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The role of proteases in the invasion of SARS-CoV-2 virus into human host cells

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Abstract

The new coronavirus, which originally broke out in Wuhan, China, in December 2019, increase quickly around the world, causing a pandemic all over the world. The virus uses its spike protein to enter the human host cells. Protein S binds to the angiotensin-converting enzyme II (ACE2) receptor and enters the host cell. For the more successful binding, several proteases facilitate and optimize this binding, the most important of which are transmembrane protease serine 2 (TMPRSS2), furin, and cathepsin L proteases. After binding of protein S to the ACE2 receptor, cleavage of protein S is required for membrane fusion by protein S, which causes viral entry into host cells. This proteolytic activity may be cathepsin L-dependent and occurs with changes in pH in cell endosomes, or it may occur through serine proteases activity at the surface of the host cell membrane or within vesicles. Finally, the cell becomes infected with the virus. Several studies have tried to reduce the rate of viral infection by using inhibitors of these proteases.

Keywords: SARS-CoV-2, TMPRSS2, Furin, Cathepsin L, COVID-19

1. Introduction

The new coronavirus, which primary started in December 2019 in Wuhan, China, caused cases of pneumonia [1], so that by February 15, 2021, more than 108 million 400 thousand people worldwide infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and about 2 million people died due to coronavirus disease (COVID-19) [2]. There are many diverse kinds of coronaviruses. Some of them can cause colds or other mild respiratory (nose, throat, and lung) sicknesses. Further coronaviruses can cause more severe diseases, as well as severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). The name of Corona derives from the Latin name crown meaning circular crown [3]. This virus belongs to the beta-coronavirus family based on Phylogenetic studies [1]. Investigation of the SARS-CoV-2 RNA sequence showed that it is most similar to the coronavirus isolated from bat species [4, 5]. Bats are natural reservoirs of coronaviruses, and human-to-human transmission of the disease has been confirmed [6, 7]. This positive single-stranded virus structurally requires four vital proteins to form a whole viral particle: spike (S) protein, nucleocapsid (N) protein, membrane (M) protein, and envelope (E) protein [8]. This virus binds to its surface cell receptor through its spike protein, causing the virus to enter the host cell. This S protein has two domains, S1 and S2. Proteases cut the link between N terminal S1 and C

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terminal S2 [9]. S1 subunit is the receptor-binding motif (RBM) located in the receptor-binding domain (RBD), which interacts straight with host cell receptor angiotensin-converting enzyme II (ACE2) and mediates virus binding to host cells. Sub-unit S2 exists membrane-terminal C-terminal and amphipathic heptad repeats that predict to be involved in coiled-coil formation in virus-cell binding [9]. SARS-CoV-2 enters the cell by binding to the ACE2 cell receptors [10, 11]. The virus detects human ACE2 with high efficiency and therefore increases the ability of SARS-CoV-2 to be transmitted from person to person [12]. ACE2 is a carboxypeptidase that converts angiotensin II to angiotensin-(1-7), anti-fibrosis, anti-hypertrophy, and vasodilation [11, 13, 14]. The ACE2 gene locates in the Xp22 chromosomal area with a length of 39.98 kb of genomic DNA. This gene produces two transcripts that finally form a protein with 805 amino acids. High levels of polymorphisms observed in this gene, several single nucleotide polymorphisms (SNPs) are associated with vulnerability to diseases such as type 2 diabetes and hypertension [15, 16]. Figure 1 (https://www.proteinatlas.org), shows ACE2 expression in different tissues, suggesting that a lot of organs may be the host for viral attack due to their ACE2 receptor. Numerous host proteases can degrade S protein in SARS-CoV-2, including transmembrane protease serine 2 (TMPRSS2), cathepsins, and furin. Among these proteases, TMPRSS2 is necessary for viral entry and occurrence in the SARS-CoV2 infected host [1]. In this review, we will deal with the most important protease involved in the cleavage of protein S.

