



Defense of COVID-19 by Human Organoids

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Abstract

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has created an immense menace to public health worldwide, exerting huge effects on global economic and political conditions. Understanding the biology and pathogenesis mechanisms of this novel virus, in large parts, relies on optimal physiological models that allow replication and propagation of SARS-CoV-2. Human organoids, derived from stem cells, are three-dimensional cell cultures that recapitulate the cellular organization, transcriptional and epigenetic signatures of their counterpart organs. Recent studies have indicated their great values as experimental virology platforms, making human organoid an ideal tool for investigating host–pathogen interactions. Here, we summarize research developments for SARS-CoV-2 infection of various human organoids involved in multiple systems, including lung, liver, brain, intestine, kidney and blood vessel organoids. These studies help us reveal the pathogenesis mechanism of COVID-19, and facilitate the development of effective vaccines and drugs as well as other therapeutic regimens.

Keywords COVID-19 · Human organoids · SARS-CoV-2 infection · Multi-organ damage · Drug discovery

Introduction

In December 2019, a novel beta-coronavirus which causes coronavirus disease-19 (COVID-19) was identified as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (Randhawa et al. 2020; Lu et al. 2020; Claude-Rosny 2020). This bat originated virus crossed species to infect human, transmitted from person to person (Lu et al. 2020). SARS-CoV-2 infected patients have clinical manifestations ranging from asymptomatic infection, mild influenza like symptoms to severe lung injury (Dai et al. 2020; Gao et al. 2020). Yet

symptoms are noted in a wide range of other organs, such as heart, brain, lung, liver, kidney, intestine and coagulation system, accompanying symptoms like fever, cough, myalgia, fatigue, odynophagia, and dyspnea (Meng et al. 2021; Norma and Raul 2020; Wang et al. 2020a; Xiong et al. 2020; Yang et al. 2020a). This pandemic has caused over 157 million people infected and more than 3 million deaths worldwide as of 9 May 2021 (WHO 2021).

The establishment of robust infection models is essential for investigating COVID-19. Transformed immortalized cancer-derived cell lines are one of the most commonly used types of in vitro models to study viral infections. For instance, Caco-2 (colorectal adenocarcinoma), Calu-3 (lung adenocarcinoma), HEK293T (embryonic kidney), Vero (monkey kidney epithelial cells) and Huh7 (hepatocellular adenocarcinoma), have all been applied in SARS-CoV-2 infection experiments (Takayama 2020). However, the architectural complexity and physiological functionality of tissues could not be properly mimicked in those cell lines consisting of just one cell type. In addition, low titer of SARS-CoV-2 is often generated within cell lines due to potential absence of the target cell type (Takayama 2020; Ramani et al. 2018).

Mouse model, as a classical animal model, is generally utilized to study emerging human viral diseases. In most

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cases, genetical modification must be made prior to inoculation with human pathogenic virus, because virus needs an adaptation to cross specie-barrier due to species-restricted tropism. This also applies to SARS-CoV-2 (Johansen et al. 2020). Several studies have reported that SARS-CoV-2 uses the SARS-CoV receptor angiotensin I converting enzyme 2 (ACE2) for host cell entering, and transmembrane serine protease 2 (TMPRSS2) for viral spike (S) protein priming (Burgueno et al. 2020; Hoffmann et al. 2020) (Fig. 2B). Interestingly, SARS-CoV-2 was found to interact with ACE2 proteins in a wide range of species except for mouse (Bao et al. 2020). To overcome this barrier, Bao et al. (2020) used transgenic mice expressing human ACE2 for SARS-CoV-2 infection, and found that the infected mice showed weight loss and interstitial pneumonia caused by virus propagation in lung, making this genetically modified mouse line a sound model for COVID-19 studies. Furthermore, some large animals, including ferrets and cats which are reported as highly susceptible to SARS-CoV-2, have also shown great potentials to be candidate animal models for evaluating the efficacy of antiviral drugs or vaccines against COVID-19 (Shi et al. 2020).

