OPEN ACCESS

DOI: 10.25040/ntsh2021.01.05

For correspondence:
P. O. Box 1583, 71345 Karafarin Avenue, Shiraz, Iran
E-пошта: f.tavakoli99@yahoo.com

Received: Dec, 12, 2020
Accepted: Apr, 4, 2021
Published online: Jun, 29, 2021

ORCID IDs
Fateme Tavakoli Far
https://orcid.org/0000-0002-1783-0460
Ehsan Amiri-Ardekani
https://orcid.org/0000-0001-8948-9153

Disclosures. The authors declared no conflict of interest

Author Contributions:
Conceptualization: Fateme Tavakoli-Far, Ehsan Amiri-Ardekani
Results of study: Fateme Tavakoli-Far, Ehsan Amiri-Ardekani
Writing: original draft Fateme Tavakoli-Far.
Review & editing: Ehsan Amiri-Ardekani

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

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.

Spike protein and involved proteases in SARS-COV-2 pathogenicity and treatment

Fateme Tavakoli Far[1], Ehsan Amiri-Ardekani[2,3,4*]

[1] Faculty of Pharmacy, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran
[2] Department of Phytopharmaceuticals (Traditional Pharmacy), Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
[3] Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
[4] Student Association of Indigenous Knowledge, Shiraz University of Medical Sciences, Shiraz, Iran

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 by Scopus, PubMed, and Google Scholar from December 2019 to April 2020. Based on this study, it is concluded 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 into 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 the ACE 2 receptor and spike protein mediated by TMPRSS2, Cathepsin B/L, and other enzymes is responsible for the entry of the virus into 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, COVID-19, ACE2, Spike protein, TMPRSS2, Furin.

Спайковий білок і залучені протеази в патогенність та лікування SARS-COV-2

Фатеме Таваколі Фар[1], Ехзан Амірі-Ардекані[2,3,4*]

[1] *Фармацевтичний факультет, Філія аятоли Амолі, Ісламський університет Азад, Амол, Іран*
[2] *Кафедра фітофармацевтики (традиційної фармацевтики), Фармацевтичний факультет, Ширазький університет медичних наук, Шираз, Іран*
[3] *Комітет студентських досліджень, Ширазький університет медичних наук, Шираз, Іран*
[4] *Студентська асоціація традиційних знань, Ширазький університет медичних наук, Шираз, Іран*

Резюме

Від грудня 2019 року світом шириться новий бета-коронавірус. Цей вірус може викликати тяжкий гострий респіраторний синдром (SARS). У цьому дослідженні ми розглянули протеолітичні ферменти вірусу SARS-CoV-2 на основі пов'язаних статей, опублікованих у журналах, які індексуються в базах Scopus, PubMed і Google Scholar, з грудня 2019 року до квітня 2020 року. На підставі цього дослідження можна стверджувати, що цей коронавірус має генотип, що на близько 76% схожий із коронавірусом SARS (SARS-CoV). Крім того, між двома вірусами виявлено подібність у механізмі потрапляння в клітини-господаря та патогенності. Ангіотензинперетворювальний фермент (АПФ-2) відіграє роль у ренін-ангіотензин-альдостероваій системі (РААС) та регуляції артеріального тиску. Повідомляється про роль деяких механізмів АПФ-2 у патогенності SARS-CoV-2. Для прикладу, взаємодія між рецептором АПФ 2 і спайковим білком через TMPRSS2. Катепсин B/L та інші ферменти відповідають за потрапляння вірусу в клітини людини й патогенність. Деякі ендосомальні ферменти клітин-господарів необхідні для розщеплення спайкового білка та спричиняють зв'язування з їхнім загальним рецептором. Тому зроблено висновок, що такі молекули, як антитіла, чи невеликі молекули, як-от блокатори АПФ-2 та розчинний АПФ-2, можна використовувати як можливі кандидати для запобігання SARS-CoV-2.

Ключові слова: SARS-CoV-2, АПФ-2, спайковий білок, TMPRSS2, Furin.

OPEN ACCESS

DOI: 10.25040/ntsh2021.01.05

Для листування:
П/С 1583, 71345 прос. Карафарін, Шіраз, Іран
Е-пошта: ehsanamiri@sums.ac.ir

Стаття надійшла: 16.12.2020
Прийнята до друку: 04.04.2021
Опублікована онлайн: 29.06.2021

ORCID IDs
Fateme Tavakoli Far
https://orcid.org/0000-0002-1783-0460
Ehsan Amiri-Ardekani
https://orcid.org/0000-0001-8948-9153

Конфлікт інтересів: Автори декларують, що немає конфлікту інтересів.