2. TMPRSS2

SARS-CoV-2 uses ACE2 to enter the cell, and TMPRRS2 is a serine protease involved in the cleavage of protein S [17]. Early preparation of S protein by TMPRSS2 is necessary for the entry and spread of the SARS-CoV-2 virus by its mediator with ACE2 [18]. The TMPRSS2 gene is located on human chromosome 21q22.3, encodes a 492-amino acid polypeptide with five different domains: serine proteinase, scavenger receptor, low-density lipoprotein, transmembrane, and cytoplasmic [18]. The TMPRSS2 gene essentially expresses in the adult prostate [19]. Regulation of TMPRSS2 gene expression is affected by androgen signaling [20]. Androgen receptor activity required for transcription of the TMPRSS2 gene, no other regulatory element found for the TMPRSS2 promoter up till now [20, 21].

The human TMPRSS2 promoter has an androgen response. Also, TMPRSS2 mRNA expression is affected by androgen regulation in prostate cells [21]. ACE2 is involved in anchoring the SARS-CoV-2 virus to the cell surface, which is also affected by androgens and is more active in men [22]. One research reported that men were at higher risk for developing the disease with severe symptoms [23]. The TMPRSS2 gene is mostly expressed in the adult prostate but also expressed in other tissues, including the adult colon, small intestine, pancreas, kidney, lung, and liver [19].

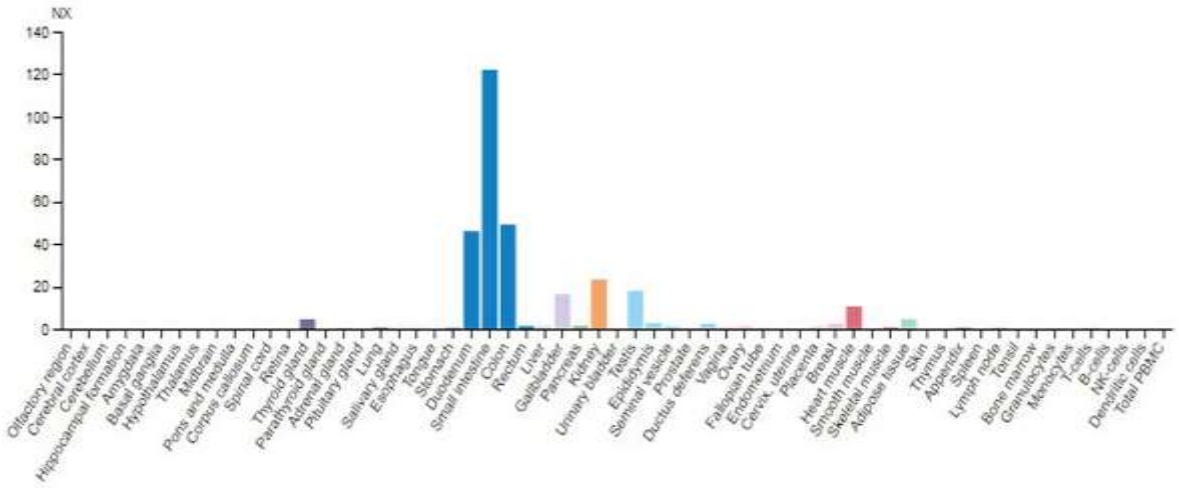

Figure 1. The expression of ACE2 in different tissues in terms of Consensus Normalized expression (NX) levels, obtained from three transcriptomics datasets (HPA, GTEx, and FANTOM5)

As well, TMPRSS2 is express in the lungs, liver, and kidneys, which are target organs for COVID-19 [24]. Expression of TMPRSS2 in the small intestine facilitates virus binding and entry into host cells, which may contribute to the increase of the disease because it provides a potential site for virus replication in the small intestine enterocytes [25]. A study showed that genetic polymorphisms ACE2 and TMPRSS2 could be associated with genetic susceptibility to COVID-19. It is better to treatment directed to personal medicine [26]. TMPRSS2 can help SARS-CoV-2 to enter host cells [18].

