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Viewpoint Article

ACE2 enzymatic role in the SARS-CoV-2 activation: a perspective through the evolutionary promiscuity and substrate diversity of enzymes

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Abstract

The SARS-CoV-2 is an RNA B type β-coronavirus that distinguishes itself from previous coronaviruses by its high infectivity and mortality rates. The mechanism of viral entry into the host cell via ACE2 is currently under research. Several proteases have been nominated to activate the virus but identifying the exact enzyme/enzymes is missing. Moreover, recent work suggests that TMPRSS2 cannot be the enzyme to cleave the SARS-CoV-2 spike or that multiple proteases contribute to SARS-CoV-2 activation. The multitude of proteases that have been nominated to activate the virus suggests that the consensual identification of the precise, key enzyme is still missing. In this context, we synthesize the current controversies regarding the putative enzymes involved in SARS-CoV-2 infectivity and analyze whether ACE2 could have unexpected enzymatic roles in this process, besides its acknowledged receptor role. We hypothesize that ACE2 plays an enzymatic role as well in SARS-CoV-2 activation. Understanding the exact roles of ACE2 in COVID-19 is capital for the future design of specific, efficient therapies and deserves dedicated research. Our conviction is therefore not "if", “but” "when" will the researchers start to wonder about what is hidden behind the apparent only role of ACE2 as a receptor for SARS-CoV-2.

Keywords: ACE2, SARS-CoV-2, COVID-19, enzymatic activity, enzymatic promiscuity, TMPRSS2, ADAM-17, Romania

Background

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemics still represent the most stringent worldwide health problem. There is still no effective and specific treatment for it, and its associated infectivity and morbidity rates are outstanding, with almost three million deaths reported [1]. SARS-CoV-2, from the family of Coronaviridae, is an RNA B type β-coronavirus that distinguishes itself by its higher infectivity rates in contrast with other previously known coronaviruses [2-4]. It has an improved mechanism of cellular entry as it recognizes and binds with increased affinity (10 to 20 times) to angiotensin-converting enzyme 2 (ACE2) receptors that are extremely abundant and widely spread in our body [5-10]. This characteristic of SARS-CoV-2 explains the multiple routes of human viral infectivity and even its putative propagation across species through vectors that possess ACE2 receptors, such as insects [11]. The multiple mobile hosts for the SARS-CoV-2 and various dissemination routes explain why the end of the pandemics cannot be obtained only via quarantine and social isolation [12] but through an active improved knowledge regarding this new type of coronavirus and its specific mechanisms of infectivity. CoV-2, along with other previous coronaviruses, has a huge registry of genomic mutations and immune escape. Therefore, a major future concern is regarding the risk of repeated pandemics; also, that of new viral strains occurrence rendering the design of future vaccines a very complex target [11]. In this context, only a detailed knowledge of the precise mechanism of viral infectivity can be at the base of an efficacious therapy and prevention. However, the detailed mechanism of SARS-CoV-2 infectivity still has many gaps and represents a source of controversies, explaining why a final effective treatment is currently missing. In this context, one of the significant persisting controversies is regarding the enzymes responsible for the viral activation, essential for the viral fusion to the host cells.

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The current controversies regarding the proteases involved in SARS-CoV-2 activation

