The Role of SARS-CoV-2 Spike Protein in Human Endothelial Cell-Cell Fusion.

S. Yasin Tabatabaei Dakhili,[1] Preety Panwar,[1] and Dieter Brömme

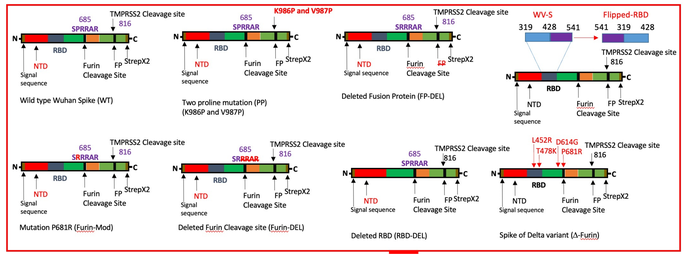
Faculty of Dentistry, The University of British Columbia, Vancouver, Canada

OBJECTIVES

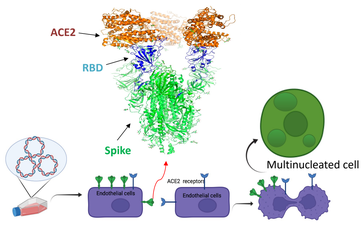
COVID-19 is a multi-tissue disease caused by an infection with SARS-CoV-2. In contrast to most other respiratory viruses, SARS-CoV-2 has a strong impact on the cardiovascular system. Spike (S) is required for the virus particle to enter the host cell. Virus infection has also been shown to result in syncytia, the formation of dysfunctional giant cells by cell-cell fusion. In this study, we show that the expression of the S-protein alone in endothelial cells (ECs) can induce cell-cell fusion. Different modifications of S-protein have been made in order to examine how they affect cell-cell fusion.

METHODS

From a commercial source, we obtained chemically synthesized and codon optimized SARS-CoV-2 spike (Wuhan variant, WT) in a mammalian expression vector (pTwist). Different parts of the S-gene likely required for cell-virus (or cell-cell) fusion events have been altered with molecular biology tools. The following constructs were then used to transfect human endothelial cells:



ECs were transfected with different vector constructs and an empty control vector. Cell fusion was monitored after 72 h post-transfection and the the expression of the S-protein and the inflammatory marker, TNF-α, was determined.



RESULTS

We demonstrated that the expression of WTS, Δ-Furin, and the double proline-prefusion-stabilized WT-version (PP) of the S-protein as used in the Pfizer and Moderna vaccines induced syncytia and the upregulation TNF-α. Empty vector (control) did not cause any cell fusion. S-protein receptor-binding-domain-deleted plasmid (RBD-DEL) and RBD-flipped construct did not express stable S-proteins and thus did not show fusion events. Deletion of the fusion peptide region or of the furin-cleavage site allowed the expression of the S-protein and led to a strong reduction of the fusion events.

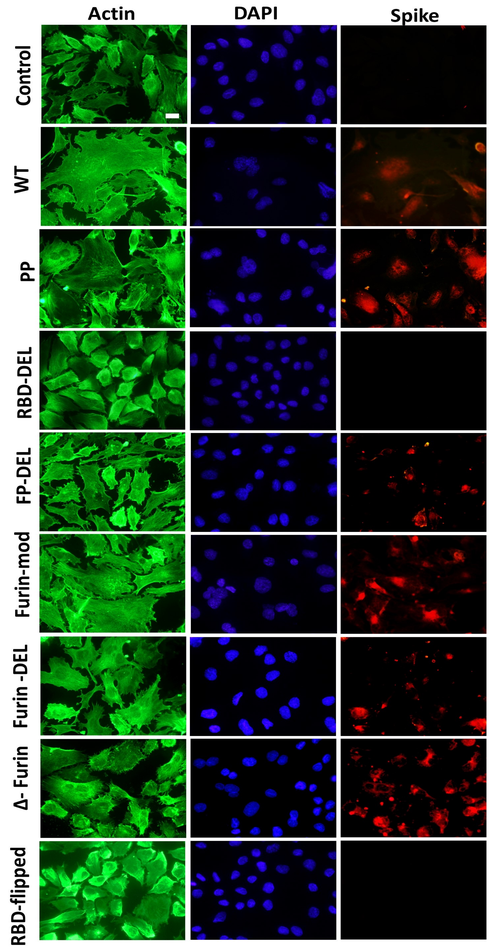
REFERENCES

Zhou, P., et al., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *nature*, *579*(7798), pp.270-273.

