**Name of Journal:** *World Journal of Gastroenterology*

**Manuscript NO:** 66507

**Manuscript Type:** MINIREVIEWS

**Cathepsin L, transmembrane peptidase/serine subfamily member 2/4, and other host proteases in COVID-19 pathogenesis – with impact on gastrointestinal tract**

Berdowska I *et al*. Proteases in SARS-CoV-2 gastrointestinal tract invasion

Izabela Berdowska, Malgorzata Matusiewicz

**Izabela Berdowska, Malgorzata Matusiewicz,** Department of Biochemistry and Immunochemistry, Wroclaw Medical University, Wroclaw 50-368, Lower Silesia, Poland

**Author contributions:** Berdowska I performed the literature research and wrote the manuscript; Matusiewicz M performed the literature research, created the graphs and revised the manuscript; both authors discussed, designed the general concept of the work, read and approve the final manuscript.

**Corresponding author: Malgorzata Matusiewicz, PhD, Senior Lecturer,** Department of Biochemistry and Immunochemistry, Wroclaw Medical University, Chalubinskiego 10, Wroclaw 50-368, Lower Silesia, Poland. malgorzata.matusiewicz@umed.wroc.pl

**Received:** March 29, 2021

**Revised:** June 28, 2021

**Accepted:** September 19, 2021

**Published online:**

**Abstract**

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) seems to employ two routes of entrance to the host cell; *via* membrane fusion (with the cells expressing both angiotensin converting enzyme 2 (ACE2) and transmembrane peptidase/serine subfamily member 2/4 (TMPRSS2/4)) or *via* receptor-mediated endocytosis (to the target cells expressing only ACE2). The second mode is associated with cysteine cathepsins (probably cathepsin L) involvement in the virus spike protein (S protein) proteolytic activation. Also furin might activate the virus S protein enabling it to enter cells. Gastrointestinal tract (GIT) involvement in SARS-CoV-2 infection is evident in a subset of COVID-19 patients exhibiting GIT symptoms, such as diarrhea, and presenting viral-shedding in feces. Considering the abundance and co-localization of ACE2 and TMPRSS2 in the lower GIT (especially brush-border enterocytes), these two receptors seem to be mainly involved in SARS-CoV-2 invasion of the digestive tract. Additionally, *in vitro* studies have demonstrated the virions capability of infection and replication in the human epithelial cells lining GIT. However, also furin and cysteine cathepsins (cathepsin L) might participate in the activation of SARS-CoV-2 spike protein contributing to the virus invasiveness within GIT. Moreover, cathepsin L (due to its involvement in extracellular matrix components degradation and remodeling, the processes enhanced during SARS-CoV-2-induced inflammation) might be responsible for the dysregulation of absorption/digestion functions of GIT, thus adding to the observed in some COVID-19 patients symptoms such as diarrhea.

**Key Words:** COVID-19; SARS-CoV-2; Angiotensin converting enzyme 2; Transmembrane peptidase/serine subfamily member 2/4; Cathepsin L; Gastrointestinal tract

Berdowska I, Matusiewicz M. Cathepsin L, transmembrane peptidase/serine subfamily member 2/4, and other host proteases in COVID-19 pathogenesis – with impact on gastrointestinal tract. *World J Gastroenterol* 2021; In press

**Core Tip:** Gastrointestinal tract (GIT) is believed to participate in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) dissemination. The current research shows the abundance and co-localization of angiotensin converting enzyme 2 (ACE2) and transmembrane peptidase/serine subfamily member 2 receptors in the lower GIT. Furthermore, about half of COVID-19 patients present with GIT symptoms, such as diarrhea, and exhibit viral-shedding in feces. Additionally, *in vitro* studies have demonstrated the virions capability of infection and replication in the human epithelial cells lining GIT. This paper reviews the possible routes of the virus infection with respect to the host-enzymatic systems responsible for the proteolytic priming of SARS-CoV-2.

**INTRODUCTION**

Coronaviruses may employ several host proteases for their invasion into target cells. The enzymes participating in the viruses activation include: Proprotein convertases (PCs) (mainly furin), transmembrane serine proteases, especially transmembrane peptidase/serine subfamily member 2 (TMPRSS2), the lysosomal cathepsins (mainly cathepsin L), elastase, and coagulation factor Xa[1].

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus responsible for COVID-19 pandemic exhibits many similarities when compared to SARS-CoV. It employs a similar mechanism of host cells invasion; recognizes and binds the same type of angiotensin converting enzyme 2 (ACE2) receptors to enter host cells (but with higher affinity[2]), and comparable processing of spike protein (S protein) seems to be necessary for the virion fusion with the host cell membrane[3-7] (Figure 1).

Two proteolytic events need to be conducted for SARS-CoV-2 activation; initially spike protein is cut in the specific cleavage site between S1 and S2 domain, then the second cleavage within S2 domain (S2’ site) allows for the exposition of the fusion peptide, which enables membrane fusion. The first proteolytic step can happen in the producer cell, in the extracellular space, or within the host cell’s endosome. This cleavage site in SARS-CoV-2 spike protein is recognized by various proteases, including furin (unlike in SARS-CoV lacking furin-cleavage site between S1 and S2)[6,8] and TMPRSS2[3]. The second cleavage can be either performed by TMPRSS2 on the surface of the host cell, or in the endolysosomes by lysosomal proteases, most probably cathepsin L[9].

Therefore, as proposed by Pislar *et al*[10], two routes of SARS-CoV-2 entry to the host cell are likely; *via* membrane fusion with the host cells which expose both ACE2 and TMPRSS2 (and/or other transmembrane serine proteases such as TMPRSS4[11]) proteins, or *via* receptor-mediated endocytosis (RME) to the target cells expressing only ACE2 receptors. In the first case, both processing steps performed by TMPRSS2 before the virus entry enable membrane fusion, whereas in the second mechanism, the virion binding with ACE2 receptors induces endocytosis followed by spike protein activation by cathepsin L (and/or other cysteine cathepsins)[4,10]. As a result of either of these pathways, viral RNA is released in the host cell and undergoes the processes of replication (Figure 2).

**TYPES OF HOST PROTEASES IN SARS-CoV-2 INVASION**

The findings of several studies support the notion that, apart from ACE2 receptors, the main host peptidases involved in SARS-CoV-2 spike protein processing include: TMPRSS2 (and/or TMPRSS4), lysosomal cysteine cathepsins (mainly cathepsin L), as well as furin-like PCs. They may participate in SARS-CoV-2 activation independently, or their actions may overlap or complete one another, depending on the pattern of the virion-recognized proteins exposed on the host cells (e.g. expressing or not TMPRSS2).

