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Review Article

ACE-2 receptors blockers: A novel therapeutic line of attack against COVID-19

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ABSTRACT

A novel infectious severe acute respiratory syndrome-like coronavirus (SARS-CoV 2) has caused an outbreak in Wuhan city, Hubei province, China, starting from December 2019 that swiftly spread across all nations around the world. Comprehending how SARS-CoV-2 enters humans is the first preference for perceiving its enigma and restraining its spread. Coronavirus is a positive-stranded enveloped, pleiomorphic viruses whose surface transmembrane spike protein mediates SARS-CoV2 entry into cells. To attain its purposes, SARS-CoV2 spike binds to its receptor hACE2(human angiotensin-converting enzyme 2) through its receptor-binding domain. But as per the phylogenetic analysis, the coronaviruses has revealed that SARS-CoV2 is a member of the Betacoronavirus genus, which encompasses SARS-CoV, MERS-CoV, SARSr-CoV and as well as others identified in humans and animal species.Bat coronavirus seems to be the closest relative of SARS-CoV2 sharing more than 93.1% sequence identity in the spike (S) gene. SARS-CoV2 shares less than 80% sequence identity with SARS-CoV and SARSr-CoV. Based on crystal structure analysis, the SARS-CoV spike proteins have a strong binding affinity to the human ACE2 receptor.SARS-CoV2 and SARS-CoV spike proteins share 76.5% identity in amino acid sequence, and hence importantly, the SARS-CoV and SARS-CoV2 spike proteins have a high degree of homology. According to the cell entry mechanisms of SARS-CoV2, first, to aid entry into host cells, coronaviruses first bind to the cell surface receptor for viral attachment and ultimately fuse viral and lysosomal membranes to enter the endosomes consequently. The interplay initiates infection when a virus surface anchored homotrimeric spike protein mediates coronavirus entry. However, as per the cell entry mechanisms, the S1 protein consists of RBD(receptor binding domain) that concedes explicitly (ACE-2) receptor. These coronavirus cell entry mechanisms depend upon cellular proteases, which include cell surface proteases TMPRSS2, lysosomal proteases cathepsins and human airway-trypsin like proteases (HAT). These are the features of SARS-CoV-2 entry which contribute to its severe symptoms and high pathogenicity.

Keywords: SARS-COV-2, ACE-2 receptor, therapy, transmission.

INTRODUCTION

The emergence and rapid expanse of the highly pathogenic severe acute respiratory syndrome (SARS) like- coronavirus SARS CoV2 is destroying global health, economy and has also posed a serious global public health emergency. These coronaviruses gained peculiar ill fame when the severe acute respiratory syndrome (SARS) outbreak quivered the world in 2002-2003 [1]. Diverse members of the family Coronaviridae persistently circulating in the human population and typically cause mild respiratory disease [2]. But before gaining importance for public health in 2003, the illnesses associated with coronaviruses were mainly of veterinary interest [3]. In contrast, severe acute respiratory syndrome (SARS-CoV) and middle east respiratory syndrome (MERS-CoV) are transmitted from animals to humans, which are called zoonotic infections and cause severe respiratory diseases in oppressed individuals, SARS and MERS respectively [2]. SARS appeared in November 2002 in Guangdong province, Southern China, with its subsequent global spread. The natural reservoir hosts for SARS-CoV was Chinese horseshoe bats. Human transmissions were expedited by intermediate hosts like civet cats and raccoon dogs, which are regularly sold as food sources in Chinese wet markets [2]. Since the initial outbreak on 31st December 2019, a new infectious respiratory disease appeared in Wuhan, Hubei province, China.SARS-CoV2 has expanded throughout China with more than 80 countries worldwide [3]. This initial cluster was linked to the Huanan seafood market, conceivably due to animal contact. After several humans to human transmission, the International Committee on Taxonomy of Viruses named the virus as SARS-CoV2 and the disease as COVID-19 [4]. A novel coronavirus which is nearly related to SARS-CoV was recognised in patients and is believed to be the causative agent of the new lung infection.

