IL11 involvement in inflammatory and pro-fibrotic alterations via STAT3-WNT5A signaling activation by SARS-CoV-2 accessory proteins

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INTRODUCTION

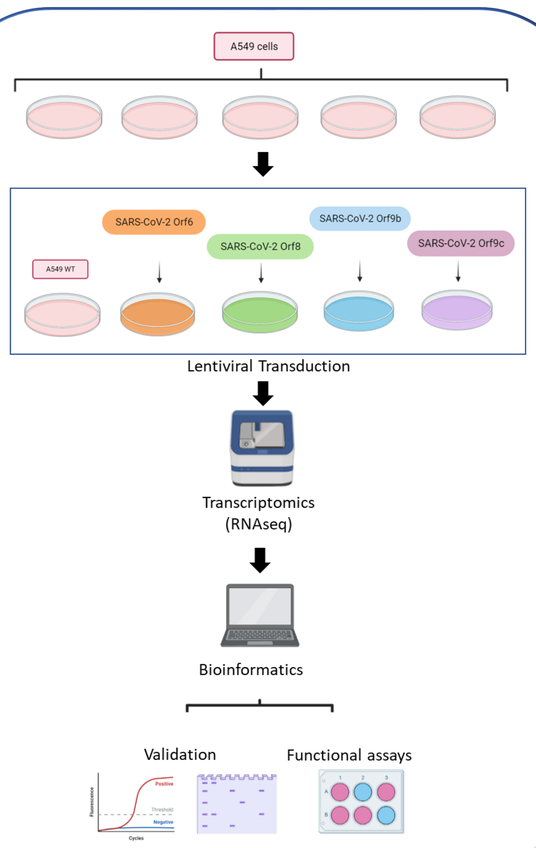
The coronavirus disease 2019 (COVID-19) is a potentially fatal respiratory disease caused by a new SARS-CoV-2. The clinical course of COVID-19 exhibits a broad spectrum of severity and progression patterns. It is known that the underlying cause of severe disease is a cytokine dysregulation and hyperinflammation status[1]. SARS-CoV-2 genome encodes for eleven accessory proteins: ORF3a, ORF3b, ORF3c, ORF3d, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c and ORF10[2]. As their name suggests, accessory proteins are dispensable for viral replication, but may importantly mediate host responses to the virus, which can affect pathogenicity and virulence. This suggests that accessory proteins play a key role in pathogenesis not observed in less virulent coronavirus infections.

WNT5A is a member of WNT family proteins which plays critical roles in a myriad of processes in both health and disease, such as embryonic morphogenesis, fibrosis, inflammation or cancer[3]. Several IL-6 family members induce Wnt5A by amplifying Wnt5A signal in a feedback loop[3]. Among those, IL-11 is a pleiotropic cytokine with effects that overlap with IL-6, particularly relevant in epithelial cells. IL-11 is upregulated in a wide variety of fibro-inflammatory diseases[4]. Indeed, it is known that WNT5A and IL-11 have the ability of activating STAT3 signaling and, this ability, has been postulated as a possible mechanism to link WNT5A gene with immunomodulation[3].

GOAL:

To study the involvement of SARS-CoV-2 accessory proteins ORF6, ORF8, ORF9b and ORF9c in inflammatory and/or pro-fibrotic responses.

METHODS



RESULTS

1 Cellular localization of ORF6, ORF8, ORF9b or Orf9c

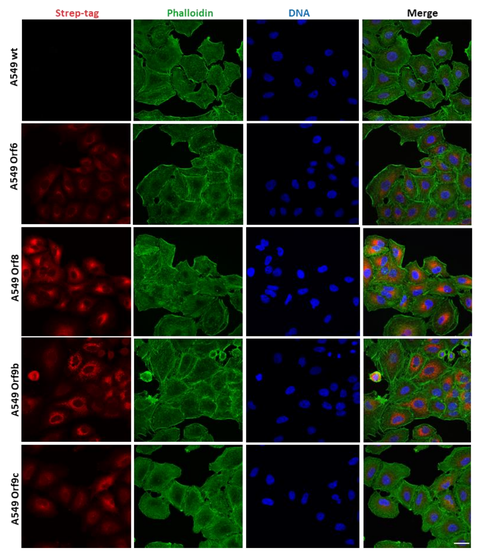


Figure 1. A549 transduced cells with Strep-tagged viral proteins were imaged by confocal microscopy. Red: Strep-tag antibody signal; Green: Phalloidin; Blue: DAPI (nuclei staining). Objective 63x, scale bar 25 µm.

