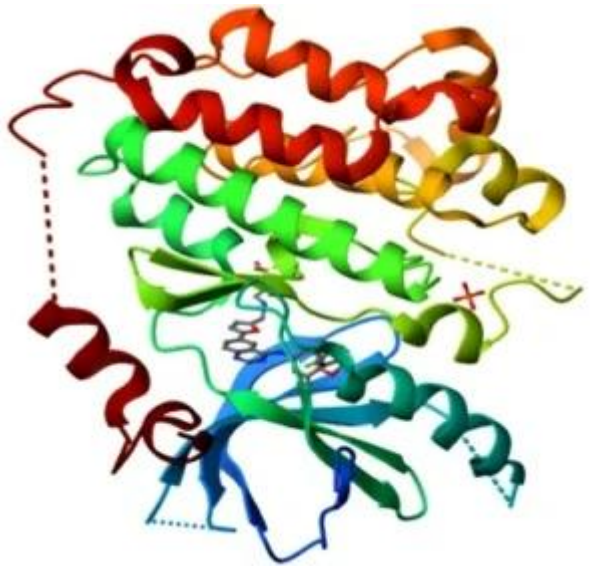
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) protein. Researchers from the United Arab Emirates and Qatar conducted this study to determine whether the SARS-CoV-2 spike protein activates the epidermal growth factor receptor (EGFR), its associated downstream signaling pathways, and the anti-apoptotic protein survivin. They hypothesized that the virus may use the EGFR expressed on epithelial lung cells as a receptor/co-receptor for its entry.

The S protein, which appears to be a major pathogenic factor, is composed of S1 and S2 subunits, separated by host cell proteases. The S1 is composed of the N-terminal domain (NTD), receptor-binding domain (RBD) with a receptor binding motif (RBM), and two C-terminal domains. The RBD in the S1 subunit is responsible for attachment to host cells. Two host-cell factors are important for SARS-CoV-2 viral entry: angiotensin-converting enzyme 2 (ACE2), which is bound by S-protein, and transmembrane serine protease 2 (TMPRSS2), which cleaves S-protein, allowing this binding to take place.

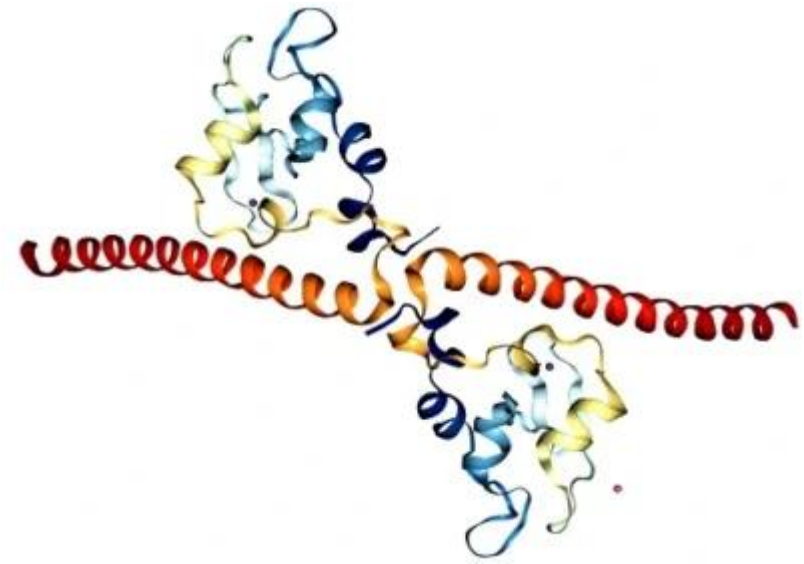
However, other factors and alternative cell surface receptors may also be involved in viral entry. It has been suggested that EGFR, expressed on lung epithelial cells, serves as a receptor/co-receptor for viral entry. Of note, EGFR was found to be important during influenza or Salmonella infection. Salmonella Rck membrane protein binds and activates EGFR and its signaling pathways, resulting in receptor/bacteria co-internalization and cell infection. The binding of EGF (epidermal growth factor) to its receptor EGFR activates several signaling pathways, including protein kinase B (AKT) and extracellular signal-regulated kinase (ERK), thereby leading to control of cell survival and metabolism.



