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SARS-COV-2 SPIKE PROTEIN DESTABILIZES MICROVASCULAR HOMEOSTASIS

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BACKGROUND

Corona virus disease (COVID19), caused by SARS-CoV-2 infection has potential to compromise respiratory function and initiate fatal thrombotic events. SARS-CoV-2 spike protein (SpkPr) binds and down-regulates angiotensin converting enzyme 2 (ACE2) on cells and decrease the enzymatic activity of ACE2 which results in increased concentrations of pro-inflammatory molecules, angiotensin II and Bradykinin (BK), contributing to the pulmonary pathogenesis of COVID19.

METHODS

Immunofluorescence microscopy and digital image data quantification of COVID19 +/- FFPE lung tissue and Human Aortic Endothelial Cells (HAoEC) receiving indicated treatments *in vitro*; Computer assisted molecular docking analyses using 'ZDOCK' and 'AutoDock Vina' software suit; ELISA; and Western immunoblotting.

Human Aortic Endothelial Cells on Chamber Slides

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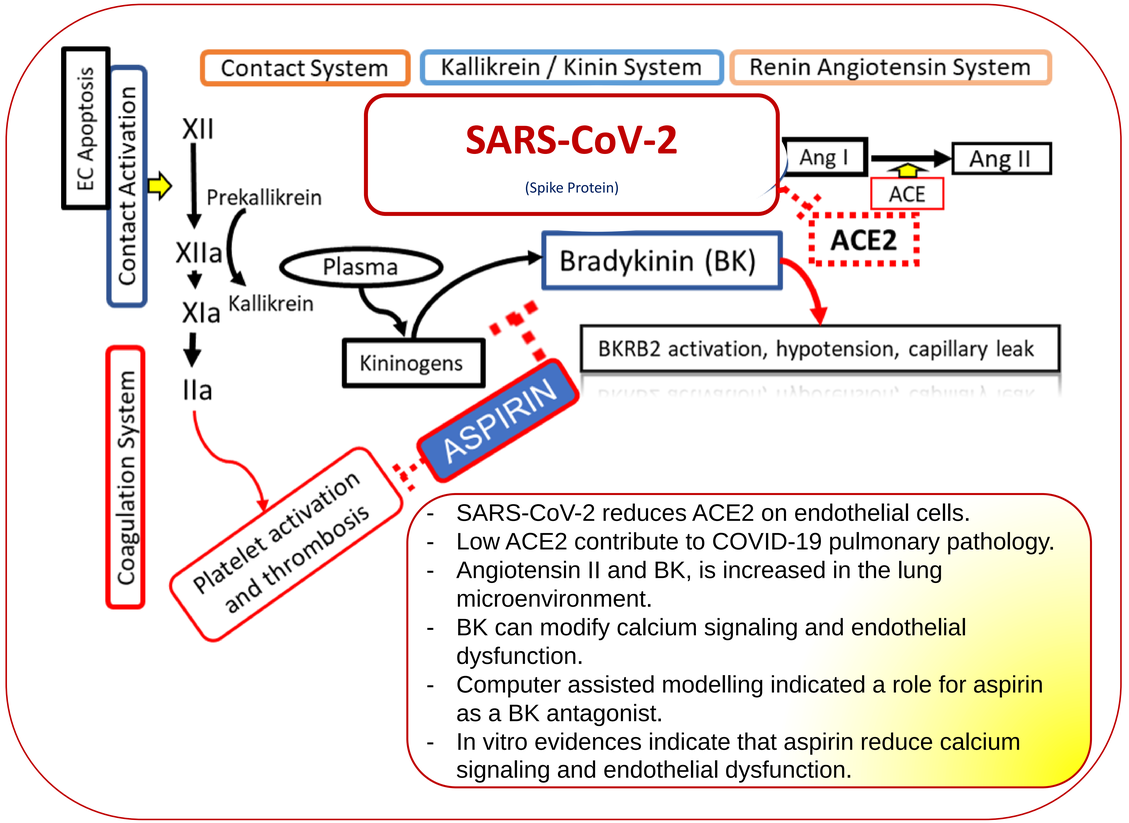
Tx as indicated (24h)

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Immuno-fluorescence staining and microscopy

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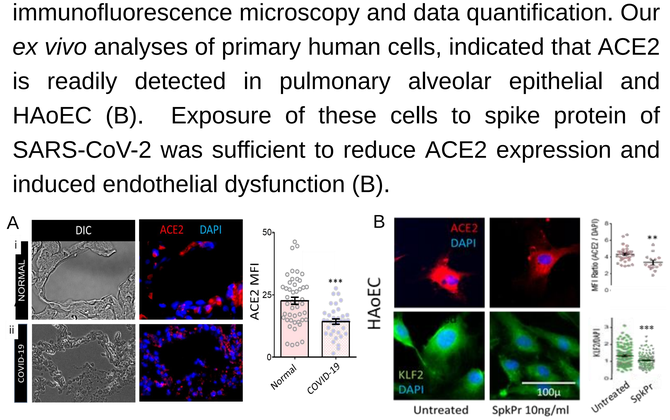
Digital Image data Analyses by ImageJ®

