Decoding Human Norovirus Tropism via Single-Cell RNA seq Analysis

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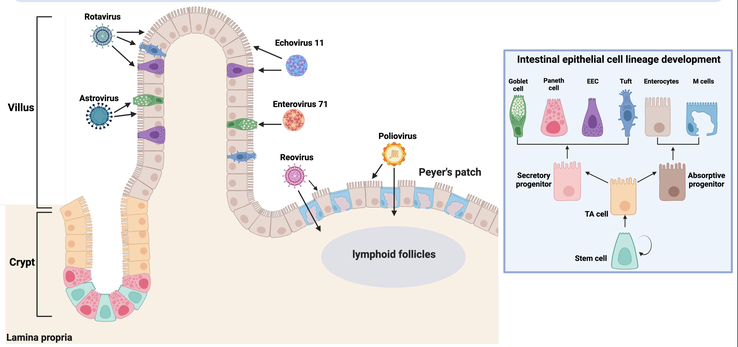
INTRODUCTION

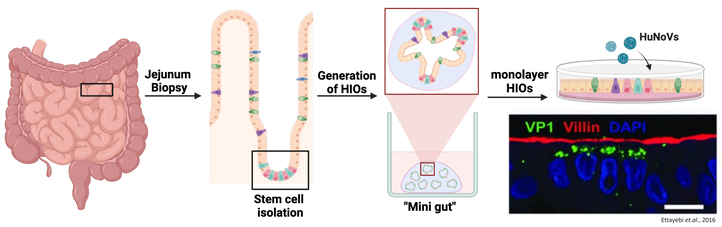
The gut epithelial cells are the targets for many human enteric viruses



Human Norovirus (HuNoV) causes a substantial health burden worldwide

- HuNoV is the leading cause of acute gastroenteritis worldwide in all aged groups
- No FDA-approved vaccine or treatment
- The cellular tropism of HuNoVs is less studied

Human Intestinal Organoid (HIO) is a powerful tool to study HuNoV infections



Question:
What is the cellular tropism of HuNoV?

METHOD

Generating a single cell transcriptomic atlas of HIOs infected with HuNoVs

RESULTS

A unique infection-responding (IR) cluster emerges following HuNoV infection and expands at 24 hours post-infection

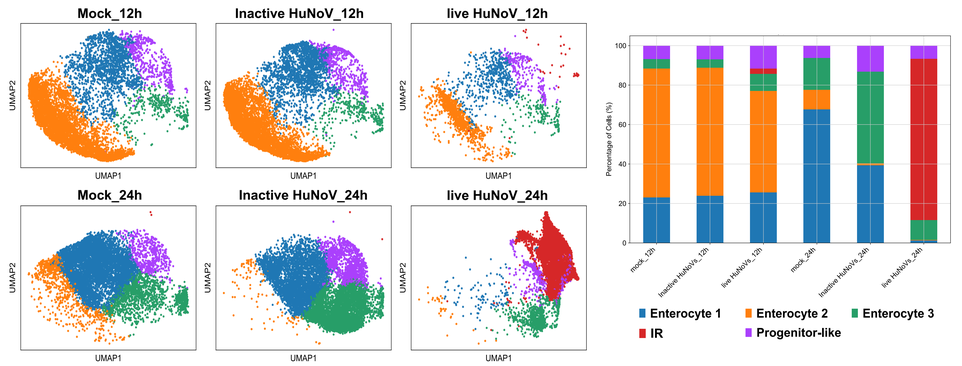


Figure 1. Changes of cluster types in HIOs treated with medium control (MC), or inoculated with inactive HuNoV and live HuNoVs at 12 hours and 24 hours post infection. Cluster identities were determined using intestinal epithelial markers.

High expressions of HuNoV transcripts indicate the IR cluster contains infected cells

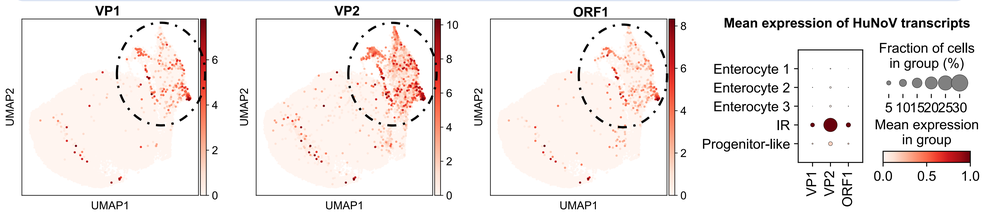


Figure 2. Expressions of HuNoV genes (VP1, VP2, VP3) displayed by UMAPs or the Dotplot. The IR cluster is highlighted in the black circles on the UMAPS.

