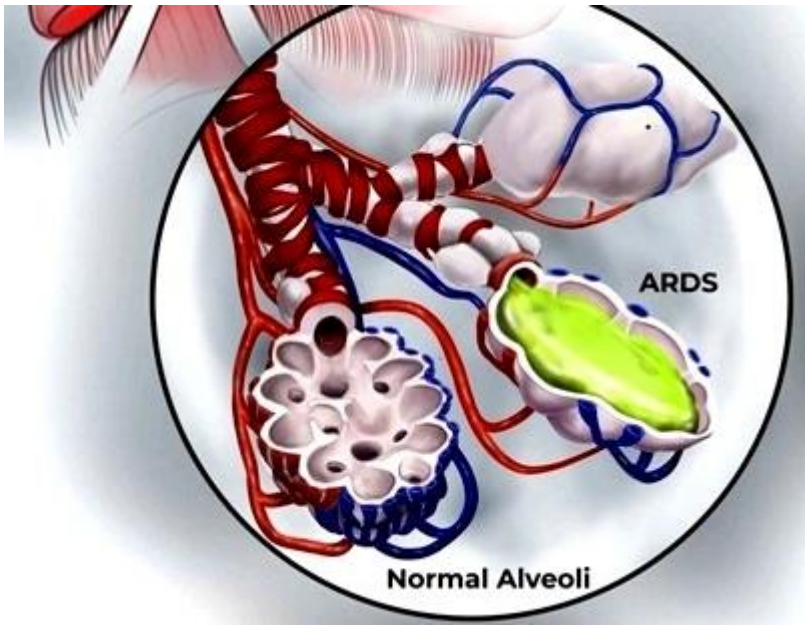
As molecular mimicry is an evolutionary strategy adopted by viruses to exploit the host cellular machinery, some authors hypothesized that the virus may mimic host substrates to achieve proteolysis. In 2020, Anand *et al* in their *in silico* analysis identified that, among all proteins encoded in the human genome, the only one that contains a motif 100% identical to the furin cleavage site of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein is the alpha subunit of the human epithelial sodium channel (ENaC-alpha). Based on these results of molecular mimicry between SARS-CoV-2 and human EnaC-alpha, a recent study conducted by authors from Mexico and the United States experimentally tested the hypothesis that the hijacking of furin-like proteases by the SARS-CoV-2 S protein may affect ENaC processing and activity and contribute to COVID-19 pathogenesis.

The genome of SARS-CoV-2 encodes four structural proteins, the spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins. The S protein is a membrane-bound glycoprotein, forming a homotrimer that binds to the membrane-bound angiotensin-converting enzyme 2 (ACE2) in host cells. It comprises two subunits: S1, responsible for receptor binding, and S2, which mediates membrane fusion. The receptor binding motif (RBM) located within the S1 subunit interacts specifically with the host cell ACE2 receptor.

After the initial interaction between the S protein and the host cell ACE2, SARS-CoV-2 entry to the host cell is mediated by the proteolysis of the S protein. The S protein is cleaved at two positions, the S1/S2 site at Arginine-667/Serine-668 and the S2 site located several residues downstream within the S2 portion. Proteolytic processing is mediated by membrane-bound serine proteases such as transmembrane serine protease 2 (TMPRSS2), cathepsins, and furin-like enzymes found in cell types such as type II pneumocytes, proximal tubule cells, arterial and venous endothelial cells, and brain cells. As a result, the S proteins display cleaved forms of S1 and S2 subunits. The efficiency of viral infection is determined by co-expression of ACE2 with the molecular machinery for proteolytic processing.

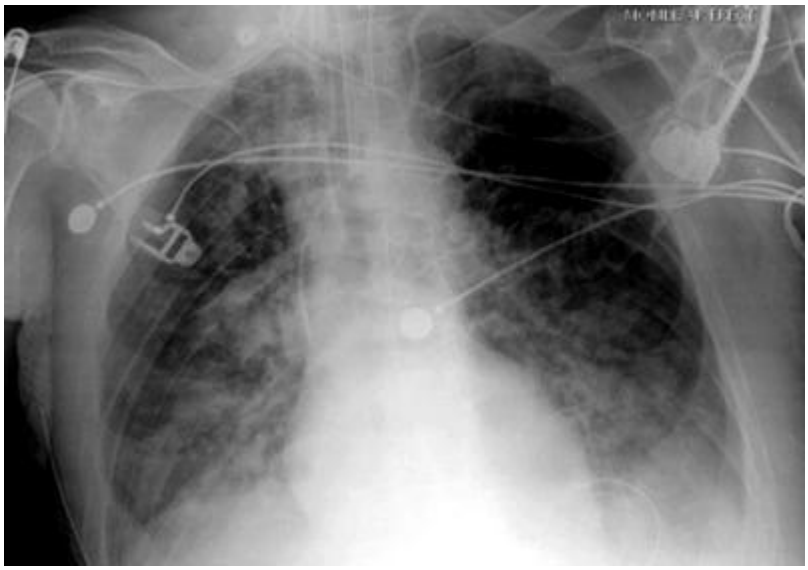
Numerous scientists emphasize that compared to the S proteins of closely related coronaviruses, the SARS-CoV-2 S protein has a unique sequence insertion at the S1/S2 site, tribasic 8-mer peptide (RRARSVAS). This cleavage site, as a novel feature of this virus, seems to contribute to the high pathogenicity and transmissibility of SARS-CoV-2. In 2020, Anand *et al* found a striking molecular mimicry between this unique S1/S2 cleavage site of the SARS-CoV-2, absent in previous coronaviruses, and the human EnaC-alpha. Their study of more than ten million peptides (8-mers) from 20,350 canonical human proteins revealed that the furin-cleavable peptide on human EnaC-alpha is the only one that contains a motif 100% identical to the furin cleavage site of SARS-CoV-2 and that the peptide of interest (RRARSVAS) is exclusively present in the furin-cleavable peptide on human ENaC-alpha. As

