REGULATORY MECHANISMS OF ANGIOTENSIN-CONVERTING ENZYME 2 AND THEIR SIGNIFICANCE IN THE DEVELOPMENT OF SEVERE COVID-19

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ABSTRACT

From 2019 to the present day, the coronavirus infection COVID-19 continues to be a serious health problem. Scientists and clinicians from all over the world have joined efforts in studying the molecular interaction mechanisms between SARS-CoV-2 and ACE2, including virus-induced changes in ACE2 transcription, expression, and functionalities leading to disruption of basic regulatory pathways for vascular homeostasis, and reprogramming of key proteases, co-receptors and adhesion molecules.

Here, we aimed to clarify the mechanisms and signals that would restore the virus-induced imbalance between destructive and protective effects of ACE2.

Understanding why only certain individuals are predisposed to infection with SARS-CoV-2, and development of severe pathology is at the center of scientific interest, and the strategies for prevention and therapy.

Keywords: ACE2, COVID-19, SARS-CoV-2

INTRODUCTION

Over the past 20 years, society has witnessed an increasing number of pandemics that have led to the hospitalization and death of millions of people worldwide, causing an unprecedented strain on the healthcare systems: the severe acute respiratory syndrome (SARS) in 2002, the Middle East respiratory syndrome (MERS) in 2012 and the COVID-19 infection in 2019, all caused by beta-coronaviruses, SARSCoV, MERS-CoV and SARS-CoV-2, respectively.[1] Despite the enormous efforts of the research community, the new SARS-CoV-2 virus has infected globally more than 771,679,618 people worldwide (as of 2 November 2023) and caused the death of more than 6,977,023.[2] A large percentage of those had multiorgan dysfunction including pulmonary, cardiovascular, neurological and endocrine symptoms with a powerful cytokine storm unfolding.[2] Understanding the mechanisms underlying the individual predisposition to infection with SARS-CoV-2, as well as the reasons for the development of severe pathology only among certain individuals is at the center of scientific interest, strategies for prevention and therapy.[3] Despite the vast amount of data illuminating the effects of SARS-CoV-2 on the human body, there are still numerous unanswered questions. The mechanisms and signals that would restore the virus-induced imbalance between destructive and protective effects of ACE2 are of interest, as well as the intimate interactions between the regulators of receptor-bound signaling cascades.

Specificity of SARS-COV-2

The mechanism underlying the attachment of SARS-CoV-2 to the cell membrane is well studied and described. The receptor-binding domain (RBD) of S1-subunit of the spike protein of SARS-CoC-2, exhibits a 10- to 20-fold greater affinity for ACE2 than SARS-CoV, which is explained by a greater number of matches between the N-terminus of ACE2 and SARS-CoV-2 (1204 Å) as compared to SARS-CoV (998 Å) (4). Kowever, in order to attach to the receptor and the cell membrane, the spike protein of SARS-CoV-2 needs to be previously "functionalized" by separating the S1 and S2 subunits: a process known as priming. Unlike SARS-CoV-1, the SARS-CoV-2 virus cannot self-attach. The attachment is realized by using the human transmembrane serine protease 2 (TMPRSS2) through proteolytic-mediated activation of the virus. The enzyme FURIN, a type 1 membrane-bound protease, also assists in the process of separating the

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two subunits of SARS-CoV-2 and plays an alternative role in the process of attachment of the virus to ACE2. In this way, SARS-CoV-2 is less dependent on TMPRSS2 and has a "backup plan" in its pathophysiological endocytosis program. This gives SARS-CoV-2 the advantage to interact with cells with low TMPRSS2 expression, and to adhere to membranes with high FURIN expression.[4] This is another distinguishing feature of SARS-CoV-2 as compared to SARS-CoV-1, which lacks a FURIN-binding domain. The cellular co-expression of ACE2, TMPRSS2 and FURIN and tissue localization of the proteases are important factors in the pathogenetic mechanism of cell binding (4).