In order to become infective, by fusing to the host cell membranes, the spike protein of the SARS-CoV-2 has to be cleaved by proteases that will separate the S1 and S2 subunits [13-15]. However, currently, there is a lack of consensus between studies regarding the exact sheddase to cleave and activate the SARS-CoV-2 – to cite some authors (Samavati L. and Uhal BD., 2020): "the exact protease has not been identified" [16]. The lack of knowledge regarding the specific protease/proteases to play a role in activating the SARS-CoV-2 is one of the current major drawbacks in our understanding of the viral mechanisms of host infectivity. In the absence of a clear understanding of this aspect, the design of specific and efficient therapies in this infection remains complicated. Also, the anticipation of the effects of existing various medications in COVID-19 patients gains an unexpected level of complexity [17]. Instead of a simplified model, too many putative enzymes have been proposed to activate the SARS-CoV-2. Some authors present the transmembrane protease serine two or epitheliasin (TMPRSS2) as the final truth. In contrast, others name totally different proteases to activate the SARS-CoV-2 for host cell fusion, such as the disintegrin and metalloprotease 17 (ADAM-17), also known as TACE (tumor necrosis factor-α-converting enzyme), TMPRSS4, furin, human airway trypsin-like protease, trypsin, cathepsin, various types of membrane-associated serine proteases (MASPs) and others [13, 16-20]. These proteases are zinc-dependent metalloproteases activated by many stimuli and are essential for intracellular signaling, cell proliferation, and growth, playing roles in many physiological and pathological processes [21, 22]. However, other authors consider that SARS-S per se induces ACE2 shedding [23]. The multitude of putative proteases potentially responsible for the SARS-CoV-2 activation highlights much uncertainty about the exact sheddase activating the viral conformational switch, its site, and mechanism of action. The affirmation that a sheddase, such as TMPRSS2, ADAM-17, furin, or other, determines SARS-CoV-2 activation could have been precipitated by previous studies on other coronaviruses, but truth cannot always be extrapolated, especially when dealing with a different type of coronavirus [24], that shares only an 80%- nucleotide identity to SARS-CoV [9]. SARS-CoV-2 differs from other viruses as it has the distinctive ability to bind ACE2 receptors with higher affinity, contrasting with previous coronaviruses where the main mechanism of cell entry was via the endosomal pathway. Even in the case of the previous SARS-CoVs, the experiments have not clearly nominated which sheddase (TMPRSS2, ADAM-17, furin, or another) is responsible for the virus activation as their KO or inhibition did not terminate receptor-mediated viral entry [23, 25]. After the inhibition/in the absence of TMPRSS2 or ADAM-17, ACE2-expressing cells presented membrane fusion to the viral envelope, indicating that these proteases are not the sole ones in the process of viral activation [21, 25]. Some papers concluded that TMPRSS2 is the enzyme to activate the SARS-CoV-2 based on the observation that its inhibition prevents viral cell entry and is beneficial for patient treatment [18]. Instead, other authors state that, due to its small dimension, TMPRSS2 could not be the protease to cleave the SARS-CoV-2 [26]; also, it appears that TMPRSS2 could not cleave the virus between S1 and S2. However, some researchers see ADAM-17 as the activating protease, while different researchers consider that a furin-pre-cleavage would be required or that an" interplay" between ADAM-17-TMPRSS2 is likely to take place; also, a cathepsin role appeared to be required in the MERS activation [27-29]. Some researchers consider the requirement of the activity of two enzymes: cathepsin L protease and TMPRSS2, to activate the SARS-CoV-2 [30]. There are also reports regarding the existence of a commonly present furin recognition motif at the S1/S2 cleavage region of the spike protein in the SARS-CoV-2; moreover, it also presents a cleavage site at the S2' position, that is near to the S1/S2 site. Furin inhibitors and the deletion of the furin cleavage sites decrease the SARS-CoV-2 replication in hamster respiratory cells and kidney cells, respectively. The ubiquitous expression of furin with a wider distribution than TMPRSS2 in our bodies represents a solid argument to presume that furin is an essential protease for the SARS-CoV-2 activation [13]. Furin-recognition motifs in the SARS-CoV-2 would represent a "gain of function", increasing the viral infectivity. However, in the case of nonfunctional furin-containing SARS-CoV-2 mutants, the viral fusion capacity can be rescued in the presence of high concentrations of human airway trypsin [14], suggesting that multiple enzymes can actually intervene simultaneously or alternatively in the viral infectivity process. Other researchers consider that multiple proteases could actually synergistically activate the SARS-CoV-2, along with or without the TMPRSS2, furin playing a pre-activation role in the viral priming [20]. In this context, important aspects remain to be discovered: identifying the exact enzyme/enzymes that normally activate the SARS-CoV-2 and investigating whether ACE2 (physiologically an enzyme) does not actually play an additional role to that of receptor in SARS-CoV-2 infection. This aspect appears to have been omitted until now. An explanation could be in the paucity of unifying studies to highlight the controversies over the exact sheddase to activate SARS-CoV and SARS-CoV-2. We could not find unanimous, dedicated studies to demonstrate that ACE2 enzymatic activity does not intervene additionally in the SARS-CoV-2 virus activation. Therefore, this aspect should be investigated in the future as too little is clearly known about this type of virus. Until now, if all the studies were to nominate the same enzyme, there would be no place for other possibilities or speculations. However, in the given context, such an uncertainty leaves place to hypothesize even that ACE2 could intervene in the SARS-CoV-2 activation. Such a hypothesis on the potential role of ACE2 as an activating enzyme for SARS-CoV-2, besides its recognized role as a viral receptor, deserves attention and further research, as too little is clearly known on this new type of virus is different from previous coronaviruses.

