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MINI REVIEW ARTICLE

ACE2 role in SARS-CoV-2 infectivity and Covid-19 severity

Elena AZIZAN[1]*, Morris BROWN[2]

[1]Department of Medicine, The National University of Malaysia (UKM) Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000, Kuala Lumpur, Malaysia. [2]Barts Heart Centre, William Harvey Research Institute, Queen Mary University London, United Kingdom

Abstract

In 2003, it was discovered that the entry receptor for the Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) is a protein called the angiotensin-converting enzyme 2 (ACE2). This protein is present in a number of cell types, including those from the respiratory tract. Soon after the emergence of SARS-CoV-2 that is responsible for the disease Covid-19, scientists found that ACE2 was also used by the new coronavirus to infect cells. This opened some interesting possibilities to explain the striking variation in risks of catching and dying from Covid-19. The best recognised of these are the much higher risk of serious illness in older than younger people, in men than women, and in those with pre-existing comorbidities such as hypertension and cardiovascular diseases. There are several ways in which the ACE2 protein might contribute to this variation. The most obvious would be if there is more ACE2, there would be more entry points for the virus to infect the cell, e.g. in older people or in men. However, the evidence for this is rather small, partly because it is not that easy to obtain representative healthy tissues. Alternatively, it could be related to ACE2 membership of a family of proteins that has one end of the protein anchored inside the cell while most of the protein protrudes from the outside of the cell which therefore can be shed when cleaved by proteases at the cell membrane. Herein we review current evidence and theories of ACE2 role on SARS-CoV-2 infectivity and Covid-19 severity.

Keywords: angiotensin-converting enzyme 2, ACE2, SARS-CoV-2, Covid-19, coronavirus

INTRODUCTION

The angiotensin converting enzyme-2 (ACE2) is a transmembrane glycoprotein which acts as a type 1 zinc mono-carboxypeptidase that efficiently inactivates angiotensin II, a pro-inflammatory mediator, by removing its terminal a-amino acid phenylalanine to generate the anti-inflammatory heptapeptide angiotensin 1-7.[1,2] ACE2 can also cleave angiotensin I to angiotensin 1-9 and other biological peptides involved with inflammation such as des-Arg9-bradykinin, albeit inefficiently.[3] Despite these known beneficial biological effects, ACE2 is currently most notoriously known for its role as the entry protein into human cells for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) similar to its role that have been documented in other coronavirus such as SARS-CoV and HCoV-NL63.[4-7] This is because viruses like SARS-CoV-2 are too large to enter the cells which they infect without engaging help from one of the cell's own proteins. Like a key in a lock, one of the virus's proteins (the key) fits into one of the cell proteins (the lock). It was in 2003, that scientists discovered the lock for the SARS-CoV was ACE2.[6] Soon after emergence of the SARS2 Covid-19 virus, scientists tested whether ACE2 is used by the new virus, and the answer was a clear yes. If anything SARS2 fits even better than the original SARS virus.

Expression of ACE2 in human cells

ACE2 is present on the surface of a number of cell types, including those in the respiratory tract. ACE2 expression in human tissues is high in the adipose tissue, heart, kidneys, small intestine, testis, and thyroid, medium in the adrenal gland, bladder, colon, liver, and lungs, relatively low in the blood, blood vessels, bone marrow, brain, muscle, and spleen, and to a

**Address for correspondence:* Dr Elena Azizan, Department of Medicine, The National University of Malaysia (UKM) Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000, Kuala Lumpur, Malaysia. Tel: +603-91455582. Fax: +603-91712640. E-mail: *elena.azizan@ukm.edu.my*

lower extent in respiratory apparatus such as the bronchi, nasopharynx, olfactory epithelium, and trachea.[8-10] In the lungs, most ACE2 are expressed in type 2 pneumocytes, small cylindrical alveolar cells which produce pulmonary surfactant and work as stem cells by differentiating into larger and flattened type 1 pneumocytes in case of damage.[8,11] The heterogeneity of expression suggests some interesting possibilities to explain the striking variation in risks of catching and dying from Covid-19. The best recognised of these are the much higher risk of serious illness in older than younger people, and 3-fold higher risk in men than women.