Особистий внесок авторів:
Концепція — Фатеме Таваколі Фар, Ехзан Амірі-Ардекані;
Збір та обробка даних — Фатеме Таваколі Фар, Ехзан Амірі-Ардекані;
Написання тексту — Фатеме Таваколі Фар, Ехзан Амірі-Ардекані;
Рецензування — Фатеме Таваколі Фар, Ехзан Амірі-Ардекані

Фінансування: *Автори декларують відсутність фінансування у підготовці даної статті.*

1. Introduction

The SARS-CoV-2 is a zoonotic virus from the family of coronaviruses (CoVs). It has a 76% genome similarity with SARS-CoV, which broke out in 2003 and had a 10% mortality rate [1]. 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 incubation period is 2–14 days [2].

Virus pathogenesis begins with the virus attaching to the host receptor and entering of its genome into the host cell [3]. The virus employs host cell machinery such as proteolytic enzymes to encode its proteins and infect the host cell [3]. The role of proteases in SARS-CoV virulence has been investigated. These proteolytic enzymes play a role in various steps of the viral life-cycle such as attachment and entry [4], endocytosis [5], fusion [6, 7], protein biosynthesize, assembly, and egress [4, 5]. As the SARS-CoV-2 and SARS-CoV have a 76% similarity in their genomes, it seems, this similarity may be found in the ways of pathogenicity. These enzymes are so major that inhibiting them may be used as a therapeutic approach. In this narrative review, we discussed the roles of the most major proteolytic enzymes in more detail.

2. Objectives

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.

3. Methods

We searched titles, abstracts, 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 entry into the host cell. 101,724 articles were found based on search engine reports. Afterwards, full texts of 300 articles were studied. we included 20 of them based on inclusion criteria.

4. Results and discussion

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

4.1. Coronavirus structure

All CoVs have a specific structure consisting of the envelope (E), nucleocapsid (N), spike protein (S) integrated into the membrane (Figure 1). M protein is an ionic channel that

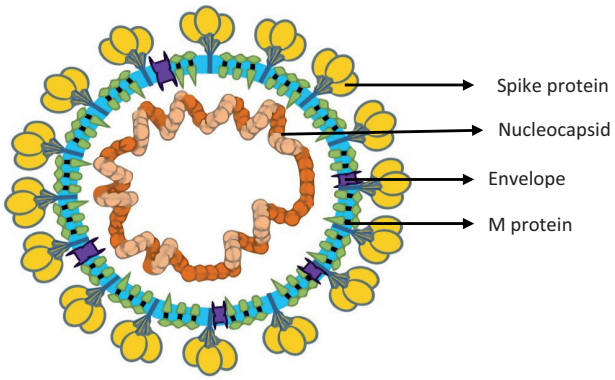

Fig. 1: A schematic image of SARS-CoV-2 and its structure

is essential for 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 [8, 9]. Also, studies have shown that viruses cultured in a tunicamycin-rich medium can be formed without spike; they contain M and Free-S

[10–12]. E protein has a facilitative effect on virus assembling and exit. Furthermore, this protein has ionic channel activity in SARS-CoV-2 pathogenicity [9, 13, 14].

SARS-CoV-2 genome is a single-stranded and positive-sense RNA [12]. 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 (Fig. 2). 16NSPs can be made of these PPs via three chymotrypsin-like proteases and papain protease [12, 15], while ORF2-10 encodes structural proteins [1]. The structural proteins (E, M, S, and N) are functional proteins essential for the virus' life and replication [12]. Host-cellular proteases' action locations and timing are partial since initiating the endoproteolytic cleaving of the spike protein takes place just after ACE2 engagement [16–18].

4.1.1. Spike protein and virus entry

4.1.1.1. *Structure*

The spike glycoprotein is a trimeric integrated glycosylated massive protein (150kDa) [19, 20]. Protein parts of spike glycoprotein consist of a short C-terminal intracellular tail, a single-pass transmembrane anchor, and a large N-terminal ectodomain [21, 22]. Amino acid residues are essential for the interaction between the SARS spike protein and target receptor (ACE2) [6, 21]. Eight of 14 important amino acids in the SARS-CoV-2 spike protein Receptor-Binding Domain (RBD) are similar to those of SARS-CoV spike proteins which indicated a similar host receptor for both viruses [23].

