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Current Opinion in Virology

Human organoid cultures: transformative new tools for human virus studies

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Studies of human infectious diseases have been limited by the paucity of functional models that mimic normal human physiology and pathophysiology. Recent advances in the development of multicellular, physiologically active organotypic cultures produced from embryonic and pluripotent stem cells, as well as from stem cells isolated from biopsies and surgical specimens are allowing unprecedented new studies and discoveries about host–microbe interactions. Here, we summarize recent developments in the use of organoids for studying human viral pathogens, including intestinal infections with human rotavirus, norovirus, enteroviruses and adenoviruses (intestinal organoids and enteroids), neuronal infections with Zika virus (cerebral organoids) and respiratory infections with respiratory syncytial virus in (lung bud organoids). Biologic discovery of host-specific genetic and epigenetic factors affecting infection, and responses to infection that lead to disease are possible with the use of organoid cultures. Continued development to increase the complexity of these cultures by including components of the normal host tissue microenvironment such as immune cells, blood vessels and microbiome, will facilitate studies on human viral pathogenesis, and advance the development of platforms for pre-clinical evaluation of vaccines, antivirals and therapeutics.

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Current Opinion in Virology 2018, **29**:79–86

This review comes from a themed issue on **Viral pathogenesis**

Edited by **Diane Griffen** and **Harry Greenberg**

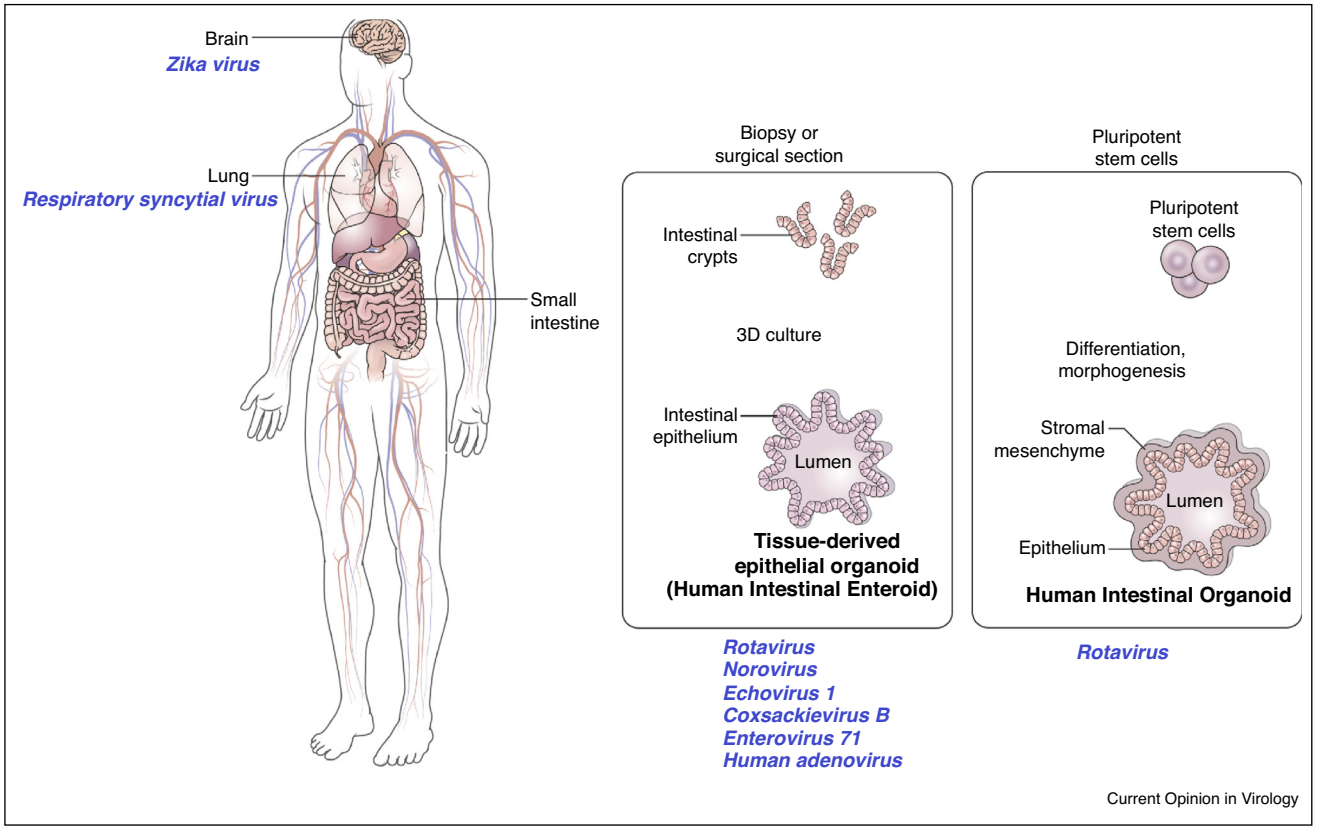
https://doi.org/10.1016/j.coviro.2018.04.001

