Spike protein and its proteases role in SARS-COV-2 pathogenicity and treatment; a review

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Abstract

Since December 2019, a novel beta coronavirus has spread around the world. This virus can cause severe acute respiratory syndrome (SARS). In this study, we reviewed proteases of SARS-CoV-2 based on related articles published in journals indexed in Scopus, PubMed, and Google Scholar from December 2019 to April 2020. Based on this study we can claim that this coronavirus has about 76% genotype similarity to SARS coronavirus (SARS-CoV). Also, similarities between these two viruses have been found in the mechanism of entry to host cells and pathogenicity. ACE 2, the angiotensin convertase enzyme 2, has roles in the Renin-Angiotensin-Aldosterone system (RAAS) and blood pressure regulation. Some mechanisms have been reported for the role of ACE 2 in the pathogenicity of SARS-CoV-2. For example, the interaction between ACE 2 receptor and spike protein mediated by TMPRSS2, Cathepsin B/L, and other enzymes is responsible for the entry of virus to human cells and pathogenicity. Some host cell endosomal enzymes are necessary to cleavage coronavirus spike protein and cause binding to their common receptor. So, we conclude that molecules like antibodies or small molecules as ACE2 antagonists and soluble ACE 2 can be used as a good therapeutic candidate to prevent SARS-CoV-2.

Keywords: SARS-CoV-2, ACE2, Spike protein, TMPRSS2, Furin
1. Introduction

The SARS-CoV-2 is a zoonotic virus from the coronaviruses (CoVs) family (1). It has a 76% similarity in the genome with SARS-CoV, which broke out in 2003 with 10% mortality rate (1-3). Clinical manifestations of SARS-CoV-2 include fever, nonproductive cough, dyspnea, myalgia, fatigue, normal or decreased leukocyte counts, and pneumonia, which are similar to the symptoms of SARS-CoV infection. SARS-CoV-2 commune period is 2–14 days (4). Some factors are involved in the pathogenesis process, which are introduced in this narrative review.

Because of the rapid widespread of this novel virus and the lack of efficient treatments, we decided to review the SARS-CoV-2 host receptor, spike protein, and enzymes that are involved in
pathogenicity processes. Molecules that can inhibit the spike protein attachment to ACE2, spike protein, or involved enzymes could be a choice for treatment.

2. Method

We searched title, abstract, and keywords for related English articles in Google Scholar, Scopus, and PubMed databases for all types of articles from December 2019 to April 2020. The search was performed by FA and EA separately and then checked by both of them. The following keywords were searched: [(COVID-19 AND ACE2) OR (COVID-19 AND Spike protein) OR (COVID-19 AND Protease) OR (SARS-CoV-2 AND ACE2) OR (SARS-CoV-2 AND Spike protein) OR (SARS-CoV-2 AND Protease)]. We searched keywords in the mentioned databases to find basic information about the structure of SARS-CoV-2 and the mechanisms of protease-associated spike protein cleavage that provide the viral entrance to the host cell. 101724 articles were found based on search engines reports. Afterward, 300 articles full texts were evaluated. we included 20 of them based on inclusion criteria.

3. Result and discussion

SARS-CoV-2 spike glycoprotein facilitates virus entry to host cells by attaching to the ACE2 receptor. Spike cleavage occurs at different stages of the virus life cycle like entry, pathogenesis, and release from host cells. Beta coronavirus spike protein may be cleaved. In many cases, furin cleaves the S1/S2 sites. Cleavage sites such as S1/S2 site, S2’ site, and furin-like cleavage sites have their role in virus entry and pathogenicity. S1 peptide is responsible for receptor binding and S2 peptide causes virus and host membrane fusion.