4.1.1.2. Function

Spike protein has two sites to be cleaved by cellular proteases; S1/S2 and S2' [24, 25]. S1/S2 site consists of arginine amino acid residues that are associated with proteolytic processing of spike protein and have an important role in the interaction between the RBD ectodomain of spike protein and ACE2 receptor [25, 26]. S1/S2 is cleaved into two polypeptides; three head S1 and trimeric stalk S2 [21, 27]. The N-terminated S1 polypeptide, which cleaves from spike protein, is responsible for attachment to the ACE2 receptor and makes the RBD [25, 28]. The C-terminated membrane-anchored S2 is an attendant for membrane fusion and virus entry [6, 29, 30]. Generally, S1 acts as a specific RBD and the fusion between virus membrane and membrane of the host cell via S2 enables the virus to release its single-stranded RNA into the host cell. S2 polypeptide consists of four parts which are a fusion peptide (FP), a secondary proteolytic site (S2'), an internal fusion peptide (IFP), and two heptad-repeat (HR) chains [5, 30]. IFPs are similar in SARS-CoV and SARS-CoV-2. Both IFP and PF play roles in viral entry. However, the molecular mechanism of cell entry has not been fully understood yet [5].

A furin site cleavage is located between the S1/S2 site [31–33]. Research suggests that the furin-like cleavage site can be cleaved during virus endocytosis for spike priming. Thus, these sites may result in a higher SARS-CoV-2 virulence in the human populations [1, 34].

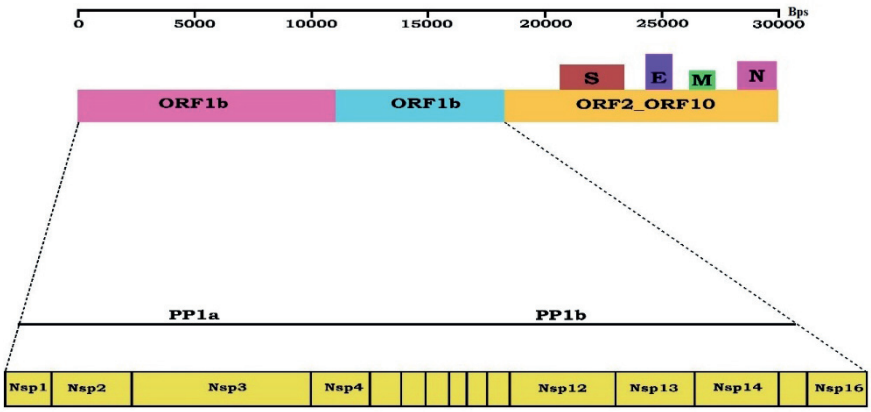

Fig. 2: The genome of CoVs with open reading frames (ORFs) and zones of encoding structural and functional proteins

4.2. ACE2, SARS-CoV-2 host receptor

The host receptor for SARS-CoV-2 is the same as the receptor that SARS-CoV uses to enter the cell [7]. SARS-COV-2 penetrates host cells through ACE2 with a conformational change in the spike protein structure after the attachment to ACE2 occurs [35, 36]. However, the binding affinity of the SARS-CoV-2 spike protein to ACE2 is 10–20 folds higher than for SARS-CoV [37]. It was reported that the injection of SARS-CoV spike protein into mice induced acute lung injury, which was reduced by the Renin-Angiotensin-Aldosterone system (RAAS) suppression [6]. Anti-serum against ACE2 can block SARS-COV-2 and reveals that the ACE2 is the main target for SARS-CoV-2[6].

ACE2 is a soluble mono carboxypeptidase enzyme in the RAAS which converts Angiotensin II (Ang) to Ang 1–7 with anti-inflammatory, vasodilative, and anti-apoptotic activities [34]. 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 [37, 38]. Testis, gastrointestinal tract, and kidney can express ACE2. So, the fecal-oral route may be one of the transmission routes. The human protein atlas database shows that the ACE2 mRNA is mainly identified in the duodenum, small intestine, colon, testis, kidney, and gallbladder. Although the ACE2 expression level in the lung is minimum [21, 39, 40], various conditions can regulate ACE2 expression in lung cells. For example, SARS-CoV can down-regulate ACE2 expression via spike protein attachment to the receptor [37, 41–43].

