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Insights into the Interaction of SARS-COV-2 with ACE2 Receptor

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ABSTRACT

The causative agent for the ongoing coronavirus disease 2019 (COVID-19) is the severe acute respiratory syndrome coronavirus 2 (SARS-COV-2). SARS-COV-2 makes entry into host cell via ACE2 receptor. The binding interaction of SARS-COV-2 with ACE2 receptor has been presented along with the mechanism of viral infection. In addition to lungs, other tissues viz heart, brain, kidneys etc. become potential targets of SARS-COV-2 infection owing to the expression of ACE2 in them. Understanding of damage in such tissues has been addressed along with some preventive therapeutics.

1. Introduction

Pathogens are the disease causing microorganisms which include bacteria, viruses, parasites and fungi. For spreading infection, all these primarily require to invade the host cell [1-3]. Among them, viral infections have been found to be more severe. Viruses are the small infectious agents that do not grow by cell division but uses the host cell metabolism for complete self-replication [4-5]. They consist of (i) the genetic material, i.e. long molecules of DNA or RNA that encode the structure of the proteins by which the virus acts; (ii) a protein coat, the *capsid*, which surrounds and protects the genetic material; and in some cases (iii) an outside envelope of lipids. Among different classes of viruses based on genome type, coronaviruses belong to one such class which consists of a single-stranded positive-sense RNA genome encapsulated within a membrane envelope. The viral membrane is studded with glycoprotein spikes that give coronaviruses their crown like appearance (Fig. 1).

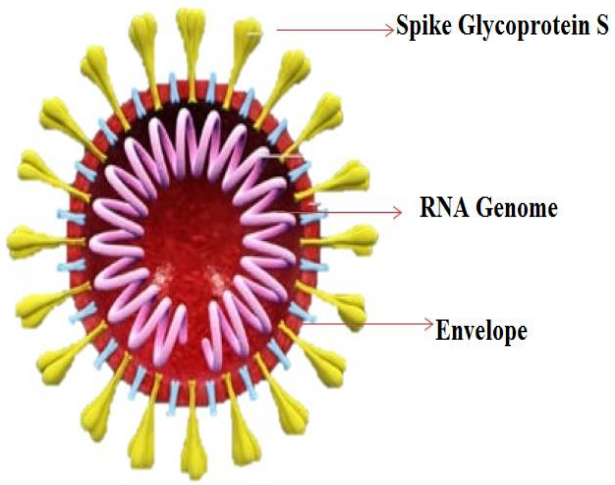

Fig 1. Virion

2. Discovery of SARS-COV-2

The initial reports of coronavirus infection have been observed in late December 2019 in Wuhan, the capital of Hubei, China where in patients were admitted with fever, myalgia, cough, shortness of breath, and other symptoms [6-7]. These patients were subjected to Computed Tomography (CT) imaging which revealed varied opacities (denser, more profuse, and confluent) in lungs in comparison to images of healthy lung [8]. This initial assessment led to the diagnosis of Pneumonia. However, samples from infected patients were screened by the use of a multiplex polymerase chain reaction (PCR) panel of known pathogens that reflect negative results. This suggested that the cause of Pneumonia has been of some unknown origin. By January 10, 2020 the bronchoalveolar lavage fluid from patients was sequenced to reveal the pathogen that showed its genome to resemble with *β*-Coronaviruses [9]. It was discovered that the genome sequence of new pathogen has~ 80%,~ 50% and~ 96% similarity with the genome sequence of SARS-COV (2002-03 in China), MERS-COV (2012 in Saudi Arabia) and RaTG13 (Bat coronaviruses) respectively [6, 10]. Clearly, the origin of this new virus might be from bats like other *β*-Coronaviruses. This has been named as SARS-COV-2, though previously termed as 2019-nCOV or Wuhan coronavirus [11-12]. However, the intermediate host animal is yet unclear that brought the bat coronavirus to human hosts. Some studies show that Malayan pangolin may be the intermediate host [13-14] while some propose it to be the natural source [15-16]. From transmission electron microscopy, SARS-COV-2 appeared to be spherical in shape with some pleomorphism having diameter 60-140 nm. There are four types of structural proteins that include the spike surface glycoprotein (*S*), small envelope protein (*E*), matrix protein (*M*), and nucleocapsid protein (*N*). In coronaviruses, the *S* gene codes for the receptor-binding spike protein that enables the virus to infect

