00164

Molecular Determinants of SARS-CoV-2 Cellular Tropism In Vitro

Maritza Puray-Chavez[1], Kyle M. LaPak[2], Mohammed, Shawn[1], Dana Q. Lawson[1], Dennis Goldfarb[2,3], M. Ben Major[2,4], and Sebla B. Kutluay[1,5*]

[1]Department of Molecular Microbiology, Washington University in St. Louis, St. Louis, MO, USA, [2]Department of Cell Biology and Physiology, Washington University in St. Louis, St. Louis, MO, USA, [3]Institute for Informatics, Washington University in St. Louis, St. Louis, MO, USA, [4]Department of Otolaryngology, Washington University in St. Louis, St. Louis, MO, USA, [5]Lead Contact, *Correspondence: kutluay@wustl.edu (S.B.K.).

BACKGROUND

Lung cell lines to model SARS-CoV-2 replication in vitro are greatly limited hampering the rigorous study of SARS-CoV-2-host interactions. We analyzed a panel of 10 airway cell lines with various levels of ACE2 expression to identify models of SARS-CoV-2 infection. We found that none of the ACE2 expressing cell lines supported replication, whereas the H522 human lung adenocarcinoma cells were naturally permissive to SARS-CoV-2 infection despite undetectable expression of ACE2. We confirmed that SARS-CoV-2 replication is indeed completely independent of ACE2 in H522s but dependent on heparan sulfates and the E484D substitution within the Spike. Further, we show that many of the ACE2 positive non-permissive cell lines express high basal levels of interferon-stimulated genes, which can be overcome by inhibition of the JAK/STAT pathway or by ACE2 overexpression. Together, our findings highlight ACE2-independent pathways can control the cellular tropism of SARS-CoV-2.

METHODS

Conventional molecular virology assays have been conducted to study the permissiveness of a panel of 10 cell lines expressing various levels of ACE2. ACE2 independence of SARS-CoV-2 replication was validated by antibody blocking, Fc-ACE2 decoy peptide and CRISPR-based approaches in H522 cells. RNA sequencing was used to study the basal level of genes in the type-I IFN pathway in the panel of 10 cell lines, which was further validated by western blotting and qRT-PCR. A panel of 5 cell lines, with varying expression levels of ACE2 and TMPRSS2, were pre-treated with Ruxolitinib, a JAK inhibitor, and infected with SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020 and spike variants. Viral replication was detected through analysis of cell associated RNA.

ACE2 positive non-permissive cell lines, H596, Detroit562, SCC25 and OE21, express ***high basal levels*** of ***interferon-stimulated genes*** which can be overcome by inhibition of the JAK/STAT pathway.

RESULTS

H522 human lung adenocarcinoma supports SARS-CoV-2 replication in a completely ACE2-independent manner (Fig. 1A and 1B). Transcriptomic analysis revealed basal high level of expression of interferon response pathway genes in some ACE2-positive cells recalcitrant to SARS-CoV-2 infection (Fig. 1C, 2A). Infection of OE21 and SCC25 cells required blocking of the IFN response pathway (Fig. 2B and 2C) or ACE2 overexpression to allow SARS-CoV-2 infection.