Blocking the interaction between ACE2 and spike protein is a promising approach and a potential prevention method of Covid-19 disease. For this purpose, designing biocompatible polymeric dendrimers may prevent spike protein attachment to ACE2. However, this method has some limitations. For instance, biocompatible polymers should not degrade in the physiological medium rapidly to have the most efficacy in trapping the virus. Additionally, these polymers should not attach to the body's endogenous molecules and the physiological processes of the body should not be affected by them.

Up to date, though there is no reliable evidence on ACE2 expression variations relating to age, smoking, sex, and gender [44], some hypotheses suggest that the death rate due to COVID-19 depends on sex and this rate is higher in men [45, 46]. Higher prevalence of smoking in men [47], more intense and stronger immune response in women [48], estrogen hormone in women which can up-regulate ACE2 expression [49], and over-expression of genes like ACE2 which are located in XCL site in XX cells [50] are probable factors that may result in higher death rate in men compared to women.

However, medications such as Angiotensin receptor blockers (ARBs) and ACE-inhibitors can increase ACE2 expression [44], but methyldopa and molsidomine decrease ACE2 expression, significantly [51]. (Table 1)

4.3. Effective Enzymes on spike Priming

4.3.1. Serine proteases

Serine proteases of the trypsin-like family have been identified to have significant effects in biological processes such as digestion, blood coagulation, fibrinolysis, and immunity. One of the serine proteases is the transmembrane serine

Table 1

Drugs modifying ACE2 expression

Factors	Effects on the ACE2 expression	Reference
ARBs	Up-regulation	[44]
ACE-inhibitors	Up-regulation	[44]
Thiazolidinedione	Up-regulation	[44]
Ibuprofen (NSAID)	Up-regulation	[44]
Methyldopa (alpha2 agonist)	Down-regulation	[51]
Molsidomine (vasodilator)	Down-regulation	[51]
Anti-adrenergics other than alpha/beta-blockers	Down-regulation	[51]
Calcium channel blockers (CCBs)	No significant affect	[51]

protease type 2 (TMPRSS2), which is essential for spike priming [7, 34]. Also, TMPRSS2 has proteolytic effects on the spike protein allowing virus and cell membrane fusion [4]. TMPRSS2 Overexpression increases the susceptibility of cells to MERS-CoV [52]. Based on the previous research, SARS-COV can be activated with TMPRSS2 through proteolytic mechanisms. TMPRSS2 expression has a notably more magnificent impression on entry propitiated by the SARS-CoV-2 S protein than by SARS-CoV [53]. In a study, transient transfection of HEK293T-ACE2 cells with TMPRSS2 resulted in a significant increase (up to 100-fold) in SARS-CoV-2 entry, when TMPRSS2 was expressed [53]. VeroE6/TMPRSS2 cells which are more vulnerable to SARS-COV-2, express ACE2 more. This observation illustrates the definite action of TMPRSS2 in SARS-COV-2 infection [4]. The same study showed that SARS-CoV-2 isolated from Vero E6 and TMPRSS2 cells was 10 times more than normal human lung tissue [4]. Since TMPRSS proteases group is highly expressed in the respiratory tract [34], the pathogenesis in the respiratory tract is expected.

Since TMPRSS2 has physiological functions in the body, inhibiting this enzyme cannot be proposed as a treatment option. Consequently, designing some agents to lyse the link between enzyme and virus represents a more feasible approach.

Trypsin is one of the endosomal serine peptidases, and its role in viral glycoprotein priming has been investigated extensively [4, 54]. Trypsin prefers arginine and lysine amino acids to other amino acids for cleavage [55]. As the function of this peptidase is not substrate selective, different regions within the spike protein can be cleaved by trypsin. Trypsin as a TMPRSS is expressed in the respiratory system; however, it has a digestive function in the small intestine. Trypsin can directly cleave spike proteins of many enteric coronaviruses; therefore, respiratory coronaviruses can use trypsin as a surrogate for proteases such as TMPRSS family members, because of similar substrate specificities [54, 56]. VeroE6 are cells where SARS-CoV is assembled and where trypsin revokes the necessity for cathepsin-mediated cleavage directing the virus to a low-pH independent entry route, probably at the plasmic membrane [54, 56]. Based on the information provided, originating or designing substances that can break the linkage between trypsin and its substrate may be helpful. However, it should be noted that these materials should be specific and selective to the cleavage site of the spike protein.

4.3.2. Proprotein convertases

Proprotein convertases (PCs) are 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 [5, 57]. They cleave the envelope glycoprotein for viral fusion with the host cell membrane [5].

