Development of an intestinal organoid-based platform for screening antiviral agents

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1 Background

Enteric viruses are a major source of human diseases, particularly in neonates and young children. Despite being a pressing medical problem, very little is known regarding the events associated with the infection with these viruses on the human gastrointestinal (GI) tract. The interaction of such pathogens with the intestinal barrier depends on host-specific mechanisms that affect the success of an active viral infection in vitro. The use of immortalized tumour cell lines, typically limited by the presence of only one cell type, have hampered our understanding of the host/pathogen interactions and the development of novel interventions against the infection with such pathogens. Thus, a more reliable in vitro model is urgently needed to also reduce the work with animal models. Here, we have used human iPSC-derived intestinal organoids (hiPSC-IO) to establish a new platform for antiviral drug discovery. Intestinal organoids not only retain the genetic background of the patients from which they are derived, but also the differentiation of the adult stem cells can be steered towards all the major cell types that populate the GI epithelium, allowing to recapitulate the viral infection in vitro and better predict drug responses.

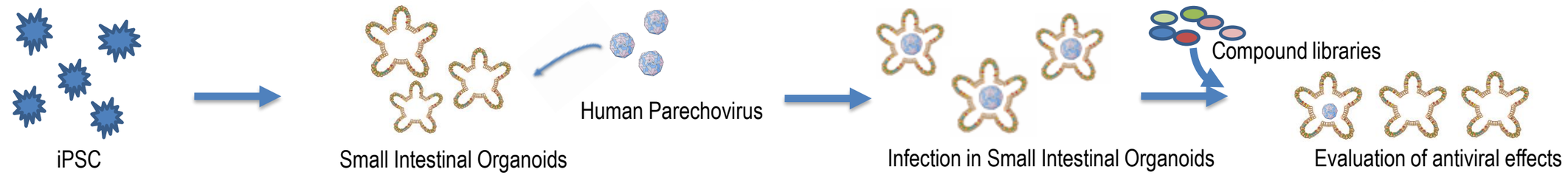

Figure 1. Schematic overview of the project goal.

2 Generation of small intestinal organoids from hiPSC

hiPSC was differentiated into small intestinal organoids using StemCell Technologies STEMdiff™ kit. The hiPSC-IOs were characterized using immunofluorescence and RT-PCR. These organoids were used to study the enteric virus Parechovirus genotype 1 (PeV-A1) infection and the innate immunity response.

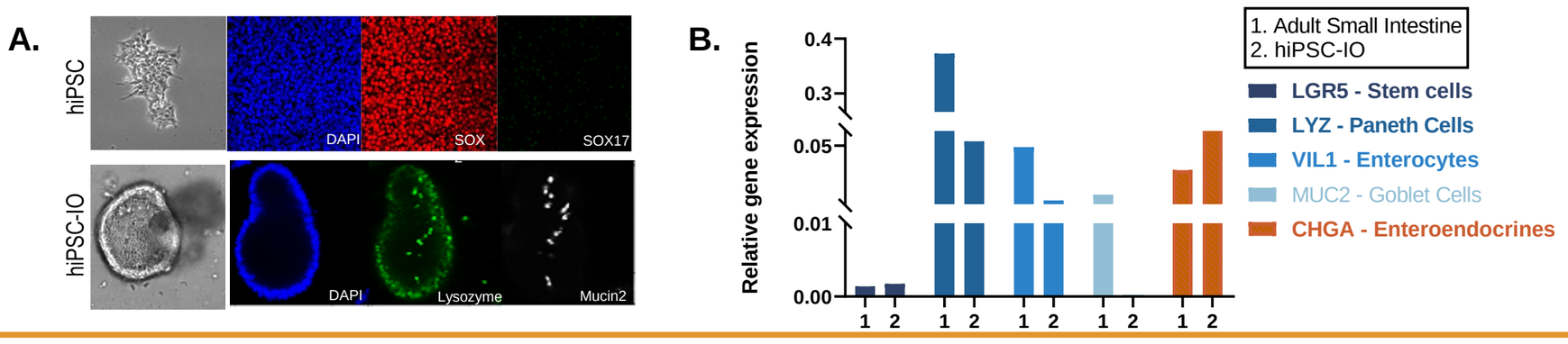

Figure 2. (A) Images represent hiPSCs: Dapi (blue) for nucleus, SOX2 (red) for pluripotency transcription factor, SOX17 (green) for intestinal transcription factor, and differentiated small intestinal organoids: Dapi (blue) for nucleus, Lysozyme (green) for paneth cells, Mucin 2 (white) for goblet cells. **(B)** Relative expression of total RNA of adult small intestine as control and hiPSC-IO. The expression level of each gene was calculated relative to EEF1A1 gene expression.