TMPRSS2 is useful at neutral pH and loses its activity in acidic situations [27]. Therefore, TMPRSS2 can play a critical function in the proteolysis of the S1 subunit of the virus at the surface of the host cell, while membrane-bound or released cathepsin L (CatL) should also target the same substrate [28]. SARS-CoV viruses are pH-sensitive viruses, and their intracellular trafficking requires an acidic environment [29]. TMPRSS2 acts locally on the plasma membrane of the host cell and probably acts along with the endocytotic vesicle trafficking [30]. Also, mechanisms suggest for virus entry, including the breakdown of ACE2 by membrane-bound serine proteases, which leads to increased virus entry [30]. Regarding the role of serine proteinases, success in inhibiting serine proteinase may be efficient in virus entry to the cell in vitro [18]. SARS-CoV-2 requires TMPRSS2 and CatL to enter. Camostatmesylate, an inhibitor of TMPRSS2 performs a critical role in reducing virus entry into the Calu-3 lung cell line [18]. Hoffman et al. have recently shown that the TMPRSS2 inhibitor blocks the entry of the virus and may be a treatment option [18]. They reported that Camostatmesylate a serine proteinase inhibitor accepted in Japan for the treatment of distinct diseases blocks the activity of TMPRSS2 [31, 32].

3. Cathepsin L

SARS-CoV-2 also enters the cells through endocytosis. Phosphatidylinositol 3-phosphate 5-kinase and CatL are significant for endocytosis [33]. After binding, protein S cleavage, required for fusion membrane fusion with protein S, which causes the virus to enter host cells. This proteolytic activity may be CatL-dependent and occurs with pH changes in cell endosomes, or it may bind via serine proteases to the surface of the host cell membrane or within vesicles [33, 34]. After SARS-CoV-2 endocytosis, the S protein is cleaved by CatL, which permits the virus membrane to combine with the endosomal membrane. Following that, the viral genome is released into the host cell [35].

Endosomal proteases for instance cathepsins can cause viruses to enter the host cell through an endosomal pathway that occurs at low pH. As well, the virus can enter the cells through cell surface proteases, particularly TMPRSS2 [36-38]. Both endosomal and non-endosomal pathways are involved in the entry of the SARS-CoV-2 virus, and low pH is essential for virus activity within the cell [33]. CatL, with 220 amino acids, is an enzyme involved in other pathologies, including osteoporosis and periodontal disease. As a result, a host of CatL inhibitors are available [39]. This protein is a lysosomal cysteine peptidase and has a double chain (L and R) [40].

CatL is involved in protein turnover and cell apoptosis. Excessive expression of CatL in cancer cells has made it suitable as a target for anti-cancer strategies [41]. It has been found that in some food proteins and peptides naturally have the inhibitory activity of CatL, some peptides in food proteins may be able to inhibit CatL and therefore help prevent COVID-19 [42]. Inhibition of CatL may be practical in reducing infection by SARS-CoV-2. Many inhibitors of CatL, such as the Epoxydipeptide ketones used to suppress SARS-CoV, are fundamentally peptidomimetic [35].

By inhibiting the activation of protein S, teicoplanin prevents CatL in pseudoviruses [43]. SID26681509 is also a CatL inhibitor that reduces the entry of SARS-CoV-2-like viruses, demonstrating the importance of CatL in priming the SARS-CoV-2 protein [33]. Chloroquine can prevent the activity of proteases and S protein, neutralize endolysosomal pH. Then reduce viral entry into the host. It can also trap ACE2 in nuclear vacuoles [44]. The anti-malarial drug chloroquine can efficiently block SARS-CoV-2 infection in cultured cells [45]. But to date, there is no recorded clinical trial to support this inference. Chloroquine affects ACE2 terminal glycosylation [46]. While chloroquine may have short-term benefits for COVID-19 patients by inhibiting CatL activity, it can influence to cardiac arrhythmia [47]. The mechanism of chloroquine is related to the function of CatL, due to which its activity increases the endosomal pH [48]. Chloroquine prevents proteolysis of the S1 subunit in endosomes by raising endosomal pH and decrease the

