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TMPRSS2 AND CD147: NOVEL THERAPEUTIC TARGET FOR THE PREVENTION OF SARS-COV-2 INVASION INTO HOST CELLS

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ABSTRACT
The ongoing outbreak of coronavirus disease-2019 (COVID 19) is a serious concern for public health. It is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). This viral infection which causes severe respiratory distress syndrome was declared as a pandemic on 11th March, 2020. More than 250 countries of the world now suffer in COVID-19. At present more than 171 million confirmed cases and more than 3.6 million people were died in COVID-19 worldwide. It is characterised by acute respiratory distress, pneumonia and multi-organ failure. Viral entry into cells is a major target for host immune surveillance and human intervention strategies. SARS-CoV-2 binds with membrane bound Angiotensin Converting Enzyme 2 (ACE2) via its spike protein and gain entry to host cell. ACE2 widely distributed in various tissues and acts as primary receptor for SARS-CoV-2. CD147 acts as second receptor particularly in target immune cells of SARS-CoV-2 which is lack of ACE2. Viral entry via surface receptor recognition and membrane fusion is the most potent route of viral entry. Trans-membrane serine protease 2 (TMPRSS2) that cleaves SARS-CoV-2 and ACE2 facilitates viral entry by membrane fusion. Thus ACE2, CD147 and TMPRSS2 are three major players in pathogenesis of COVID 19. Blocking of host cell receptor and or inhibition of protease could be a therapeutic approach against SARS-CoV-2 infection. ACE2 provides protection against acute cardiovascular and lung injury and protection against disseminated intravascular coagulation caused by COVID-19. Thus treatment with blocking ACE2 probably has a negative impact. CD147, is a target for COVID-19 treatment as CD147 antibodies efficiently improve the recovery from COVID-19. TMPRSS2 inhibitor block viral entry into host cells. Thus prevention of SARS-CoV-2 entryby using TMPRSS2 blocker would be beneficial against COVID-19.

KEYWORDS: SARS-CoV-2, ACE2, CD147, TMPRSS2, Spike protein.

INTRODUCTION
At present the world is going through an outbreak of Corona virus disease 2019 (COVID-19)caused by severe acute respiratory syndrome coronavirus-2 (SARS CoV-2). The disease was first observed in Wuhan, China in December 2019 and since then spread globally. WHO declared the 2019-2020 coronavirus outbreak as a pandemic on 11th March 2020. It is highly transmissible from person to person through respiratory droplets. More than 250 countries of the world now suffer in COVID-19.[1] At present (1st June, 2020) more than 171 million confirmed cases and more than 3.6 million people were died in COVID-19 worldwide. The most of the COVID-19 cases are asymptomatic or show mild symptoms include fever, cough, breathing difficulty, fatigue and shortness of breath.but low percentage suffer in severe respiratory failure in spite of same viral load like symptomatic patients.[2] Death from COVID-19 is due to viral pneumonia, intravascular coagulation and multi-organ failure.[3]

Mortality rate was higher in older patients with diabetes and hypertension.[4] Global health and economy is now destroying from rapid spread of COVID-19.[5,6] There is no potent drugs nor vaccine for treatment of COVID-19. Thus prevention strategy should be considered to minimize complications of COVID-19.Entry of coronavirus into host cells is the determinant of viral infectivity and pathogenesis.[7]

Viral entry into cells is a major target for host immune surveillance and human intervention strategies. The aims of this study was to identify mechanisms through reanalysis of publicly available data by which COVID-19 enter into host cells.

SARS-CoV-2 Receptor
Receptor recognition is crucial in viral infectivity and

pathogenesis. Corona viruses use variety of receptors to enter the cells. For instance MERS-CoV uses dipeptidyl protease 4 or CD26[8] and SARS CoV uses angiotensin converting enzyme 2 (ACE2) and CD209L.[9] Like SARS-CoV, SARS CoV-2 uses ACE2 as its receptor[10], through which SERS CoV-2 enter into cells.[11,12] The pattern of ACE2 expression in different tissues can determine susceptibility, symptoms and outcome of SARS-CoV2 infection.[13] Oral infection of SARS- CoV2 is common as ACE2 is expressed on the mucosa of oral cavity and epithelial cells of tongue.[10] ACE2 is distributed on the surface of various cells including CNS, Upper air way and lungs, Liver, Kidney, pancreas, heart and endothelial cells.[14]