Furin is expressed in a broad spectrum of cell types, including lung tissue. Additionally, a furin-like cleavage site is located on the spike protein of pathogenic influenza viruses [56]. Thus, 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 of infectious bronchitis virus (IBV) by adding a furin-like cleavage site to spike protein IBV [58]. 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. Effectual furin-mediated cleavage of the SARS-CoV-2 Spike protein results in more virus pathogenicity due to an enhanced affinity to the ACE2 receptor [34]. Different furin-like proteases would cleave the S2′ site [5], and although it is supposed that S2′ processing is a significant stage in final spike protein activation, the enzyme(s) involved in this process have not been determined.

According to these data, designing antagonist molecules that can fill the furin-like cleavage site and avoid the interaction between this site and enzymes is a potential treatment.

4.3.3. Cysteine proteases

Cysteine proteases such as cathepsin B and L (cat B/L) are important to spike priming in SARS-CoV-2 [7, 56]. These enzymes activate virus glycoproteins [54]. As mentioned, the SARS-CoV-2 entry into host cells is dependent on TMPRSS2 activity. In contrast, cat B/L activity is dispensable[6, 17, 59, 60]. Cat L is

Table 2

Stages of coronavirus life cycle and proteases related to each stage

Virus life cycle stage / Relevant Proteases	Free virus Particle	Attachment & entry	Endocytosis	Fusion	Protein Bio-synthesis	Assembly & Egress	Refe-rence
Furin			✔		✔	✔	[5]
TMPRSS group		✔			✔	✔	[4]
Trypsin	✔						[6, 54]
Cathepsin				✔			[6, 7]

more common for the CoVs entry than cat B. However, the intracellular acidity required for viral entry in cat B is higher than for cat L pH [61]. Notably, low endosomal pH is essential for virus uncoating. Ammonium chloride increases intracellular pH [61, 62]. Experiments show that SARS-CoV and SARS-CoV-2 entry into 239T cells (TMPRSS2-) was blocked by ammonium chloride. Although, ammonium chloride treatment shows low efficacy in entry inhibition on Caco-2 cells (TMPRSS2+) [6]. 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, full inhibition of the virus entry happens when E-64d as a cat B/L inhibitor is added to camostat mesylate. We can understand that SARS-CoV-2 uses of both cat B/L and TMPRSS2 to spike priming [6].

4.4. Possible and suggested treatments

Medications such as ARBs and ACE-inhibitors, thiazolidinediones, and ibuprofen (NSAID) increase ACE2 expression [44]. Investigations have revealed that losartan as an AT1R blocker decreases the severity of COVID-19 [12]. No evidence can support changing the treatment regimen in patients who received ACE-inhibitors, ARB, and thiazolidinediones [44]. Chloroquine and hydroxychloroquine as weak bases prevent the virus fusion to host cell by increasing endosomal pH [63, 64]. Umifenovir blocks the interaction between the virus and the host cell and inhibits the viral attack on host cells [65, 66]. Baricitinib is a Janus kinase inhibitor (JAK) that binds to AP2-associated protein kinase 1 (AAK1) and cyclin G-associated kinase (GAK) with higher affinity [67]. AAK1 and GAK are amongst the main regulators of endocytosis[68]. So, inhibiting them can prevent the virus entry into cells and also the intracellular gathering of viral particles [69]. Remdesivir has known antiviral activity against coronavirus, in vitro. It can diminish the viral load in the lung tissue of MERS-CoV infected mice, significantly. Remdesivir prevents interference with SARS-CoV-2 with a high selectivity index[70].

5. Conclusion

SARS-CoV-2 spike glycoprotein facilitates virus entry into host cells by attaching to the ACE2 receptor. A wide range of enzymes involved in spike priming 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. Protease inhibitors such as camostat mesylate, a TMPRSS2 inhibitor, E-64d, as a cat B/L inhibitor can be evaluated more as SARS-CoV-2 treatments. Further research on these mechanisms and blocking the function of E and M proteins may lead to hopeful results in SARS-CoV-2 prevention and treatment.

Acknowledgments

We thank Dr. Zohreh Esam for her great assistance in writing this manuscript.