Although animal models maintain the advantage of vividly exhibiting the internal complex physical conditions, it is still challenging to address human-specific physiological and pathological events, not to say the laborious and technically difficult methodologies when performing *in vivo* experiments and the ethical concerns that they pose (Kim et al. 2020a). To accelerate the development of effective treatments for COVID-19, it is urgent to establish optimal *in vitro* models that faithfully resemble human physiology to complement existing animal models and two-dimensional (2D) cell lines.

Human organoids are emerging tools that allow modeling of viral pathogenesis (Dutta et al. 2017), promising for progressing the understanding of SARS-CoV-2 biology. Because of the advent of stem cell technology over last decades, human organoids derived from pluripotent stem cells (PSCs) or adult tissue stem cells (ASCs) can faithfully recapitulate structural organization and functionality of their *in vivo* organ counterparts in many aspects. Organs involved in multiple systems, including lungs, brain, intestine, stomach, esophagus, liver, pancreas, prostate, kidneys, retina, ovary, etc. (Dutta et al. 2017), have been so far successfully established with corresponding organoids which are readily infected with SARS-CoV-2 as a viral platform. In early 2020, Zhao et al. reported the first human organoids infected with SARS-CoV-2. Using the human liver ductal organoid infection model, Zhao et al. identified the virus-targeted human-specific ACE2⁺/TMPRSS2⁺ population of cholangiocytes, and revealed the fact that SARS-CoV-2 caused liver damage through direct entry and attacking liver tissue (Zhao et al. 2020). Plentiful studies that deploy various

human organoids to examine SARS-CoV-2 propagation and pathogenesis are reported subsequently.

Here we have reviewed the reported SARS-CoV-2 infection models based on human organoids to better understand the interaction between coronavirus and human (Fig. 1). We also give perspectives on the application and future development of human organoid, a powerful virology research platform, for realizing its potentials in fighting against COVID-19.

The Respiratory System

As a typical type of airborne virus, SARS-CoV-2 normally makes an entry into human body through the airway of respiratory system which turns out to be the initial viral infection target, and causes pneumonia and lymphopenia in infected patients (Morawska and Cao 2020). Lung is considered as the core hub center for SARS-CoV-2 colonization, propagation and transmission of the whole body (Fig. 2A). The clinical manifestations differ from mild, self-limiting upper respiratory tract infection symptoms to acute respiratory distress, even cardiopulmonary arrest and death (Melley et al. 2020). Particularly, lung is the major target of SARS-CoV-2, and lung disease is the major cause for mortality. Acute respiratory distress syndrome (ARDS), which is recognized as the most severe form of lung injury caused by SARS-CoV-2, is thought to emerge in the setting of COVID-19 pneumonia, while epithelium of the distal lung is being gradually infected (Huang et al. 2020a; Zhu et al. 2020; Hou et al. 2020; Qi et al. 2020).

The human adult lung is made up of two zones: conducting zone (airways) and respiratory zone (alveoli) (Weibel 1963). The conducting zone is composed of a system of highly branched epithelial tubes, including the trachea, bronchi and bronchioles. The major functions of the conducting zone are to bring air into the alveoli, to humidify the incoming air, and to expel foreign particles via the mucociliary escalator to protect the lung. The airway epithelium contains a combination of cell types, including ciliated, club, goblet, basal and neuroendocrine cells. As the destination of the bronchioles, alveoli are found deep inside the lung where gas exchange takes place between the air and the blood (Ochs et al. 2004). The alveoli epithelium is composed of alveolar type 1 (AT1) and alveolar type 2 (AT2) cells. AT1 facilitates gas exchange with the associated vasculature, and AT2 maintains dual roles for acting both as the facultative progenitors and as surfactant secretory cells to enable alveoli expansion and prevent collapse (Barkauskas et al. 2013). Furthermore, AT2 predominantly expresses ACE2 which is host cell surface receptor for SARS-CoV-2, making it a potential target for viral attachment and infection (Katsura et al. 2020).