Introduction

Studies of infectious agents have traditionally focused on *in vitro* culture systems using transformed cell lines and animal models. While these systems have enabled historic progress in the understanding of microbial pathogenesis and host–pathogen interactions, and facilitated the development of vaccines and therapeutics, the physiological relevance of these models for human pathogens and diseases can be limited. Routinely used cell lines are immortalized and cancer-derived and may not adequately reflect responses of normal human cells [1–3]. Many signaling pathways are altered in cancer cells and have profound effects on the core metabolism in cancer-derived lines [4]. Furthermore, cell lines are typically comprised of a single cell type and do not mimic the architecture, environmental complexity and functionality of tissues which are comprised of many different cell types. While some of these drawbacks are overcome in animal models, these have other restrictions for studying human infectious diseases. Many human pathogens display unique human specificity that precludes their study in animals while in other cases, animal models fail to reproduce human pathophysiology. Further, findings and effects seen in animal models do not always translate to humans, as has been observed in many different fields [5–7].

The development of *in vitro* organoid cultures offer remarkable new model systems to study infectious agents and disease pathogenesis [8]. While organotypic cultures comprised of three dimensional (3D) cell aggregates have been in existence for decades, the term organoids now largely refers to self-organizing, propagatable 3D cultures derived from stem cells that recapitulate the organization, functionality, and genetic signature of the specific tissue or organ and host from which they are derived. Organoids can be derived from embryonic and pluripotent stem cells (ESCs and PSCs, respectively) as well as stem cells isolated from specific human tissues. Through new understanding of defined developmental cues and growth factors, stem cells can be directed to grow *ex vivo* into organ-like structures. Unlike transformed cells lines and animal models, organoid cultures are multicellular, reflect the cellular heterogeneity of specific human organs and are physiologically active; thus, they are increasingly being validated as relevant models for studies of infectious disease, particularly of human-specific pathogens. Organoids have been established for multiple organs including the intestine, stomach, esophagus, liver, kidneys, lungs, brain, prostate, pancreas, retina and ovary [8]. Although the use of this new technology is in its infancy, paradigm shifting results and unexpected discoveries have been made with several human viruses (Figure 1)

Figure 1



Human organoids currently used for studies of viral pathogens.

and select data are summarized below. Other recent articles address aspects of organoids for disease studies that are not covered in this review [8–13].

Human intestinal organoids and enteroids

The human gastrointestinal tract is a complex organ with a polarized epithelial layer that contains different cell types including enterocytes, enteroendocrine cells, tuft cells, goblet cells, Paneth cells and stem cells. Distinct regions of the intestine (duodenum, jejunum and ileum, proximal and distal colon) perform unique functions and demonstrate segment-specificity in terms of transport, protein expression and interactions with pathogens [14,15]. The breakthrough in intestinal organoid cultures began with the successful culture of murine epithelial organoids from Lgr5+ intestinal stem cells in 2009, followed by the development of organoids from human PSCs and biopsy samples [16•,17•,18]. Two types of intestinal organoids have been used for virus studies; organoids derived from PSCs, that are epithelial cultures associated with mesenchyme, and human tissue-derived organoids (also called mini-guts) that are epithelial only cultures derived from stem cells isolated from biopsies or surgical tissues. To distinguish epithelial/mesenchymal cultures from epithelial only cultures, the nomenclature organoids and enteroids, respectively, was proposed by the intestinal stem cell consortium in 2012 [19]. The terminology human intestinal organoids (HIOs) and human intestinal enteroids (HIEs) are used henceforth to describe these cultures. Apart from 3D cultures, HIEs can also be plated as monolayers that allow for easy access to apical and basolateral compartments as well as measurement of epithelial barrier function [20]. HIOs and HIEs have been documented to functionally recapitulate normal human gastrointestinal pathophysiology [21], and both 3D and monolayer cultures are being used to study enteric viruses including human rotavirus, norovirus, enteroviruses, and adenoviruses (Figure 1).

Rotaviruses are a leading cause of severe, dehydrating gastroenteritis in young children worldwide, resulting in about 215 000 death annually [22]. Live, attenuated rotavirus vaccines were introduced in 2006 and have significantly reduced the burden of rotavirus disease. However, these vaccines are less effective in developing countries with the greatest disease burden [23]. Both HIOs and HIEs have been used for studying rotavirus infections. Proof-of-principle studies showing utility of organoid cultures for human rotavirus infection was first shown with HIOs [24•]. The replication of 12/13 clinical