***Cysteine cathepsins (CCs) in pathology***

Cysteine cathepsins belonging to the papain-like family of cysteine proteases (containing cysteine in their catalytic center) comprise 11 cathepsins (B, C, F, H, K, L, O, S, V, X and W) in the human organism[12,13]. They belong to lysosomal proteases involved mainly in intracellular protein breakdown, antigen processing, MHC-II mediated immune response, and apoptosis. However, their functions go far beyond this; they participate in various physio-pathological processes not only intracellularly, but also in the extracellular matrix (ECM), because, except for their endolysosomal sequestration, they have been observed in the nucleus, cytosol, mitochondria, at the plasma membranes and in the extracellular milieu[13,14]. Their secretion is observed in physiological conditions (e.g. in bone remodeling – conducted by osteoclasts-secreted cathepsin K, in wound healing performed by keratinocytes-secreted cathepsin B, in prohormone processing – thyroid hormones released from thyroglobulin by cathepsins B, L and K secreted from the thyroid epithelial cells). However, an excessive secretion of cysteine cathepsins is mostly observed in pathological states associated with inflammatory processes, such as cancer diseases (cathepsins B, C, K, L, S, H, X), cardiovascular diseases (cathepsins C, K, L, S, V), joint and bone diseases (cathepsins K, B, L, S, H), inflammatory bowel disease (cathepsin L), and many other disorders (summarized in[12-15]). In these pathological states CCs typically act extracellularly, where they participate in collagen, elastin, and other ECM components degradation directly or indirectly (activating other proteases) after being secreted from recruited immune cells (mostly), as well as from inflamed tissue cells (to the lesser extent). Macrophages and other immune cells infiltrating tissues seem to be the main extracellular source of CCs whose secretion is stimulated by inflammatory factors like cytokines. However, also other cell types secrete excessive amounts of CCs, including osteoclasts or chondrocytes (oversecreting cathepsin K and/or S in arthritis and osteoporosis), or cancer cells and tumor-associated fibroblasts (oversecreting cathepsin S, L and/or B in cancer invasion)[14]. Apart from degradation of main components of ECM, also more refined processing of ECM (undergoing both intra-, and extracellularly) is ascribed to CCs. This comprises modifying and shedding cell adhesion molecules and cell membrane receptors, which affects signal transduction pathways, as well as processing cytokines and chemokines, which upregulates immune response[14]. The resulting augmentation of inflammatory processes further induces the secretion of CCs, enhancing the destruction of ECM, which eventually leads to the acceleration of the processes observed in the aforementioned disorders.

Due to their roles in multiple inflammatory-based disorders, CCs have been considered for a long time as a target for medicinal drugs design. However, because of ubiquitous expression of most CCs, their constitutive, overlapping functions, broad substrate specificities, most of the studied so far medicines have exhibited unfavorable side effects, thus not surviving clinical trials. However, the attempts to construct CCs-aimed drugs, taking advantage of the newest technology, are underway. The most clinical trials have been conducted on cathepsins K and S inhibitors, also cathepsin C seems to be a promising target, whereas the remaining cathepsins inhibitors have either got stuck in the initial stages of clinical trials or their trials have been discontinued (reviewed in[14]).

***Cathepsin L in inflammatory processes***

Similarly to other cysteine cathepsins, cathepsin L exhibits pleiotropic activities in the human organism. One of the most evident actions of this enzyme is (beside cathepsins S, K and V) its participation in inflammatory processes associated with various pathological conditions[14-16]. For example Menzel *et al*[17] have demonstrated the up to 10-fold induction of cathepsin L expression (mRNA) in intestinal macrophages derived from inflammatory bowel disease (IBD) patients, and the clear improvement of the disorder symptoms in DSS (dextran-sulphate-sodium)-induced colitis mice mode, when a simultaneous application of cathepsins L and B inhibitors was investigated. Xu *et al*[18] have exhibited the stimulatory function of cathepsin L in microglia-mediated neuroinflammation, which accompanies many neurological disorders including Parkinson’s disease. Cao *et al*[19] have shown the correlation between serum cathepsin L activity and the markers of inflammation (such as neutrophile counts and hs-CRP) in the patients with chronic kidney disease.

***Cysteine cathepsins and TMPRSS2 in SARS-CoV-2 invasion***

Cell line experimental systems creating the environment aimed at the inhibition of CCs have substantiated the function of these enzymes in the processes of SARS-CoV-2 activation. Raising pH in the endolysosomal compartments (with ammonium chloride and/or bafilomycin A), which inactivates lysosomal proteases working in acidic environment, or application of cysteine proteases inhibitors (such as E-64d inhibitor - inactivating cysteine cathepsins L, B, H, as well as cytosolic calpain) have significantly limited entry of SARS-CoV-2 in chosen cell lines[4]. Ou *et al*[20] (applying lentiviral pseudotype system) have demonstrated that the treatment of HEK-293/hACE2 cells with E-64d inhibitor reduced entry of SARS-CoV-2 S pseudovirions by over 90%. Further, the Authors compared the effect of two specific inhibitors of cathepsin L (SID 26681509), and cathepsin B (CA-074). Whereas the first inhibitor limited the pseudovirions entry by over 76%, the second one did not exhibit any significant effect, which suggests a prevalent function of cathepsin L in the receptor-mediated endocytosis mechanism of the virus invasion. RME mechanism in HEK- 293/hACE2 cells has been confirmed by the Authors in the experiments showing the inhibition of the SARS-CoV-2 S protein entry by blocking the factors inevitable in the process of endolysosomal trafficking (PI(3)P 5-kinase (PIKfyve) and two-pore channel subtype 2 (TPC2)[20]. The involvement of CCs has been also observed by Hoffmann *et al*[3] who demonstrated that the increase in pH (with ammonium chloride) almost completely inhibited SARS-2-S-driven entry into 293T cells expressing ACE2 but devoid of TMPRSS2. This observation is in agreement with Ou *et al*[20] findings confirming that the virus entry into these cells undergoes *via* RME with the involvement of cathepsin L in spike protein activation. In their experiments on the human colon cell line - Caco-2 cells overexpressing TMPRSS2, Hoffmann *et al*[3] exhibited the participation of both TMPRSS2 and CCs in the mechanism of the virus entry into these cells. Alkalization of the environment caused around 90% reduction in SARS-2-S-driven entry, whereas incubation with E-64d inhibited the virus entry by around 40%. On the other hand, the application of camostat mesylate (inhibitor of TMPRSS2 and other serine proteases) reduced the virus entry by about 90%, and when the Authors used both inhibitors simultaneously, they achieved nearly complete virus-entry inhibition to Caco-2 TMPRSS2 (+) cells. They also found that E-64 inhibitor significantly reduced the virus entry into Vero and 293T cells not expressing TMPRSS2, which indicates the endolysosomal pathway. However, the transduction of these cell lines with TMPRSS2 markedly reversed the effect of CCs inhibition, emphasizing the function of TMPRSS2 in the S protein priming, agreeably with Ou *et al*[20] findings that the expression of TMPRSS 2, 4, 11 A, 11D, and 11E on 293/hACE2 cells enhanced SARS-CoV-2 S protein-mediated cell–cell fusion. Moreover, the Authors noted that, however the addition of trypsin (like TMPRSS belonging to serine proteases) stimulated the formation of syncytia in 293/hACE2 cells (indicative of activated by trypsin SARS-2-S protein-stimulated cell-cell fusion), this process was also noticed in the experiments without trypsin. These findings indicate that binding with ACE2 receptors may be a sufficient event inducing cell-cell fusion (without the proteolytic priming with the extracellular protease). Nevertheless, it is possible that other host extracellular peptidases are involved in spike protein activation. The findings derived from Hoffmann *et al*[3] and Ou *et al*[20] experiments are depicted in Table 1.

