ScRNA-seq of HRSV/SARS-CoV-2 Co-Infection Reveals Unique Cellular Responses and lncRNA Signatures

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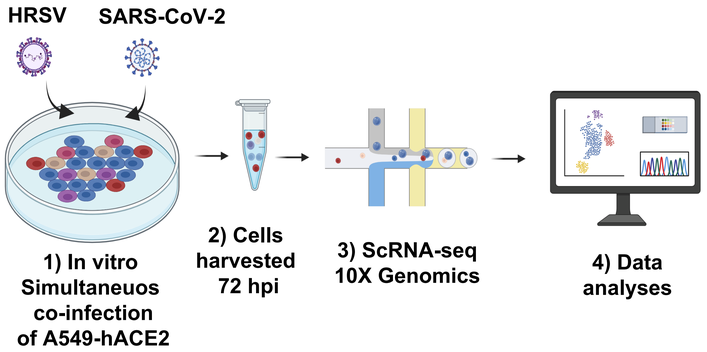
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BACKGROUND

Respiratory infections caused by human respiratory syncytial virus (HRSV) and SARS-CoV-2 often result in severe respiratory disease, particularly in vulnerable populations. During co-infections, viruses can either synergies or obstacle each other, impacting replication dynamics and promoting genetic recombination. The intricate and dynamic nature of coinfections can have profound implications in disease and viral replication evolution. Using an in vitro model of HRSV/SARS-CoV-2 co-infection, we investigated how individual cells respond to simultaneous or single infections, providing insights into synergistic/antagonistic viral interactions.

METHODS



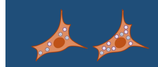
1) A549-hACE2 were co-infected with HRSV and SARS-CoV-2 (MOI: 0.01)
2) 72 hours post infection (hpi) cells were harvested and fixed in methanol
3) Cells were acquired for single cell RNA sequencing (scRNA-seq - 10X Genomics)
4) Data analyses:
   - Raw sequencing reads processed with Cell Ranger count module
   - Gene annotation was performed using the mapIds function from the AnnotationDbi R/Bioconductor package and the EnsDb.Hsapiens.v86 annotation database
   - Normalization was conducted using the sizeFactors, logNormCounts, and modelGeneVar functions (scater R/Bioconductor package).

ADDITIONAL KEY INFORMATION:

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Co-infection drives the formation of **a singular and transcriptionally unique subpopulation** of lung epithelial cells



Within the co-infected cluster, two subpopulations of cells with **differential HRSV replication capacity** are indicative of the complexity of the HRSV and SARS-CoV-2 interaction within the same cell



Co-infection induces **metabolic reprogramming** that may influence viral replication and fitness

RESULTS

1. Distinct transcriptomic signatures correspond to 4 different clusters: uninfected, SARS-CoV-2 single infected, RSV single infected and HRSV/SARS-CoV-2 co-infected cells

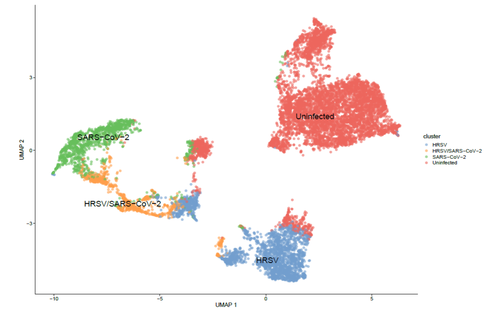


Figure 1. Visualization of the scRNA-seq dataset. Uniform Manifold Approximation and Projection (UMAP) plot showing the distribution of individual cells in a two-dimensional space. Clusters are labeled and annotated as uninfected (#1; n=6,977), SARS-CoV-2-infected (#2; n=1,505), HRSV-infected (#3; n=2,608), and SARS-CoV-2/HRSV co-infected (#4; n=895).

2. Cluster-specific marker analysis reveals molecular signatures of both protein-coding and lncRNA, with co-infected cells exhibiting a unique cellular state; GO-BP enrichment analysis heightened innate immune activation and inflammatory response in this cluster

