Calu-3 cells as New Approach Methodologie (NAM) for identification of new antiviral/anti-inflammatory drug repurposing opportunities to treat COVID-19.

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BACKGROUND

The emergence of SARS-CoV-2 that causes the 2019 coronavirus disease (COVID-19) has erupted into a global pandemic that has led to tens of millions of infections and more than one million of deaths worldwide. The exact pathogenesis of severe COVID-19 remains unclear, but it typically involves a hyperinflammatory response following viral infection and induces significant damage in the respiratory tract. The entry of SARS-CoV-2 in host cells depends on binding of its Spike protein to the Angiotensin Converting Enzyme 2 (ACE2) receptor and priming by the protease TMPRSS2, enhanced by neuropilin (NRP-1). With the growing need for antiviral therapeutics to fight COVID-19, there is a growing need to find a relevant and sensitive model for drug screening. Such a model should express SARS-CoV-2 key receptors and responds to inflammatory stimulation by increasing production of pro-inflammatory cytokines, mimicking cytokine storm seen in Covid-19 patients.

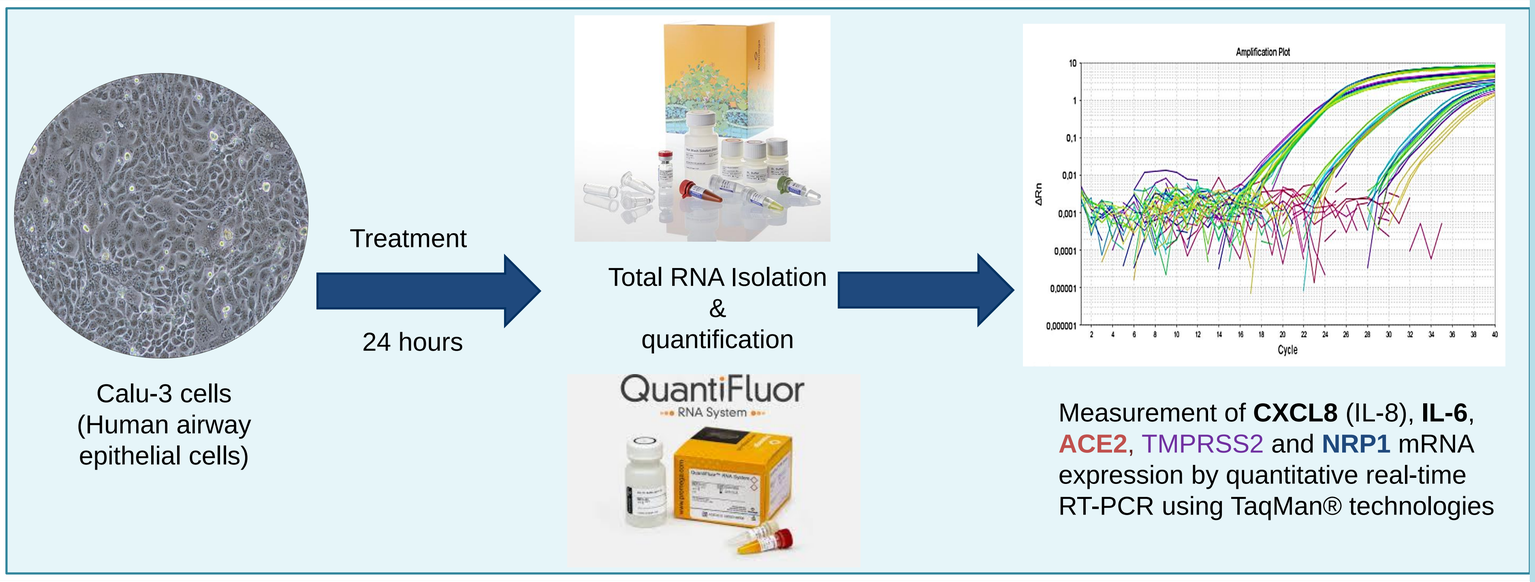
The aim of this work was to evaluate the human airway epithelial Calu-3 cells as a suitable model for screening and identification of new antiviral/anti-inflammatory drug repurposing opportunities to treat COVID-19.

