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ADAM17 inhibition may exert a protective effect on COVID-19

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During the last 2 months, a novel conoronavirus [severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)] infection or coronavirus disease 2019 (COVID-19) has been identified, producing a global threat leading to more than 40 598 deaths worldwide [1]. Recently, by genome sequencing and homology modelling, angiotensin-converting enzyme 2 (ACE2) has been found to be the receptor for SARS-CoV-2, despite amino acid variation at some key residues [2]. The extracellular domain of ACE2 was demonstrated as a receptor for the spike (S) protein of SARS-CoV [3]. ACE2 is predominantly expressed on the apical surface of well-differentiated airway epithelia, especially ciliated cells [4, 5]. Since a virus must attach to and enter cells before it can replicate, surface expression of ACE2 and the state of cell differentiation may directly influence SARS-CoV disease pathogenesis [4]. ADAM17 is a disintegrin and metalloproteinase domain 17 that was initially described in 1997 by Black *et al.* to specifically cleave the precursor of tumour necrosis factor α (pro-TNF-α) [6]. It is already known that ADAM17 can also release the ectodomains of a diverse variety of membrane-anchored cytokines, cell adhesion molecules, receptors, ligands and enzymes, such as ACE2 [7].

ACE2 shedding by ADAM17 was first described by Lambert *et al.* by experiments performed using human embryonic kidney cells (HEK293) expressing human ACE2 (HEK-ACE2) in 2005 [7]. Under phorbol myristate acetate stimulation, the ACE2 protein detected on western blot analysis changed from a polypeptide of 120 kDa to a polypeptide of 105 kDa showing a proteolytic shedding occurring in the extracellular juxtamembrane region and releasing an enzymatic active ectodomain [7]. The authors developed distinct techniques to detect the main proteinase responsible for this proteolytic shedding event. First, incubation of HEK-ACE2 cells with TNF-α protease inhibitor 1 (TAPI-1), a hydroxamic acid-based metalloproteinase inhibitor, helped to detect ADAM17 as the main enzyme responsible for ACE2 shedding. Secondly, the use of natural inhibitors of metalloproteinases such as TIMP-3 also showed a reduced stimulated ADAM17 shedding activity. Finally, the confirmation was made using small interfering RNA (siRNA) to reduce the expression of ADAM17. Analysis of media proteins collected following incubation of transfected cells demonstrated an associated decrease in stimulated ACE2 shedding. These studies suggested that the up-regulation of ACE2 shedding could modulate high levels of shed ACE2, which could inhibit the infectivity of the SARS virus [7]. In the same line, Jia *et al.* [4] demonstrated that ACE2 sheds from differentiated primary airway epithelial cells and Calu-3 cell line. When SARS S protein pseudovirions were applied to HEK cells transfected with wild-type ACE2, SARS-CoV S was efficiently transduced. In contrast, when ACE2-SEC (containing the entire ACE2 ectodomain fused to the secretion signal peptide of the human β-defensin 2 gene) and ACE2-ΔRBD (containing alanine substitution mutations that allow the interaction with SARS-S) were cotransfected into HEK293 cells, the transduction efficiency of S protein-pseudotyped virions failed to increase, demonstrating that ACE2 must be cell-associated to serve as a coronavirus receptor, and that soluble ACE2 (sACE2) competes for S protein binding [8].

However, there are some controversies regarding the role of ADAM17 and sACE2 during virus infection. In that sense, in 2008, Haga *et al.* found that SARS-S induced ADAM17-dependent shedding of ACE2 and also that the process was coupled with TNF-α production. They demonstrated that mutation in the cytoplasmic tail of ACE2 inhibited its shedding and reduced viral infection, and ACE2 or ADAM17 silencing blocked viral infection. Interestingly, mutants of cytoplasmic ACE2 were unable to activate either ACE2 shedding or TNF-α production, or even SARS-S binding. From these findings, they suggested that ADAM17 activity is modulated by the cytoplasmic tail of ACE2, facilitating both virus entry and tissue damage through TNF-α. However, the authors do not specify the intracellular pathway activated by the intracellular domain of ACE2 [9]. The authors postulated that ACE2 is down-regulated after viral infection because ACE2 shedding increases. However, administration of TAPI-0 and ADAM17 siRNA inhibited ACE2 shedding, while introduction of ADAM17 cDNA recovered SARS-S-induced shedding. Finally, this study also showed that ADAM17 siRNA reduced SARS-CoV infection, indicating that ADAM17 plays an important role in the entry of SARS-CoV. It is unclear how ADAM17 facilitates viral entry. However, it seems that the peptidase activity would be critical

for the fusion of viral particles and cytoplasmic membranes [9]. These late results suggest that ADAM17 inhibition by decreasing ACE2 shedding may have a protective effect on SARS-CoV infection by reducing the viral load. Riera *et al.* demonstrated that the ADAM17/ACE2 axis may also be regulated by some treatments such as paricalcitol, a synthetic vitamin D analogue [10]. However, these strategies such as ADAM17 inhibition/blockade and paricalcitol administration need further preclinical and clinical studies.

In contrast to those findings, Heurich *et al.* suggested that the serine-protease Transmembrane Serine Protease 2 (TMPRSS2) participates in SARS-S internalization and that ACE2 processing by TMPRSS2 is dispensable for activation of SARS-S for cathepsin L-independent entry. Only cleavage by TMPRSS2 resulted in SARS-S-driven entry, in the absence of ADAM17 contribution. Interestingly, the authors postulated that while ADAM17 participates in ACE2 ectodomain shedding, TMPRSS2 induces intracellular cleavage of ACE2, and that there was a competition between ADAM17 and TMPRSS2 for ACE2 processing [11]. Recently, it has been published that SARS-CoV-2 uses the TMPRSS2 protease for S protein processing, and that TMPRSS2 inhibitors blocked SARS-S cell entry [12]. Taking all of these results together, more studies are needed to ascertain the role of ADAM17 and other proteases on ACE2 shedding in the kidney and lungs, and to elucidate the importance of those proteases in fostering SARS-CoV-2 infection.

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CONFLICT OF INTEREST STATEMENT

None declared.

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