***Furin in SARS-CoV-2 invasion***

As determined by Shang *et al*[8], another enzyme involved in SARS-CoV-2 spike protein priming is furin belonging to proprotein convertases (PCs). PCs are eukaryotic serine proteases, and ubiquitously expressed furin belongs to the PCs subfamily present in the organelles of the constitutive protein secretion pathway. These enzymes participate in the proteolytic post-translational modification of a variety of functionally important peptides and proteins, such as growth factors and hormones, both intra- and extracellularly (in the trans-Golgi network, endosomes, and pericellular environment). The amino acid sequence specifically recognized and cleaved by PCs including furin, is found in many viral surface proteins, so different viruses (like MERS-CoV) are activated by these enzymes[1]. Unlike SARS-CoV (exhibiting PC cleavage site motif only in the S2’ site) [1], SARS-CoV-2 spike protein includes PCs specific motif at the S1/S2 boundary[8]. Shang *et al*[8] have performed experiments to examine whether this sequence is cut by furin. They demonstrated that PCs inhibitors reduced SARS-CoV-2 pseudovirus entry into three cell lines expressing hACE2 receptors; HeLa cells (human cervical cells), Calu-3 cells (human lung epithelial cells), and MRC-5 cells (human lung fibroblast cells). Moreover, the mutation of the PCs specific motif significantly reduced the pseudovirions entry to the studied cells. The Authors detected no cleavage within the spike protein, when they packaged the pseudoviruses to HEK293T cells pretreated with furin-targeting siRNA. Additionally, they excluded the participation of matrix metalloproteinases (MMPs) in the experiment with the application of MMP inhibitor. Therefore, they confirmed the involvement of furin in SARS-CoV-2 entry into chosen cells. Additionally, furin priming of SARS-CoV-2 spike protein has been associated with the virions recognition and binding by neuropilin 1 (NRP1 - receptor which binds furin-cleaved substrates). Cantuti-Castelvetri *et al*[21] have shown that, except for ACE2, also NRP1 receptors are involved in SARS-CoV-2 invasion. Although the exact mechanism of NRP1 participation in this process is not elucidated, the Authors found the receptors to markedly enhance the virus infectivity. Apart from furin involvement, Shang *et al*[8] also observed the association of other aforementioned peptidases with SARS-CoV-2 invasion. They noticed the reduction in the pseudovirus entry to the three studied cell lines, caused by the application of both serine proteases (camostat) and cysteine cathepsins (E64) inhibitors. Furthermore, the pseudovirus entry to HeLa cells was more markedly reduced when either camostat or E64d was applied in the cells pretreated with proprotein convertases inhibitor[8]. Therefore, it might be concluded that depending on the type of target cells, TMPRSS2, lysosomal cathepsins, and furin might be involved in the activation of SARS-CoV-2 entry exhibiting cumulative/overlapping final effect (Figure 2). The observations of Shang *et al*[8] and Cantuti-Castelvetri *et al*[21] are collected in Table 1.

***Cysteine cathepsins as a target in search for COVID-19 therapy***

Focusing on cathepsin L expression as a target in search for an anti-Covid-19 drug, Smieszek *et al*[22] have tested an array of medications applied in clinical practice. They found amantadine (a drug used previously to treat influenza A, and now applied in neurological diseases including Parkinson’s disease) to be a promising compound. Being able to accumulate in lysosomes and alkalize them, amantadine belongs to lysosomotropic agents. Such compounds inactivate lysosomal enzymes including CCs whose optimal pH lies below 5. The Authors demonstrated a significant reduction in cathepsin L gene expression using amantadine, however also other cysteine cathepsin genes expression was inhibited, including cathepsins B and K, with the most pronounced effect observed for cathepsin H. Therefore, it might be hypothesized that this is also cathepsin H which plays an important role in the processing of the SARS-2-S spike protein. The activity of cathepsin H would have been inhibited similarly to other lysosomal cathepsins in the aforementioned experiments (raising pH and/or using E64 inhibitor), which demonstrated a significant reduction of SARS-2-S-mediated entry to the studied cells. However, in comparison with thoroughly studied cathepsins B, L, S, and K, there is much less scientific data referring to cathepsin H.

Amantadine efficiency in COVID-19 treatment has been suggested by Rejdak *et al*[23] who documented no clinical manifestations of COVID-19 infection in 22 patients in spite of the confirmation of SARS-CoV-2 presence with rRT-PCR testing in all of these individuals. The patients had been treated with either amantadine or memantine, for at least 3 months prior to the infection exposure, due to their conditions (multiple sclerosis, Parkinson's disease or cognitive impairment). Therefore, amantadine seems to be a promising treatment for COVID-19 patients. The effect of amantadine may be associated with the down-regulation of cysteine cathepsins (L and/or H) in the endolysosomal compartment and/or the disturbance of viroporin protein channel probably involved in the viral RNA release, as suggested for SARS-CoV[24].

As discussed before, the generation of anti-CCs medicinal drugs is a problematic issue (associated with the observation of unfavorable side effects). Hence, the attitude aimed at screening the already existing therapeutics, which would lower the activity of proteases involved in SARS-CoV-2 activation, seems a rational approach.