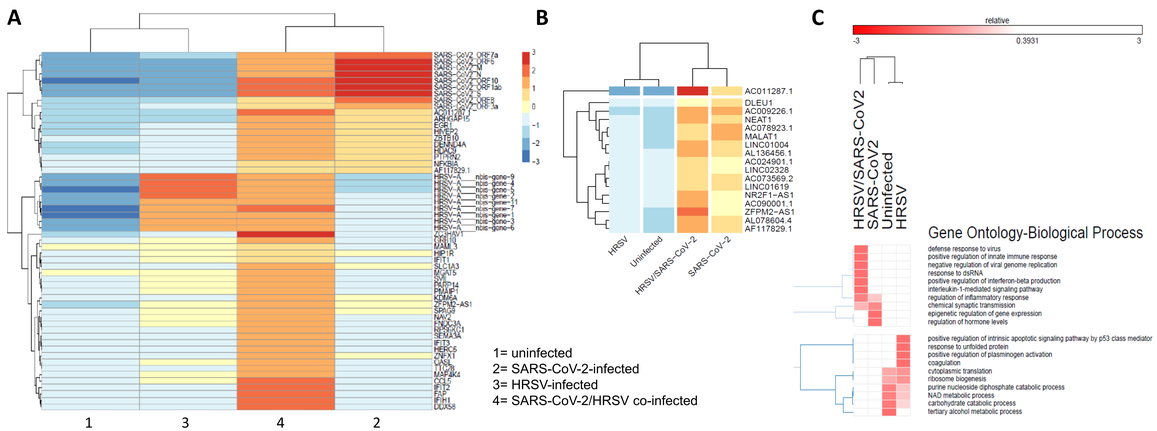


Figure 2. Heatmaps displaying **A)** the average expression of viral genes and the cellular markers per cluster, and **B)** the expression of 17 lncRNA markers. The color gradient represents relative gene expression, from high (dark red) to low (dark blue). **C)** Heatmap summarizing functional enrichment analysis, showing associations between cell clusters and biological processes. Enrichment significance is represented as -log10(P-value), from high (dark red) to low (light red). Co-infected cells were enriched with pathways like virus defense, innate immune response and viral genome replication regulation, and reduced in processes linked to ribosome biogenesis, unfolded protein response, apoptotic regulation, hinting at viral immune evasion and cellular stress.

3. HRSV-specific gene expression analysis within the co-infected cluster reveals two subclusters with differential HRSV replication capacity, enriched in distinct biological processes

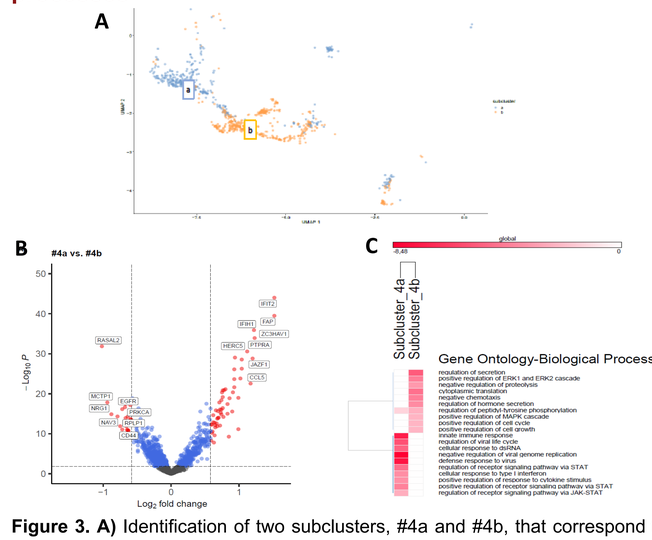


Figure 3. A) Identification of two subclusters, #4a and #4b, that correspond to low and high HRSV-specific gene expression, respectively, within coinfected cluster. **(B)** Volcano plot showing differentially expressed genes between subclusters #4a and #4b. Red dots: significant DEGs (adjusted P-value < 0.05, |log2FC| > 0.58); blue dots: nominal P-value < 0.05; grey dots: not significant. **(C)** Heatmap summarizing functional enrichment signatures for subclusters, showing their association with specific Gene Ontology terms. Enrichment significance is represented as -log10(P-value), from high (dark red) to low (light red). #4a was enriched for pathways associated with antiviral defense, innate immunity, and JAK-STAT; #4b was enriched for processes related to cell cycle progression, growth regulation, activation of MAPK/ERK1/ERK2 signaling cascades.

CONCLUSIONS

- The scRNA-seq analysis revealed that co-infection drives the formation of a transcriptionally distinct subpopulation of lung epithelial cells, potentially influencing cell fate and behavior
- Two distinct subpopulations characterized by a reduced or an enhanced HRSV replication rate, driven by a heightened antiviral response or cell cycle progression respectively, are distinguished within co-infected cluster; this is indicative of the complexity of the HRSV and SARS-CoV-2 interaction within the same cell
- Co-infection modulates biological mechanisms that may favor viral replication of both viruses or create metabolic bottleneck that impact viral fitness