Could ACE2 be the enzyme that contributes to SARS-CoV-2 activation?

ACE2, an enzyme first described in 2000 [31], is known to be a membrane-expressed monopeptidyl carboxypeptidase (metallopeptidase) and a homolog of the angiotensin-converting enzyme (ACE) (the dipeptidyl carboxypeptidase responsible for angiotensin I activation to vasoconstrictive angiotensin II) [32]. ACE2 naturally cleaves angiotensin II, converting it to angiotensin 1-7; it also converts angiotensin I to angiotensin 1-9 that will bind to Mas receptor ATR2 receptor, respectively, with vasodilator and anti-inflammatory effects [33, 34]. ACE2 has been identified in two forms: membrane-expressed ACE2 (native ACE2) - that possesses an ectodomain that binds the SARS-CoV-2 spikes and holds the catalytic site, a transmembrane domain and a cytoplasmic tail that appears to be essential for viral entry into the cell- and a soluble form (circulating ACE2) of various dimensions [7, 35]. The soluble form of various dimensions appears to be ignored or unknown by many authors and holds an unclear, confusing function to others [7].

Membrane-expressed ACE2 is cleaved by the exact various sheddases considered to activate SARS-CoV-2 (especially ADAM-17, TMPRSS2), resulting in soluble ACE (sACE2) that possesses catalytic activity [23, 32, 36, 37]. From the first discovery of the full-length ACE2 (native ACE2) of 805 amino acids (110-120 kDa), other shorter ACE2 isoforms have been described that possess catalytic functions but a different tissue distribution, titer, regulation, and substrate preferences. Smaller active ACE2 isoforms appear to be generated in various tissues (especially kidneys) by the same sheddases that have been shown to activate the SARS-CoV-2: ADAM-17, the class of TMPRSS2 proteases and TMPRSS1D [37, 38]. The description of various inconsistent dimensions of sACE2 resulting after shedding by different proteases has probably confused researchers regarding its functional significance in the SARS-CoV-2 infection. However, in the SARS-CoV-2 infection, an ACE2 shedding is described, and ACE2-S1 viral subunit complexes are probably released in the patient plasma [4]. The mechanism of viral entry into the host cell via ACE2 is still insufficiently understood in detail. The lack of knowledge of the detailed mechanism of viral infectivity explains why an effective therapy has not been designed yet and the existing controversies over the effects and risks of administration of various therapies in COVID-19. However, more and more studies suggest an increase in the ACE2 enzymatic activity upon the binding of the SARS-CoV-2 S1 subunit [39-42] that holds a yet unclear significance in the viral mechanism of infection. Furthermore, through evolutionary changes and gain of promiscuity, ACE2 could actually catalytically intervene on different substrates than originally thought, such as the SARS-CoV-2.