2 Gene expression of A549 cells transduced with SARS-CoV-2 ORF6, ORF8, ORF9b or ORF9c accessory proteins

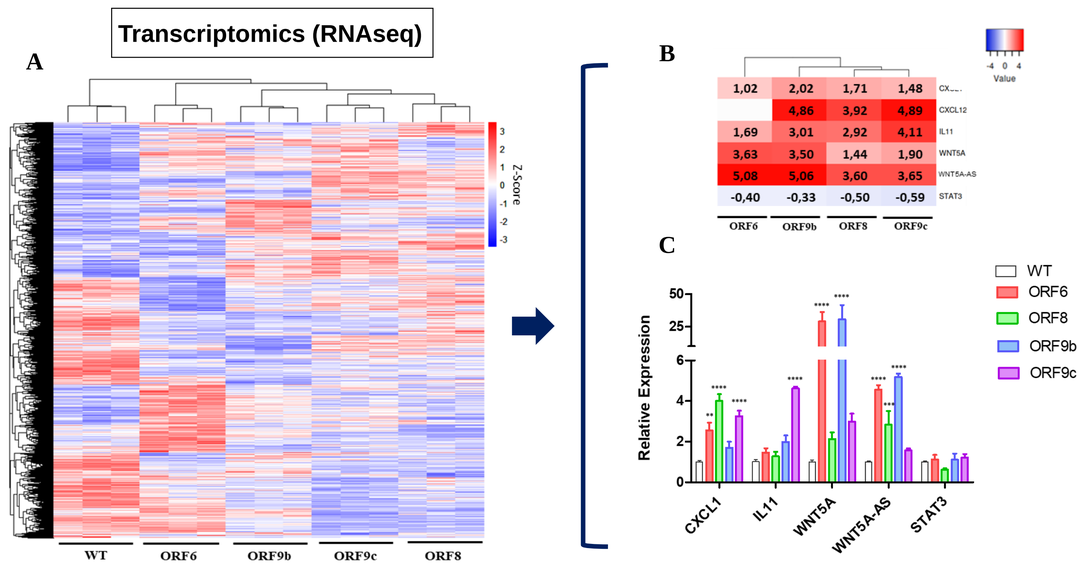


Figure 2. **A)** Heatmap of RNA-Seq analysis of transduced cells expressing viral proteins. **B)** Log2 Fold Change heatmap of WNT5A and IL11 signaling pathways related genes. **C)** Expression levels calculated with 2-ΔΔCT method by normalizing to that of GADPH. Error bars represent mean ± SD (n=3). Statistical significance is as follows: **p < 0.01, ***p < 0.001, **** p < 0.0001.