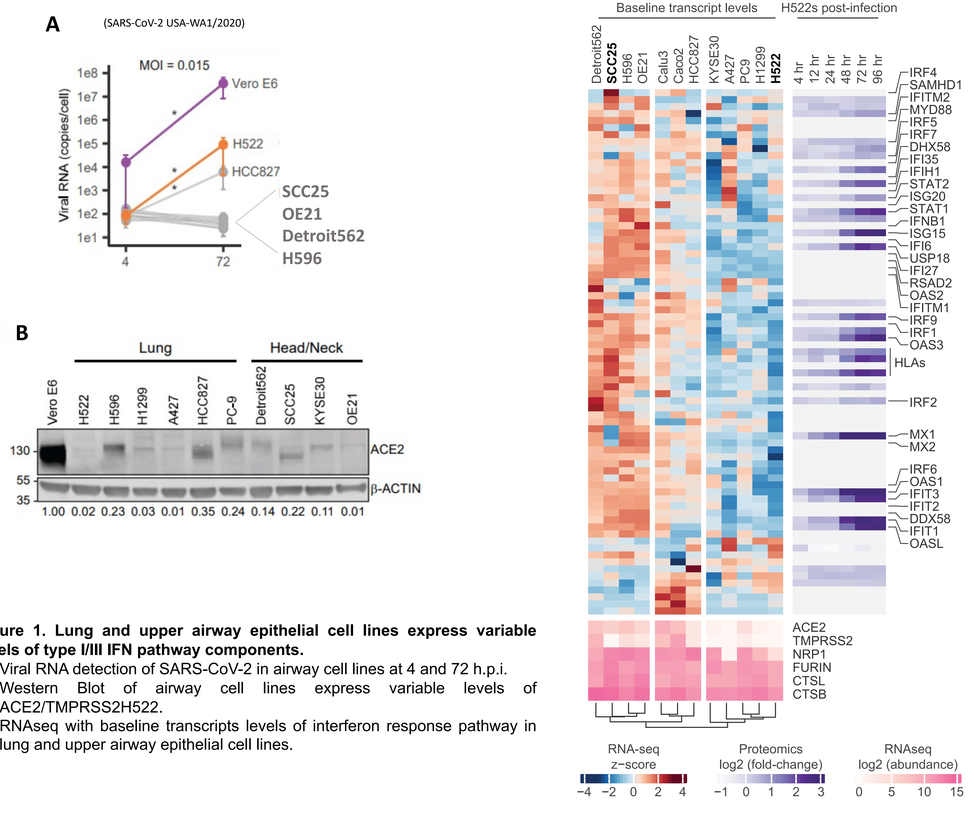


Figure 1. Lung and upper airway epithelial cell lines express variable levels of type I/III IFN pathway components.

A. Viral RNA detection of SARS-CoV-2 in airway cell lines at 4 and 72 h.p.i.
B. Western Blot of airway cell lines express variable levels of ACE2/TMPRSS2H522.
C. RNAseq with baseline transcripts levels of interferon response pathway in lung and upper airway epithelial cell lines.

RESULTS

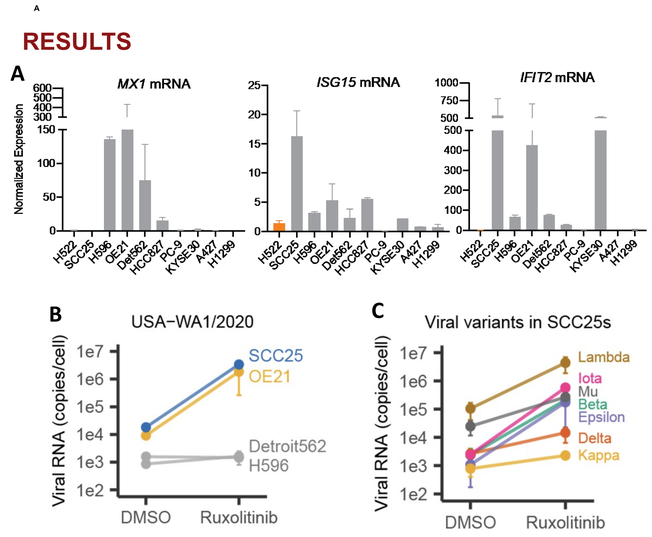


Figure 2. Inhibition of JAK1/2 by ruxolitinib treatment or ACE2 overexpression restores SARS-CoV-2 replication in a subset of ACE2(+) cells.

A. RT-qPCR with *Mx1, Isg15 and Ifit2* mRNA ISG expression levels in uninfected cells.
B. and C. Viral RNA detection of SARS-CoV-2 in airway cell lines treated with 1mM Ruxolitinib.

CONCLUSIONS

- These findings suggest that SARS-CoV-2 replication can proceed in complete absence of ACE2 and that the innate immunity is a key determinant of SARS-CoV-2 cellular tropism.
- These findings may explain the complex SARS-CoV-2 pathogenesis in vivo as it shows that factors independent of ACE2 can define cellular tropism.

ADDITIONAL KEY INFORMATION

Key words:
ACE2, SARS-CoV-2, type I/III IFN pathway components

Author Contact Information:
*Correspondence: kutluay@wustl.edu (S.B.K.)

Acknowledgements
Diamond laboratory at Washington University in St. Louis for providing SARS-CoV-2 variants