METHODS

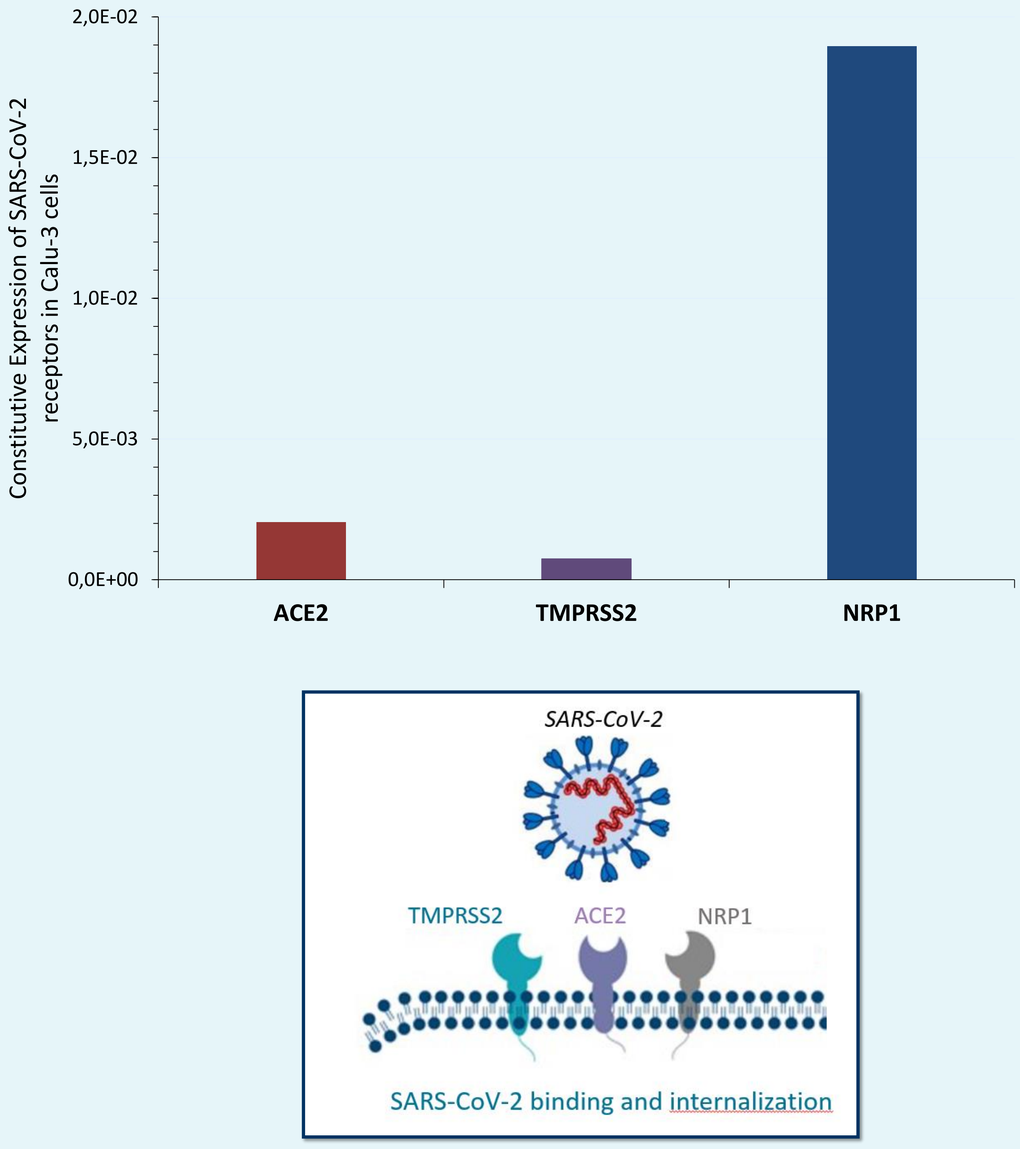
Calu-3 is a human lung cancer cell line derived from human bronchial submucosal glands. Constitutive expression of SARS-CoV-2 key receptors (ACE2, TMPRSS2 and NRP-1) was measured by quantitative real time RT-PCR, using TaqMan® technologies. GAPDH was used as housekeeping gene for normalization.