3 PeV-A1 replication in hiPSC-derived small intestinal organoids

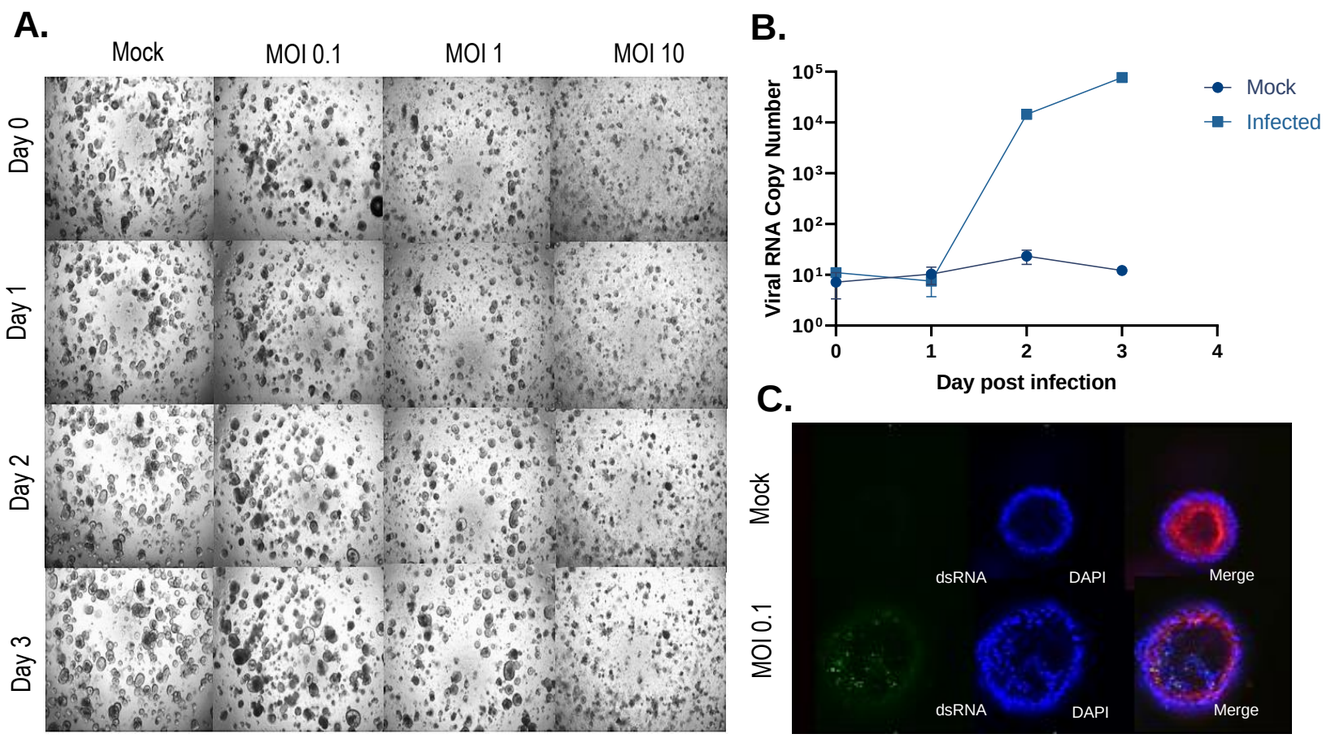

Figure 3. PeV-A1 replication kinetics in hiPSC derived small intestinal organoids. **(A)** Brightfield images of mock and infected organoids at different MOIs. **(B)** Fold changes in viral RNA copies in the supernatant of at MOI 0.1 from day 0. Data represents the mean of two technical replicates. **(C)** Confocal image z-stacks of mock as negative control and infected organoids with MOI 0.1. Expression of actin filaments (phalloidin, red), viral double-stranded RNA (dsRNA, green), and nuclei (DAPI, blue) was examined by double immunofluorescence staining.

hiPSC-IOs provide a model that support PeV-A1 replication and infection. PeV-A1 infection in hiPSC-IOs are depicted by cytopathic effect (CPE) at different multiplicity of infection (MOI), increased viral RNA load, and change in morphology.