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cells [17]. The length of spike was found to be around 8-12 nm [18]. These proteins are involved in encasing the RNA and/or in protein assembly, budding, envelope formation, and pathogenesis [19-20].

The disease caused by SARS-COV-2 is referred to as COVID-19. It has been observed that the disease is contagion and involve human to human transmission. As estimated, an infected person can approximately infect three other persons [21]. This, therefore, leads to the chain of infected persons. On March-11, 2020 WHO declared this outbreak of COVID-19 as global pandemic [22]. At the time of writing this article, over 4 million people are infected and around 3 lac deaths are witnessed globally. The worst hit by this pandemic are mostly European Countries. The biggest challenge is that no vaccine is yet available for the disease, even though mixture of some previously developed antivirals (HIV drug combination of liponavir and ritonavir) [23], chloroquine and remdesivir were under clinical trials [24-25] but that do not prove to be significant. Because of this non-availability of vaccine and partly due to asymptomatic nature of disease, its widespread occurrence takes place. This led to isolations, quarantines and lockdowns throughout the countries so as to hamper its spreading. But this causes major disruption to the economy of people and countries [26].

3. Binding Interaction and Host Cell Infection

For cross-species transmission, host cell entry espatially for *β*-coronaviruses is an essential component. Like other coronaviruses, SARS-COV-2 has been found to make use of densely glycosylated spike (*S*) protein to gain entry into the host cells via ACE2 receptor. The spike protein has two subunits, the receptor binding subunit *S1* and the membrane fusion subunit *S2*. It is the *S1* subunit of the spikes of coronaviruses that have difference in structural homology while as *S2* remain usually same [27]. The subunit *S1* is characterized by having receptor binding domain (RBD) that has a core which consist of a twisted five stranded anti-parallel *β*-sheets (*β1*, *β2, β3, β4, β7*) with short connecting helices and loops. In between *β4* and *β7* strands are the short *β5* and *β6* strands extending away from core along with *α4* and *α5* helices and loops. This part of RBD is referred to as receptor binding motif (RDM). This is the active part of RBD that develops interaction with ACE2 receptor.

ACE2 is a metalloproteinase receptor containing Zn as metal ion. Zn ion is in tetrahedral arrangement of His374, His378, Glu-402 residues and a water molecule, representing its active site [28]. This active site forms a cleft by protein folding's whose N-terminal is referred to as subdomain-I and C-terminal as subdomain-II. ACE2 has emerged to provide a dominant mechanism for negative regulation of the RAS (renin-angiotensin system), by metabolizing Ang-II (vasoconstrictor) into the beneficial peptide Ang 1–7 (vasodilator) via ACE2/Ang-1-7/Mas axis [29]. This important biochemical and physiological property is being harnessed as potential therapy for heart failure. Inhibition of ACE2 allows the well established mechanism of RAS via ACE/Ang-II/AT1R axis that lead to deleterious effects such as vasoconstriction, hypertrophy, fibrosis and cell proliferation [30]. It has been observed that inhibitor binding occurs at the active site (i.e. the inhibitor binds to Zn for activity). This process allows large hinge-bending movement (conformation change) of subdomain-I towards subdomain-II. However, with SAR-COV-2 like other coronaviruses binding do not occur at the active site.