**EFFECT OF SARS-CoV-2 ON GASTROINTESTINAL TRACT**

***Gastrointestinal symptoms and fecal virus shedding in COVID-19 patients***

A significant amount of scientific evidence accumulated so far points to gastrointestinal tract (GIT), especially its lower part, as a target organ affected by SARS-CoV-2, beside the respiratory system[25]. Apart from the typical pulmonary symptoms (cough, fever, shortness of breath), some of the patients (around 4%-50% individuals) present with digestive symptoms like diarrhea, nausea, vomiting and abdominal pain[26]. Moreover, the virus mRNA presence in stool samples has been observed in some patients, often persisting long after its disappearance from the respiratory tract. Wu *et al*[27] have documented SARS-CoV-2 mRNA presence in the feces of more than half of the studied patients, with duration for up to 5 wk after its vanishing from the respiratory tract specimens. It might suggest active proliferation of the virus in the gastrointestinal tract of some patients. Similarly, Xiao *et al*[28] have observed fecal virus shedding in more than 50% of the studied patients, and in over 20% of them the duration of positive results in stool exceeded the virus presence in the respiratory samples. Additionally, the Authors detected the protein parts of the virus (as well as the presence of ACE2 receptors) in gastrointestinal epithelial cells. The virus replication in rectal tissue derived from a COVID-19 patient has been noted by Qian *et al*[29] who detected SARS-CoV-2 components in the intestinal epithelial cells (but mainly in intestinal lymphocytes and macrophages). The Authors hypothesize that, like in the case of influenza virus, it is possible for SARS-CoV-2 virions to be transported from the respiratory tract to the GIT *via* the immune cells. In the meta-analysis of 60 studies comprising over 4000 COVID-19 patients, performed by Cheung *et al*[30], 17.6% of the patients exhibited gastrointestinal symptoms, and in almost 50% (30%-70%) the virus mRNA was detected in the feces. Most of these positive stool samples (above 70%) were collected after the loss of the virus from the respiratory specimens.

However, it is still not clear, whether the virus is transmittable *via* fecal-oral route. Whereas Wang *et al*[31] detected live virus in the fecal samples, Zang *et al*[11] demonstrated the inactivation of the virions released into the intestinal lumen in the environment simulating human colonic fluid. Moreover, the Authors did not manage to recover infectious virus from the stool specimens.

***Ability of SARS-CoV-2 to productively infect human GIT cells via ACE2 and TMPRSS***

Several studies have demonstrated the invasion and replication of SARS-CoV-2 in the human GIT cells. Chu *et al*[32] in their ex-vivo experiments on human intestinal tissues have evidenced the ability of SARS-CoV-2 to infect, proliferate and release infectious virus particles from intestinal cells. In comparison with SARS-CoV, SARS-CoV-2 replicated less efficiently and brought about less damages in the human intestinal epithelium, but evoked greater response of innate immune system (inducing the expression of proinflammatory mediators such as interferons and interleukins). Hence, the Authors suggested that the gastrointestinal tract might serve as an alternative route of virus dissemination. The vulnerability of the human GIT epithelial cells to SARS-CoV-2 invasion *via* ACE2 and TMPRSS-2 receptors has been confirmed in other studies with the application of intestinal cell lines models, as well as human small intestinal organoids – hSIOs[11,33-35]. Lee *et al*[33] investigated the growth of SARS-CoV-2 in a human GIT cell line model; C2BBe1 (a subclone of human epithelial colorectal adenocarcinoma cells: Caco-2). C2BBe1 (genetically and structurally resembling the brush border epithelial cells in the human GIT[36,37], and expressing moderate level of ACE2 and high level of TMPRSS2[33]) exhibited the greatest susceptibility to the virus. SARS-CoV-2 virions invaded and replicated in these cells, as well as in Caco-2[33,35] and T84 (human colon carcinoma) cells[35]. Furthermore, Stanifer *et al*[35] demonstrated SARS-CoV-2 infection of human colon organoids followed by active virions replication. These observations are in agreement with other studies on hSIOs[11,34]. Zang *et al*[11] reported productive infection of SARS-CoV-2 in ACE2(+) mature enterocytes, dependent on TMPRSS2 and TMPRSS4 receptors in human small intestinal enteroids. The Authors noted the role of an additional serine protease: TMPRSS4 which heightened the effect of TMPRSS2. Also, the two serine proteases enhanced SARS-CoV-2 spike protein-induced cell-cell fusion observed by the Authors.

ACE2 receptors (except for the respiratory system) are present in a variety of other organs including the gastrointestinal tract, where a great number of these proteins has been detected in the lower part of GIT[38]. Unlike in the upper segment (oral cavity, esophagus, stomach), high expression of ACE2 (both mRNA and protein) is observed in the small intestine (the greatest level), colon and rectum, as well as in the gall bladder[38,39]. Actually, ACE2 expression in the small intestine is much higher in comparison with all other organs in the human organism including the respiratory tract[11]. Specifically, ACE2 is present in the enterocyte cytoplasm and in the apical brush border, as well as in the glandular cells (in the lining epithelium of the lower GIT)[37]. The expression of ACE2 receptors has been exhibited to increase upon enterocytes differentiation. Lee *et al*[33] observed that (unlike constitutively expressed TMPRSS2), the expression of ACE2 receptors was significantly stimulated in the experiment inducing C2BBe1 enterocytes differentiation, associated with the generation of more pronounced features typical for brush border cells. Similarly, Zang *et al*[11] detected the greatest expression of ACE2 in mature brush border enterocytes, and Lamers *et al*[34] noticed around 1000-fold increase in ACE2 mRNA expression upon enterocytes differentiation. Zang *et al*[11] observed all studied receptors (ACE2, as well as TMPRSS2 and TMPRSS4) to be correlated with the virus invasiveness, similarly as Lee *et al*[33] noted a strong correlation between TMPRSS2 (although not ACE2) and viral RNA levels in the studied human epithelial cell lines (including Caco-2 and C2BBe1). Lee *et al*[33] found that the ectopic coexpression of ACE2 and TMPRSS2 in RPMI 2650 cells enhanced viral dissemination by 56.7 times (over 10-fold more in comparison with the sole ACE2 effect – 4.9 times, whereas TMPRSS2 transfection alone did not enhance the level of infectivity). It might be supposed that an effective level of ACE2 receptors is a prerequisite for the virions invasion of epithelial cells, but abundant expression of TMPRSS2 (and possibly TMPRSS4) greatly facilitates ACE2-mediated SARS-CoV-2 dissemination in the human GIT.

The involvement of both ACE2 and TMPRSS2 in SARS-CoV-2 invasion of GIT epithelial cells is in accordance with the reported co-localization of the two receptors in the lower GIT[38-40]. The most abundant expression of both proteins has been detected in the small intestine epithelial cells[38]; especially in the brush border cells[33]. Lee *et al*[39] evaluated single-cell RNA-sequencing datasets from the GIT in search for these two genes co-expression, and found the small intestine enterocytes as well as colonocytes to display the highest proportions of cells co-expressing ACE2 and TMPRSS2. Additionally, the Authors checked for the co-expression of ACE2, TMPRSS2 and TMPRSS4, and demonstrated the highest proportions of the three genes co-expression in the progenitor and stem-like epithelial cells in the small intestine. TMPRSS4 is an extra serine protease involved in SARS-CoV-2 activation and invasion, enhancing TMPRSS2 priming effect[11]. Therefore, both ACE2 and TMPRSS2 seem to be the main receptors responsible for SARS-CoV-2 invasion of GIT. The experimental data coming from Lee *et al*[33], Zang *et al*[11], and Stanifer *et al*[35] investigations are displayed in Table 1.