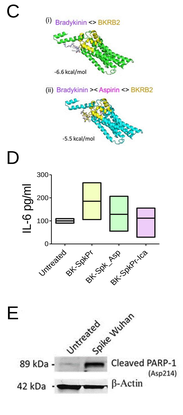


RESULTS

We confirmed that ACE2 is highly expressed in human pulmonary alveolar epithelial cells and significantly reduced along the alveolar lining of SARS-CoV-2 infected lungs (A) by immunofluorescence microscopy and data quantification. Our *ex vivo* analyses of primary human cells, indicated that ACE2 is readily detected in pulmonary alveolar epithelial and HAoEC (B). Exposure of these cells to spike protein of SARS-CoV-2 was sufficient to reduce ACE2 expression and induced endothelial dysfunction (B).

Computer assisted modeling of molecular interactions indicated that aspiring binds bradykinin receptor B2 (BKRB2), and act as a potential BK antagonist (C). In addition we show that BK and SpkPr induce release of IL6 from HAoEC which is reduced in presence of Aspirin (D).





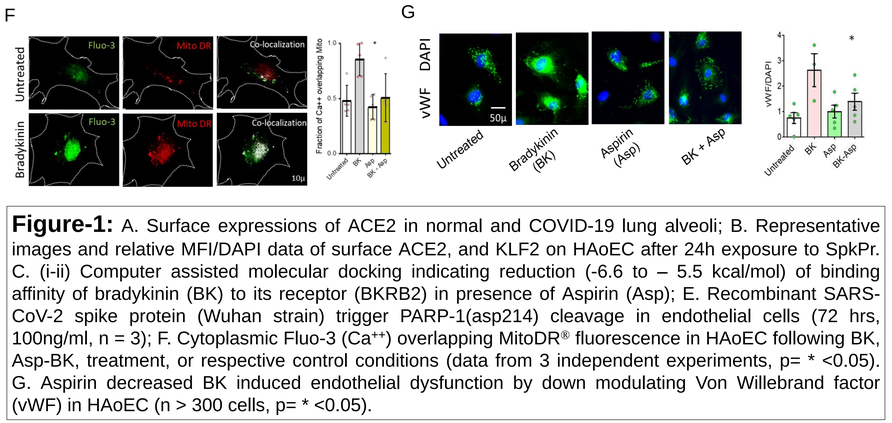


Figure-1: A. Surface expressions of ACE2 in normal and COVID-19 lung alveoli; B. Representative images and relative MFI/DAPI data of surface ACE2, and KLF2 on HAoEC after 24h exposure to SpkPr. C. (i-ii) Computer assisted molecular docking indicating reduction (-6.6 to – 5.5 kcal/mol) of binding affinity of bradykinin (BK) to its receptor (BKRB2) in presence of Aspirin (Asp); E. Recombinant SARS-CoV-2 spike protein (Wuhan strain) trigger PARP-1(asp214) cleavage in endothelial cells (72 hrs, 100ng/ml, n = 3); F. Cytoplasmic Fluo-3 (Ca^{++}) overlapping MitoDR® fluorescence in HAoEC following BK, Asp-BK, treatment, or respective control conditions (data from 3 independent experiments, p= * <0.05). G. Aspirin decreased BK induced endothelial dysfunction by down modulating Von Willebrand factor (vWF) in HAoEC (n > 300 cells, p= * <0.05).

Results (cont.)

Moreover, we showed exposure of endothelial cells to SpkPr induced pan-caspase activation, PARP-1 cleavage, and apoptosis (E). In contrary, BK caused calcium relocation in HAoECs (F) and endothelial dysfunction characterized by increased expression of von Willibrand Factor (vWF) and decreased expression of Krüppel-like Factor 2 (KLF2) but did not adversely affect viability in primary HAoEC. When tested in the *in vitro* model, we found evidences that aspirin decreases BK induced endothelial dysfunction and cell death in HAoEC (G).

CONCLUSIONS

SARS-CoV-2 causes complex detrimental effects on microvascular homeostasis that contribute to organ dysfunction and coagulopathies. Interference with interactions of SpkPr or BK with HAoEC may serve as an important strategy to stabilize microvascular homeostasis in COVID-19 disease. SARS-CoV-2 binds to, and causes down-regulation of ACE2 on cells that it infects. It is thought that reduced ACE2 enzymatic activity can contribute to inflammation and pathology in the lung. Our studies add to this understanding by providing evidence that spike protein alone can mediate adverse effects on vascular cells. Understanding these mechanisms of pathogenesis may provide rationale for novel intervention strategies which could limit microvascular events associated with SARS-CoV-2 infection.

ADDITIONAL KEY INFORMATION

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