Another key factor in this intermolecular play is ADAM17 (disinterring and metalloproteinase domain 17) popularly known as TNFα-converting enzyme as it releases proteolytically the precursor of TNFα (pro-TNFα) into the extracellular space when expressed on the cell surface membrane. There, TNFα exerts auto- and paracrine functions associated with activation of systemic pro-inflammatory cytokines (IL-1β and IL-6) as well as autonomous effects resulting in TNFαR activation. Thus a key signaling pathway is realized resulting in ADAM17 activation, proteolytical cleavage of the ectodomain of the membrane-bound long-chain ACE2 and its conversion to soluble ACE2 with a short half-life (5,6).

In this way, the first axis of influence on the pathologically low cell membrane expression of ACE2, suppressed hydrolysis of Ang II to Ang(1-7) and reduced activity of MasR is involved. The resulting excess of Ang II exerts its destructive general effects (oxidative stress through increased ROS production, hypertrophy, fibrosis, vasoconstriction and intestinal dysbiosis) (7).

On the other hand, excess AngII also causes local overactivation of AT1R, which is an essential modulator of ADAM17 overactivity through AT1R, thus realizing the second axis of suppressing membrane expression of ACE2 and deepening the effects of AngII in a vicious circle. A third ADAM17 over-activation signaling pathway related to endocytosis of the ACE2-SARS-CoV-2 complex is added to both axes (5).

The cumulative effects of suppressed ACE2 expression and function are associated with inability to metabolize AngII and, hence, increased AT1R/AngII-ADAM17/TNFα/IL-1β/IL-6 signaling with multiorgan dysfunction and cytokine storm-driven hyperinflammation (5).

Effect of SARS-COV-2 on ACE2 functions

In addition to an imbalance in the renin-angiotensin-aldosterone system (RAAS), the pathological program of SARS-CoV-2 includes adverse effects on the kallikrein-kinin system (KKS), the alpeinergic system (AS), th eamino acid transport in the gastro intestinal tract (GIT), and intestinal dysbiosis (ACE2-B0AT1). These add to and amplify the adverse effects of COVID-19. Although most studies have focused on the RAAS, due to the potent destructive effects of AngII and the wide tissue distribution of the AT1R, changes in the regulation of KKS and AS cannot be neglected. The involvement of ACE2 as a key protease makes them closely linked; they share common signaling pathways and receptor apparatus, interrelated effects and consequences in the course of the COVID-19 disease (2,8,9,10).

Hepatic angiotensinogen is hydrolyzed to AngI by the renal protease renin, then through the receptor with proteolytic function ACE, AngI is metabolized to AngII with a direct effect on AT1R and an alternative effect on AT2R. Negative regulation of AngII activity is realized by ACE2 at two points: directly on AngII to Ang(1-9) and on AngI to Ang(1-7), followed by MasR activation. An additional pathway to reduce the activity and effects of AngII is via APA to AngIII, again with effects on AT1R and AT2R. The balance between the protective and destructive effects of AngII is maintained through two signaling pathways: destructive AngI/AngII/AT1R; AngI/AngII/AT2R and protective AngI/Ang(1-9); AngII/Ang(1-7)/MasR. ACE2 plays a central role as a switch between the two opposite effects.[2,8,9,10] Accumulation of AngII and overactive AT1R lead to activation of three intracellular signaling pathways: 1) via NADPH/COX-2/ROS/cytochrome C/ to apoptosis; 2) through Caspase 3 to apoptosis; 3) via p38MAPK/JNK to hypertrophy. An alternative signaling is also discussed, which leads to overproduction of reactive oxygen species (ROS) under oxidative stress, and direct activation of transcription factor (NF)-Kb resulting in over-expression of IL-6, IL-1β and TNFα (2,10). Thus, the vicious cycle involving cell-autonomous overproduction of proinflammatory

cytokines, pathological over-activation of ADAM17, persistently suppressed ACE2 expression, and AngII accumulation is closed

The kallikrein-kinin system is also involved in the pathogenesis of COVID-19 via ACE2. Under physiological conditions, it regulates coagulation, inflammation and pain (11). The main activities are associated with the peptides bradykinin (BK), Lys-BK, [des-Arg9]-BK (DEABK) and Lys-[des-Arg9]-BK (LDEABK. After binding to the receptor pore PB2, BK and Lys-BK induce locally to increase the synthesis of nitric oxide and realize a vasodilatory effect.