3 Pulmonary fibrosis idioptahtic signaling pathway genes in A549 cells transduced

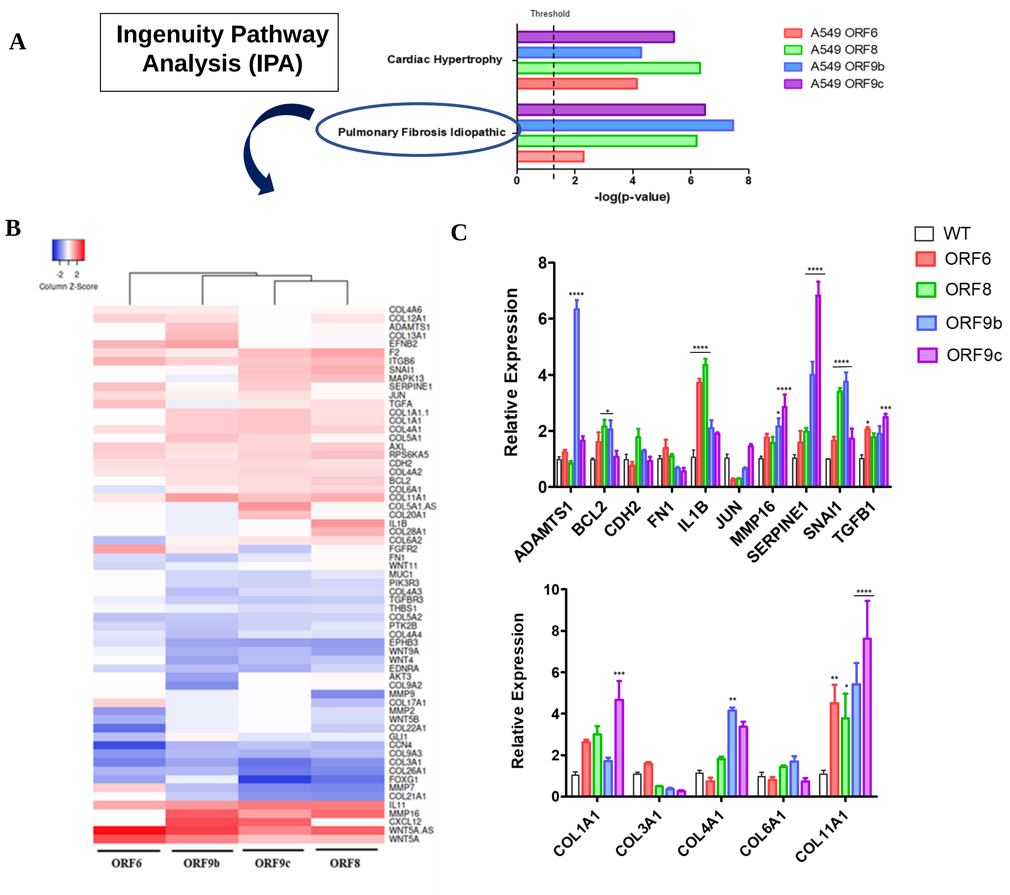


Figure 3. **A)** WNT5A and IL11 related canonical pathways common in transduced cells (IPA software analysis). **B)** Heatmap of genes involved in pulmonary fibrosis in each of the A549 transduced cell lines. **C)** Expression levels of representative genes involved in pulmonary fibrosis pathway. Error bars represent mean ± SD (n=3). Statistical significance is as follows: *p < 0.05, **p < 0.01, ***p < 0.001, **** p < 0.0001.

4 Profibrotic capacity of transduced cells

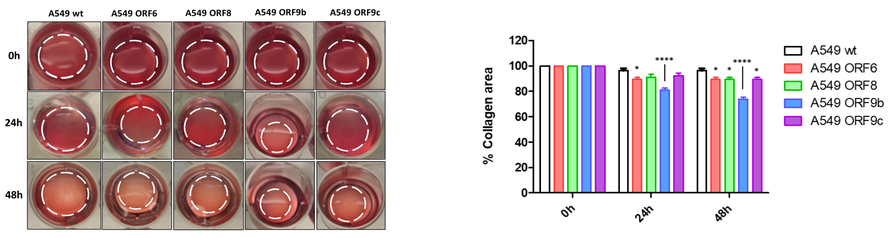


Figure 4. Cell Contraction Assay showed the ability of cells to contract a collagen matrix in vitro. Dashed lines designate the gel edges (left panel) and quantification of percentage collagen area contraction (right panel). Data are represented as mean ± SD (n=3). Statistical significance is given as follows: *p < 0.05, ****p < 0.0001 to the control group A549 wt.