Receptor Binding

COVID-19 binds with ACE2 via its spike protein (S-protein). In coronaviruses the S-protein istransmembrane glycosylated protein. Spikes appear as crown on the virion surface hence such viruses are called coronavirures. Spike is a homotrimer and composed of an extracellular domain, a trans membrane domain and a short intracellular tail. Each monomer is composed of two subunits (S1 and S2) in its extracellular domain. S1 is related to receptor recognition whereas S2 is related with membrane fusion.[15] S1 is subdivided into two domains S1A or N-terminal domain (NTD) and S1B or receptor binding domain (RBD). S1A interacts with glycoproteins that have sialic acid at the distal end of glycan portion especially if the monosaccharide is in the modified form of 5-N acetyl-9-o-acetyl- sialoside[16] whereas S1B binds to the ACE2 receptor.[17] In some coronavirus species like MERS-CoV receptor binding with S1B is potentiated by prior binding of S1A domain with sialosides.[18] For most of the corona viruses importance of both domains for viral invasion is not known. The studies from other mammalian coronaviruses suggest that sialosides may facilitate the interaction between S-protein receptor.[19] The existence of sialic acid binding site in NTD of SARS-CoV-2 was established from silico preprint analyses.[20] Sialoside moieties are noted in glycans attached to both ACE2[21] and CD147.[22] The hidden RBD may be a cause of insufficient immune response and long incubation period. Many SARS-CoV-2 patients develop low levels of neutralizing antibodies.[23]

Both SARS-CoV and SARS-CoV-2 binds with ACE2 receptor. RBD of SARS-CoV-2 has higher affinity to ACE2 than RBD of SARS-CoV. In coronavirus RBD exists either standing-up state which binds to host receptor or lying-down state which does not bind.[24] In cryo-EM studies it was suggested that RBD of SARS-CoV exists as standing state.[25] whereas in SARS-CoV-2 the RBD remains in lying-down state.[11,26] In compare with SARS-CoV binding affinity RBD of SARS-CoV-2 to ACE2 is lower as it is less accessible.

The expression of ACE2 is very ubiquitous in the nasal epithelium, lung, heart, kidney and intestine but rarely expressed in immune cells.[27] But immune cells can be infected by SARS-CoV-2 like SARS CoV.[28,29] This suggest involvement other receptor for viral invasion. CD147 also called extracellular matrix metalloproteinase inducer (EMMPRIN) acts as receptor for SARS CoV-2 in cell lines of epithelial origin.[27] SARS CoV-2 invades human host cells via CD147 binding.[30] CD147 is a transmembrane glycoprotein of the immunoglobulin superfamily which participate Plasmodium invasion and viral infection. Theloss of CD147 or blocking CD147 by meplazumab inhibits SARS-CoV-2 replication on the other hand overexpression promotes virus infection.[31] Viral loads are detectable in the lungs of hCD147 mice infected with SARS-CoV-2 but not in wild mice.[31] CD147 is ubiquitously expressed in epithelium and immune cells.[27] In addition CD147 has three asparagine glycosylation sites to which glycan bind.[32] Spike protein of SARS-Cov-2 also glycosylated[33]suggesting carbohydrate interaction between CD147 and spike protein for binding. After binding SARS-CoV-2 virion enter the host cells through CD147-spike protein route by endocytosis which is independent on clathrin.

Proteolytic Cleavage of SARS-CoV-2

SARS-CoV-2 relies on host protease for cellular entry as RBD is less assessable. Coronavirus entry is tightly regulated by the expression and activation of host proteases. S-protein is a class I viral fusion protein. Priming and activation convert the fusion protein to a fusion- competent state.[34,35] The first cleavage at S1/S2 site is called priming which gives rises to S1 and S2 functional subunit those are linked together noncovalently to a pre-fusion state.[36] Cleavage at S2 is known as activation by which fusion peptide inserted into target membrane.