***Putative association between SARS-CoV-2-mediated ACE2 disturbance and gastrointestinal symptoms development in COVID-19 patients***

The known function of angiotensin converting enzyme 2 (ACE2) is the regulation of systemic arterial blood pressure (renin-angiotensin system). ACE2 catalyzes the conversion of Ang I to angiotensin (1–9) and angiotensin II (Ang II) to angiotensin (1–7), what counteracts the effects of Ang II, leading to decrease in blood pressure and inflammatory processes attenuation (reviewed in[41]). However, the role of ACE2 in GIT (where it is abundantly expressed) seems to be rather associated with the processes occurring in this organ. The analysis of the digestive system specific functional enrichment map for ACE2 gene suggests the involvement of ACE2 in digestion (with reference to its proteolytic activity) and transport of metabolites (the regulation of amino acid transport)[38]. In fact, ACE-2 proteins have been demonstrated to be coupled with sodium-dependent amino acids and glucose transporters. ACE2 is a chaperone for the sodium-dependent amino acid transporter B0AT1 which is involved in transport of neutral amino acids[42]. Moreover, ACE2 participates in the regulation of gut microbiota homeostasis[43,44].

Therefore, as proposed by Kumar *et al*[38], it might be hypothesized that SARS-CoV-2-associated dysregulation of ACE2 receptors in the human GIT may be involved in the mechanism of GIT symptoms development in COVID-19 patients.

**CONCLUSION**

In light of the presented studies, the impact of SARS-CoV-2 virus on GIT is evident, with the most substantiated involvement of ACE2 and TMPRSS2. However, except for these two receptors (and probably TMPRSS4), also other proteases might be implicated in SARS-CoV-2 invasion of GIT, as well as the development of the observed symptoms. Ubiquitously expressed furin and cathepsin L may be involved in spike protein processing, contributing to the virus invasiveness. Cathepsin L (and other CCs) might participate in the endolysosomal processing of the spike protein following ACE2-mediated endocytosis of the virion particles. Additionally, SARS-CoV-2 – induced inflammatory cytokines could stimulate the secretion of cathepsin L. Therefore, extracellular cathepsin L may contribute both to the spike protein processing, as well as degradation/remodeling of the ECM components, as well as membrane-bound receptors including TMPRSS2/4 and ACE2. The resulting events might accelerate the inflammatory processes disturbing the digestion/absorption of nutrients yielding the observed symptoms such as diarrhea. However, more research is required, since the participation of furin and lysosomal cathepsins in SARS-CoV-2 GIT-invasion is more speculative.

**REFERENCES**

1 **Izaguirre G**. The Proteolytic Regulation of Virus Cell Entry by Furin and Other Proprotein Convertases. *Viruses* 2019; **11** [PMID: 31505793 DOI: 10.3390/v11090837]

2 **Wrapp D**, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020; **367**: 1260-1263 [PMID: 32075877 DOI: 10.1126/science.abb2507]

3 **Hoffmann M**, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; **181**: 271-280.e8 [PMID: 32142651 DOI: 10.1016/j.cell.2020.02.052]

4 **Blaess M**, Kaiser L, Sauer M, Csuk R, Deigner HP. COVID-19/SARS-CoV-2 Infection: Lysosomes and Lysosomotropism Implicate New Treatment Strategies and Personal Risks. *Int J Mol Sci* 2020; **21** [PMID: 32668803 DOI: 10.3390/ijms21144953]

5 **Zhou P**, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **579**: 270-273 [PMID: 32015507 DOI: 10.1038/s41586-020-2012-7]

6 **Walls AC**, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* 2020; **181**: 281-292.e6 [PMID: 32155444 DOI: 10.1016/j.cell.2020.02.058]

7 **Zhu G**, Zhu C, Zhu Y, Sun F. Minireview of progress in the structural study of SARS-CoV-2 proteins. *Curr Res Microb Sci* 2020; **1**: 53-61 [PMID: 33236001 DOI: 10.1016/j.crmicr.2020.06.003]

8 **Shang J**, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, Li F. Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A* 2020; **117**: 11727-11734 [PMID: 32376634 DOI: 10.1073/pnas.2003138117]

9 **Wei J**, Alfajaro MM, DeWeirdt PC, Hanna RE, Lu-Culligan WJ, Cai WL, Strine MS, Zhang SM, Graziano VR, Schmitz CO, Chen JS, Mankowski MC, Filler RB, Ravindra NG, Gasque V, de Miguel FJ, Patil A, Chen H, Oguntuyo KY, Abriola L, Surovtseva YV, Orchard RC, Lee B, Lindenbach BD, Politi K, van Dijk D, Kadoch C, Simon MD, Yan Q, Doench JG, Wilen CB. Genome-wide CRISPR Screens Reveal Host Factors Critical for SARS-CoV-2 Infection. *Cell* 2021; **184**: 76-91.e13 [PMID: 33147444 DOI: 10.1016/j.cell.2020.10.028]

10 **Pišlar A**, Mitrović A, Sabotič J, Pečar Fonović U, Perišić Nanut M, Jakoš T, Senjor E, Kos J. The role of cysteine peptidases in coronavirus cell entry and replication: The therapeutic potential of cathepsin inhibitors. *PLoS Pathog* 2020; **16**: e1009013 [PMID: 33137165 DOI: 10.1371/journal.ppat.1009013]

11 **Zang R**, Gomez Castro MF, McCune BT, Zeng Q, Rothlauf PW, Sonnek NM, Liu Z, Brulois KF, Wang X, Greenberg HB, Diamond MS, Ciorba MA, Whelan SPJ, Ding S. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Sci Immunol* 2020; **5** [PMID: 32404436 DOI: 10.1126/sciimmunol.abc3582]

12 **Berdowska I**. Cysteine proteases as disease markers. *Clin Chim Acta* 2004; **342**: 41-69 [PMID: 15026265 DOI: 10.1016/j.cccn.2003.12.016]

13 **Vizovišek M**, Fonović M, Turk B. Cysteine cathepsins in extracellular matrix remodeling: Extracellular matrix degradation and beyond. *Matrix Biol* 2019; **75-76**: 141-159 [PMID: 29409929 DOI: 10.1016/j.matbio.2018.01.024]

14 **Vizovišek M**, Vidak E, Javoršek U, Mikhaylov G, Bratovš A, Turk B. Cysteine cathepsins as therapeutic targets in inflammatory diseases. *Expert Opin Ther Targets* 2020; **24**: 573-588 [PMID: 32228244 DOI: 10.1080/14728222.2020.1746765]