5 IL11 involvement in pSTAT3 and TGFb expression mediated by SARS-CoV-2 ORFs

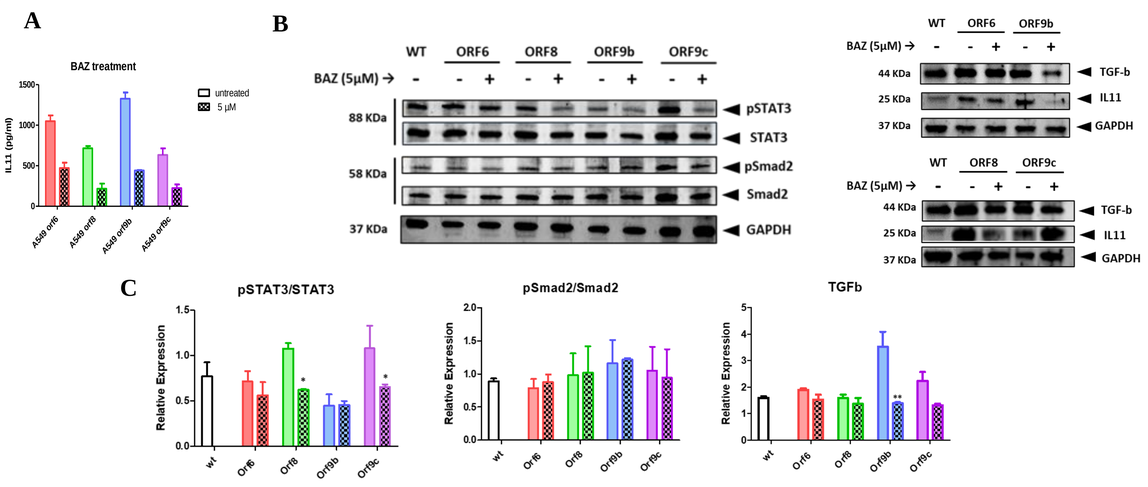


Figure 5. Decreasing IL11 entry into the A549 transduced cells restores pSTAT3 and TGFb levels. Bazedoxifene (BAZ) treatment decrease IL11 entry into the cells **A)** ELISA assay showed secreted IL11 levels after BAZ treatment. **B)** pSTAT3/STAT3, pSmad2/Smad2 and TGFb were assessed by western blot analysis **C)** Quantification of protein expression levels. Error bars represent mean ± SD (n=3). Statistical significance is as follows: *p < 0.05, **p < 0.01.

CONCLUSIONS

- Differential gene expression was identified in A549-ORF6, A549-ORF8, A549-ORF9b and A549-ORF9c transduced cells.
- ORF6, ORF8, ORF9b and ORF9c are involved in pro-fibrotic processes by IL11 and WNT5A dysregulation signaling (Figura 6):
  - ➢ ORF6 increases IL11 levels by WNT5A overexpression.
  - ➢ ORF8 increases IL11 levels in a STAT3-phosphorylation dependent manner.
  - ➢ ORF9b increases TGFb levels through WNT5A overexpression.
  - ➢ ORF9c overexpresses IL11 endogenously and does not depend on exogenous input.

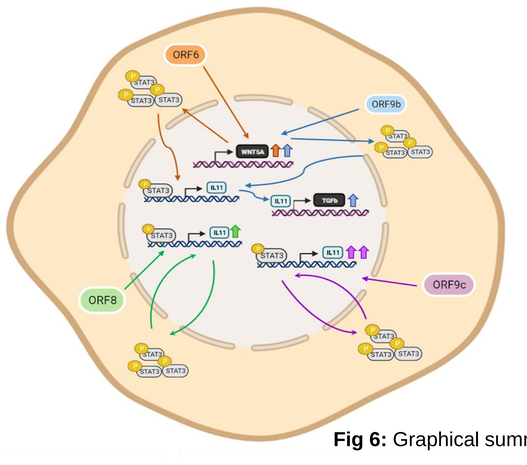


Fig 6: Graphical summary

References

1. Ramasamy S, et al., Clin Microbiol Rev. 2021
2. Redondo N, et al., Front Immunol. 2021
3. Lopez-Bergami & Barbero, Can Met Rev. 2020
4. Cook &Schafer, Annu Rev Med. 2020

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