Editorial

Modeling cellular polarity, plasticity, and disease disparity in 4D

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Most cell types are polarized with distinct structural orientations or protein localization patterns that allow cells to perform context-dependent functions in space and time in response to physiological regulation (Forte and Yao, 1996). Epithelial cell polarity is characterized by cells with apical and basolateral membrane domains, and the establishment and maintenance of cell polarity are crucial during development to specify cell fate and functions, e.g. epithelial cells undergo dynamic renewal to orchestrate the tissue homeostasis in gastrointestinal tracts for digestive physiology (Yao and Forte, 2003). However, the molecular mechanisms underlying the dynamic lineage specification and heterogenetic cell fate decision in epithelial tissues have remained elusive until the identification of leucine-rich repeat-containing, G-protein-coupled receptor 5 (Lgr5) epithelial stem cells (Sato et al., 2009). Since then, Clevers and colleagues have made paradigm shift discoveries in epithelial cell linage specification and fate determination using the mini-gut epithelial organoids comprising $Lgr5^{+}$ stem cells and all types of differentiated lineages (Sato et al., 2009; Barker et al., 2010).

Development of 3D organoids-like structure *in vitro* can be dated by the initial engineering stratified squamous epithelial colonies by Rheinwald and Green (1975). Modern organoids, expressing self-renewable and other differentiated lineages, are derived from either pluripotent (embryonic or induced) or adult stem cells from various organs (Sato and Clevers, 2013). The molecular delineation of organoid formation thus provides valuable information about the mechanisms underlying human organogenesis, tissue regeneration, and tumorigenesis, highlighting their value for mechanistic study of fundamental biology in addition to their potential application in precision medicine. Although a decade of exciting progresses in modeling organoids might leave the impression that most of the important mechanisms underlying cellular polarity and plasticity regulation have already been discovered, many fundamental questions of organoid plasticity and regulation remain to be explored. In this special issue, we collect one Perspective, four Reviews, and four research Articles (with one Highlight essay), which are related to better understanding mechanisms of action underlying cell polarity, plasticity, and cell fate decision, ranged from studying rare cell types in 3D organoids to endodermal organ development, disease modeling, chemical biology, and cellular dynamics of organoid plasticity control.

One of the characteristics of 3D organoids is the power to recapitulate the stem cell lineage and mimic the differentiated cell type heterogeneity of the *in vivo* tissue of origin. Clevers and colleagues have pioneered the establishment and modeling of cellular physiology in organoids (Sato and Clevers, 2013). However, homeostatic maintenance and enrichment of rare cell types remain bottleneck for mechanistic analysis. In this issue, using intestinal organoids as a model, Clevers and colleagues highlight their recent advances and methods used to enrich for specific cell types including stem cells, enterocytes, Paneth cells, goblet cells, micro-fold (M)-cells, tuft cells, and enteroendocrine cells in intestinal organoids. They envision how these new cell type-enriched intestinal organoids can be applied to uncover mechanisms of action in rare cell physiology and pathogenesis.

Germ layers formed in the earliest stages of embryonic development consist of the endoderm, ectoderm, and mesoderm. They form during the process of gastrulation and represent some of the first lineage-specific stem cells in embryonic development. Hence, each germ layer eventually gives rise to certain tissue types in the body. Here, Gao and colleagues summarize the advances of the technology for generating various organoids of tissues from the three germ layers and

discuss the prospects for tissue engineering. In addition, they propose to engineer current 3D organoids with different parenchymal cell types, blood vessels, and the nervous and immune systems to provide real physiologic setting mimicking *in vivo* environment in humans.

Endoderm gives origin to epithelial tissues including the lining of the gastrointestinal, urinary, and pulmonary tracts. Using pancreatic cell lineage development as a model system, Chen et al. (2009) had harnessed the differentiation of endocrine, exocrine, and ductuall cells from Pdx1-expressing cells. In this issue, they compare and contrast current strategies for generating human pluripotent stem cell (hPSC)-derived endodermal organoids. In addition, they discuss how to consolidate organoid models with genome editing and bioengineering to build robust and powerful platforms for understanding human organ development and crosstalks using 3D hPSC-organoids.

Organoids provide a powerful model not only for studying mechanisms of action during human development but also delineating the molecular paradigm during tumorigenesis and progression. Gastroesophageal cancers are leading causes of cancer death and underlying mechanisms for disease formation and progress are complex. Here, Mills and colleagues highlight modeling gastroesophageal cancers using 3D organoids. In addition, they elaborate how this exciting technology can guide the discovery of paradigm underlying carcinogenesis and thus revolutionize cancer patient care in the clinics.

The mammalian liver possesses a remarkable regenerative ability and detoxication physiology. Thus, the establishment of a long-term 3D organoid culture system for primary hepatocytes and ductual cells is significant. Hui and colleagues directly reprogrammed human hepatocytes with inactive p53 and RB to establish organoids possessing liver architecture and function. Their analyses show that excessive contacts between mitochondrion and endoplasmic reticulum prime hepatocellular carcinoma initiation (Sun et al., 2019), highlighting the importance of dynamic interaction of organelles that is essential for liver development and regeneration. In this issue, Hui and colleagues update the progress in modeling human liver organoids and envision organoid–organoid communication among the liver, gallbladder, and pancreas. On the other hand, the liver expresses heterogenetic lineages of many epithelial cells. Here, Zhao and Lin laboratories employed small-molecule probes to harness the signaling mechanisms underlying the plasticity control of liver organoids *in vitro*. Their studies show that Wnt/β-catenin, NMII–Rac, and PKA–ERK are core signaling pathways essential and sufficient for mouse liver progenitor expansion. The excitement ahead is to identify molecular switches to generate a palette of liver organoids rich in hepatocytes or ductual cells for better understanding of systems biology of the liver and for chemical biological interrogation of viral infection such as SARS-CoV-2 (Zhao et al., 2020).

