Modelling intestinal inflammation and infection using ‘mini-gut’ organoids

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In 2020, major advances to the understanding of gastrointestinal inflammatory and infectious disease have been made using ‘mini-gut’ organoids. Key findings include the discovery of somatic inflammatory gene mutations in ulcerative colitis epithelium, a unique mutational signature in colorectal cancer caused by genotoxic *Escherichia coli*, and infection of intestinal organoids by SARS-CoV-2.

In 2009, 3D organoid culture was first established when single intestinal stem cells (ISCs) were shown to grow and functionally differentiate into self-organizing ‘mini-guts’. Unlike many other 3D organotypic cultures, ISC-derived organoids are purely epithelial. Owing to their unlimited expansion capacity, organoids offer unique opportunities for disease modelling, drug screening and regenerative medicine [Fig. 1]. Over the past decade, a variety of living organoid biobanks have been generated from patients with genetic diseases and cancer. These biobanks are valuable resources for better understanding of human diseases and for personalized medicine. In 2020, three studies have further strengthened our knowledge of inflammation and infection in the gastrointestinal tract using intestinal organoids.

Ulcerative colitis is characterized by chronic inflammation in the large intestine. Patients with ulcerative colitis are predisposed to colorectal cancer (CRC), suggesting that chronic exposure to the inflammatory environment can lead to somatic evolution of neoplastic clones. To understand how inflammation shapes the genetic landscape, Nanki et al. utilized organoids to analyse somatic mutations in the ulcerative colitis epithelium. An organoid biobank was established from 55 patients with ulcerative colitis — 26 of whom had colitis-associated neoplasia — and 16 healthy individuals. Clonal organoids were expanded from single cells and subjected to whole-genome sequencing (WGS) to capture mutations at the clonal level. Analysis of single-nucleotide variants (SNVs) showed that the number of SNVs in ulcerative colitis organoids was slightly higher than in the paired control organoids derived from the uninfamed tissues of the same individuals, whereas the mutational signatures were comparable. The results suggest that inflammation leads to a moderate increase in mutation numbers in ulcerative colitis epithelium. Analysis of functional genetic mutations further revealed that recurrent truncating mutations of genes related to IL-17 signalling — *NFKBIZ*, *PIGR* and *ZC3H12A* — were found exclusively in ulcerative colitis organoids, suggesting a selection for inflammation-specific mutations. CRISPR-based knockout screening of IL-17A-treated organoids resulted in enrichment of *IL17RA* and *NFKBIZ* mutations, confirming that the in vivo-selected genetic mutations confer IL-17A resistance. Interestingly, growth factor screening in these organoids revealed that deficiency of the bone morphogenetic protein (BMP) antagonist Noggin sensitized organoids to IL-17A cytotoxicity via inducible nitric oxide synthase (iNOS). As iNOS is highly expressed at the mucosal surface of ulcerative colitis tissues, the results imply that chronic inflammation in patients with ulcerative colitis activates IL-17 signalling, which triggers iNOS-dependent apoptosis at the superficial layer of the intestinal epithelium, where BMP antagonists are absent. This process subsequently drives a pattern of somatic evolution and expansion of IL-17-resistant mutant clones to protect the ulcerative colitis epithelium from IL-17-mediated apoptosis. This study elegantly demonstrates how organoids can be applied to interrogate complex somatic evolution in ulcerative colitis tissues to adapt to the chronic inflammatory microenvironment. Indeed, a parallel study, also published in 2020, sequenced ulcerative colitis and non-ulcerative colitis crypts isolated directly from patients and similarly reported the same positive selection of mutant clones that converge on the *NFKBIZ* pathway. Together, these studies suggest that organoids are reliable disease models for genomic and functional studies of human gastrointestinal diseases.

Healthy organoids can also be used for disease modelling by exposing organoids to pathogens. This year, in an insightful study, Pleguezuelos-Manzano et al. demonstrated how organoids can be used to probe the role of the intestinal microbiota in CRC. A genotoxic *Escherichia coli* strain carrying a 50-kb hybrid polyketide-norribosomal peptide synthase operon (*pks*) produces the genotoxin colibactin, which is known to induce double-strand DNA breaks. *pks*-*E. coli* are present in ~20% of healthy individuals and 60% of patients with CRC, suggesting a link between *pks*-*E. coli* and CRC development. To study this mutagenic property, a *pks*-*E. coli* strain and a *clbQ*-deficient *E. coli* control strain that could not produce active colibactin were microinjected into the lumen of healthy human intestinal organoids. The results showed that exposure of organoids to *pks*-*E. coli* induced double-strand DNA breaks and interstrand crosslinks. To recapitulate long-term exposure of the pathogens in patients, single-cell-derived organoids were repeatedly injected with *pks*-*E. coli* over a period of 5 months, followed by WGS of the subclonal organoids. Organoids exposed to *pks*-*E. coli*, but not to the control strain, showed increased numbers of single-base substitutions (SBS), with a bias towards T>N substitutions that occurred preferentially at the middle bases of ATA, ATT and TTT, as well as a small indel signature characterized by a single T deletion at T homopolymers. Importantly, interrogation of the WGS data from 5,876 human cancers generated from two independent cohorts confirmed the enrichment and co-occurrence of both *pks*-specific SBS and indel signatures predominantly in CRCs. Notably, many driver genes, including the most commonly mutated gene, *TP53*, were found in a subset of CRCs, indicating that *pks*-*E. coli* can act as a mutagenic agent in human intestinal tissue.

**Key advances**

- Evolution of somatic inflammatory gene mutations targeting IL-17 signalling occurs during chronic tissue inflammation in patients with ulcerative colitis.
- A distinct mutational signature caused by exposure to genotoxic colibactin-producing *pks*-*Escherichia coli* is found in a subset of colorectal cancers.
- Active SARS-CoV-2 infection and replication is possible in human and bat intestinal organoids.
A recent study by Zhou et al. [2020] highlighted the importance of gastrointestinal (GI) symptoms in COVID-19 patients. They observed that a significant number of patients showed signs of GI illness, including vomiting and diarrhea, which may have been caused by the SARS-CoV-2 virus. This observation is consistent with previous studies that have suggested the GI tract might be a transmission route for SARS-CoV-2 [Li et al., 2020].

In addition, the authors noted that the virus can infect both human and bat organoids, which are cultured tissue cultures derived from human and bat intestinal cells. This finding suggests that the GI tract could be a key location for viral replication and transmission.

Furthermore, the study by Zhou et al. also showed that the virus can infect differentiated cells in organoids, which might be important for understanding the infection and transmission of SARS-CoV-2 in future pandemics.

In conclusion, the study by Zhou et al. [2020] provides valuable insights into the role of the GI tract in SARS-CoV-2 infection and transmission. It highlights the importance of investigating the viral infection in organoids and understanding the role of the GI tract in future pandemics.