Buchrieser, J., et al., 2020. Syncytia formation by SARS-CoV-2-infected cells. *The EMBO journal*, *39*(23), p.e106267

S-protein expression and cell fusion

(A) Fluorescence microscopy analysis of S-protein variant expression



(B) S-protein expression

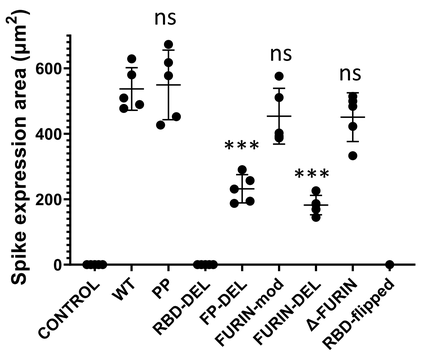


Figure 1: (A) Immunofluorescence analysis of S-protein expression transfected with S-gene-containing vectors in endothelial cells; DAPI (nuclei-blue), S-protein (red), actin (green). (B) S-protein expression is significantly reduced in cells transfected with FP- and FURIN-DEL vector constructs.

Quantification of cell fusion caused by S-protein expression

Figure 2: **Quantification of multinucleated cell formation by Spike variant expression (syncytia).** Area of (A) mononucleated and, (B) multinucleated cells; (C) % of multinucleated cells; subdivided into (D) cells with ≥ 2 nuclei; (E), cells with 2 nuclei.

- WT, prefusion-stabilized (PP), and furin-cleavage site-modified Spike variants caused syncytia leading to multinucleated cells.
- Fusion-peptide deletion, furin site deletion and Δ-Furin site reduced the size of syncytial cells, although Δ-Furin increased the number of 2-nucleated cells.
- RBD-deletion and RBD-flipped variants of the S-protein did not express stable proteins and thus had no effect on cell fusion.

Effect of S-protein variant expression on the inflammatory marker TNF-α

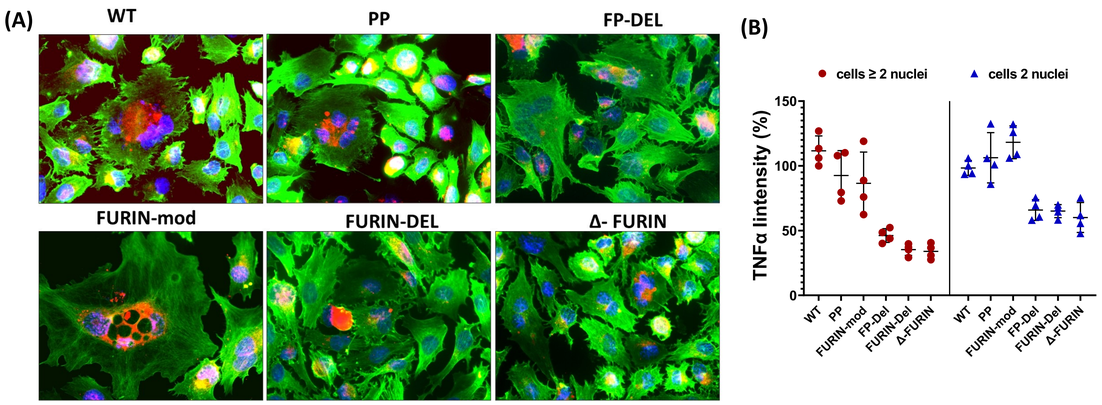


Figure 3: (A) Fluorescent microscopy images of TNF-α expression (red) in various S-protein variant-expressing human ECs. (B) TNF-α is overexpressed in WT, PP, and Δ-Furin expressing S-proteins in cells when compared to those using FP-DEL, Furin-DEL constructs.

CONCLUSIONS

- Expression of WT, Δ-Furin, and prefusion-stabilized (PP) spike expression induced syncytia and the upregulation of TNF-α, a major pro-inflammatory cytokine.
- Deletion of the fusion peptide region and modification of the furin-cleavage site led to a strong reduction of the fusion events without significantly affecting the expression of the S-protein on ECs.
- Empty vector-control or unstable S-protein variants did not cause any cell fusion.