It opposes the vasopressor effects of the RAAS by balancing them. Bradykinin regulates the secretion of tissue plasminogen (tPA) and plays an important role in thrombus formation (12). The peptides DEABK and LDEABK interact with βB1 and play an important role in inflammation (13,14). Unlike βB2, βB1 is weakly expressed in endothelial cells, but is induced after tissue injury and is overexpressed under the influence of proinflammatory cytokines IL-1B, TNFα, IL-2, and IFNγ (13). Upon activation, βB1 can exacerbate the inflammatory response by hypersecreting proinflammatory cytokines and promoting neutrophil infiltration (15). The receptor with proteolytic activity ACE2 cannot inactivate bradykinin, but is able to hydrolyze the terminal chains of DEABK and LDEABK, making them lose affinity for BPB1. Therefore, internalization of ACE2 upon SARS-CoV-2 infection will create an imbalance in the kallikrein-kinin system, causing overstimulation of the DEABK/LDEABK/BRP1 axis and subsequent severe inflammation, vascular effusion, and angioedema (16). Although KKS potentiates the pathology in COVID-19, its regulation has not been targeted by the main therapies applied to date. According to the available literature, there is only one treatment regimen that targets RAAS and KKS simultaneously (17).

The involvement of ACE2 in the apelinergic system (AS) is also at the fringes of worldwide research interest. Apelin peptides are a family of proteins that bind to the apelin receptor and mediate protective effects on the cardiovascular system (18). The apelin signaling pathway leads to increased ACE2 mRNA transcription and ACE2 expression (4). On the other hand, however, ACE2 hydrolyzes the C-terminus of phenylalanine in apelin and inactivates it. Thus, mutual regulation between apelin and ACE2 is realized. In addition, the pyr-apelin-13 peptide suppresses Ang II-mediated free radical (superoxide) production, myocardial hypertrophy, dysfunction, and fibrosis (19). Through its mono-carboxypeptidase activity, ACE2 cleaves and inactivates apelin-13 and apelin-36 peptides (20). The interrelationship between the apelin receptor and the AT1R responsible for the destructive effects of AngII is interesting. Active receptor leads to heterodimerization of the AT1R and sequesters it, thereby disrupting the AngII/AT1R axis (4).

The B0AT1 receptor is expressed in the intestinal epithelium and kidney and has a role in the absorption of neutral amino acids. The ACE2-B0AT1 complex occurs as a dimer or heterodimer (21). Thus, ACE2 performs a completely different function, regulating amino acid transport, and antimicrobial peptides expression, and interacting with the GIT microbiome (22). The regulation of ACE2-B0AT1 in SARS-CoV-2 infection remains unexplored.

ACE2 tropism: physiological conditions after SARS-COV-2 infection

The expression of ACE2 under physiological and pathological conditions (SARS-CoV-2 infection, chronic cardiovascular, pulmonary, hepatic, and endocrine diseases) in various human tissues has been the subject of scientific interest due to its key role as a gateway to COVID-19. The influence of the demographic, and geographic factors on the occurrence, progression and prognosis of the disease is interesting. High expression of ACE2 was found in the small intestine, testis, kidney, heart, thyroid gland, adipose tissue and salivary glands. Moderate expression is described in the pancreas, esophagus, lung, colon, liver, adrenal gland, while low expression of ACE2 is found in nervous tissue, stomach, uterus, blood vessels, bone marrow, and spleen (23). No gender- or age-related differences in receptor expression were found (23). Other similar studies have focused on ACE2 gene expression specifically targeting the respiratory system and found similar tissue tropism (24). High expression of ACE2 was found in type II epithelial cells of alveoli and nasal epithelium, in which very high co-expression of ACE2 and TMPRSS2 was detected. This would explain the predisposition of these cells as a gateway in SARS-CoV-2 pathogenesis (24).