15 **Vidak E**, Javoršek U, Vizovišek M, Turk B. Cysteine Cathepsins and their Extracellular Roles: Shaping the Microenvironment. *Cells* 2019; **8** [PMID: 30897858 DOI: 10.3390/cells8030264]

16 **Gomes CP**, Fernandes DE, Casimiro F, da Mata GF, Passos MT, Varela P, Mastroianni-Kirsztajn G, Pesquero JB. Cathepsin L in COVID-19: From Pharmacological Evidences to Genetics. *Front Cell Infect Microbiol* 2020; **10**: 589505 [PMID: 33364201 DOI: 10.3389/fcimb.2020.589505]

17 **Menzel K**, Hausmann M, Obermeier F, Schreiter K, Dunger N, Bataille F, Falk W, Scholmerich J, Herfarth H, Rogler G. Cathepsins B, L and D in inflammatory bowel disease macrophages and potential therapeutic effects of cathepsin inhibition in vivo. *Clin Exp Immunol* 2006; **146**: 169-180 [PMID: 16968411 DOI: 10.1111/j.1365-2249.2006.03188.x]

18 **Xu S**, Zhang H, Yang X, Qian Y, Xiao Q. Inhibition of cathepsin L alleviates the microglia-mediated neuroinflammatory responses through caspase-8 and NF-κB pathways. *Neurobiol Aging* 2018; **62**: 159-167 [PMID: 29154036 DOI: 10.1016/j.neurobiolaging.2017.09.030]

19 **Cao Y**, Liu X, Li Y, Lu Y, Zhong H, Jiang W, Chen AF, Billiar TR, Yuan H, Cai J. Cathepsin L activity correlates with proteinuria in chronic kidney disease in humans. *Int Urol Nephrol* 2017; **49**: 1409-1417 [PMID: 28534128 DOI: 10.1007/s11255-017-1626-7]

20 **Ou X**, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J, Qian Z. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun* 2020; **11**: 1620 [PMID: 32221306 DOI: 10.1038/s41467-020-15562-9]

21 **Cantuti-Castelvetri L**, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, van der Meer F, Kallio K, Kaya T, Anastasina M, Smura T, Levanov L, Szirovicza L, Tobi A, Kallio-Kokko H, Österlund P, Joensuu M, Meunier FA, Butcher SJ, Winkler MS, Mollenhauer B, Helenius A, Gokce O, Teesalu T, Hepojoki J, Vapalahti O, Stadelmann C, Balistreri G, Simons M. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science* 2020; **370**: 856-860 [PMID: 33082293 DOI: 10.1126/science.abd2985]

22 **Smieszek SP**, Przychodzen BP, Polymeropoulos MH. Amantadine disrupts lysosomal gene expression: A hypothesis for COVID19 treatment. *Int J Antimicrob Agents* 2020; **55**: 106004 [PMID: 32361028 DOI: 10.1016/j.ijantimicag.2020.106004]

23 **Rejdak K**, Grieb P. Adamantanes might be protective from COVID-19 in patients with neurological diseases: multiple sclerosis, parkinsonism and cognitive impairment. *Mult Scler Relat Disord* 2020; **42**: 102163 [PMID: 32388458 DOI: 10.1016/j.msard.2020.102163]

24 **Torres J**, Maheswari U, Parthasarathy K, Ng L, Liu DX, Gong X. Conductance and amantadine binding of a pore formed by a lysine-flanked transmembrane domain of SARS coronavirus envelope protein. *Protein Sci* 2007; **16**: 2065-2071 [PMID: 17766393 DOI: 10.1110/ps.062730007]

25 **Sahu T**, Mehta A, Ratre YK, Jaiswal A, Vishvakarma NK, Bhaskar LVKS, Verma HK. Current understanding of the impact of COVID-19 on gastrointestinal disease: Challenges and openings. *World J Gastroenterol* 2021; **27**: 449-469 [PMID: 33642821 DOI: 10.3748/wjg.v27.i6.449]

26 **Pan L**, Mu M, Yang P, Sun Y, Wang R, Yan J, Li P, Hu B, Wang J, Hu C, Jin Y, Niu X, Ping R, Du Y, Li T, Xu G, Hu Q, Tu L. Clinical Characteristics of COVID-19 Patients With Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *Am J Gastroenterol* 2020; **115**: 766-773 [PMID: 32287140 DOI: 10.14309/ajg.0000000000000620]

27 **Wu Y**, Guo C, Tang L, Hong Z, Zhou J, Dong X, Yin H, Xiao Q, Tang Y, Qu X, Kuang L, Fang X, Mishra N, Lu J, Shan H, Jiang G, Huang X. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol* 2020; **5**: 434-435 [PMID: 32199469 DOI: 10.1016/S2468-1253(20)30083-2]

28 **Xiao F**, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for Gastrointestinal Infection of SARS-CoV-2. *Gastroenterology* 2020; **158**: 1831-1833.e3 [PMID: 32142773 DOI: 10.1053/j.gastro.2020.02.055]

29 **Qian Q**, Fan L, Liu W, Li J, Yue J, Wang M, Ke X, Yin Y, Chen Q, Jiang C. Direct evidence of active SARS-CoV-2 replication in the intestine. *Clin Infect Dis* 2020 [PMID: 32638022 DOI: 10.1093/cid/ciaa925]

30 **Cheung KS**, Hung IFN, Chan PPY, Lung KC, Tso E, Liu R, Ng YY, Chu MY, Chung TWH, Tam AR, Yip CCY, Leung KH, Fung AY, Zhang RR, Lin Y, Cheng HM, Zhang AJX, To KKW, Chan KH, Yuen KY, Leung WK. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis. *Gastroenterology* 2020; **159**: 81-95 [PMID: 32251668 DOI: 10.1053/j.gastro.2020.03.065]

31 **Wang W**, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA* 2020; **323**: 1843-1844 [PMID: 32159775 DOI: 10.1001/jama.2020.3786]

32 **Chu H**, Chan JF, Wang Y, Yuen TT, Chai Y, Shuai H, Yang D, Hu B, Huang X, Zhang X, Hou Y, Cai JP, Zhang AJ, Zhou J, Yuan S, To KK, Hung IF, Cheung TT, Ng AT, Hau-Yee Chan I, Wong IY, Law SY, Foo DC, Leung WK, Yuen KY. SARS-CoV-2 Induces a More Robust Innate Immune Response and Replicates Less Efficiently Than SARS-CoV in the Human Intestines: An Ex Vivo Study With Implications on Pathogenesis of COVID-19. *Cell Mol Gastroenterol Hepatol* 2021; **11**: 771-781 [PMID: 33010495 DOI: 10.1016/j.jcmgh.2020.09.017]

33 **Lee S**, Yoon GY, Myoung J, Kim SJ, Ahn DG. Robust and persistent SARS-CoV-2 infection in the human intestinal brush border expressing cells. *Emerg Microbes Infect* 2020; **9**: 2169-2179 [PMID: 32969768 DOI: 10.1080/22221751.2020.1827985]