HuNoV infection increases the expression and activity of the tuft cell transcriptional factor *POU2F3* in the Progenitor and IR cluster

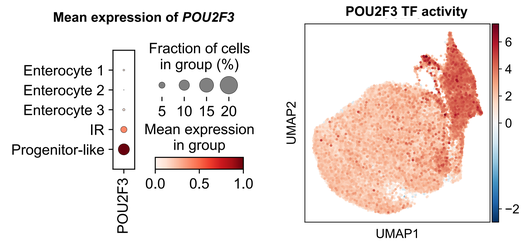


Figure 3. The expression of *POU2F3* by different treatments and the transcriptional factor (TF) activity of *POU2F3* displayed by the UMAP.

The **first evidence** showing HuNoV infection induces *POU2F3* expression, suggesting tuft cell expansion in HIOs!

Co-localization of *POU2F3* and viral transcripts in the IR cluster indicate HuNoV infects tuft cells

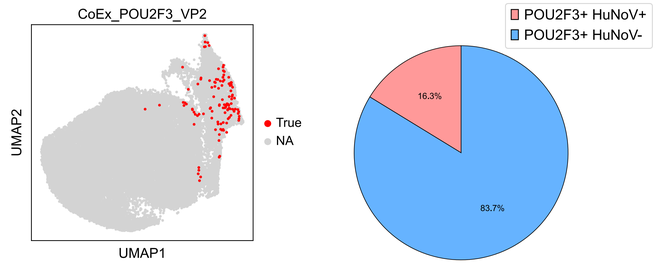


Figure 4. Left: Individual cells expressing both POU2F3 and VP2 transcripts (labeled as True) in the IR cluster

Right: The percentage of infected POU2F3+ cells (Red: HuNoV+POU2F3+) and uninfected POU3F3+ cells (Blue: HuNoV-POU2F3+) in the IR cluster

IL-32 is a key proinflammatory cytokine gene in induced POU2F3+ cells

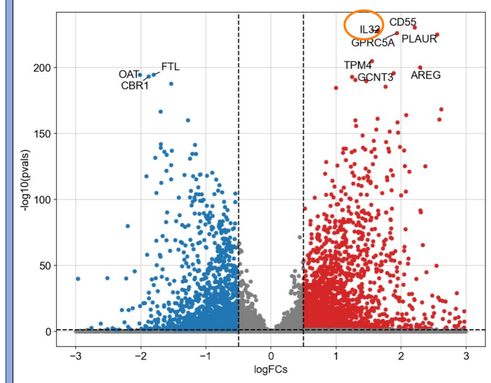


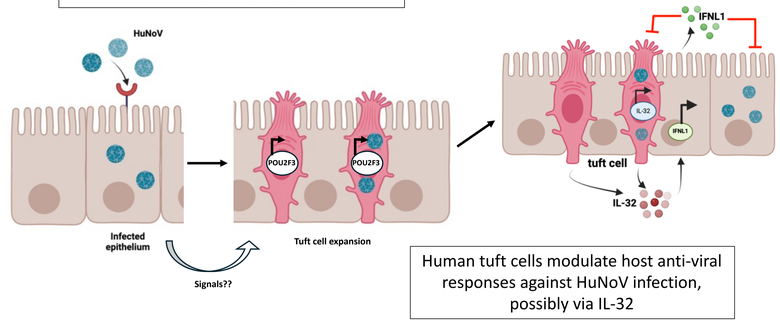
Figure 4. Top 20 DEGs in POU2F3+ cells compared to POU2F3- cells in the IR cluster. IL32 is highlighted in the **orange circle**.

Why IL-32 is interesting?

- IL-32 can modulate IFNL-1 mediated anti-viral responses in many viral infections
- IFNLs have potent antiviral effects on HuNoV infections

Working model: a potential role of human tuft cell in HuNoV infections

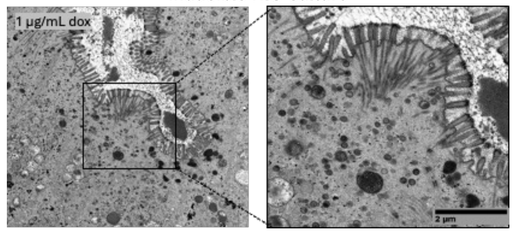
HuNoV infection induces tuft cell expansion in the HIO culture



Human tuft cells modulate host anti-viral responses against HuNoV infection, possibly via IL-32

FUTURE DIRECTIONS

EM confirmation of mature tuft cells in the inducible HIO culture



- We will use genetically modified HIO cultures to establish a human tuft cell model in vitro to further delineate their role in HuNoV infection
- **This work will significantly enhance our understanding of HuNoV tropism and tuft cell biology, informing potential therapeutic strategies for HuNoV infection**

ACKNOWLEDGMENTS

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