References

1. Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharm Sin B. 2020;10(5):766-88.
2. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. J Pharm Anal. 2020;10(2):102-8.
3. Gioia M, Ciaccio C, Calligari P, De Simone G, Sbardella D, Tundo G, et al. Role of proteolytic enzymes in the COVID-19 infection and promising therapeutic approaches. Biochem Pharmacol. 2020;182:114225.
4. Matsuyama S, Nao N, Shirato K, Kawase M, Saito S, Takayama I, et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proc Natl Acad Sci. 2020;117(13):7001-3.
5. Coutard B, Valle C, de Lamballerie X, Canard B, Seidah N, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Res. 2020;176:104742.
6. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181(2):271-80.
7. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 2020;181(2):281-92.e6.
8. Armstrong J, Niemann H, Smeekens S, Rottier P, Warren G. Sequence and topology of a model intracellular membrane protein, E1 glycoprotein, from a coronavirus. Nature. 1984;308(5961):751-2.
9. Boopathi S, Poma AB, Kolandaivel P. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. J Biomol Struct Dyn. 2020:1-10.
10. de Haan CAM, Kuo L, Masters PS, Vennema H, Rottier PJM. Coronavirus Particle Assembly: Primary Structure Requirements of the Membrane Protein. J Virol. 1998;72(8):6838-50.
11. Woo PCY, Huang Y, Lau SKP, Yuen K-Y. Coronavirus Genomics and Bioinformatics Analysis. Viruses. 2010;2(8):1804-20.
12. Mousavizadeh L, Ghasemi S. Genotype and phenotype of COVID-19: Their roles in pathogenesis. J Microbiol Immunol Infect. 2020.
13. Godet M, L'Haridon R, Vautherot J-F, Laude H. TGEV corona virus ORF4 encodes a membrane protein that is incorporated into virions. Virology. 1992;188(2):666-75.
14. Nieto-Torres JL, DeDiego ML, Verdiá-Báguena C, Jimenez-Guardeño JM, Regla-Nava JA, Fernandez-Delgado R, et al. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. PLoS Pathog. 2014;10(5):e1004077.
15. van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, et al. Genomic Characterization of a Newly Discovered Coronavirus Associated with Acute Respiratory Distress Syndrome in Humans. mBio. 2012;3(6):e00473-12.
16. Shulla A, Heald-Sargent T, Subramanya G, Zhao J, Perlman S, Gallagher T. A Transmembrane Serine Protease Is Linked to the Severe Acute Respiratory Syndrome Coronavirus Receptor and Activates Virus Entry. J Virol. 2011;85(2):873-82.
17. Zhou Y, Vedantham P, Lu K, Agudelo J, Carrion R, Nunneley JW, et al. Protease inhibitors targeting coronavirus and filovirus entry. Antiviral Res. 2015;116:76-84.
18. Khan MKA, Pokharkar NB, Al-Khodairy FM, Al-Marshad FM, Arif JM. Structural Perspective on Molecular Interaction of IgG and IgA with Spike and Envelope Proteins of SARS-CoV-2 and Its Implications to Non-Specific Immunity. Biointerface Res Appl Chem. 2020:10923-39.
19. Johari YB, Jaffé SRP, Scarrott JM, Johnson AO, Mozzanino T, Pohle TH, et al. Production of trimeric SARS-CoV-2 spike protein by CHO cells for serological COVID-19 testing. Biotechnol Bioeng. 2021;118(2):1013-21.
20. Hargett AA, Renfrow MB. Glycosylation of viral surface proteins probed by mass spectrometry. Curr Opin Virol. 2019;36:56-66.
21. Chen Y, Guo Y, Pan Y, Zhao ZJ. Structure analysis of the receptor binding of 2019-nCoV. Biochem Biophys Res Commun. 2020.
22. Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. Annu Rev Virol. 2016;3(1): 237-61.
23. Liu Z, Xiao X, Wei X, Li J, Yang J, Tan H, et al. Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. J Med Virol. 2020;92(6):595-601.
24. Cannalire R, Stefanelli I, Cerchia C, Beccari AR, Pelliccia S, Summa V. SARS-CoV-2 Entry Inhibitors: Small Molecules and Peptides Targeting Virus or Host Cells. Int J Mol Sci. 2020;21(16):5707.
25. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;426(6965):450-4.