Recent laboratory and clinical evidence suggesting an increase in ACE2 enzymatic activity upon SARS-CoV-2 spike binding

Some authors consider that ACE2 does not appear to play an enzymatic role in the SARS-CoV-2 mechanism of infection [43]. It is a review paper where the authors do not appear to provide a reference for their allegation. However, other researchers disagree, and recent research suggests that SARS-CoV-2 binding to ACE2 receptors determines an increase in ACE2 enzymatic activity (a three to increase tenfold) [40]. Some authors have also described an increase in ACE2 enzymatic activity upon binding the SARS-CoV-2 spike, demonstrated by an increase in the cleavage of one of the ACE2 known substrates des-Arg9-bradykinin that may play a role in the COVID-19 associated symptoms and complications [41]. Also, the incubation of SARS-CoV-2 spike trimer with ACE2 appears to be followed by the viral spike trimer dissociation [44], suggesting an enzymatic role on the virus. These laboratory research results appear even more convincing as clinical results strengthen them. In this regard, more authors are describing an important rise in the serum ACE2 enzymatic activity (40-times higher ACE2 activity levels than normal) in real patients, with severe forms of COVID-19 [42]. Even more, the SARS-CoV-2 patients have higher serum ACE2 activity levels, but the ACE2 enzymatic activity appears to be correlated to the disease severity and to be higher in male patients [39]. As authors report an ACE2 important conformational change upon the SARS-CoV-2 spike binding to an allosteric site that is distal to the enzymatic site [10], the ACE2 binding to the virus could trigger the enzymatic activity of further importance for the viral infectivity. These findings regarding the ACE2 enzymatic activity are in contrast with the earlier different SARS-CoV, where it was considered that ACE2 activity would not be important in SARS-CoV-2 entry into the cells [45], as this earlier type of coronaviruses did not express such affinity for ACE2 receptors, and their main mechanism of cell entry was via the endosomal pathway. Even in the case of earlier coronaviruses, there were authors to disagree, clearly stating that SARS-CoV binding to ACE2 determines the activation/processing of ACE2 by ADAM-17/ TMPRSS2 or other enzymes, and therefore the shedding of the catalytic part of ACE2 into the blood. In the process of SARS-CoV-2 infectivity, ACE2 activation as an enzyme and shedding of the catalytic part of ACE2 appear to be required [35]. ACE2 expression increases in hypertensive, diabetic, aging individuals, patients with chronic kidney or cardiovascular disease, which means an increased infection risk for these categories of patients [2]. If ACE2 is more than a SARS-Co-V-2 receptor and a viral activator, it would explain why these particular categories of individuals have a higher risk of SARS-CoV-2 infectivity.

ACE2 is a similar peptidase to TMPRSS2 and ADAM-17. Evolutionary gain of enzyme promiscuity and substrate diversity

Some recent studies conclude that the effects triggered by the SARS-CoV-2 attachment to the ACE2 receptors on the ACE2 activity as a peptidase are currently insufficiently known. [35]. It is not yet understood how ACE2 binding to the SARS-CoV-2 spike triggers an important proteolytic cleavage at the S1/S2 site [15], essential to viral activation and fusion to the cell membranes. At the same time, the exact role of soluble ACE2 is currently unclear. If ACE2 activation by various sheddases takes place in the SARS-CoV-2 activation process (a process that authors do not dispute), with a demonstrated release of the soluble catalytic part of ACE2 (after SARS-CoV-2 linkage to the integral ACE2) [26, 46], which would be its functional significance? Which further effects could have the activated enzymatic profile ACE2 on the SARS-CoV-2 host cell entry? As ACE2 is physiologically a peptidase, a question to arise is whether the catalytic part of ACE2 - soluble ACE2 - is not an enzyme to play a role in the SARS-CoV-2 cleavage and activation for cell entry. The attachment of a substrate to a protease would normally lead to a reaction. It is already known that ACE2 does not exert its enzymatic actions only on angiotensin II and I, but also on a large array of other substrates than those classically described, such as bradykinin, neurotensin 1-13, kinetensin, dynorphin, apelin, des-Arg9- bradykinin, ghrelin [32-34, 41]. The fact that SARS-CoV-2 binds to the ectodomain of ACE2 that also holds its catalytic domain suggests that it is similar to other ACE2 substrates, including angiotensin II. Therefore, if it is similar to the natural substrates of ACE2, a logical possibility would be that ACE2, a peptidase, could cleave it at distinctive residues, not only bind it. ACE2 is similar to ADAM-17 and TMPRSS2, being a zinc-dependent metalloprotease as well [22, 47]. If similar, could ACE2 act on SARS-CoV-2 as an enzyme too? Studying enzyme evolution is a positive answer comes regarding the possibility of ACE2 functioning as an enzyme on other substrates than those already described, including a virus. Enzymologists have found that enzymes (as members of superfamilies of homologs) can change their substrate and reaction type selectivity and specificity with evolutionary selection and mutation. Also, enzymes gain promiscuity; they can develop supplementary enzymatic functions that are not related to their normal physiological activity. Enzymes also function on a broader substrate than initially described, catalyzing substrates for which they did not originally have specificity. Therefore, the

