TARGETING THE HOST-VIRUS INTERFACE TO BLOCK SARS-COV-2 ASSEMBLY IN AIRWAY CELLS

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BACKGROUND

- The rapid emergence of the SARS-CoV-2 Omicron variant that evades many therapies illustrates the need for antiviral treatments with high genetic barriers to resistance.
- The small molecule PAV-104, identified through a moderate-throughput screen involving cell-free protein synthesis, was recently shown to target a subset of host protein assembly machinery in a manner specific to viral assembly with minimal host toxicity.
- PAV-104 shows broad activity against respiratory viral pathogens with low susceptibility to evolutionary escape.

OBJECTIVE

- To investigate the capacity of PAV-104 to inhibit SARS-CoV-2 replication in human airway epithelial cells (AECs).

METHODS

- Dose-dependent cytotoxicity of PAV-104 in Calu-3 cells was determined by MTT assay.
- Calu-3 cells were infected with SARS-CoV-2 isolate USA-WA1/2020 (MOI=0.01).
- Primary AECs were isolated from healthy donor lung transplant tissue, cultured at air liquid interface (ALI), and infected with SARS-CoV-2 Gamma, Delta, and Omicron variants (MOI=0.1).
- SARS-CoV-2 replication was assessed by RT-PCR quantitation of the *N* gene, immunofluorescence assay (IFA) of nucleocapsid (N) protein, and titration of supernatant ($TCID_{50}$).
- Transient co-expression of four SARS-CoV-2 structural proteins (N, M, S, E) to produce virus-like particles (VLPs) was used to study the effect of PAV-104 on viral assembly. VLPs were detected by western blot and nanoparticle tracking analysis (NTA).
- Drug resin affinity chromatography was performed to study the interaction between PAV-104 and N.
- Glycerol gradient sedimentation was used to assess N oligomerization.
- Total RNA-seq and the REACTOME database were used to evaluate PAV-104 effects on the host transcriptome.

PAV-104 is a pan-respiratory virus small molecule inhibitor with promising activity against SARS-CoV-2 in human airway epithelial cells that should be explored in animal models and clinical studies.

RESULTS

1) PAV-104 treatment potently inhibits SARS-CoV-2 replication in AECs, including primary AECs in ALI culture.

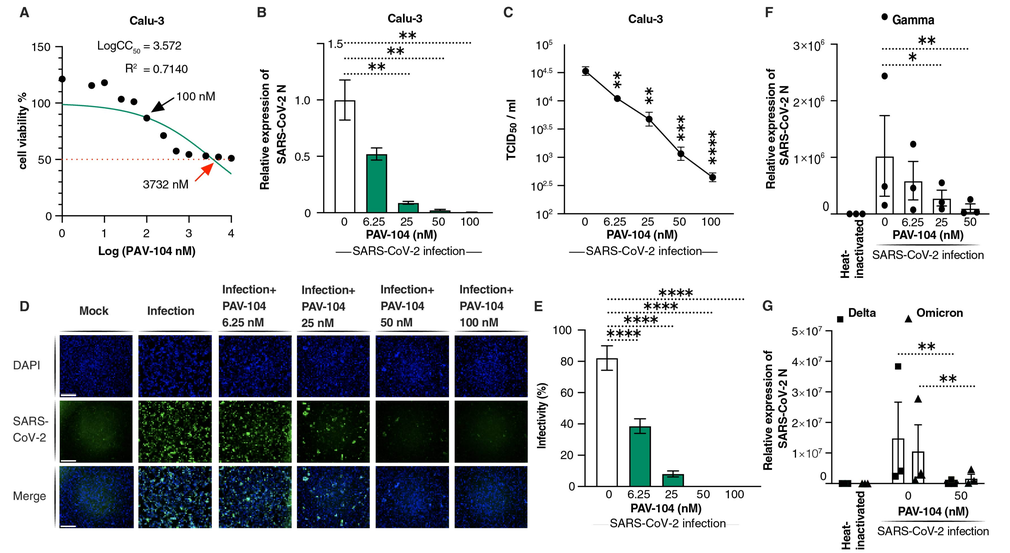


Figure 1. PAV-104 decreases SARS-CoV-2 replication in airway epithelial cells. A. Cellular toxicity was examined in Calu-3 cells using MTT assay and was expressed as relative cell viability normalized to DMSO control. B. SARS-CoV-2 *N* gene expression was measured by qRT-PCR in Calu-3 cells in the presence of PAV-104 treatment at specified concentrations. Results were normalized to *RNaseP* and are shown as the fold change compared to DMSO control. C. Viral titer ($TCID_{50}$/ml) of supernatants collected after SARS-CoV-2 infection in the presence of PAV-104 at the indicated concentrations. D. Calu-3 cells were stained with DAPI nuclear marker (blue) and anti-SARS-CoV-2 N protein antibody (with FITC-conjugated secondary antibody) to measure SARS-CoV-2 infection (green) and PAV-104 effects. E. Quantification of SARS-CoV-2 infected cells (FITC positive cells) in Calu-3 cells (shown in panel D). F and G. qRT-PCR analysis of SARS-CoV-2 *N* gene from ALI-cultured primary airway epithelial cells with or without PAV-104 treatment in six different donors. Each color represents data from a single donor. Results were normalized to *RNaseP* and are shown as the fold change compared to heat-inactivated control (Heat-in). Statistical analysis was performed by Student's *t*-test or paired *t*-tests. Bar = mean ± SEM. $p \leq 0.05$ [*], $p \leq 0.01$ [**], $p \leq 0.001$ [***], $p \leq 0.0001$ [****].

2) PAV-104 inhibits SARS-CoV-2 replication post entry, and blocks SARS-CoV-2 viral assembly and release.