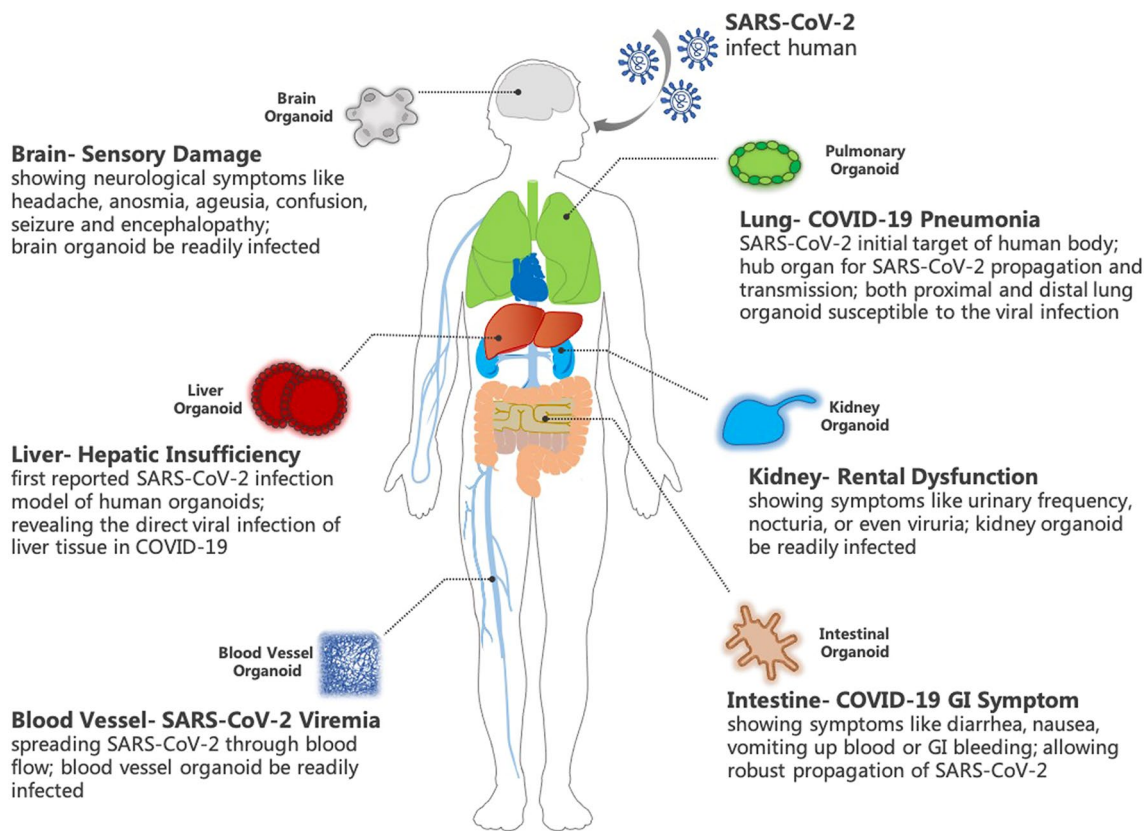


Fig. 1 Human organoids modeling SARS-CoV-2 infection in COVID-19. Human organoids are associated with a wide range of human body counterparts, including respiratory system organ-lung, digestive organs-liver and intestine, nervous system organ-brain, as well as other organs such as blood vessel, kidney, etc. Human organoids

are readily infected with SARS-CoV-2, exhibiting great potentials as a viral platform to be applied for revealing the pathogenesis mechanism of COVID-19 and facilitating the development of effective vaccines and drugs

Pulmonary 3D cultures can be efficiently derived from isolated human embryonic stem cells (Nikolic and Rawlins 2017), or adult lung tissue stem cells (Butler et al. 2016; Rock et al. 2009; Mou et al. 2016; Sachs et al. 2019) including airway secretory (club) cells and AT2 cells (Barkauskas et al. 2013). Lung organoids have potential applications in the studies of lung disorders or diseases. Due to COVID-19 pandemic, organoid models to study human airways have become increasingly important for drug and vaccine development.