result of evolutionary changes is that enzymes can have the same catalytic function but on different substrates from initially described physiologically. Enzymes can even start performing other catalytic reactions than originally ascertained. In time, these new catalytic properties of the enzyme become normal, physiological [48, 49]. In fact, promiscuity (catalytic of the substrate, or conditions) is already described for a very large array of enzymes, representing a much wider characteristic than originally thought [50]. As the enzymatic activity of the ACE2 was found to be increased upon SARS-CoV-2 spike binding, there is a logical possibility that the sACE2 could further catalytically act on the SARS-CoV-2, playing a role in its activation and increasing its host cell entry.

Could ACE2 bind the SARS-CoV-2 at its catalytic site as well? The movable hinge region of the catalytic region of ACE2

ACE2 has only recently been discovered, and knowledge on its structure, function, and substrates still has many gaps [47]. Not even for the earlier SARS-CoVs, the ACE2 binding sites have not been described up to now. ACE2 catalytic domain contains two subdomains, S1 and S1', that form a relatively deep groove and are connected by a movable, a hinge motion-binding, there is a hinge motion that brings in closer vicinity subdomains and helps in grasping better the substrate that will be proteolytically processed. It appears that the SARS-CoV does not bind to the S1 domain that is small; however, this does not mean that it is considerably the S1 subdomain that is considerably larger. Mutations in S1 subdomain appear not to inhibit the SARS-CoV binding to the ACE2, showing that the much larger S1'subdomain could be a potential binding site in the catalytic domain of ACE2 for coronaviruses of this type. The MLN-4760, a metallopeptidase inhibitor, that binds to S1 subdomain of ACE 2 determines a conformational change that appears to inhibit SARS-CoV binding to ACE2 [32, 47]. Therefore, an inhibition in the catalytic subdomain of ACE2 prevents viral binding. As no binding sites have been clearly described even for earlier SARS-CoV further research is required to prove or disprove a potential linkage of the SARS-CoV-2 to the catalytic S1 subdomain of ACE2. Other authors consider that the ML effects on SARS-CoV infectivity cannot be extrapolated to the novel SARS-CoV-2, as these viruses differ by several mutations [10]. As there are authors already considering that, due to its important flexibility, SARS-CoV-2 could be cleaved by multiple/various proteases [28] and considering the flexibility of the hinge region of the catalytic domain of ACE2 as well [47], a new aspect to investigate appears: is there a putative enzymatic role for ACE2 in SARS-CoV-2 activation?

Fitting the TMPRSS2 findings in the SARS-CoV-2 activation mechanism

As an increase in TMPRSS2 activity was shown to augment the SARS-CoV-2 viral entry, a question to arise is whether TMPRSS2 acts directly and solely on the virus, or it may act indirectly via ACE2 activation? Therefore, the conclusion of some authors that TMPRSS2 is the activating enzyme for SARS-CoV-2 [18] can be true if TMPRSS2 or other similar sheddases like ADAM-17 activates directly both SARS-Co-V2 and ACE2 (ACE2 activation by ADAM-17/TMPRSS2 and other sheddases is not disputed) (figure 1, a and b).