Priming Cleavage Initial SARS-CoV-2 proteolytic processing in human cells has been associated with recognition of polybasic (several arginine residues) furin site at S1/S2cleavage site.[37]

CoV S-proteins have multiple motifs and cleavage sites. Thus proteins act as substrate for various host proteases like cathepsin[38], transmembrane serine protease[39,40] and furin like proprotein convertases.[41] A furin sensitive motif at the junction between S1 and S2 subunit was noted in SARS-CoV-2 which is absent in other SARS-CoVs. Such furin sensitive motif may be responsible for high pathogenecity of SARS-CoV-2.[42] Furin preactivation enhances SARS-CoV-2 pseudovirus entry in different hAEC2 –expressing cell lines including lung epithelial cells.[43] The furin cleavage site is conserved among all sequenced SARS- CoV-2 virions[44] and its abrogation reduced the efficency of viral entry.[45]

Furin is a type of proprotein convertage. It is located in in the transgolgi network and activated by acidic pH.[46] It can cleave precursor protein with specific motifs to produce biological active protein. The first and fourth amino acid at the N-terminus of the substrate cleavage

site must be Arginine. Furin, host protease cleaves SARS-CoV-2 S-protein at the S1/S2 junction.[47] TMPRSS2 serves as substitute protease. Arginine 667 is critical for initialSARS-CoV-2 protein priming.

Activation Cleavage: S2 cleavage i.e activation of SARS CoV-2 in human airway cells occursvia TMPRSS2 after priming at the S1/S2 priming site.[48] TMPRSS2 shows expression in various tissues including brain, lungs, bronchial epithelial cells, heart, liver and gastrointestinal tract.[49] Expression is high in adult lungs than fetal counterpart.[50]

Expression was noted in type-II alveolar epithelial cells and alveolar macrophages but not in type-I alveolar epithelial cells (51, 52). As both ACE2 and TMPRSS2 are expressed in human corneal epithelial cells suggesting that ocular surface is a potential route of COVID-19 infection.[53]

The S-protein undergoes further cleavage at the S2 site by host proteases to make irreversible conformational change of later for membrane fusion.[54] Transmembrane serine protease-2 (TPMRSS2) and lysosomal cathepsins both have cumulative effects with furin for viral entry. However, furin preactivation allows viral entry with relatively low expression of TPMRSS2 and lysosomal cathepsins.[47] TMPRSS2 processing of SARS CoV-2 is principally at the cell membrane whereas furin-mediated processing occurs at the cell surface and in the early endosome.[55,56]

S2 contains activation proteolytic site and fusion peptide. Activation cleavage event gives rise to the mature hydrophobic fusion peptide that is inserted into the host target cell membrane[57] and causes membrane fusion. In SARS-CoV S-protein-induced membrane fusion is calcium dependent. Higher calcium concentration enhancing membrane fusion.[58]

Viral Entry Into Host Cells

Membrane fusion and endocytosis are two main entry modes for virus infection.[59] SARS CoV entry is either late of early depending on entry route. Viral entry via surface receptor recognition and membrane fusion is referred to as early[60] whereas entry occur through endosome is referred to as late entry.[61] In late entry the virus is first endocytosed and subsequenty cleaved by furin proprotein convertases[62], cathepsin L[63] and or Cathepsin-B.[64]

Theoritical Preventive Approaches

Inhibition of host cell receptor and or protease could be a promising route for the development of therapeutic approach against SARS-CoV-2 infection. Because a host receptor or protease is targeted, this strategy would avoid the resistance due to mutation ofvirus.

Blocking of cell recognition: SARS-CoV-2 invades host cells via two receptor: ACE2 and CD147. Thus inhibition of ACE2 and or CD147 should be a effective therapeutic approach against COVID-19 infection. ACE2 is the main receptor for coronavirus as viral replication is inhibited by anti-ACE2 antibody. SARS-CoV-2 binds with surface ACE2 by their S-protein and then internalized along with ACE2[65,66] Internalization of ACE2 reduces surface tissue expression of it. After SARS-CoV infection ACE2 mRNA expression is inhibited.[67] Thus SARS-CoV-2 infection-induced down regulation of ACE2 is associated with decrease Ag-1-7 and increase Ag-II which leads to cardiovascular and pulmonary injury disseminated intravascular coagulation.[68,69] However ACE2 play an important role in controlling bloodpressure and preventing heart failure and kidney injury.[70] Thus treatment with blocking ACE2 probably has a negative impact.