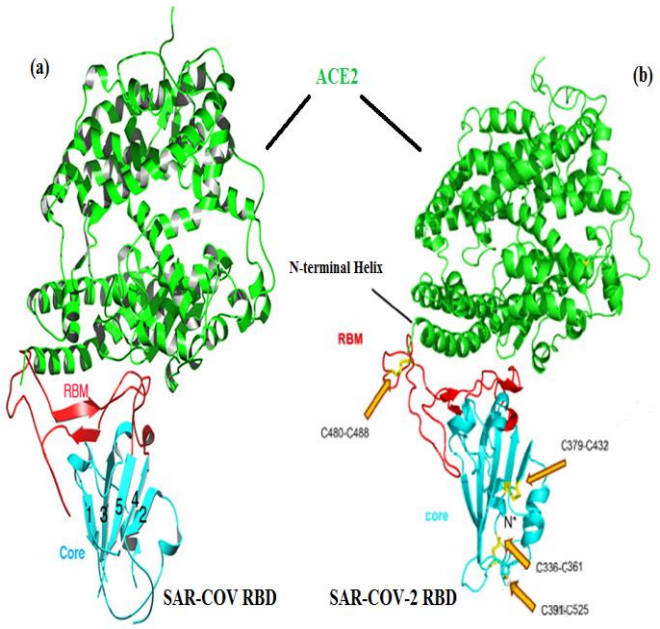

Fig 2. Binding interaction between ACE2 protein moieties with RBM's of SARS-COV (a) and SARS-COV-2 (b).

This was confirmed by mutating the ACE2 zinc-coordinating residues His374 and His378 to asparagines no effect on syncytial formation was observed [31], suggesting that interfering with the active site has no effect on viral spike protein binding to ACE2. Rather, the attachment of RBM of virus takes place at subdomain-I (N-terminal helix) of ACE2 receptor via protein-protein interaction. This is illustrated in Fig.2.

Since inhibitor binding to ACE2 causes large (16°) hinge-bending movement of subdomain-I, this may prove to be unfavorable for viral binding [29]. Thus, the use of metallopeptidase inhibitors may prove to be useful for preventing viral binding to ACE2 and hence may block viral entry. However, it has been observed that ACE inhibitors lead to the upregulation of the expression of ACE2 which results into the increased chances of viral entry for COVID-19. This may be the reason hypertensive and diabetic patients were prone to COVID-19.

From Fig. 2, the densely packed RBM of SARS-COV-2 with furin-like cleavage site (similar to MERS-COV and human coronavirus OC43) as compared to SARS-COV may be responsible for its high binding affinity (10 to 20 fold increase) [32]. By this conformation, it may put all important residues into place for better interaction with ACE2. However, the difference in protein assay in RBM compared to SARS-COV can also be the reason for high binding affinity of SARS-COV-2 for ACE2 receptor. This enhanced binding of SARS-COV-2 with ACE2 may contribute to the faster spread of infection from human to human.

After the binding of SARS-COV-2 spike with ACE2 receptor, priming of *S1* subunit (in its metastable prefusion conformation) of spike by cellular surface proteases, such as transmembrane protease serine 2 (TMPRSS2), takes place. This is followed by the transition of *S2* subunit in its stable postfusion conformation, thereby allowing cell entry for SARS-COV-2 [33]. Inside the cell, the released viral RNA uses host cell metabolism for further processing to form many copies of viruses, thus evolve into a full blown infection. This can be schematically shown in Fig.3.