26. Jaimes JA, André NM, Millet JK, Whittaker GR. Structural modeling of 2019-novel coronavirus (nCoV) spike protein reveals a proteolytically-sensitive activation loop as a distinguishing feature compared to SARS-CoV and related SARS-like coronaviruses. bioRxiv. 2020:2020.02.10.942185.
27. Cheng J, Zhao Y, Xu G, Zhang K, Jia W, Sun Y, et al. The S2 Subunit of QX-type Infectious Bronchitis Coronavirus Spike Protein Is an Essential Determinant of Neurotropism. Viruses. 2019;11(10):972.
28. de Groot RJ, Luytjes W, Horzinek MC, van der Zeijst BAM, Spaan WJM, Lenstra JA. Evidence for a coiled-coil structure in the spike proteins of coronaviruses. J Mol Biol. 1987;196(4):963-6.
29. Davidson AM, Wysocki J, Batlle D. Interaction of SARS-CoV-2 and other Coronavirus with ACE (Angiotensin-Converting Enzyme)-2 as their main receptor: therapeutic implications. Hypertension. 2020;76(5):1339-49.
30. Jamwal S, Gautam A, Elsworth J, Kumar M, Chawla R, Kumar P. An updated insight into the molecular pathogenesis, secondary complications and potential therapeutics of COVID-19 pandemic. Life Sci. 2020;257:118105.
31. Abraham S, Kienzle TE, Lapps W, Brian DA. Deduced sequence of the bovine coronavirus spike protein and identification of the internal proteolytic cleavage site. Virol J. 1990;176(1):296-301.
32. Cheng Y-W, Chao T-L, Li C-L, Chiu M-F, Kao H-C, Wang S-H, et al. Furin inhibitors block SARS-CoV-2 spike protein cleavage to suppress virus production and cytopathic effects. Cell reports. 2020;33(2):108254.
33. Luytjes W, Sturman LS, Bredenbee PJ, Charite J, van der Zeijst BAM, Horzinek MC, et al. Primary structure of the glycoprotein E2 of coronavirus MHV-A59 and identification of the trypsin cleavage site. Virology. 1987;161(2):479-87.
34. Lukassen S, Chua RL, Trefzer T, Kahn NC, Schneider MA, Muley T, et al. SARS□CoV□2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. EMBO J. 2020;30(10).
35. Jamwal S, Gautam A, Elsworth J, Kumar M, Chawla R, Kumar P. An updated insight into the molecular pathogenesis, secondary complications and potential therapeutics of COVID-19 pandemic. Life sciences. 2020:118105.
36. Müller WE, Neufurth M, Schepler H, Wang S, Tolba E, Schröder HC, et al. The biomaterial polyphosphate blocks stoichiometric binding of the SARS-CoV-2 S-protein to the cellular ACE2 receptor. Biomater Sci. 2020;8(23):6603-10.
37. Sanchis-Gomar F, Lavie CJ, Perez-Quilis C, Henry BM, Lippi G, editors. Angiotensin-Converting Enzyme 2 and Antihypertensives (Angiotensin Receptor Blockers and Angiotensin-Converting Enzyme Inhibitors) in Coronavirus Disease 2019. Mayo Clin Proc; 2020: Elsevier.
38. Monteil V, Kwon H, Prado P, Hagelkrüys A, Wimmer RA, Stahl M, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. Cell. 2020;181(4): 905-13.e7.
39. Vieira Braga FA, Kar G, Berg M, Carpaij OA, Polanski K, Simon LM, et al. A cellular census of human lungs identifies novel cell states in health and in asthma. Nat Med. 2019;25(7):1153-63.
40. Xu Y, Mizuno T, Sridharan A, Du Y, Guo M, Tang J, et al. Single-cell RNA sequencing identifies diverse roles of epithelial cells in idiopathic pulmonary fibrosis. JCI Insight. 2016;1(20):e90558-e.
41. Haga S, Yamamoto N, Nakai-Murakami C, Osawa Y, Tokunaga K, Sata T, et al. Modulation of TNF-α-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-α production and facilitates viral entry. Proc Natl Acad Sci. 2008;105(22):7809.
42. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature. 2005;436(7047):112-6.
43. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. Nat Med. 2005;11(8):875-9.
44. Gracia-Ramos AE. Is the ACE2 Overexpression a Risk Factor for COVID-19 Infection? Arch Med Res. 2020;51(4):345-6.
45. [The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2020;41(2):145-51.
46. Baughn LB, Sharma N, Elhaik E, Sekulic A, Bryce AH, Fonseca R, editors. Targeting TMPRSS2 in SARS-CoV-2 infection. Mayo Clin Proc; 2020: Elsevier.
47. Singh O, Bhardwaj P, Kumar D. Association between climatic variables and COVID-19 pandemic in National Capital Territory of Delhi, India. Environ Dev Sustain. 2020:1-15.
48. Ghosh S, Klein RS. Sex Drives Dimorphic Immune Responses to Viral Infections. Journal of immunology (Baltimore, Md : 1950). 2017;198(5):1782-90.
49. Bukowska A, Spiller L, Wolke C, Lendeckel U, Weinert S, Hoffmann J, et al. Protective regulation of the ACE2/ACE gene expression by estrogen in human atrial tissue from elderly men. Exp Biol Med. 2017;242(14):1412-23.
50. Tukiainen T, Villani A-C, Yen A, Rivas MA, Marshall JL, Satija R, et al. Landscape of X chromosome inactivation across human tissues. Nature. 2017;550(7675):244-8.