Coronaviruses are the members of the subfamily Coronavirinae (family Coronaviridae; order Nidovirales), which contains four genera, historically based on genetic studies as well as serological analysis: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, Deltacoronavirus. Alpha and Betacoronaviruses are known to cause illness in humans and animals, whereas Gamma and Deltacoronaviruses generally infect birds, and some of them cause infection in mammals [5,1]. Nevertheless, Betacoronaviruses are the most important group because they include highly pathogenic viruses toward humans, including SARS-CoV2, MERS-CoV, and SARS-CoV. Coronaviruses are spherical or pleomorphic and are enveloped with typical sizes varying from 80nm to 120nm. The genome of CoV is a single-stranded positive-sense RNA (+ssRNA) (~30kb) with a 5’ capped structure and 3 ’- poly-A tail. The virus is named as the longest known RNA virus with a length of between 26.2 to 31.7kb. The genome includes six to ten open reading frames (ORFs). The first ORF(ORF1a/b) comprises two-third of the genome and encodes the replicase proteins, whereas the last third of the genome near the 3 ’ terminus contains the structural protein genes in a fixed order:- (HE)-S-E-M-N. The genome is wrapped into a helical nucleocapsid envelope by a host-derived lipid bilayer. The virion envelope contains three viral proteins named: (S) spike protein, (M) membrane protein, and (E) envelope protein. Furthermore, some coronavirus also contains a (HE) hemagglutinin esterase. Whereas M and E proteins are involved in virus assembly, the spike protein is the mediator for the virus entry [1].

As per the phylogenetic analysis, the coronavirus has revealed that SARS-CoV2 is a member of the Betacoronavirus genus, which encompasses SARS-CoV, MERS-CoV, SARSr-CoV and as well as others identified in humans and animal species.Bat coronavirus seem to be the closest relative of SARS-Cov2 sharing more than 93.1% sequence identity in the spike (S) gene.SARS-CoV2 shares less than 80% sequence identity with SARS-CoV and SARSr-CoV [3]. The entry of the virus depends on a subtle interaction between the host cell and the virion. Infection is instigated by the interplay of the viral particle with a particular protein on the cell surface. After the inceptive binding of the receptor, enveloped viruses need to fuse their envelope with the host cell membrane to deliver their nucleocapsid to the target cell. This spike protein plays a dual role in entry by mediating the membrane fusion and receptor binding. The fusion includes the substantial conformational changes of the spike protein [1].

There are many similarities between SARS-CoV and SARS-CoV2. Practising computer modelling found that spike proteins of SARS-CoV2 and SARS-CoV have about the same 3-D structure in the receptor-binding domain (RBD). Based on crystal structure analysis, the SARS-CoV spike proteins have a strong binding affinity to the human ACE2 receptor.SARS-CoV2 and SARS-CoV spike proteins share 76.5% identity in amino acid sequence, and hence importantly, the SARS-CoV and SARS-CoV2 spike proteins have a high degree of homology [6]. In this review, we will address the ACE2 receptor, the entry portal of SARS-CoV2 like coronavirus in a human body.

SPIKE PROTEIN OF CORONAVIRUS:

The spike protein is a broad type 1 transmembrane protein varying from 1,160 amino acids for avian infectious bronchitis virus (IBV) and up to 1,400 amino acids for feline coronavirus (FCoV). Moreover, spike protein is highly glycosylated as it comprises 21-35 N- glycosylation sites. Spike proteins are assembled into trimers that are present on the virion surface and form the distinctive “corona” or crown-like appearance. The ectodomain of all coronavirus spike proteins distribute the same organisation in two domains: a N terminal domain named S 1 that is bound for receptor binding and a C terminal S 2 domain responsible for fusion. A noteworthy contrast between the spikes proteins of different coronavirus is whether it is cleaved or not in the course of assembly and exocytosis of virions. With some anomalies, in most alphacoronavirus and betacoronavirus SARS-CoV, the virions harbour an uncleaved spike protein, whereas, in some betacoronavirus and all gammacoronavirus, the cleaved protein is found in between S1and S2 domains, typically by a Golgi-resident host protease called furin. The spike protein is a class 1 fusion protein, and the formation of alpha-helical coiled-coil structure is the attribute of this class of fusion protein, which covers in their C- terminal part regions prognosticated to have alpha-helical secondary structure and to form coiled-coils. The S1 includes two subdomains, a N-terminal domain (NTD) and a C-terminal domain (CTD). Both these subdomains bind a diversity of proteins and sugars and function as a receptor-binding domain (RBD). Coronavirus spike proteins consist of two heptad repeats in their S2 domain, a characteristic typical of a class I viral fusion protein. Heptad repeats include a repetitive heptapeptide abcdefg with a and d being a hydrophobic residues feature of the development of coiled-coil that participate in the fusion process [1].