3.1. Coronavirus structure

All CoVs have a specific structure consist of the envelope (E), nucleocapsid (N), spike protein (S) integrated into the membrane (Figure 1). M protein is an ionic channel that is essential for the virus uncoating. M protein is an integral protein with a glycosylated N-terminal in ectodomain and a C-terminal in the endo domain. M protein has an important role in virus structure formation in host cells (5). Also, studies have shown that viruses cultured in a tunicamycin-rich medium can be formed without spike; contains M and Free-S (6). E protein has facilitative effect on virus assembling and exit. Furthermore, this protein has ionic channel activity in SARS-CoV-2 pathogenicity (5).
SARS-CoV-2 genome is a single-stranded and positive-sense RNA. The open reading frame (ORF) is a part of the genome that encodes the nucleoside salvage pathway (NSP). There is a region between ORF1a and ORF1b that produces Protein phosphatase (PPI) including PP1a and PP1b (Figure 2). 16NSPs can be made of these PPs by three chymotrypsin-like proteases and papain protease (6). ORF2-10 makes structural proteins (2). Besides, the structural proteins (E, M, S, and N), are functional proteins essential for the virus life and its replication such as enzymes (6). Host-cellular proteases action locations and timings are partial because initiating Spike protein endoproteolytic cleavages take place just after ACE2 engagement (7, 8).

Fig.1: A schematic image of SARS-CoV-2 and its structure

Fig.2: The genome of CoVs with open reading frames (ORFs) and zones of encoding structural and functional proteins
3.1.1. Spike protein and virus entry

3.1.1.1. Structural fractures
The spike glycoprotein is a trimeric integrated glycosylated massive protein (150kDa) (5). A large N-terminal ectodomain, a single-pass transmembrane anchor, and a short C-terminal intracellular tail are in the protein parts of spike glycoprotein (9). Amino acid residues are essential for the interaction between the SARS spike protein and target receptor (ACE2) (9, 10). Eight of 14 amino acids important in the SARS-CoV-2 spike protein Receptor-Binding Domain (RBD) are similar to SARS-CoV spike protein, so the host receptor is the same(11).

3.1.1.2. Function
Spike protein has two sites to be cleaved by cellular proteases; S1/S2 and S2’ (5, 12). S1/S2 site consists of Arginine amino acid residues that are associated with proteolytic processing of spike protein and has an important role in the interaction between the RBD ectodomain of spike protein and ACE2 receptor (10, 13). S1/S2 cleaved into two polypeptides; three head S1 and trimeric stalk S2 (9, 14). The N-terminated S1 polypeptide which cleaves from spike protein is responsible for attachment to the ACE2 receptor. C-terminated membrane-anchored S2 is an attendant for membrane fusion and virus entry (10, 12). Generally, S1 binding to the specific receptor by RBD and fusing the virus membrane and membrane of the host cell with S2 enable the virus to enter its single-stranded RNA into the host cell. Fusion peptide (FP), a secondary proteolytic site (S2’), an internal fusion peptide(IFP), and two heptad-repeat (HR) chains form the S2 polypeptide (11, 13). IPFs are correspondent between SARS and SARS-CoV-2. Both IPF and PF are present in viral entry. But the molecular mechanism of cell entry is not totally known yet(11).

Further, S2’ is located within S2 upstream. A furin site cleavage is located between the S1/S2 site (13). Investigations suggest this furin-like cleavage site can be cleaved till virus endocytosis for spike priming. Also, it may provide result in more SARS-CoV-2 contagion in the human populations (2, 15).

3.2. ACE2, SARS-CoV-2 host receptor
As we know, the host receptor for SARS-CoV-2 is the same as the receptor that SARS uses to enter the cell (16). SARS-CoV-2 penetrates the host cells by ACE2 with a conformational change in the spike protein structure when attachment to ACE2 occurs (17, 18). However, the binding affinity of the SARS-CoV-2 spike protein to ACE2 is 10-20 times higher than SARS-CoV (17). During the researches, the injection of the spike protein of SARS to mice induced the lung acute injury that was reduced by the Renin-Angiotensin-Aldosterone system (RAAS) suppression. Anti-serum against ACE2 can block SARS-COV-2 and reveals that the ACE2 is the main target for SARS-CoV-2 (10).