In 2017, Chen et al. developed a method which can directly differentiate hPSCs into definitive endoderm (DE) and anterior foregut endoderm (AFE) to form lung bud organoids (LBOs) (Chen et al. 2017). Compared with alveolar and airway spheroids that specifically represent a defined regional space of the lung, both proximal and distal cell populations can be detected in LBOs in vitro (Strikoudis et al. 2019). LBOs mainly contain the fully functional AT2 cells which can uptake and secrete surfactant actively, approximating the human second trimester of gestation at transcription level.

Cells from different parts of adult human lung tissue can also be used for 3D culture. Primary cells can be isolated and cultured directly in extracellular matrix (ECM) (Rock et al. 2009; Tata et al. 2013). The methodology for basal cell-derived organoid generation is a two-stage approach, in which cells are maintained under 2D culture condition for a period of time to elevate basal cell ratio before transferring to 3D. Genetic manipulation, which are used to select and clone specific mutants, can readily be performed at the 2D stage. Isolated single-cell suspensions are then seeded into 3D culture in vitro, with or without feeder cells (Barkauskas et al. 2017). Human Basal cells make up ~30% of the pseudostratified mucociliary epithelium, lining most of the conducting airways of the human lung and the trachea. Basal cell-derived organoids have been used for high- and medium-throughput screens (Tadokoro et al. 2014). Organoids from human basal cells have been used to screen for cytokines and other proteins that affect the ratio of ciliated and secretory cells, and therefore might be potential therapeutic agents for disorders in which the balance is disrupted (Whitsett and Alenghat 2015). The club cell is generally