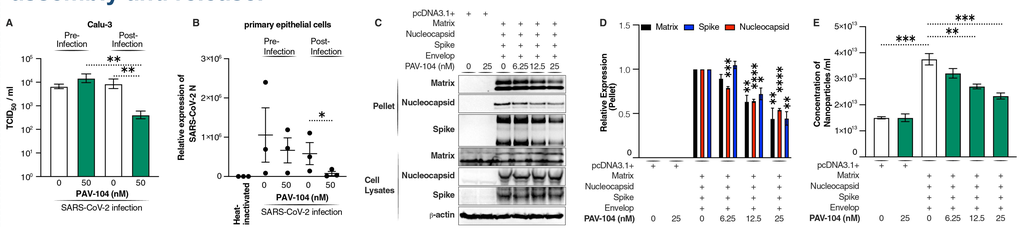


Figure 2. PAV-104 specifically inhibits SARS-CoV-2 post entry, and suppresses production of SARS-CoV-2 VLPs without affecting viral protein synthesis. A. Viral titer in Calu-3 cells treated with PAV-104 at indicated doses pre-infection or post-infection. B. Virus production in ALI-cultured primary AECs treated with PAV-104 at indicated doses pre-infection or post-infection. C. Total proteins in cell lysates and in ultra-centrifuged pellets were measured by western blot. Anti-β-actin antibody was used as a loading control for the cell lysates. D. Densitometry analysis of western blot data (normalized to DMSO control). E. Quantification of SARS-CoV-2 VLPs by nanoparticle tracking analysis (Malvern NanoSight NS300). Data are representative of the results of three independent experiments (mean ± SEM). Statistical significance was analyzed by *t* test or paired *t* test. $p \leq 0.05$ [*], $p \leq 0.01$ [**], $p \leq 0.001$ [***], $p \leq 0.0001$ [****].

3) PAV-104 interacts with SARS-CoV-2 N and interferes with N oligomerization.

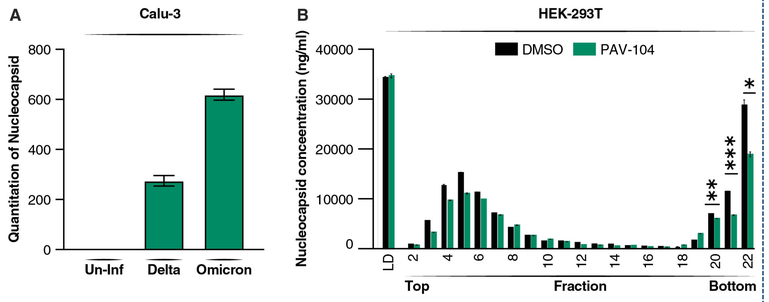


Figure 3. PAV-104 interacts with nucleocapsid and interferes with nucleocapsid oligomerization. A. Quantitation of resin-bound N band density detected by western blot. B. Concentration of SARS-CoV-2 N in each fraction (protein content analyzed using a commercial SARS-CoV-2 N sandwich ELISA kit). Data are representative of the results as mean ± SEM. Statistical significance was analyzed by *t* test. $p \leq 0.05$ [*], $p \leq 0.01$ [**], $p \leq 0.001$ [***], $p \leq 0.0001$ [****].

4) PAV-104 treatment reverses SARS-CoV-2 induction of the interferon and 'maturation of nucleoprotein' signaling pathways.

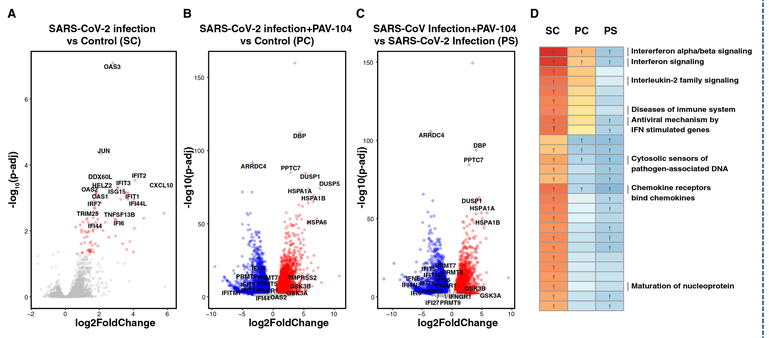


Figure 4. Impact of SARS-CoV-2 infection and PAV-104 treatment on the transcriptome of primary AECs. A-C. Volcano plots showing differentially-expressed genes (DEGs) in the setting of A. SARS-CoV-2 infection (SARS-CoV-2 infection vs Control (SC)), B. SARS-CoV-2 infection in the presence of PAV-104 (SARS-CoV-2 infection+PAV104 vs Control (PC)), and C. SARS-CoV-2 infection in the presence of PAV-104 vs SARS-CoV-2 infection (PS). D. Top enriched REACTOME pathways in response to SARS-CoV-2 infection or PAV-104 treatment identified using gene set enrichment analysis (GSEA). The orange and blue-colored bars in the bar chart indicate predicted pathway activation or predicted inhibition, respectively, based on enrichment-score. Y represents FDR<0.25.

CONCLUSIONS

- PAV-104 is effective against circulating SARS-CoV-2 variants in human cell lines and in human primary airway epithelial cells.
- PAV-104 interferes with SARS-CoV-2 N oligomerization and inhibits viral assembly/budding.
- Our data suggest that PAV-104, a host-targeted pan-viral small molecule inhibitor, could be a promising therapeutic candidate for SARS-CoV-2.

ADDITIONAL KEY INFORMATION

- This study was supported by The Sandler Program for Breakthrough Biomedical Research (UCSF PBBR) and NIH grant R01MH112457 (to SKP).
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