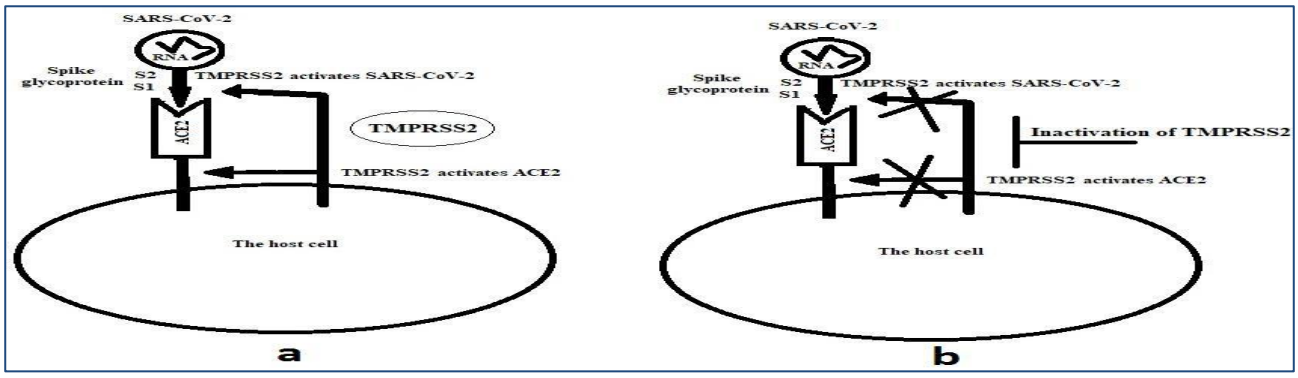

Figure 1. a) The mechanism considered by some authors for SARS-CoV-2 activation: TMPRSS2/ ADAM-17/ other enzyme activates SARS-CoV-2; it is also known that TMPRSS2/ ADAM-17 also activates ACE2; b) the effects of TMPRSS2/ ADAM-17 inhibition if we considered the same mechanism of SARS-CoV-2 mechanism of cell entry: no ACE2 activation and no SARS-CoV-2 activation.

However, this process can take place even if ACE2 would activate the virus; in this case, TMPRSS2/ ADAM-17 would not directly but indirectly activate SARS-CoV-2 via ACE2 activation (in this scenario, TMPRSS2 or ADAM-17 inhibition would still suppress viral entry) (figure 2, a and b). A confounder factor could therefore explain this process. It is already known that upon SARS-CoV-2 binding to its receptor, ACE2 activation is achieved via its cleavage by the same sheddases incriminated to activate SARS-Co-V-2, such as TMPRSS2, ADAM-17, and other possible proteases (a process shown to occur in previous SARS-CoVs as well) [23, 25]. ADAM-17/ TMPRSS2 inhibition will lead to a decrease in ACE2 activation, and if ACE2 is an enzyme to activate the virus, its decreased activity/inhibition via TMPRSS2 or ADAM-17 blocking would also prevent SARS-CoV-2 activation and cell entry (figure 2, a and b). Therefore, a conclusion that TMPRSS2 /ADAM-17 activates the SARS-CoV-2 cannot be drawn based only on the observation that TMPRSS2/ADAM-17 inhibition reduces/prevents viral activation and cell entry. Other authors consider that TMPRSS2 or ADAM-17, a promiscuous enzyme [51], has to be the activating enzyme for SARS-CoV-2 as the inhibition of one or another appears to exert beneficial effects in COVID-19 [18, 19]. But we could also speculate that ADAM-17 and/or TMPRSS2 inhibition is beneficial in COVID-19, not in a direct manner, but indirectly, as ADAM-17/TMPRSS2 inhibition would determine a decrease in ACE2 activation [46] (see figure 2,a and b).