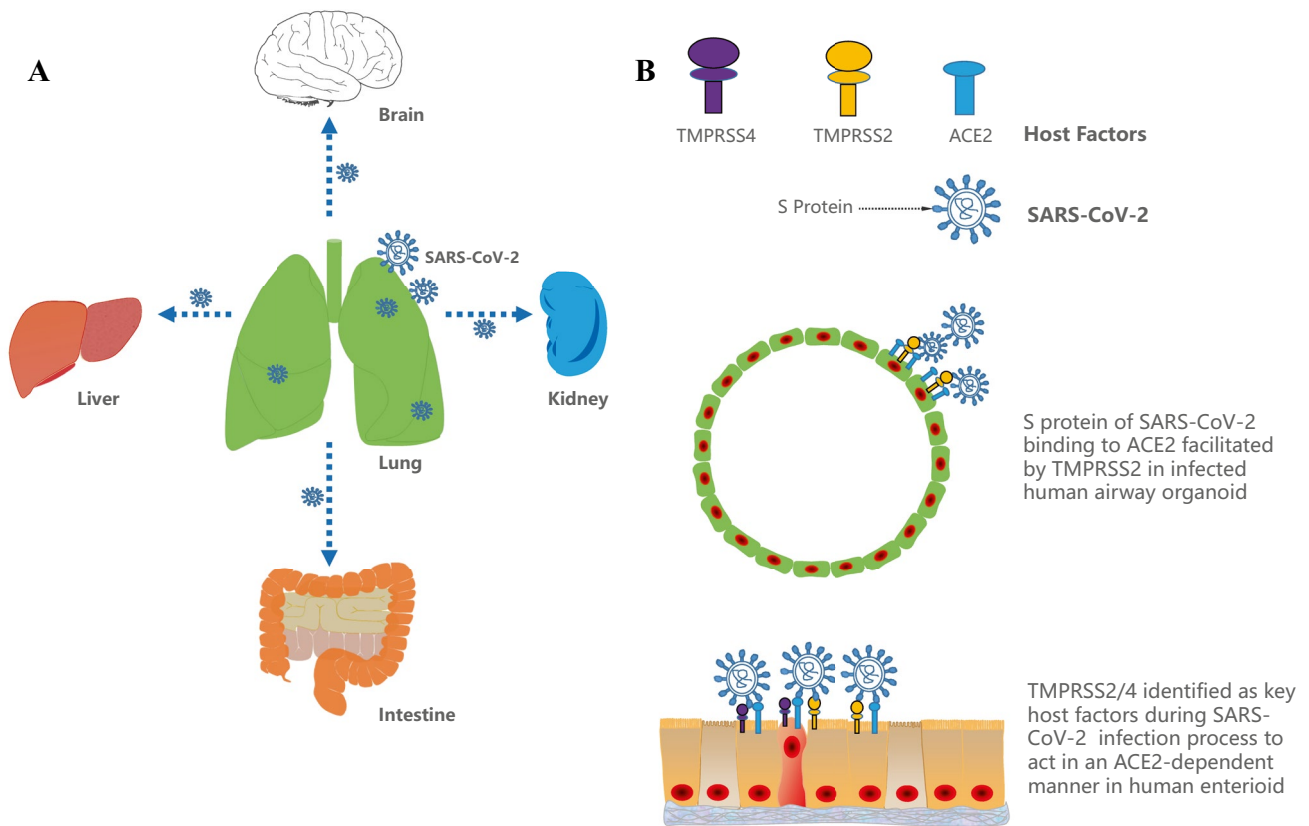


Fig. 2 The entry mechanism and transmission path of SARS-CoV-2. **A** SARS-CoV-2 infects lung as the initial target, are then quite probably spread through blood circulation to other parts of the human body, including brain, kidney, liver and intestine. **B** The recognition

and entry mechanism of SARS-CoV-2: SARS-CoV-2 uses ACE2 for host cell entering and TMPRSS2 for viral spike protein priming, and TMPRSS4 is identified as a novel SARS-CoV-2 host factor, like TMPRSS2, to facilitate viral entry into intestinal epithelial cells

known as the second stem cell in the airway epithelium, retaining potentials of regeneration and differentiation depending on their location on the tracheal–bronchial axis (Rawlins et al. 2009). Attempts to use club cells to generate bronchioalveolar spheroids rely exclusively on co-culturing with underlying stromal populations. Currently isolated club cells could not form into spheres, unless when co-cultured with other cell types such as endothelial cells or fibroblasts population (Teisanu et al. 2011). Human AT2s can be specifically isolated using HTII280 antibody to identify HTII280⁺ cells in both normal and diseased lung tissues (Gonzalez et al. 2010). Isolated AT2 cells can be adopted to generate alveolospheres through co-culturing with some necessary feeder cells (McQualter et al. 2010). Either AEC2-derived alveolospheres or club cell-derived bronchioalveolar spheroids rely on feeder cells during their generation process with existed protocols, which implies the pressing need for a more advanced practicable culture system.

Katsura et al. recently reported a feeder-free, long-term and chemically-defined modular culture system to generate alveolosphere from human lung tissue primary AT2

cells. Alveolosphere generated using this method could be infected with SARS-CoV-2, which led to the loss of surfactant protein secreting ability, cell apoptosis and the production of inflammatory responses mediated by Type I (IFN-I) and Type III (IFN-III) interferons within organoids. In this study, they also found that to pretreat alveolosphere with low dose IFNs could reduce viral replication (Katsura et al. 2020). Salahudeen et al. developed two types of human distal lung organoids: AT2 organoids derived clonally from adult human single AT2 cells which exhibits AT1 trans-differentiation potential, and basal cell organoids derived from KRT5⁺ basal cells which maintain lumens lined by club and ciliated cells. Using the established pulmonary organoid culture system, they discovered the SCGB1A1⁺ club cell as a novel SARS-CoV-2 target in distal lung (Salahudeen et al. 2020).

In the work by Han et al., a high-throughput drug screen performed using hPSCs-derived lung organoid identified imatinib, mycophenolic acid and QNHC as inhibitors of SARS-CoV-2 for significantly reducing viral RNA replication and weakening SARS-CoV-2 infection (Han et al. 2021;

Pei et al. 2020). Huang et al. developed hPSC-derived AT2 organoid and 2D air–liquid interface (ALI) lung cultures using the technique of directed differentiation, and then stimulated the initial apical infection of alveolar epithelium with SARS-CoV-2. Drug screening using this model has also been described (Table 1) (Huang et al. 2020b). Tiwari et al. revealed a tissue-specific SARS-CoV-2 infection and host responses using PSC-Derived lung organoids, and identified camostat and EK1 peptides as effective inhibitors for COVID-19 (Tiwari et al. 2021). Using PSC-derived human alveolar organoids, Wang et al. discovered that Cholesterol 25-Hydroxylase could inhibit SARS-CoV-2, as well as other coronaviruses, by depleting membrane cholesterol (Wang et al. 2020b). Samuel et al. built a SARS-CoV-2 infection model with hESC-derived human lung organoids to investigate the infection mechanism, implying the crucial role of androgen signaling in regulating SARS-CoV-2 receptor Levels (Samuel et al. 2020).