ACE2 (ANGIOTENSIN-CONVERTING ENZYME 2)-

Enveloped virus invades cells by binding with enveloped glycoprotein to cell surface receptors reflected by conformational changes heading to membrane fusion and delivery of the genome in the cytoplasm. Very recently, the receptor was perceived as a functional receptor of SARS-CoV(Coronavirus) [7]. ACE2(also known as ACE related carboxypeptidase) is a monocarboxypeptidase that is chiefly signified in the renal tubular epithelium and vascular endothelial cells. ACE 2 is a type I transmembrane, an 805 amino acid protein that includes an intracellular tail, extracellular domain(amino-acid 18-739), a transmembrane region (amino-acids 740-768). The extracellular part of ACE2 consists of the catalytic domain of (amino-acid 147-555), has a substrate-binding area(amino-acids 273-345), and a typical metalloproteinase zinc-binding site HEMGH of amino-acids 374-378. Apart from this, ACE 2 mediated catabolism plays a significant and vital role in cardiovascular protection [8].

DISTRIBUTION OF ACE2 RECEPTOR:

The expression of the ACE2 receptor is located in many extrapulmonary tissues, together with the kidney, endothelium, intestine, and heart. The ACE2 receptors are mostly present in alveolar epithelial type II cells (AECII), and these cells can serve as a reservoir for viral invasion. This expression of the ACE2 receptor makes the lung further susceptible to the inhaled virus and a more vulnerable target organ. Moreover, gene ontology enrichment analysis described that ACE2 expressing AECII has significant levels of multiple viral-process- related genes, including regulatory genes for viral genome replication, viral life cycle, and viral assembly and these expedite coronaviral replication in the lungs [6].ACE2 tissue distribution in other organs could explain the multiple-organ dysfunction in patients. High ACE2 expression was recognised in type II alveolar cells of the lung, absorptive enterocytes from the ileum and colon, oesophagus upper and stratified epithelial cells, myocardial cells, cholangiocytes, urothelial bladder cells, and kidney proximal tubule cells. These conclusions depicted that these organs with high ACE2 expressing cells should be regarded as high risk for 2019n-CoV infection [9].

ENTRY AND BINDING:

The coronaviruses, cellular entry depends on the binding of the spike (S) protein to a particular cellular receptor and following S protein priming by cellular proteases. Likewise to SARS-CoV, SARS-CoV2 applies ACE2 as a receptor for coronavirus cellular entry. However, a significant determinant of the SARS-CoV replication rate and disease severity was the endowed binding affinity of the S protein and the ACE2 [5]. The viral entry also depends on the cathepsin B/L and TMPRSS2 protease activity and may be able to substitute for TMPRSS2 [10,4]. As time passed, coronaviruses had modified their spike proteins, which lead to a variety of triggers used to activate their fusion. These conformational changes can be started by receptor binding but may need supplementary triggers like pH acidification [1]. Coronavirus entry into host cells is a significant target for human intervention strategies and host immune surveillance. To aid entry into host cells, coronaviruses first bind to the cell surface receptor for viral attachment and ultimately fuse viral and lysosomal membranes to enter the endosomes consequently.