51. Sinha S, Cheng K, Aldape K, Schiff E, Ruppin E. Systematic cell line-based identification of drugs modifying ACE2 expression. 2020.
52. Shirato K, Kawase M, Matsuyama S. Middle East Respiratory Syndrome Coronavirus Infection Mediated by the Transmembrane Serine Protease TMPRSS2. J Virol. 2013;87(23):12552-61.
53. Ou T, Mou H, Zhang L, Ojha A, Choe H, Farzan M. Hydroxychloroquine-mediated inhibition of SARS-CoV-2 entry is attenuated by TMPRSS2. PLoS Pathog. 2021;17(1):e1009212.
54. Millet JK, Whittaker GR. Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. Virus research. 2015;202:120-34.
55. Halfon S, Baird T, Craik C. Trypsin, handbook of proteolytic enzymes. Cysteine, Serine and Threonine Peptidases; 2004.
56. Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, et al. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell. 2020;181(4):894-904. e9.
57. van Spronsen FJ, Bijleveld CM, van Maldegem BT, Wijburg FA. Hepatocellular carcinoma in hereditary tyrosinemia type I despite 2-(2 nitro-4-3 trifluoro-methylbenzoyl)-1, 3-cyclohexanedione treatment. J Pediatr Gastroenterol Nutr. 2005;40(1):90-3.
58. Kido H, Okumura Y, Takahashi E, Pan H-Y, Wang S, Yao D, et al. Role of host cellular proteases in the pathogenesis of influenza and influenza-induced multiple organ failure. Biochim Biophys Acta Proteins Proteom BBA-PROTEINS PROTEOM. 2012;1824(1):186-94.
59. Iwata-Yoshikawa N, Okamura T, Shimizu Y, Hasegawa H, Takeda M, Nagata N. TMPRSS2 Contributes to Virus Spread and Immunopathology in the Airways of Murine Models after Coronavirus Infection. J Virol. 2019;93(6):e01815-18.
60. Shirato K, Kanou K, Kawase M, Matsuyama S. Clinical Isolates of Human Coronavirus 229E Bypass the Endosome for Cell Entry. J Virol. 2017;91(1):e01387-16.
61. Belouzard S, Chu VC, Whittaker GR. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. Proc Natl Acad Sci. 2009;106(14):5871-6.
62. Follis K, York J, Nunberg J. Furin cleavage of the SARS coronavirus spike glycoprotein enhances cell–cell fusion but does not affect virion entry. Virology. 2006;350:358-69.
63. Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, et al. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Clin Infect Dis. 2020;71(15):732-9.
64. Moussa TAA, Sabry NM. A new proposed mechanism of some known drugs targeting the sars-cov-2 spike glycoprotein using molecular docking. Biointerface Res Appl Chem. 2021;11(5):12750-60.
65. Blaising J, Polyak SJ, Pécheur EI. Arbidol as a broad-spectrum antiviral: an update. Antiviral Res. 2014;107:84-94.
66. Kadam RU, Wilson IA. Structural basis of influenza virus fusion inhibition by the antiviral drug Arbidol. Proc Natl Acad Sci. 2017;114(2):206-14.
67. Richardson P, Griffin I, Tucker C, Smith D, Oechsle O, Phelan A, et al. Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. Lancet (London, England). 2020;395(10223):e30-e1.
68. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet (London, England). 2020;395(10224):565-74.
69. Sorrell FJ, Szklarz M, Abdul Azeez KR, Elkins JM, Knapp S. Family-wide Structural Analysis of Human Numb-Associated Protein Kinases. Structure (London, England : 1993). 2016;24(3):401-11.
70. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020;30(3):269-71.