Despite the extensive applications, it is undoubtedly that the hPSCs/hESCs-derived organoids have inherent defects, such as their low efficiency, feeder dependence and fetal-like gene expression mode (Aurora and Spence 2016). Using human lung adult stem cells, Mulay et al. used two types of lung organoids to develop infection models: the differentiated proximal airway epithelium culture of ALI and 3D alveolar organoids. The differentiated proximal airway epithelium is pseudostratified mucociliary epithelium mainly composed of the four epithelial cell types—club cells, basal cells, ciliated cells and goblet cells. In 3D alveolar organoids, abundant expression of AT2 Marker-HTII-280 could be detected. Both types of organoids were used as tools to screen effective drugs against SARS-CoV-2, with the screening results indicating that remdesivir strongly suppressed viral infection or replication (Mulay et al. 2020). Huang developed airway organoids from human bronchial/tracheal epithelial cells, and confirmed that the humanized COVID-19 decoy antibody could effectively block viral entry and prevent SARS-CoV-2 infection (Huang et al. 2021). Lamers et al. generated an organoid-derived bronchioalveolar model for SARS-CoV-2 infection of human alveolar type II-like cells, and proved the inhibitory effects of interferon lambda 1 using this model (Lamers et al. 2021). There are stills plenty of other studies using ASC-derived human lung organoids to investigate SARS-CoV-2, revealing the infection mechanisms and mining the therapeutics for COVID-19 (Lamers et al. 2021; Mykytyn et al. 2020; Li et al. 2020).

Although these established organoids derived from primary human lung tissues have partly mimicked the attack of SARS-CoV-2 on lung epithelium, they still cannot fully represent the pathogenesis process because of incomplete cell-type composition compared to the epithelium of physiological lung. Tindle et al. presented a scalable, long-term, cost-effective human lung organoid culture system derived

from adult stem cell completing with both proximal and distal airway epithelia. Proximal-predominant monolayers and distal-predominant monolayers infected by SARS-CoV-2 respectively could be achieved through different differentiation patterns. They found that proximal cells were critical for recreating sustained viral infectivity, and the differentiation from AT2 to AT1 in distal alveolar was essential to emulate host response (Tindle et al. 2020).

In addition to these established lung organoids described above, the upper respiratory tract relevant organoids have also been established. Han Kyung *et.al* developed tonsil organoids and nasal cavity organoids as *ex vivo* models for SARS-CoV-2 infection (Kim et al. 2020b). Various organoids prove as valuable resources to screen for potential drugs that might be repurposed and considered for COVID-19 clinical trials.

The Digestive System

Up to 50% of COVID-19 patients show gastrointestinal symptoms such as nausea, vomiting or diarrhea, even sometimes before developing fever, and lower respiratory tract signs and symptoms linked with prolonged disease duration and increased severity (Luo et al. 2020; Wang et al. 2020c; Wei et al. 2019; Xiao et al. 2020). Of note, that liver damage was reported as a co-existed predominant symptom in patients with COVID-19 has attracted the attention of the researchers, for its association with the uses of related antiviral drugs and the prognosis of individual patients.

It was reported that 75 out of 148 (50.7%) COVID-19 patients presented liver function abnormality in an epidemiologic study in Shanghai (China), indicated by a list of key liver function parameters above the normal range, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) or total bilirubin (TBIL) (Fan et al. 2020). Although clinical association has been implicated, further studies are needed to find out whether the direct virus attack on the liver or systematic dysfunction caused by cytokine storm lead to liver damage.