SARS-CoV-2 exploits host furin for its activation, and ENaC-alpha also requires proteolytic activation for its function, it was hypothesized that hijacking of furin-like proteases by the SARS-CoV-2 S protein in infected cells may compromise ENaC processing and activity, thereby contributing to reduced alveolar fluid clearance in COVID-19 patients. (Anand P, Puranik A, Aravamudan M. SARS-CoV-2 strategically mimics proteolytic activation of human EnaC. *Elife* 2020; 9:e58603) https://doi.org/10.7554/eLife.58603



ENaC-alpha is a sodium permeable, non-voltage-sensitive ion channel belonging to the amiloride-sensitive sodium channel (TC 1.A.6) family, SCNN1A subfamily. It regulates sodium ion (Na+) and water homeostasis by controlling the luminal sodium electrodiffusion (and water that follows osmotically) through the apical membrane of epithelial cells. ENaC-alpha expression levels are controlled by aldosterone and the associated renin-angiotensin-aldosterone system. ENaC-alpha is expressed in the kidney, pancreas, and liver (at intermediate levels), and heart (at low levels). It is also expressed in the female reproductive tract, from the fimbrial end of the fallopian tube to the endometrium, and in the placenta (at low levels). In the skin, ENaC-alpha is expressed in keratinocytes, melanocytes, intradermal adipocytes, and cells of the sebaceous gland and eccrine sweat glands. ENaC-alpha controls sodium reabsorption in the kidney, colon, lung, and eccrine sweat glands and has a role in taste perception.

Besides the essential role in electrolyte and blood pressure homeostasis, ENaC-alpha has a role in airway surface liquid homeostasis, which is important for proper mucus clearance. ENaC-alpha is expressed in the bronchial epithelium and type II pneumocytes (at high levels). This channel plays a key role in controlling fluid reabsorption from the alveoli, allowing gas exchange to occur. As proteolytic processing of ENaC-alpha by furin-like proteases is essential for channel activity, competition of EnaC-alpha in type II pneumocytes infected with SARS-CoV-2 with high levels of the S protein may result in decreased ENaC-alpha activity and reduced reabsorption of alveolar fluid at the air-liquid interface. This leads to the accumulation of alveolar fluid, an important lung pathology of acute respiratory distress syndrome in COVID-19 patients.



Acute respiratory distress syndrome

About the Study and Results

In vitro results obtained in the *Xenopus laevis* system showed that reduced cleavage of ENaC-alpha in the presence of the S protein decreases channel activity. Furthermore, the observed effect was bidirectional; the S protein prevented ENaC-alpha cleavage, and *vice versa,* ENaC-alpha prevented the S protein cleavage. These results confirm that the SARS-CoV-2 S protein competes with EnaC-alpha for the same processing protease and that hijacking of furin-like proteases by the S protein in infected cells reduces ENaC-alpha activity. As the negative effect on ENaC-alpha cleavage was only slightly prevented with the

mutation of the S protein cleavage site, the authors stated that the additional residues of the S protein may be involved in protease binding, or that the S protein may inhibit ENaC cleavage by another mechanism besides competition.

In *in vivo* experiment conducted in transgenic mice infected with a lethal dose of SARS-COV2 and uninfected control mice, immunofluorescence staining of ENaC-gamma and surfactant protein A (a type II pneumocyte cell marker) revealed that ENaC-gamma was present in type II pneumocytes of both groups. However, there was no significant difference in ENaC-gamma expression in the lungs of control and SARS-Cov-2 infected mice. The authors stated that they could not assess ENaC cleavage using western blots due to safety reasons and that these *in vivo* results should be taken with reserve because of several limitations of immunofluorescent staining. They recommended that further work should investigate whether *in vivo* SARS-CoV2 infection affects ENaC function in type II pneumocytes.

Conclusion

The data suggest that high levels of the S protein may compete with EnaC-alpha, a sodium permeable non-voltage-sensitive ion channel that controls fluid reabsorption at the air-liquid interface, for the same processing protease. By hijacking the cutting mechanism for ENaC-alpha, SARS-CoV-2 interferes not only with ENaC-alpha cleavage but also with the activity of this peptide in the lung.

Reduced transepithelial sodium ion (Na^+) reabsorption (and water that follows osmotically) can lead to reduced alveolar fluid reabsorption at the air-liquid interface and pulmonary edema in COVID-19 patients.

Journal Reference

Magaña-A´ vila GR, Moreno E, Plata C, et al. Effect of SARS-CoV-2 S protein on the proteolytic cleavage of the epithelial Na^+ channel EnaC. PLoS ONE 2024;19(4): e0302436. https://doi.org/10.1371/journal.pone.0302436.s001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