An interesting finding in a study by Xu et al., who found ACE2 expression in oral mucosa, the epithelial cells of the dorsum of the tongue and in lymphocytes (25). This also provides a logical explanation for the serious lymphocytopenia especially in severe cases of COVID-19 (26). Despite local oral lesions associated with SARS-CoV-2 infection such as taste loss, xerostomia, mucosal ulcerations, enanthemas, and macules, the role of the oral mucosa in the pathogenesis of the disease is poorly understood. In a March 2021 publication in Nature Medicine, Huang and colleagues presented a detailed comparative multi-methodology study of ACE2 and the TMPRSS family (TMPRSS2, TMPRSS4, TMPRSS11D). They found that the investigated biomarkers are expressed in the gingiva, minor and major salivary glands, buccal mucosa, ventral and dorsal part of the tongue, soft and hard palate, palatine tonsils (27). The oral cavity is an important portal of entry for SARS-CoV-2, and saliva is a potential route for the virus to spread, including extraoral, oral-pulmonary, and oral-intestinal transmission (27).

Studies on the cellular expression of ACE2 are linked to the study of ACE2 gene polymorphism. More than 1,700 gene variations in ChinaMAP and 1KGP databases are annotated,[27,28] seeking to answer how do the structural and spatial variations of proteins in the binding domains of ACE2 affect (in the protective or destructive way) the adherence of SARS-CoV-2 (28,29).

Molecules modulating ACE2 expression and activity

Despite an intensive scientific research, there is still uncertainty about the pathogenetic mechanism of SARS-CoV-2 binding to the cell wall. An additional curious fact raising discussion is the low expression of ACE2 in the respiratory system on the one hand, and the severe lung pathology on the other. Trying to explain this lack of correlation, alternative modulators, co-receptors and adhesion factors were investigated: neurothelin's, heparan sulfate (HS), sialic acids (SA), CD147 and GRP78 (24,25).

The neurothelin receptor NRP1 (VEGF165R) may be a co-receptor for SARS-CoV-2 along with ACE2, potentiating virus binding (29). Heparin sulfate binds to the Spike protein of SARS-CoV-2 and induces a conformational change that stabilizes the open configuration of the S1 subunit. Since only in this form a binding to ACE2 occurs, HS promotes infection.[30] A similar role was described for SA (31). Hoffmann et al. found that ammonium chloride, an endosomal pH modulator, could block the activity and inhibit the entry of SARS-CoV-2 in TMPRSS2–293T cell lines (32). Another TMPRSS2 inhibitor, camostat mesylate, partially blocks the virus, and in combination with E-64d (inhibitor of cathepsin B and L), it can completely stop the attachment of SARS-CoV-2 to the cell membrane and realize a protective effect (32).

The alternative receptor CD147 was found to be highly expressed by IHC in alveolar epithelium, and a direct association with the spike protein was demonstrated (33,34). The chaperone BiP (HSPA5, GRP78), which is expressed upon cellular stress, can also bind SARS-CoV-2, although literature data are debatable (32).

Recently, due to the great interest in ACE2 and with the help of bioinformatics and RNA-sequencing, the whole human ACE2 genomic region was revised and a new isoform of ACE2, called deltaACE2 (δACE2), was discovered, which is different from the full-length ACE2 (full-length ACE2, flACE2) in the 356 aa N-terminal region (35,36). This renders deltaACE2 unable to bind to SARS-CoV-2 (36). Analysis of flACE2 and dACE2 found that IFNα, IFNγ, and IFN-l3 could induce δACE2 expression in human bronchial epithelial cells and prevent binding to SARS-CoV-2 (32). The interferon-dependent regulation of ACE2 mRNA remains to be elucidated, though low plasma levels of IFNα were found in critically ill patients with COVID-19 as compared to those with milder symptoms, and IFNα plasma levels remained stable for 17 days in mild cases (37,38).

CONCLUSION

The RASS system and the ACE2/angiotensin-(1–7)/MAS axis play important roles in various physiological and pathophysiological processes.

Since ACE2 is a major player in the SARS-CoV-2 binding and host cell entry, and is highly expressed in various organs and tissues, it is particularly important to trace the signaling pathways through which this connection may be affected.

Knowing the main mechanisms of action would allow the development of appropriate cytoprotective

substances blocking critical points or reducing the multiorgan dysfunction and hyperinflammation.

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