The adult liver is mainly composed of hepatocytes and ductal cells that work in conjunction with endothelial and mesenchymal cells. Both hepatocytes and ductal cells are derived from hepatoblasts, which are known as liver embryonic progenitor cells (Miyajima et al. 2014). hPSCs or hASCs can be programmed to differentiate towards hepatocyte-like cells in 3D culture that mimic the *in vivo* liver tissue in many aspects.

As a stem cell marker, Lgr5 expression cannot be detected in the normal adult liver under homeostatic conditions, however, the epithelial Lgr5⁺ cell population always appears in the injured liver tissue (Huch et al. 2013). Isolated single Lgr5⁺ cells from the adult mouse liver demonstrate their

Table 1 COVID-19 drug screening on human organoid models

	Organoids	Drugs screen	Brief description and references
Respiratory system	hPSC/hESC-derived alveolar organoids	Bestatin	None inhibition effects (Pei et al. 2020)
		Camostat	Some found it inhibiting SARS-CoV-2 infection (Huang et al. 2020b; Tiwari et al. 2021), while other suggested none inhibition effects (Pei et al. 2020)
		Dutasteride	Decreasing ACE2 levels and attenuating viral infection (Samuel et al. 2020)
		EK1 peptide	Inhibiting SARS-CoV-2 infection (Tiwari et al. 2021)
		E-64d	None inhibition effects (Huang et al. 2020b)
		Finasteride	Decreasing ACE2 levels and attenuating viral infection (Samuel et al. 2020)
		Imatinib	Inhibiting SARS-CoV-2 entry and infection (Han et al. 2021)
		Ketoconazole	Decreasing ACE2 levels and attenuating viral infection (Samuel et al. 2020)
		Mycophenolic acid (MPA)	Inhibiting SARS-CoV-2 infection (Han et al. 2021)
		Nafamostat	Inhibiting SARS-CoV-2 infection (Tiwari et al. 2021)
		Neutralizing antibody CB6	Inhibiting SARS-CoV-2 replication (Pei et al. 2020)
		Quinacrine dihydrochloride (QNHC)	Inhibiting SARS-CoV-2 infection (Han et al. 2021)
		Remdesivir	Strongly inhibiting SARS-CoV-2 replication and infection (Pei et al. 2020; Huang et al. 2020b)
		25-hydrocholesterol;	Blocking entry and spike-mediated membrane fusion of SARS-CoV-2 (Wang et al. 2020b)
	hESC-derived airway organoids	Bestatin	None inhibition effects (Pei et al. 2020)
		Camostat	Slightly inhibiting SARS-CoV-2 replication (Pei et al. 2020)
		Neutralizing antibody CB6	Inhibiting SARS-CoV-2 replication (Pei et al. 2020)
		Remdesivir	Inhibiting SARS-CoV-2 replication and infection (Pei et al. 2020)
	hASC-derived alveolar organoids	Enzalutamide	Failing to inhibit viral infection (Li et al. 2020)
		Hydroxychloroquine	Suppressing SARS-CoV-2 infection and replication (Mulay et al. 2020)
		IFNB1	Suppressing SARS-CoV-2 infection and replication (Mulay et al. 2020)
		IFN α	Pre-treatment with IFNs reducing SARS-Cov-2 replication (Katsura et al. 2020)
		IFN γ	Pre-treatment with IFNs reducing SARS-Cov-2 replication (Katsura et al. 2020)
		Enzalutamide	Failing to inhibit viral infection (Li et al. 2020)
		Remdesivir	Strongly inhibiting SARS-CoV-2 replication and infection (Mulay et al. 2020)

Table 1 (continued)