4 Comparison of innate immune response in organoids and cell line

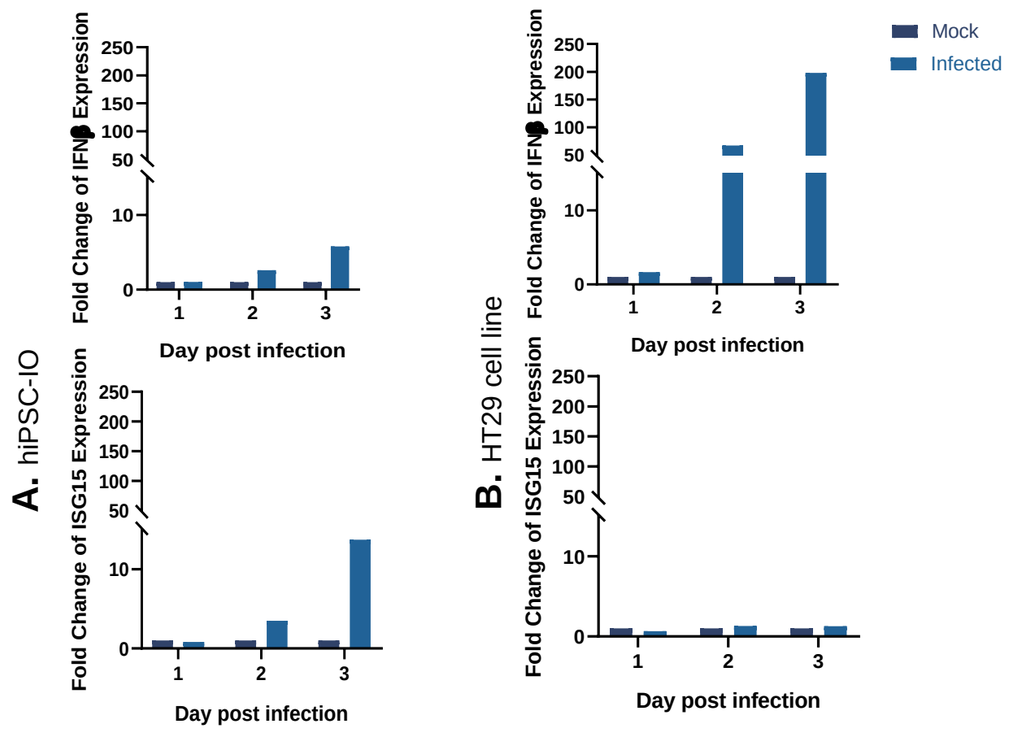

Figure 4. Innate immune response to HPeV1 infection in hiPSC-IO and HT29 cell line. qRT-PCR was used to measure the innate immune response to HPeV1 infection at MOI 0.1 by collecting mRNA of IFNβ and ISG15 **(A)** in hiPSC-IO **(B)** and HT29 cell line. The expression level of each gene was calculated relative to EEF1A1 gene expression and normalized to mock-treated organoids or cells.

hiPSC-IOs show a different expression of IFNβ and ISG15 upon HPeV1 infection compared to PeV-A1-infected HT29 cells. In hiPSC-IO there is mild innate immune response compared to the HT29 cell line.

5 Antiviral compound screen

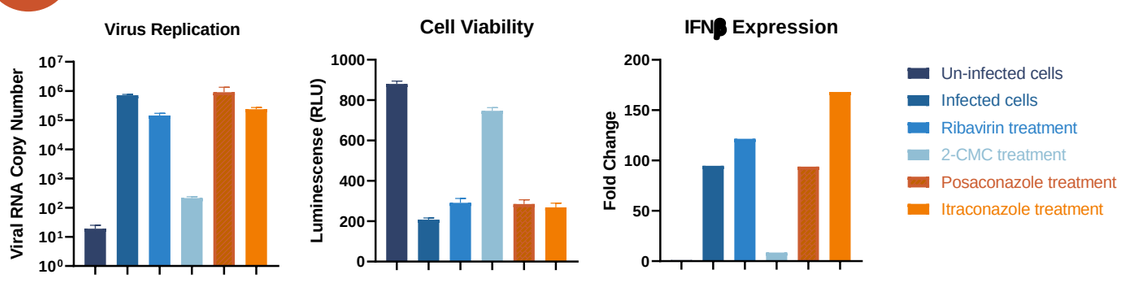

Figure 5. Compound screening for PeV-A1 inhibition in HT29 cell line at MOI 1 on Day 3. qRT-PCR was used to measure the amount of PeV-A1 virus in supernatant. Cell viability was measured using Cell-Titer Glo assay. qRT-PCR was used to measure the expression level of IFNβ and calculated relative to EEF1A1 gene expression and normalized to mock-treated cells.

HT29 cells infected with PeV-A1 were treated with 4 different compounds but only one compound, 2-CMC, has antiviral effect. The inhibition of PeV-A1 infection with 2-CMC is described by lower viral RNA load, higher cell viability, and lower IFNβ expression in infected cells.

6 Conclusions and future directions

- hiPSC are differentiated into small intestinal organoids that are amenable to PeV-A1 infection and replication
- hiPSC-IO show a different innate-immune response to PeV-A1 infection compared to HT29 cells
- hiPSC-IO would allow further study of PeV-A1 infection and reliable antiviral compound screen