Precision oncology aims to adapt treatment decisions to the molecular and genetic characteristics of an individual tumor, thereby increasing the chance of a successful outcome. The cancer genome atlas (TCGA) projects have uncovered many context-dependent genomic codes, which offers opportunities to reveal protein–protein interactions for functional and therapeutic significance (Cancer Genome Atlas Research Network, 2014). Fu and colleagues previously singlehanded a line of exciting discovery to uncover cancer-centered protein–protein interaction network, termed OncoPPi (Li et al., 2017). Here, to harness OncoPPi for precision therapeutics, they developed an ultra-high throughput screen in a 1536-well plate containing 3D organoids and demonstrated its robust performance for large-scale library compound screening. This provides a unique stepping stone to pinpoint and rewire context-dependent OncoPPi for precision drug discovery.

Polycystic kidney disease (PKD) is a prevalent genetic disorder, characterized by the formation of kidney cysts that progressively lead to kidney failure. However, the underlying mechanism of action remains elusive, while the current clinic regime is not well tolerated. As shown in this issue, Peters and colleagues developed 3D organoids from PKD patients for a phenotype-based screen. Significantly, celastrol, a triterpenoid from *Tripterygium wilfordii*, was identified as a potent inhibitor of 3D cyst growth *in vitro*, which was validated by a mouse model for PKD. It would be exciting to see how medicinal chemistry further optimizes the therapeutic efficacy of celastrol while functional analyses identify the effectors of celastrol in PKD.

Visualization of specific molecules and their interactions in real space and time is essential to delineate how cellular plasticity is achieved and orchestrated, as perturbation of cellular dynamics is detrimental to health. Given the prevalence of chromosome instability phenotype in gastric tumorigenesis (Yao and Smolka, 2019), Liu and colleagues developed a strategy to model context-dependent cell division, using a combination of light sheet microscope and 3D gastric organoids, and present in this issue. High-resolution light sheet microscopic image analyses of 3D organoids show that CENP-E inhibits cells undergoing aberrant metaphase–anaphase transition and exhibiting chromosome segregation errors during mitosis. Using correlative light and electron microscopic analyses, they show that CENP-E-mediated PRC1 assembly to the central spindle constitutes a temporal switch to organize dynamic kinetochore microtubules into stable midzone arrays. As Chen and colleagues highlighted, this study suggests that metazoans evolved an elaborate multicellularity to ensure accurate cell division and renewal control for epithelial tissue homeostasis, because CENP-E only emerges in metazoans.

Coronavirus disease 2019 (COVID-19) is a pandemic that has caused significant morbidity and mortality worldwide. So far, there are no clinically effective strategies for curing or preventing COVID-19. Using intestinal organoids, Clevers and colleagues have established a powerful platform and toolkit to delineate the coronavirus infection and the molecular mechanisms underlying the vulnerability and disease disparity in different racial populations (Lamers et al., 2020). Given the fact that SARS-CoV-2 interrogates host epithelial cell entry into mitosis (Bouhaddou, et al., 2020), it would be exciting to apply strategies reported here and examine how SARS-CoV-2 hijacks

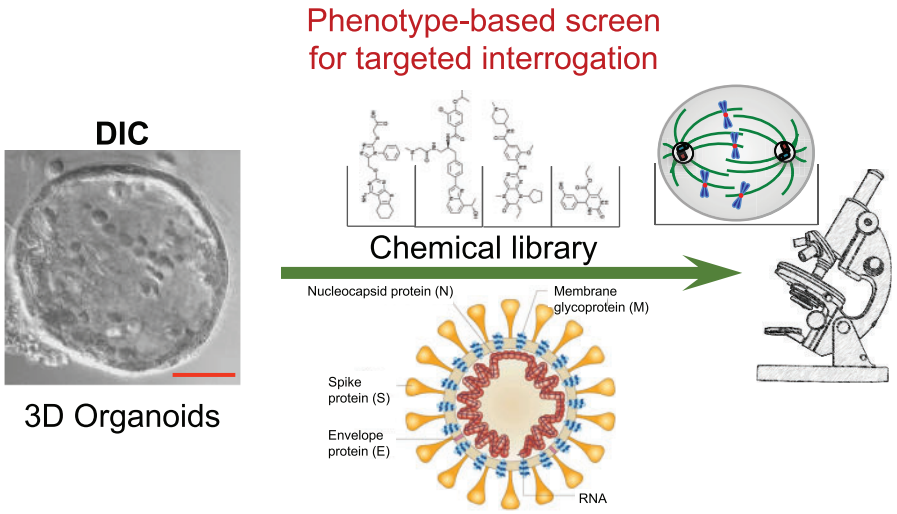

Figure 1 Modeling cellular polarity, plasticity, and pathogen–host interactions in 3D organoids. 3D organoids, shown here with phase contrasted image, provide a physiological model to delineate molecular mechanisms underlying cellular dynamics and plasticity control of self-renewal stem cells and differentiated cell lineages in response to pathogens such as SARS-CoV-2. The dynamic interactions between pathogen and host cells in space and time can be used for chemical screen of targeted interrogation. Scale bar, 50 μm.

mitotic machinery for host cell fate decision (Figure 1). There is no doubt that consolidation of protein–protein interaction network and circuitry combined with illumination of molecular dynamics in context-dependent organoids will enable us to delineate the molecular mechanisms underlying pathogen–host cell interaction, host cell polarity, plasticity, and disease disparity in space and time.

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