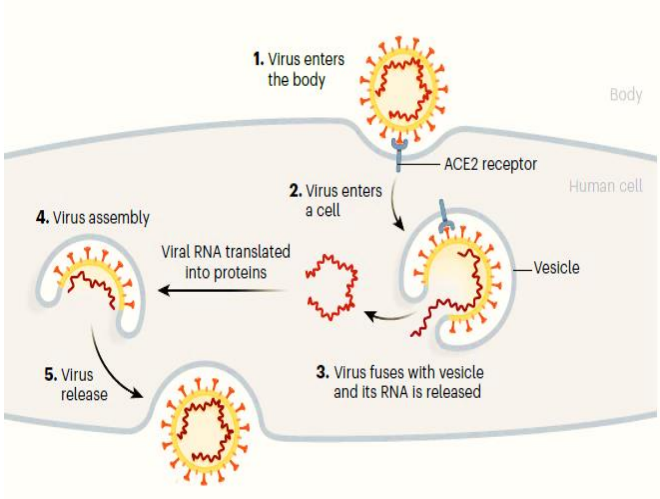

Fig 3. Mechanism of Viral Infection

4. Potential targets of SARS-COV-2

Since ACE2 forms the binding point for SARS-COV-2, therefore it is imperative to identify all those tissues where ACE2 expression is taking place. Although COVID-19 is predominantly a respiratory illness, but due to the wide distribution of ACE2 in lungs, heart, kidney, enterocytes of small intestines and cerebral cortex, striatum, hypothalamus, and brainstem of brain [34], these organs become potential targets for SARS-COV-2.

The presence of ACE2 in lung type II alveolar cells and macrophages and modestly in bronchial tracheal epithelial cells [35] leads to acute lung injury. The mechanism behind this can be similar to SARS-COV in which infection downregulates ACE2 thereby shifts ACE2/Ang-1-7/Mas system to ACE/Ang-II/AT1R system in the lung. This leads to the accumulation of non-competing Ang-II that result into the acute lung injury through AT1R activation [36]. Accumulation of Ang-II, a vasoconstrictor, can in turn lead to heart failure. In addition to this, the expression of ACE2 in myocytes can result into myocardial injury. As observed that patients with cardiovascular diseases (CVD's) were more prone to COVID-19. This might be associated with the increased secretions of ACE2 receptor as compared to healthy individual [37-39]. Moreover, the expression of ACE2 in neurons can give access to SARS-COV-2. The entry of virus has been attributed to the sluggish movement of blood (containing virus) within the microcapillaries that favor the interaction of the SARS-COV-2 spike protein with ACE2 expressed in the capillary endothelium. Subsequent budding of the viral particles from the capillary endothelium and damage to the endothelial lining can favor viral access to the brain. From where, the expression of ACE2 on neurons can further initiate the viral production to cause neuronal damage. This may be the reason of resulting anosmia in patients [22].

5. Preventive Measures

In addition to PPE for preventing viral entry into human body, at molecular level several pathways can help to prevent the damage caused by SARS-COV-2. One pathway is to develop therapeutics to block the host target ACE2 receptor or TMPRSS2. For blocking ACE2 receptor, some compounds as approved for other indications, for example baricitinib for rheumatoid arthritis, could be used to inhibit ACE2 mediated endocytosis [40]. Similarly, ruxolitinib is under clinical trials for treating COVID-19 [41]. Nafamostat mesylate [42-43] and camostat mesylate [33], the known inhibitors of TMPRSS2, could be utilized to prevent COVID-19 as has been observed to prevent viral entry into lung cells when camostat mesylate was tested on SARS-COV-2 isolated from a patient [33, 44]. Moreover, the use of recombinant soluble forms of ACE2 may act as a decoy receptor that binds SARS-COV-2 and competitively inhibits viral entry mediated by membrane ACE2. This has been observed to mitigate infection in kidney organoids and capillary organoids [45]. Many other membrane mimicking decoys can be explored to treat COVID-19 [46].

Conclusion

ACE2 being the receptor site for SARS-COV-2 does not utilize its active site (tetrahedral arranged Zn) for virion binding rather allows its N-terminal subdomain-I for interaction. This leads to the interaction between amino acid residues of subdomain-I with the residues of RBM of glycoprotein *S* spike of virion. As inhibitor binding lead to hinge-bending movement of subdomain-I towards subdomain-II, virion binding may be unfavorable. Although COVID-19 is predominantly a respiratory illness, yet the expression of ACE2 in different tissues like heart, kidneys, brain etc. may lead to deleterious effects. Thus patients suffering from COVID-19 demand in-depth examination.

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