ACE2 is a soluble mono carboxypeptidase enzyme in blood circulation in the RAAS which converts Angiotensin II (Ang) to Ang 1–7 with anti-inflammatory, vasodilative, and anti-apoptotic activities (15). ACE2 has been detected on type 2 alveolar cell (AT2) surface in the lungs and other
cell types like heart, central nervous system, and liver (17, 19). Whereas, testis, gastrointestinal tract, and kidney can express ACE2, so the fecal-oral route may be one of the routes of transmission. The human protein atlas database shows the ACE2 mRNA is mainly detected in the small intestine, colon, duodenum, kidney, testis, and gallbladder. Although, the ACE2 expression level in the lung is not significant (9). Some specific situations can regulate ACE2 expression in the lung cells. For example, SARS can down-regulate ACE2 expression via spike protein attachment to the receptor(17).

Up to date, there is no reliable evidence on ACE2 expression variations relating to age. And no observation verifies ACE2 expression dependency on smoking, sex, and gender. Also, researchers recognized that the expression of ACE2 in all lung specimens in women has increased. Medications such as Angiotensin receptor blockers (ARBs) and ACE-inhibitors, thiazolidinediones, and ibuprofen (NSAID) can increase ACE2 expression (Table 1) (20).

| Factors      | ACE2 expression dependency |
|--------------|-----------------------------|
| Age          | Independent                 |
| Sex          | Independent                 |
| Gender       | Independent                 |
| Smoking      | Independent                 |
| Medication:  |                             |
| ARBs         | Positive                    |
| ACE-inhibitors | Positive                  |
| Thiazolidinedione | Positive              |
| Ibuprofen(NSAID) | Positive               |

Table 1: Effective factors on ACE2 expression

3.3. Effective Enzymes on spike Priming

3.3.1. Serine proteases

Serine proteases of the trypsin-like family have been identified to have significant effects in biological processes like digestion, blood coagulation, fibrinolysis, and immunity.
One of the serine proteases is the transmembrane serine protease type 2 (TMPRSS2), that is essential for spike priming (15, 16, 21). Also, TMPRSS2 has proteolytic effects on the spike protein to allow virus and cell membrane fusion. Based on some researches, SARS-COV can be activated with TMPRSS2 through proteolytic mechanisms. VeroE6/TMPRSS2 are more vulnerable to SARS-COV-2 expression ACE2 more. This observation illustrates the definite action of TMPRSS2 in SARS-COV-2 infection. Some studies have shown that SARS-CoV-2 isolation from Vero E6 and TMPRSS2 cells is 10 times more than normal human lung tissue(21). TMPRSS proteases group is highly expressed in the respiratory tract (13).

Trypsin is one of the endosomal serine peptidases. The trypsin role in viral glycoprotein priming has been extensively investigated (13, 22). Trypsin potently prefers arginine and lysine amino acids to others for cleavage. As the function of this peptidase is not selective on substrates, there are many different zones at spike protein that potentially can be cleaved by trypsin. Trypsin as a TMPRSS is expressed in the respiratory tract; however, it has a digestive function in the small intestine. Trypsin can directly cleave the spike proteins of many enteric coronaviruses. Respiratory coronaviruses can use trypsin as a surrogate for proteases such as TMPRSS family members, because of similar substrate specificities. SARS-CoV is assembled in VeroE6 cells where trypsin can revoke the need for cathepsin-mediated cleavage and shifts the virus to a low-pH independent route of entry, probably at the plasma membrane (13).

3.3.2. Proprotein convertases

Proprotein convertases (PCs) make a group of serine secretory proteases that modulate virus biological processes. PCs, especially furin, are involved in viral infection because of their role in cell surface protein processing. They cleave the envelope glycoprotein for viral fusion with the host cell membrane (11).