Relationship between ACE2 expression and COVID-19 risk

There are several ways in which the ACE2 expression might contribute to this variation. The most obvious would be if there is more ACE2 – more locks for the virus to open – in older people and in men (FIG. 1). Currently, the evidence for this is rather small partly because it is not that easy to obtain representative tissues from healthy people, though to note testis does highly expressed ACE2. Furthermore, the gene which encodes the ACE2 protein is on the X chromosome, of which women have 2 copies but men only 1. Although only one copy functions in each cell, it is much less likely for women than men to inherit low levels of ACE2 in all their cells. To confirm this idea, we are now doing studies in people who had Covid-19 infection to see whether low levels are likelier in men than women, and whether there are lower levels of ACE2 in those who become very sick after infection.

The alternative way that ACE2 expression can contribute to the severity of SARS-COV-2 infection stems from ACE2 membership of a club with at least 1000 members, all a particular type of cell protein. The members of this club have two things in common. One is that the proteins straddle the membrane around the cell, with one end of the protein anchored inside the cell, while most of the protein protrudes from the outside of the cell. The other common feature of this club is that the part of the protein outside the cell is shed from the cell, containing the lock within it. Is this good or bad for the chances of the cell being protected from the virus? We do not know. If the cell is left with much lower numbers of locks, the virus may find it difficult to penetrate the cell. In addition, we know that some of these shed proteins circulate in the bloodstream where they can act as a decoy, trapping the key in the blood, so that it never reaches the cell surface. This is the basis for one of the standard treatments of rheumatoid arthritis. Could it be that the Covid-19 virus is kept away from attacking cells by this mechanism?

ACE2 are mostly anchored to cell membranes with their catalytically active site exposed on the external site, but can be cleaved and released in the circulation for effects of some proteolytic enzymes such as the tumour necrosis factor-α convertase ADAM17 which we know is responsible for the shedding of the ACE2 club of proteins from the cell surface.[12] Shed ACE2 generally circulates in very small amounts (i.e. undetectable) but can be up-regulated by angiotensin II.[13] Similarly, shed ACE2 is increased in pathological conditions such as hypertension, cardiovascular diseases, obesity, and diabetes.[14] Interestingly, a meta-analysis of seven studies with a total of 1,576

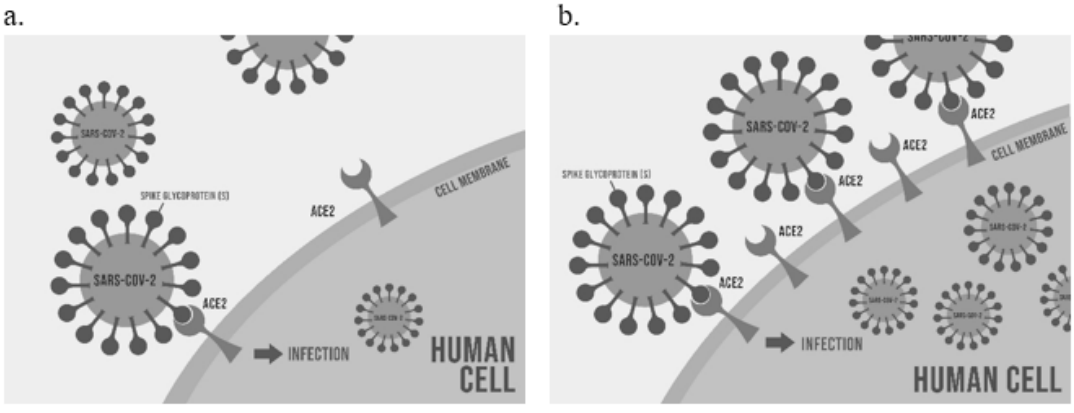

FIG. 1: Schematic on how different levels of cell expression of ACE2 may affect level of infectivity of SARS-CoV-2 and severity of COVID-19. (a) A cell with low expression of ACE2. (b) A cell with high expression of ACE2.