A virus surface-anchored homotrimeric spike protein mediates coronavirus entry. On mature viruses, the spike protein is present as a three-receptor binding trimer where S1 heads resting on top of a trimeric membrane merging S2 stalk. However, as per the cell entry mechanism, the SARS-CoV S1 consists of (RBD) receptor-binding domain that recognises explicitly (ACE2) angiotensin-converting enzyme 2 receptor. The receptor-binding domain continuously switches

between standing up position for receptor binding and lying down position for immune evasion. Furthermore, for the membrane fusion, the SARS-CoV spike needs to be proteolytically activated at the S1/S2 boundary so that S1 dissociates, and S2 experiences a dramatic structural change [11]. These coronavirus entry mechanisms depend upon cellular proteases, which include cell surface proteases TMPRSS2, lysosomal proteases cathepsins, and human airway trypsin like-protease (HAT) [4]. The following characteristics of SARS-CoV entry contribute to its severe symptoms and high fatality rates of ill patients.

There were many contradictory conclusions on the cell entry mechanisms of SARS-CoV2. Similar to SARS-CoV, SARS-CoV2 also recognises human ACE2 as its receptor. But according to the latest determined crystal structure of SARS-CoV2 RBD complexed with human ACE2, divulge precise but functionally essential differences between SARS-CoV and SARS-CoV2 in receptor recognition. These contrasts depict that SARS-CoV2 RBD to have a high binding affinity with hACE2 than SARS-CoV RBD [2].The receptor-binding domain (RBD) of SARS-CoV2 has a twisted five stranded antiparallel β-sheet (β 1,2,3,4 and β 7) with short connecting helices and loops that form the core. Among the β4 and β7 strands in the centre, with an extended insertion containing the short β5 and β6 strands,α4 and α5 helices, and loops. This expanded insertion is the RBM, which consists of the utmost of the contacting residues of SARS--CoV2 that bind ACE2 [3]. Albeit recent studies also highlighted that RBD is a crucial purpose within the S1subunit that is efficient for the binding of SARS-CoV2 by ACE2. Moreover, the cyro-EM structure divulges that SARS-CoV2 RBD is mostly in the lying downstate, a state-affiliated with weak receptor binding. Besides, there have been many opposing reports on the binding affinities of SARS-CoV2 and SARS-CoV spikes with hACE2.But as per the report, TMPRSS2 and lysosomal proteases are both critical for SARS-CoV2 entry [11].In avian influenza viruses, the proprotein convertase (PPC) motif is a symbol of pathogenesis in the surface glycoprotein. Nevertheless, coronavirus spike proteins comprise the PPC motif at the S1/S2 boundary; it was stated that spike protein PPC cleavage did not intensify coronavirus entry into cells. Therefore as per the result, SARS-CoV2 had been cleaved during viral packaging [11]. The article information states that neutralising antibodies represent a vital component to fight against SARS-CoV2. This coronavirus could be cross-neutralized by anti-horse CoV serum and convalescent serum from a patient with SARS-CoV infection [3].

CONCLUSIONS

In conclusion, the severe acute respiratory syndrome 2 is a highly infectious and pathogenic virus which has posed a critical global public health emergency. Similar to people who were infected by SARS-CoV in 2003 and MERS-CoV in 2012, public affected by SARS-CoV2 showed a range of symptoms as well as pneumonia, dry cough, headache, fever, dyspnoea, tiredness, sore throat, diarrhoea,conjunctivitis and loss of smell and taste etc. The coronavirus is the members of the subfamily Coronavirinae and family Coronaviridae, whose natural reservoir was Chinese horseshoe bats and intermediate hosts like civet cats and raccoon dogs expedited human transmission [2]. The entry of the virus depends on the subtle interaction between the host cell and the virion. Infection is initiated by the interplay of the viral particle with a particular protein on a cell surface [1]. A virus surfaced-anchored homotrimeric spike protein mediates coronavirus entry. However, as per the cell entry mechanisms, the S1 protein consists of RBD(receptor binding domain) that concedes explicitly (ACE2) receptor [11]. These coronavirus cell entry mechanisms depend upon cellular proteases, which include cell surface proteases

TMPRSS2, lysosomal proteases cathepsins and human airway-trypsin like proteases (HAT) [4]. These are the following features of SARS-CoV entry which contribute to its severe symptoms and high fatality rates of ailing patients.

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