EGFR

Protein survivin is the smallest member of the inhibitor of apoptosis protein family. It plays a crucial role in regulating cell division and inhibiting apoptosis by inhibiting caspase activation. Survivin is considered a crucial marker for activating the survival pathway in cancer cells.

Survivin expression is controlled by precise mechanisms at the transcriptional and post-translational levels. It is highly expressed in the majority of human cancers, including lung, pancreatic, and breast cancers. Survivin aberrant expression is associated with tumor cell proliferation, progression, angiogenesis, therapeutic resistance, and a poor prognosis. Also, survivin expression is associated with the aberrant activation of many receptors, like the EGFR and insulin-like growth factor-1 (IGF-1), and various cell survival signaling pathways. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4747886/



Survivin

About the study

The authors used cancer cell lines known for their expression of EGFR and differential expression of ACE-2, including ACE-2-expressing lung (A549) and colon (HT-29) carcinoma cells, and non-ACE2-expressing (HeLa) cervix adenocarcinoma cells.

Cells were treated with either the full S1 protein or the RBD for 5, 15, 30, and 60 minutes, using the EGF stimulation as a positive control.

Results

The administration of the S1 subunit to ACE2-expressing lung cancer cells (A549) for 5 minutes resulted in a strong phosphorylation of EGFR, AKT, and ERK1/2, which was either comparable (EGFR and ERK1/2) or even greater (AKT) than stimulation with the maximal dose of EGF. The time-course analysis of the EGFR, AKT, and ERK1/2 phosphorylation demonstrated that S1 induced a rapid, but transient phosphorylation of EGFR and ERK1/2, whereas AKT activation was more sustained in time.

Under similar conditions, RBD showed little effect on EGFR phosphorylation, but significantly induced AKT and ERK1/2 phosphorylation. Although RBD did not induce clear EGFR phosphorylation, it promoted rapid and robust activation of ERK1/2 and AKT, which remained persistent after 60 minutes of stimulation.

These findings show that full S1 protein, but not RBD, specifically targeted EGFR. Of note,

the RBD-mediated activation of AKT and ERK1/2 was independent of EGFR activation, suggesting the involvement of other molecular targets and/or intracellular mechanisms.

The researchers then used highly ACE2-expressing HT-29 colon carcinoma cells and non-ACE2-expressing HeLa cervix adenocarcinoma cells to examine the relation of the ACE2 expression and the targeting of EGFR and its downstream signaling pathways.

In the non-ACE2-expressing HeLa cells, RBD failed to induce phosphorylation of AKT or ERK1/2, irrespective of the duration of stimulation. In the highly ACE2-expressing HT-29 colon carcinoma cells, RBD induced rapid and sustained phosphorylation of AKT and ERK1/2, similar to the results observed in the ACE2-expressing A549 cells. These results strongly support the involvement of ACE2 in the phosphorylation of AKT and ERK1/2 mediated by RBD.

The authors then examined the activation of the anti-apoptotic protein survivin and discovered that both S1 and RBD induced a time-dependent increase in survivin expression, with maximal response after 30 and 60 minutes of stimulation. The EGFR antagonist, AG1478, significantly diminished the activation of survivin mediated by S1 or RBD, which confirmed the role of EGFR.

Conclusion

These results suggest that the S1 subunit and RBD activate the EGFR and its downstream signaling pathways in the lung cancer cell line. The activation of EGFR mediated by S1 was ACE2-dependent.

The S1 and RBD also elicited the activation of the survival pathway and expression and activation of the anti-apoptotic protein survivin, which is considered a crucial marker for the activation of the survival pathway in cancer cells.

The authors emphasized that further research is required to determine whether S protein directly binds to EGFR. Also, they believe that the expression and activation of the anti-apoptotic protein survivin may be a molecular basis for the complications and severity of COVID-19 in cancer patients, as reported by several research groups.

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Journal Reference

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