34 **Lamers MM**, Beumer J, van der Vaart J, Knoops K, Puschhof J, Breugem TI, Ravelli RBG, Paul van Schayck J, Mykytyn AZ, Duimel HQ, van Donselaar E, Riesebosch S, Kuijpers HJH, Schipper D, van de Wetering WJ, de Graaf M, Koopmans M, Cuppen E, Peters PJ, Haagmans BL, Clevers H. SARS-CoV-2 productively infects human gut enterocytes. *Science* 2020; **369**: 50-54 [PMID: 32358202 DOI: 10.1126/science.abc1669]

35 **Stanifer ML**, Kee C, Cortese M, Zumaran CM, Triana S, Mukenhirn M, Kraeusslich HG, Alexandrov T, Bartenschlager R, Boulant S. Critical Role of Type III Interferon in Controlling SARS-CoV-2 Infection in Human Intestinal Epithelial Cells. *Cell Rep* 2020; **32**: 107863 [PMID: 32610043 DOI: 10.1016/j.celrep.2020.107863]

36 **Peterson MD**, Mooseker MS. Characterization of the enterocyte-like brush border cytoskeleton of the C2BBe clones of the human intestinal cell line, Caco-2. *J Cell Sci* 1992; **102 ( Pt 3)**: 581-600 [PMID: 1506435 DOI: 10.1242/jcs.102.3.581]

37 **Klijn C**, Durinck S, Stawiski EW, Haverty PM, Jiang Z, Liu H, Degenhardt J, Mayba O, Gnad F, Liu J, Pau G, Reeder J, Cao Y, Mukhyala K, Selvaraj SK, Yu M, Zynda GJ, Brauer MJ, Wu TD, Gentleman RC, Manning G, Yauch RL, Bourgon R, Stokoe D, Modrusan Z, Neve RM, de Sauvage FJ, Settleman J, Seshagiri S, Zhang Z. A comprehensive transcriptional portrait of human cancer cell lines. *Nat Biotechnol* 2015; **33**: 306-312 [PMID: 25485619 DOI: 10.1038/nbt.3080]

38 **Kumar A**, Faiq MA, Pareek V, Raza K, Narayan RK, Prasoon P, Kumar P, Kulandhasamy M, Kumari C, Kant K, Singh HN, Qadri R, Pandey SN, Kumar S. Relevance of SARS-CoV-2 related factors ACE2 and TMPRSS2 expressions in gastrointestinal tissue with pathogenesis of digestive symptoms, diabetes-associated mortality, and disease recurrence in COVID-19 patients. *Med Hypotheses* 2020; **144**: 110271 [PMID: 33254575 DOI: 10.1016/j.mehy.2020.110271]

39 **Lee JJ**, Kopetz S, Vilar E, Shen JP, Chen K, Maitra A. Relative Abundance of SARS-CoV-2 Entry Genes in the Enterocytes of the Lower Gastrointestinal Tract. *Genes (Basel)* 2020; **11** [PMID: 32545271 DOI: 10.3390/genes11060645]

40 **Gkogkou E**, Barnasas G, Vougas K, Trougakos IP. Expression profiling meta-analysis of ACE2 and TMPRSS2, the putative anti-inflammatory receptor and priming protease of SARS-CoV-2 in human cells, and identification of putative modulators. *Redox Biol* 2020; **36**: 101615 [PMID: 32863223 DOI: 10.1016/j.redox.2020.101615]

41 **Wiese O**, Zemlin AE, Pillay TS. Molecules in pathogenesis: angiotensin converting enzyme 2 (ACE2). *J Clin Pathol* 2021; **74**: 285-290 [PMID: 32759311 DOI: 10.1136/jclinpath-2020-206954]

42 **Singer D**, Camargo SM. Collectrin and ACE2 in renal and intestinal amino acid transport. *Channels (Austin)* 2011; **5**: 410-423 [PMID: 21814048 DOI: 10.4161/chan.5.5.16470]

43 **Camargo SM**, Singer D, Makrides V, Huggel K, Pos KM, Wagner CA, Kuba K, Danilczyk U, Skovby F, Kleta R, Penninger JM, Verrey F. Tissue-specific amino acid transporter partners ACE2 and collectrin differentially interact with hartnup mutations. *Gastroenterology* 2009; **136**: 872-882 [PMID: 19185582 DOI: 10.1053/j.gastro.2008.10.055]

44 **Hashimoto T**, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, Wild B, Camargo SM, Singer D, Richter A, Kuba K, Fukamizu A, Schreiber S, Clevers H, Verrey F, Rosenstiel P, Penninger JM. ACE2 Links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* 2012; **487**: 477-481 [PMID: 22837003 DOI: 10.1038/nature11228]

**Footnotes**

**Conflict-of-interest statement:** Dr. Matusiewicz has nothing to disclose.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Corresponding Author's Membership in Professional Societies:** American Association for the Advancement of Science, 00494429; Polish Biochemical Society member from April 10, 2010.