release of viral genetic material [49]. Treatment with CatL inhibitors or protease inhibitor cocktails can have compensation over chloroquine. Patients or cells treated with specific CatL inhibitors without Camostatmesylate do not show a decrease in the optimal activity of further endosomal proteases, unlike those treated with chloroquine [50].

4. Furin

In the SARS-CoV-2 genome, although it's many similarities to the SARS-CoV genome, the furin protein cleavage site was not found in other SARS-like CoVs sequence, which may play a role in virus entry into the cell and pathogenicity [51]. The study of proteolytic activation of glycoproteins in enveloped viruses shows the relationship between viral infection and furin protein, which indicates the role of this protein in the activation of spike protein [52]. This protein can consider as a mediator in the processing and maturation of SARS-CoV-2 virus S protein [53].

Regarding the role of furin in the processing of essential surface proteins and its role in viral infectivity has been identified [52]. Coronaviruses attach to the host cell receptor to enter the cell through their S protein, and then fuse their envelope to the cell membrane to release its genome into the host cell cytoplasm and then replicate its genome inside the cell [54]. Observations indicate that the furin-cleavage site reduces the constancy of protein S in SARS-CoV-2 and provides the essential conformational for binding to the ACE2 receptor [55]. Furin is present in all vertebrates as well as many invertebrates and requires calcium for its enzymatic activity [56]. This enzyme is a type of serine protease that cleaves the amino acid sequence -Arg-X-X-Arg- consensus cleavage site R - X - K / R - R ↓, where X represents any amino acid except cysteine and rarely proline [56].

Seven distinct families from the family of proprotein convertases find out in mammalian species, furin is one of them. This family processes and activates essential biological functions. Furin is an endoprotease that expresses in all tissues. It is located in the trans-Golgi network, and some proprotein located between other parts of the cell and at the cell surface. Furin can cleave protein precursors labeled with specific sequences, including proteases of the blood-clotting, complement systems, matrix metalloproteinase, receptors, viral-envelope glycoproteins, and bacterial exotoxins [57]. This protein requires the activity of pathogens such as bacterial toxins, enveloped viruses such as the Human Immunodeficiency Virus (HIV), Ebola, and SARS-Cov-2. Certainly, it is also involved in increasing the pathogenicity of the virus because cutting the target site, activates the virus functionally [58]. *in vitro* studies have shown that suppression of furin can decrease viral infections [59]. The use of furin suppressors has been shown to protect host cells against furin-dependent viral infections, providing a new basis for host cell-based treatment for acute diseases [60]. One study showed that the use of the MI-1851 inhibitor in Calu-3 epithelial cells strictly concealed SARS-CoV-2 replication, and the mixture of TMPRSS2 and furin inhibitors had a better result against the activity of this virus [61]. Consequently, furin inhibitors can use as a preventative and therapeutic agent against this virus [62].

5. Conclusion

Various proteolytic enzymes either of the host or the virus act in a serious fashion to manage and organize definite steps of the viral replication and assembly, such as the entry of the virus, the maturation of the polyprotein, and the assembly of the secreted virions for further dispersal. So proteases are vital targets, envisaging that molecules that interfere with their activity are promising therapeutic compounds. These enzymes are thus exceptional targets for antiviral intervention.

Author Contributions

All authors contributed equally in drafting and revision of the article, and read and approved the final version.

Conflicts of Interest

The authors declare no potential conflicts of interest.

Ethical declarations

Not applicable.

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