV-2. CNS Neurosci Ther 2020; 26(5):499-501. doi:10.1111/cns.13372.
66. Baig AM, Khaleeq A, Ali U, Syeda H. Evidence of the COVID-19 Virus Targeting the CNS: tissue distribution, host-virus interaction, and proposed neurotropic mechanisms. ACS Chem Neurosci 2020; 11(7):995-8. doi:10.1021/acschemneuro.0c001 22.
67. Sironi M, Hasnain SE, Rosenthal B, Phan T, Luciani F, Shaw MA, *et al*. SARS-CoV-2 and COVID-19: A genetic, epidemiological, and evolutionary perspective. Infection, Genetics and Evolution 2020; 84:104384. https://doi.org/10.1016/j.meegid.2 020.104384