**Peer-review started:** March 29, 2021

**First decision:** June 14, 2021

**Article in press:**

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Poland

**Peer-review report’s scientific quality classification**

Grade A (Excellent): A

Grade B (Very good): 0

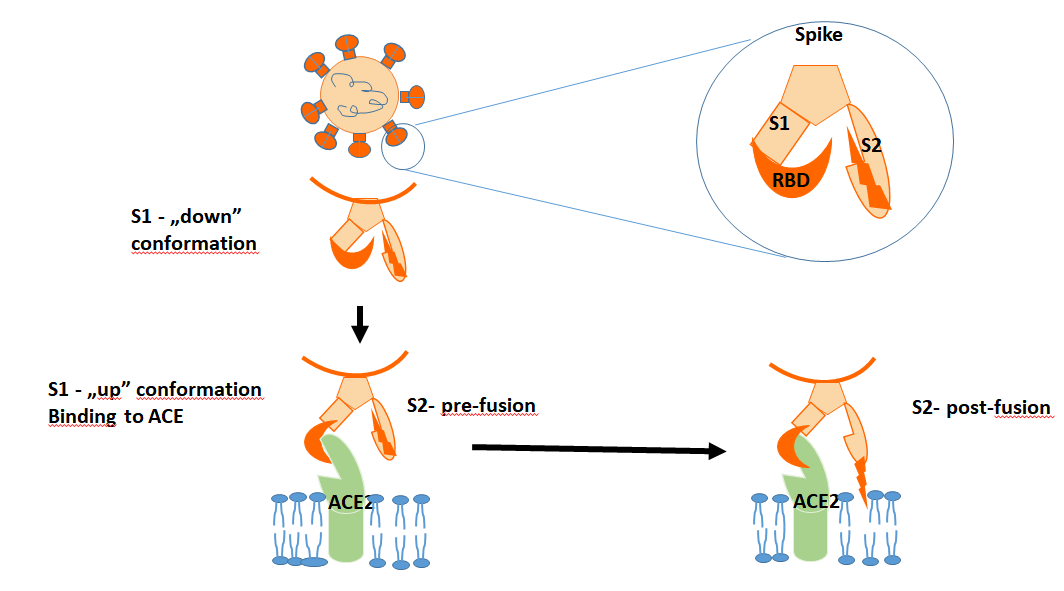
Grade C (Good): C

Grade D (Fair): 0

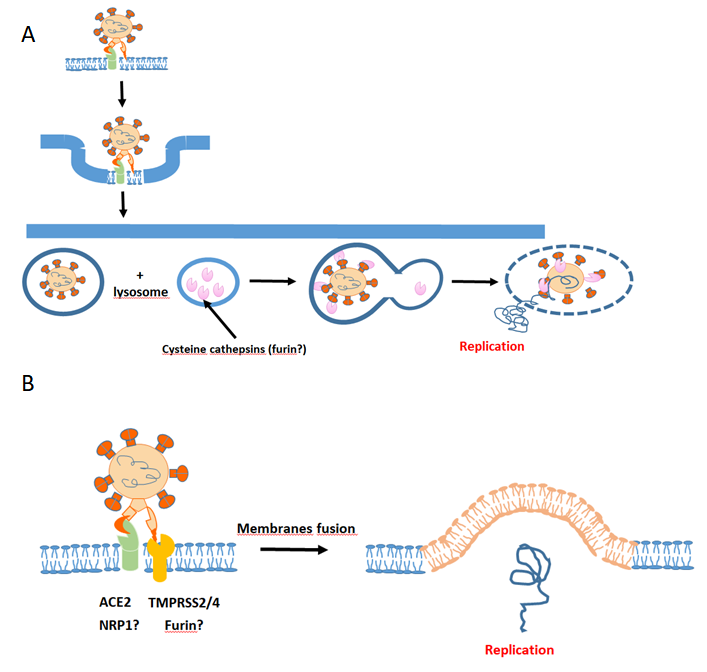
Grade E (Poor): 0

**P-Reviewer:** Khachfe H, Khan MKA **S-Editor:** Wang LL **L-Editor:** **P-Editor:**

**Figure Legends**



**Figure 1 Alternations in spike proteins conformation upon binding to ACE 2.** Spike S1 subunit contains receptor binding domain, that has to change from “down-conformation” state to “up-conformation” state to be accessible for ACE2. Changes in S1 subunit trigger conformational changes in S2 subunit, causing exposition of hydrophobic domain, changing it from “pre-fusion” to “post-fusion” state. This enables fusion of the virus with host membrane (after Zhu *et al*[7]).



**Figure 2 Two modes of virus entry.** A: Receptor-mediated endocytosis of Severe acute respiratory syndrome coronavirus-2. After binding to ACE2 and formation of endosome, lysosomal cathepsins activate spike protein, which leads to the release of the viral RNA into host cell. B. Membrane fusion mechanism - priming of the spike proteins is mediated by transmembrane peptidase/serine subfamily member 2/4, which leads to fusion of viral and host membranes and release of the viral RNA into host cell.

**Table 1 Receptors/proteases involved in Severe acute respiratory syndrome coronavirus-2 invasion of human cells**

|  |  |  |  |
| --- | --- | --- | --- |
| **Receptor/protease** | **Experimental model** | **Observation** | **Ref.** |
| ACE2 and TMPRSS2/4 | Human small intestinal enteroids; HEK-293T cell line transfected with ACE2, TMPRSS2, or TMPRSS4 | Productive infection of SARS-CoV-2 in ACE2 (+) mature enterocytes; Correlation of ACE2, TMPRSS2 and 4 with SARS-CoV-2 invasiveness | Zang *et al*[11] |
| ACE2 and TMPRSS2 | ACE2 and TMPRSS2 expressing C2BBe1, Caco-2, and Calu-3 cell lines | Persistent invasion and replication of SARS-CoV-2 in the cells; Correlation of TMPRSS2 (but not ACE2) with SARS-CoV-2 RNA | Lee *et al*[33] |
| ACE2, TMPRSS2, and CCs | HEK-293T cell line transfected with ACE2; Caco-2 cells overexpressing TMPRSS2 | Inhibition of SARS-CoV-2 pseudovirus entry into HEK-293T ACE2(+)/TMPRSS2(-) by pH increase and E-64 inhibitor;  Inhibition of SARS-CoV-2 pseudovirus entry into Caco-2 TMPRSS2(+) by pH increase, CCs inhibitor (E-64d), and TMPRSS2 inhibitor (camostat mesylate) | Hoffmann *et al*[3] |
| ACE2, TMPRSS 2, 4, 11A, 11D, 11E, and CCs (cathepsin L) | HEK-293T cell line transfected with ACE2 | Inhibition of SARS-CoV-2 pseudovirus entry into the cells with CCs inhibitor (E-64d) and cathepsin L inhibitor (but not cathepsin B inhibitor); Intensification of SARS-CoV-2 S protein-mediated cell–cell fusion, caused by expression of TMPRSS 2, 4, 11A, 11D, and 11E on 293/hACE2 cells | Ou *et al*[20] |
| ACE2 and TMPRSS2 | ACE2 and TMPRSS2 expressing Caco-2 and T84 cell lines | Persistent invasion and replication of SARS-CoV-2 in the cells | Stanifer *et al*[35] |
| ACE2, furin, TMPRSS2, and CCs | ACE2-expressing HeLa, Calu-3, and MRC-5 cell lines | Reduction of SARS-CoV-2 pseudovirus entry into cells by inhibitors of PCs, CCs and TMPRSS2 | Shang *et al*[8] |
| NRP1, ACE2, and TMPRSS2 | HEK-293T cell line transfected with ACE2, TMPRSS2, or NRP1 | Augmentation of SARS-CoV-2 infectivity when NRP1 was coexpressed with ACE2 and TMPRSS2 | Cantuti-Castelvetri *et al*[21] |

ACE2: Angiotensin converting enzyme 2; NRP1: Neuropilin 1 (receptor which binds furin-cleaved substrates); TMPRSS2/4: Transmembrane peptidase/serine subfamily member 2/4; CCs: Cysteine cathepsins; HEK-293T: Human embryonic kidney 293T cells; Caco-2: Human colorectal adenocarcinoma cell line; C2BBe1: A subclone of Caco-2; T84: Human colon carcinoma cell line; HeLa: Human cervical cell line; Calu-3: Human lung epithelial cell line; MRC-5: Human lung fibroblast cell line; PCs: Proprotein convertases.