Furin is expressed in a broad spectrum of cell types including lung tissue. So an enveloped virus causing a respiratory infection like SARS-CoV-2 can employ furin to cleave its surface spike glycoprotein. The pathogenicity of some CoVs depends on the presence of a furin-like cleavage site. For instance, researchers have elevated the rate of pathogenicity by adding a furin-like cleavage site to spike protein of infectious bronchitis virus (IBV). Also, a furin-like cleavage site is located on the spike protein of pathogenic influenza viruses (11). SARS-CoV-2 spike protein priming with furin would potentially provide 25% more susceptibility of cells to infection in comparison with TMPRSS2 spike protein cleavage. Efficient cleavage of the SARS-CoV-2 Spike protein by furin that results in more pathogenicity of the virus due to an enhanced affinity to the ACE2 receptor (15). Different furin-like proteases would cleave the S2’ site (11). It’s supposed that S2’ processing is a significant stage in final spike protein activation. However, the enzyme(s) involved in this process have not been determined absolutely.

3.3.3. Cysteine proteases

Cysteine proteases like cathepsin B and L (cat B/L) are important to spike priming in SARS-CoV-2 (10, 16). These enzymes activate virus glycoproteins (13). As mentioned, the SARS-CoV-2 entry to host cells is dependent on TMPRSS2 activity. In contrast, cat B/L activity is dispensable (10). Cat L is more common for the CoVs entry than cat B but the intracellular acidity required for viral entry in cat B is higher than cat L pH. Notably, endosomal low pH is essential
for virus uncoating (23). Ammonium chloride increases intracellular pH. Experiments show that SARS-CoV and SARS-CoV-2 entrance to 239T cells (TMPRSS2-) is blocked by ammonium chloride. Though ammonium chloride treatment shows low efficacy in entrance inhibition on Caco-2 cell (TMPRSS2+). Inhibition of both proteases is required to block virus entry. A known TMPRSS2 inhibitor is the camostat mesylate that can block SARS-CoV-2 entry into both Caco-2 and Vero/TMPRSS2 cells. However, virus entry full inhibition happens when E-64d as a cat B/L inhibitor is added to camostat mesylate. We can understand SARS-CoV-2 uses of both cat B/L and TMPRSS2 to spike priming(10).

3.3.4. Other proteases

Plasmin, as a key enzyme in clot lysis is is produced by neutrophils during the inflammatory responses. This enzyme is effective on spike protein of SARS activation. More investigations on these enzymes may prove the efficiency of elastase and plasmin in spike priming in SARS-CoV-2 (Table 2) (13, 22).

| Virus life cycle stages | Relevant Proteases | Free virus Particle | Attachment & entry | Endocytosis | Fusion | Protein Biosynthesis | Assembly & Egress |
|------------------------|--------------------|---------------------|-------------------|-------------|--------|---------------------|------------------|
| Furin                  | ✔                  | ✔                   | ✔                 | ✔           | ✔      | ✔                   |                  |
| TMPRSS group           | ✔                  |                      |                   | ✔           | ✔      |                     |                  |
| Trypsin                | ✔                  |                      |                   |             |        |                     |                  |
| Cathepsin              | ✔                  |                      |                   |             |        |                     |                  |

Table 2: Stages of coronaviruses life and proteases related to each stage.

3.4. Possible and suggested treatments

Medications such as ARBs and ACE-inhibitors, thiazolidinediones, and ibuprofen (NSAID) increase ACE2 expression (20). Investigations have revealed losartan as an AT1R blocker decreases the severity of COVID-19 (6). No evidence can support changing the treatment regimen in patients who received ACE-inhibitors, ARB, and thiazolidinediones (20).
4. Conclusion

SARS-CoV-2 spike glycoprotein facilitates virus entry to host cells by attaching to the ACE2 receptor. A wide range of enzymes are involved in spike priming that includes the TMPRSS group (specially TMPRSS2), cathepsin B/L, furin, trypsin, etc. There is still no definitive treatment for SARS-CoV-2 but structures that can inhibit the mentioned enzymes and processes can be used as a treatment choice. Proteases inhibitors such as camostat mesylate, a TMPRSS2 inhibitor, E-64d, as a cat B/L inhibitor can be evaluate more as SARS-CoV-2 treatments. Further researchs on these mechanisms and blocking the function of E and M proteins may cause to hopeful results in SARS-CoV-2 prevention and treatment.

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6. Authorship

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

7. Compliance with ethical guidelines

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

8. Conflict of interest

None.

9. References

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