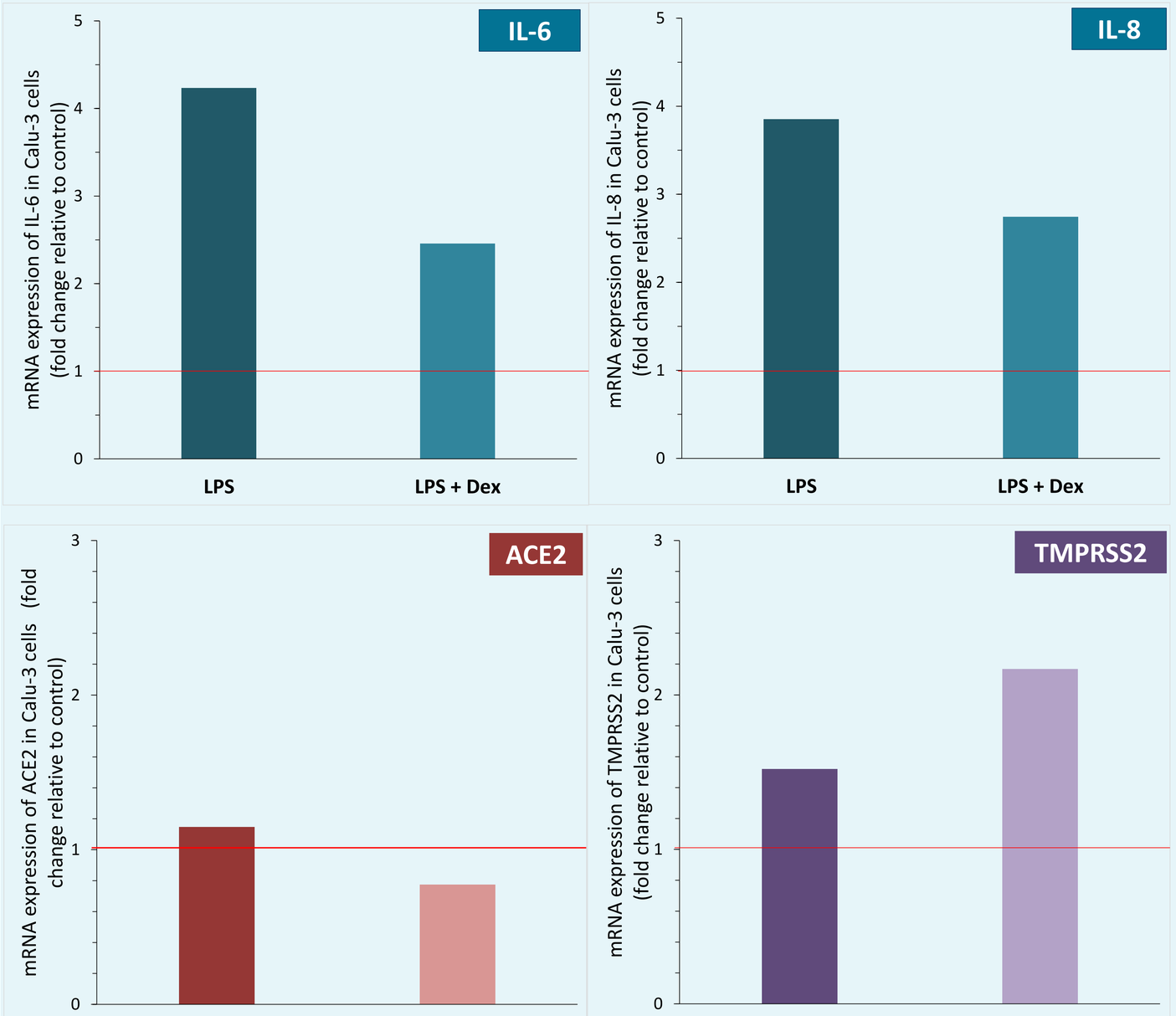
On the other hand, Calu-3 cells, seeded in 6-well plate, were treated for 24 hours with the well-known inflammatory inducer lipopolysaccharide (LPS), or with LPS plus the anti-inflammatory drug dexamethasone (Dex). Untreated cells were used as control. Incubations were done in cell incubator set at 37°C, 5% CO_2 and saturated humidity.

mRNA expression of pro-inflammatory cytokines (CXCL8 (IL-8) and IL-6), and SARS-CoV-2 receptors (ACE2, TMPRSS2, and NRP1) was measured by quantitative real time RT-PCR, using TaqMan® technologies.



RESULTS





- **Expression and modulation of SARS-CoV-2 receptors in Calu-3 cells:**
  - The three key SARS-CoV-2 receptors, ACE2, TMPRSS2 and NRP1, are expressed in Calu-3 cells, and NRP1 expression is the highest among the 3 receptors.
  - mRNA expression of ACE2 and TMPRSS2 is modulated by dexamethasone treatment in Calu-3 LPS treated cells. Assessment of modulation of NRP1 in Calu-3 cells is ongoing.
  - mRNA expression of both pro-inflammatory cytokines (IL-8 and IL-6) is increased in Calu-3 cells by LPS treatment.
  - Dexamethasone reduces the LPS-induced mRNA expression of both IL-8 and IL-6 in Calu-3 cells.

CONCLUSIONS

This study shows that SARS-CoV-2 receptors are expressed in Calu-3 cells and that ACE2 and TMPRSS2 are sensitive to pharmacological agents. Increase of pro-inflammatory cytokine expression induced by LPS did not change the expression of ACE2 and TMPRSS2 in Calu-3 cells. However, dexamethasone treatment that reduced cytokine expression decreased mRNA expression of ACE2 and increased that of TMPRSS2 in calu-3 cells. Further investigations are needed to confirm our findings, particularly using more complex 3D human airway models, like MucilAir™ model (Epithelix) or EpiAirway® model (MatTek).

Overall, this study shows that Calu-3 model could be a suitable and cost-effective human airway epithelial cell model for screening and identification of new antiviral/anti-inflammatory drug repurposing opportunities or natural products to defeat SARS-CoV-2.

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