CD147 is a new receptor for SARS-CoV-2 as ACE2 deficient T cell can be infected with SARS-Cov-2 pseudovirus in dose dependent manner which is inhibited by meplazumab, a blocker of CD147.[31] SARS-CoV virion was observed in peripheral lymphocytes and causingdestruction of later.[71] Thus one of the reason of COVID-19 induced lymphopenia is viral invasion into lymphocytes via CD147 receptor.[72] CD147 antibodies like Meplazumab efficiently improve the recovery from COVID-19.[73] Azithromycin a potential anti COVID- 19 agent work through CD147 protein pathway[74] Rodrigues-Diez et al. (2020) reported that atorvastatin down regulates CD147 in human cells and diminishes viral infectivity[75], These evidences shows that CD147 is a novel for SARS-CoV-2 infection as well as a novel target for COVID-19 treatment.[76]

Pulmonary fibrosis is an important complication due to COVID-19 (74). Pulmonary fibrosis is associated with deposition of myofibroblasts in lung alveoli. Angiotensin-II is necessary to promote lung fibrosis. ACE2 cleaves angiotensin-II and inhibits lung fibrosis in experimental animal[77] Thus SARS-CoV-2 induce down regulation of ACE2 causes lung fibrosis. CD147, second entry receptor of SARS-CoV-2, present in type-II pnemocytes and macrophages at the edges of the fibritic zones. It-induces proliferation fibroblasts to myofibroblasts. Anti CD147 antibody inhibits proliferation and differentiation of fibroblasts to myofibroblasts[78] Therefore blocked of CD147 could also play a beneficial role in pulmonary fibrosis due to COVID-19. Thus CD147 blockers not only inhibit SARS-CoV-2 invasion into host cell but also protect the host from COVID-19-induced pulmonary fibrosis.

***Prevention of fusion*:** TMPRSS2 is an androgen-responsive serine protease that cleaves SARS-CoV-2 and facilitating viral entry.[79]TMPRSS2 is expressed in tissues those are important target of SARS-CoV-2 like cardiac endothelium, respiratory system, kidney, digestive tract and microvascular endothelial cells.[80] ACE2 and TMPRSS2 are co-expressin lung AT2 cells, type-II pneumocytes and lung alveoli.[81,82] TMPRSS2

cleaves the transmembrane C-terminal domain of ACE2 (residue 697 to 716) and releases c-terminal fragment into intracellular compartment. Such cleavage of ACE2 leads to conformational changes to S1-ACE2 complex and facilitates viral entry.[83] It also cleaves S-protein at arginine rich positions (arginine 667, arginine 797) at S1-S2 fusion site which is crucial for SARS-CoV-2 entry in host cells.[84] Such S1-S2 cleavage is called priming. This priming generate a mature peptide for membrane fusion with the release of S1 domain in extra cellar compartment.[85]Thus released S1 fragment from S-protein by TMPRSS2 bind with humoral antibodies and incapacitate the latter to neutralize virus.

The most critical stage of viral entry into host cell is priming of S-protein where S-protein is cleaved by cellular proteases like TMPRSS2. S-protein priming by TMPRSS2 is crucial for SARS-CoV infection of host target cells.[84] Thus prevention of SARS-CoV-2 entry by using protease inhibitor would be beneficial.[86] TMPRSS2 is shown to be blocked by serine protease inhibitors such as camostat.[87]

CONCLUSION

ACE2 is the main receptor of coronavirus. SARS-CoV-2 binds with ACE2 via its spike protein. CD147 acts as second receptor for SARS-CoV-2 particularly in target immune cells of SARS- CoV-2 which is lack of ACE2. Membrane fusion and endocytosis are two main entry modes for virus invasion into host cells. Viral entry via surface receptor recognition and membrane fusion is the most potent route. Host protease is essential for membrane fusion. TMPRSS2 is an androgen-responsive serine protease that cleaves SARS-CoV-2 and facilitating viral entry. ACE2 and TMPRSS2 are co-express in lung AT2 cells, type-II pneumocytes, lung alveoli and many other target cells of SARS-CoV-2. TMPRSS2 cleaves the C-terminal domain of ACE2 resulted conformational changes to S1-ACE2 complex and facilitates viral entry. It also cleaves S-protein at S1-S2 fusion site to generate a mature peptide for membrane fusion.

Blocking of host cell receptor and or inhibition of protease could be a therapeutic approach against SARS-CoV-2 infection as it avoid the resistance due to mutation of virus. ACE2 not only involve in viral entry into host cells but also provide protection against acute cardiovascular and lung injury and protection against disseminated intravascular coagulation caused by COVID-19. Thus treatment with blocking ACE2 probably has a negative impact. Second SARS-CoV-2 receptor, CD147, is a target for COVID-19 treatment as CD147 antibodies efficiently improve the recovery from COVID-19. S-protein priming and activation by TMPRSS2 is crucial for SARS-CoV-2 infection of host target cells. Thus prevention of SARS-CoV-2 entry by using protease inhibitor would be beneficial.

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