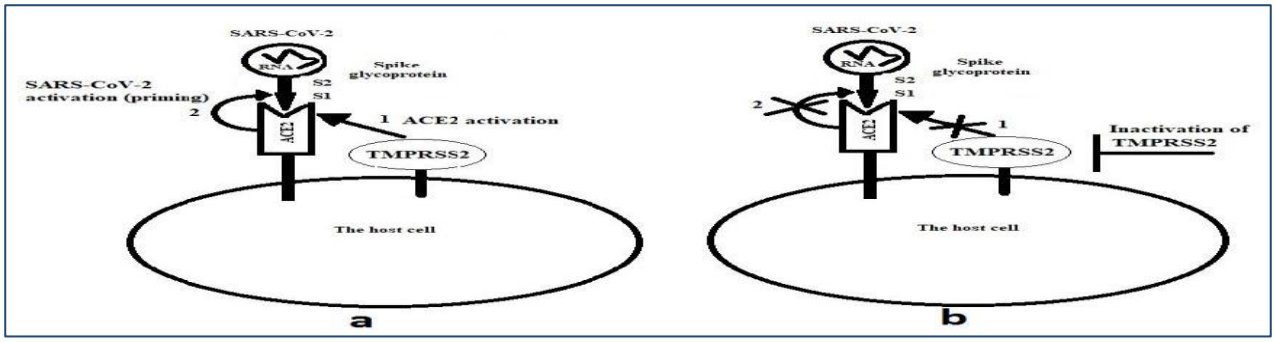

Figure 2. a) The mechanism proposed by us for SARS-CoV-2 activation: TMPRSS2/ ADAM-17/other sheddase activates ACE2 and ACE2 cleaves and activates SARS-CoV-2. **b)** TMPRSS2/ADAM-17 inhibition will still prevent SARS-CoV-2 activation even if our hypothesis is true: TMPRSS2/ADAM-17 blockage will mean no ACE2 activation and therefore no SARS-CoV-2 priming for host cell entry.

Future perspectives

There is a clear need for further studies to identify the exact protease involved in the SARS-CoV-2 activation to investigate whether ACE2 does not play an enzymatic role in SARS-CoV-2 infectivity as well and to clarify the integrin roles and their interaction with the virus and ACE2. There are currently too many controversies, unclear aspects, and unanswered questions regarding the peptidases involved in the SARS-CoV-2 activation for host cell fusion (Table 1). A clarifying of these aspects will be possible only when the scientific community considers the necessity of studying other putative roles of ACE2 in SARS-CoV-2 infectivity, following the analysis of the therapeutic results obtained via clinical studies and after further dedicated rigorous laboratory research. Important aspects of being studied remain: describing the exact crystal structure of ACE2 and of the SARS-CoV-2, which is currently not-perfected, but under work; exploring the effects of various sACE2 concentrations under different expressions/ absence of TMPRSS2 or ADAM-17 on SARS-CoV-2 entry; in silico studies, various computational tools (substrate-docking prediction and molecular dynamic simulations, interaction prediction based on bond energy analysis, thermodynamics of ligand-protein interaction or other methods; also, the use of enzymatic transformer models and computer-assisted synthetic planning [52] could be useful in predicting ACE2 intervention in SARS-CoV-2 mechanism of host cell entry; the design and testing of various ACE2 inhibitors, as well as various sheddase and integrin inhibitors.

Table 1. A synthetic view on the controversial aspects regarding the peptidases involved in the SARS-CoV-2 activation and unanswered questions.