participants observed a higher risk for patients with hypertension, respiratory diseases and cardiovascular diseases to clinically present the severe phenotype of COVID-19 (odds ratio 2.36 [95% confidence interval 1.46 to 3.83], odds ratio 2.46 [95% confidence interval 1.76-3.44] and odds ratio 3.42 [95% confidence interval 1.88 to 6.22], respectively), while the largest case series from the China Center for Disease Control and Prevention's report of 44,672 confirmed cases (diagnosis based on positive viral nucleic acid test result on throat swab samples) found fatality rate to be elevated in cases with pre-existing cardiovascular diseases (10.5%), diabetes (7.3%), chronic respiratory diseases (6.3%), hypertension (6%), and cancer (5.6%).[15-16]

The striking similarities between pathological conditions that increases ACE2 shedding and the risk factors for severe disease and death due to COVID-19 seems a paradox as more ACE2 shedding should have meant less ACE2 expression on the cell for SARS-CoV-2 to enter. However, interestingly, angiotensin II modulators such as the angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) that are widely used in the management of hypertension, cardiovascular diseases, and diabetes have been shown in several studies using animal models of the disease to upregulate ACE2 expression in addition to their main pharmacological target of inhibiting angiotensin II. For example, in rats with heart failure, enalapril an ACEI restored left ventricular ACE2 expression.[17] Similarly, in rats with myocardial infarction, the ARBs losartan and olmesartan increased the expression of ACE2 mRNA in the heart.[18] These effects were also found in rats with normal hearts, where the ARB losartan and the ACEI lisinopril increased ACE2 mRNA.[19] This has prompted the speculation that baseline ACE2 abnormalities due to pathological conditions such as treatment of prior hypertension, cardiovascular diseases, and diabetes might be the cause for severity of SARS-CoV-2 infection.

A supportive (though speculative) evidence that suggest ACE2 expression may play a role in severity of COVID-19, is the apparent counter-intuitive lack of significant association between active smoking and severity COVID-19.[20-21] A number of explanations have been suggested for this observation such as poor data quality, sampling bias, differential access to hospitals, and statistical confounding factors, but it could also perhaps be related to the effect of smoking on ADAM17 as cells treated with cigarette smoke had enhanced ADAM17 mediated shedding.[22] This could thus result in larger amounts of ACE2 being shed into the circulation, acting as a decoy to protect the lung and other tissues from being infected. But in all the above, we have talked of ACE2 as though it has been waiting for the arrival of COVID-19 (and SARS before it). However, ACE2 did not evolve as a portal for virus infection, but as a tool the body uses to dispose rapidly of angiotensin II, which has numerous beneficial functions, but becomes a poison if not removed within a few minutes of being formed. Some of angiotensin II's potentially toxic effects, especially in the lung, resemble features of serious Covid-19 infection. Cardinal features of COVID-19 severe symptoms such as diffuse pulmonary inflammation, endothelial inflammation and enhanced thrombosis, are reminiscent of adverse reactions triggered by angiotensin II.[23-30] In fact, several experimental models of lung injury have documented the causative relation between down regulation of ACE2 and pulmonary inflammation mediated by an imbalance between angiotensin II over-activity and of angiotensin1-7 deficiency.[31-34] This idea is supported further by a recent study conducted in patients with COVID-19 that found viral load and lung injury were strongly associated with circulating levels of angiotensin II.[35]

SUMMARY

So while at the outset of infection the lung may be protected by having low levels of ACE2 at the cell surface – fewer locks for the virus key to turn – the small percentage of patients admitted to hospital may need their ACE2 to protect lungs from the body's own chemical, angiotensin II. Indeed, this difference between the first and subsequent weeks of infection appears common to a number of the body's defences. Early on the objective is to avoid or reduce the presence of large amounts of virus that can penetrate into lung cells. Later the virus itself may be less important than the forces it has unleashed, such as angiotensin and the so-called cytokines.

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