A synthetic view on the controversial aspects regarding the peptidases involved in the SARS-CoV-2 activation and unanswered questions.	
1. Which is the precise peptidase for the SARS-CoV-2 cleavage and activation for host cell fusion?	8. How much do confounder factors hinder the discovery of the exact peptidases involved in the SARS-CoV-2 activation? (see figures 1 and 2)
2. Is there only one peptidase to activate the virus or is there an interplay-synergism or alternative action of various peptidases in the SARS-CoV-2 activation?	9. Is ACE2 only a receptor for the SARS-CoV-2 or could its enzymatic activity intervene as well in the SARS-CoV-2 activation? Is there a place for a dual role of ACE2 in such a process?
3. Why have so many proteases been named to intervene in the SARS-CoV-2 activation by various authors? Can we draw a conclusion regarding the exact enzyme if there is no unanimity amongst the studies?	10. Which is the significance of the increase in ACE2 enzymatic activity seen in COVID-19 patients?
4. Can TMPRSS2 be the enzyme/the only enzyme to activate the SARS-CoV-2 or there are confounder factors that hinder our knowledge on the exact peptidases that intervene in the SARS-CoV-2 activation?	11. Also, which is the significance of the increase of the soluble, plasma ACE2 in COVID-19 patients?
5. If TMPRSS2 would be the only key enzyme for the SARS-CoV-2 activation for host cell entry, why administration of MLN-4760, a metallopeptidase inhibitor, did not become an official treatment?	12. Why is it required that membrane ACE2 and soluble, plasma ACE2 have the catalytic domain to bind to the SARS-CoV-2?
6. Knowing that MLN-4760 is a nonspecific inhibitor of metallopeptidase (it also inhibits ACE2 enzymatic activity) can we draw conclusions that TMPRSS2 is the activating enzyme for the SARS-CoV-2 based on the information that MLN-4760 treatment decreases viral infectivity?	13. Via the evolutionary promiscuity of enzymes could ACE2 peptidase already act on a substrate such as the SARS-CoV-2 spike?
	14. Could the important 3D-flexibility of both ACE2 and the SARS-CoV-2 hide an enzymatic action of ACE2 on the virus?
	15. Is there a clear need for dedicated research (taking into attentive consideration the putative confounder factors) to study whether ACE2 does not actually intervene as a peptidase for the SARS-CoV-2 activation as well?

Conclusion

SARS-CoV-2, along with other previous coronaviruses, has an impressive registry of genomic mutations and immune escape, rendering the design of future vaccines a very complex target. In this context, only a detailed knowledge of the precise mechanism of viral infectivity can be the basis of an efficacious therapy, as well as prevention. However, currently, the mechanism of SARS-CoV-2 infectivity is insufficiently known. Too many proteases have been named to activate the SARS-CoV-2. It is not clear yet whether there is only one enzyme for the viral activation, a synergy between several proteases or multiple enzymes could activate the virus alternatively. Therefore, knowledge of the precise enzymes that activate the virus for fusion to the host cells and entry becomes essential to design efficient therapies, prevent viral infectivity, and even identify population categories that are at higher risk of developing more severe forms of infection. If TMPRSS2 has initially been considered the sole "culprit" enzyme for the viral infectivity, more and more data point towards other enzymes as well, while the proofs in favor of TMPRSS2 diminish. Also, more and more experimental data suggest a putative dual role for the ACE2 in viral infectivity: not only as a receptor but as an enzyme that contributes to viral activation. Our conviction is not „if", but

„when" the researchers will start to wonder about what is hidden behind the apparent only role of ACE2 as a receptor for SARS-CoV-2.

Abbreviation

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; COVID-19: the coronavirus disease 19; ACE2: angiotensin-converting enzyme 2; RAAS: renin-angiotensin-aldosterone system; ADAM 17: disintegrin and metalloprotease 17 (ADAM-17), also known as TACE (tumor necrosis factor-α-converting enzyme); TMPRSS2: transmembrane protease serine two or epitheliasin; MASPs: membrane-associated serine proteases; RBM: recognition binding motif; ACE: angiotensin-converting enzyme; sACE2: soluble angiotensin-converting enzyme 2; SARS-CoV: severe acute respiratory syndrome coronavirus.

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Availability of data and materials

Data will be available by emailing angelalazar.2008@yahoo.com

Authors' contributions

Angela Madalina Lazar (AML) is the principal investigator of this manuscript (Viewpoint). AML is the responsible author for the study concept, design, writing, reviewing, editing, and approving the manuscript in its final form. AML has read and approved the final manuscript.

Ethics approval and consent to participate

We conducted the research following the Declaration of Helsinki. However, Viewpoint Articles need no ethics committee approval.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interest.

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