	Organoids	Drugs screen	Brief description and references
	hASC-derived airway organoids	Camostat	Inhibiting entry and replication of SARS-CoV-2 (Mykytyn et al. 2020)
		E-64d	None inhibition effects (Mykytyn et al. 2020)
		Hydroxychloroquine	None inhibition effects (Mulay et al. 2020)
		Humanized COVID-19 decoy antibody	Blocking viral entry and preventing SARS-CoV-2 infection (Huang et al. 2021)
		IFNB1	None inhibition effects (Mulay et al. 2020)
		IFN-λ1	Suppressing SARS-CoV-2 infection and replication (Lamers et al. 2021)
		Remdesivir	Strongly suppressing SARS-CoV-2 infection/replication (Mulay et al. 2020)
Digestive system	Intestinal organoids	EK1	Inhibiting SARS-CoV-2 replication (Kruger et al. 2020)
		Famotidine	None inhibition effects (Kruger et al. 2020)
		IFN-β1	Inhibiting SARS-CoV-2 infection (Stanifer et al. 2020)
			Inhibiting SARS-CoV-2 infection (Stanifer et al. 2020)
			Reducing viral entry and replication (Bozzo et al. 2020)
			Inhibiting SARS-CoV-2 replication (Kruger et al. 2020)
	Colonic organoids	Imatinib	Effectively reducing SARS-CoV-2 viral RNA levels (Han et al. 2021)
		Mycophenolic acid (MPA)	Effectively reducing SARS-CoV-2 viral RNA levels (Han et al. 2021)(Duan et al. 2020)
		Quinacrine dihydrochloride (QNHC)	Effectively reducing SARS-CoV-2 viral RNA levels (Han et al. 2021)(Duan et al. 2020)
Nervous system	Cortical organoids	Sofosbuvir	Reducing SARS-CoV-2 viral RNA levels (Mesci et al. 2020)
Other organs and systems	Vascular	Human recombinant soluble ACE2 (hrsACE2)	Blocking the infection of SARS-CoV-2 (Monteil et al. 2020)
	Kidney	Human recombinant soluble ACE2 (hrsACE2)	Blocking the infection of SARS-CoV-2 (Monteilet al. 2020)
		Remdesivir; ACE2(hrsACE2)	hrsACE2 improving the effects of remdesivir (Monteil et al. 2020, 2021)
	Tonsil	Remdesivir	Greatly inhibiting SARS-CoV-2 infection (Kim et al. 2020b)

clonogenic potential to form hepatic organoids *in vitro*. In addition, Epcam⁺ bile duct-derived bipotent progenitors can also expand into human hepatic organoids (Huch et al. 2015). These hepatic-like cells maintain the differentiation potential and keep the karyotype stability.

An optimized culture medium is necessary for the expansion of human liver ductal organoid. Under expansion conditions, hepatic organoids exhibit ductal phenotypic features characterized by the expression of Krt19. In addition, the epidermal growth factors and signaling factors, like Wnt, FGF, HGF, are also very important for *in vitro* liver organoid generation (Huch et al. 2015; Sato et al. 2009).

In 2013, Takebe group generated the vascularized and functional liver organoid from hPSCs by 3D culturing *in vitro*, which can be directed via the exposure to specific signaling pathways to form liver ductal organoids (Takebe et al. 2013). In 2015, Sampaziotis and Ogawa et al. generated protocols for differentiating hPSCs into cholangiocyte-like cells. Bile duct cells polarized into cystic organoids and exhibited a morphology and functionality reminiscent of primary bile duct cells under 3D culture conditions (Ogawa et al. 2015; Sampaziotis et al. 2015).

In 2020, Zhao and his colleagues built the first SARS-CoV-2 infection model with human liver ductal organoids,

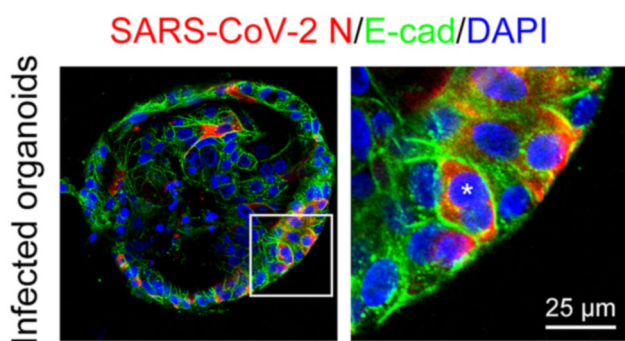


Fig. 3 Confocal image of a SARS-CoV-2 infected human liver ductal organoid. Immunofluorescence staining for SARS-CoV-2N protein and E-cadherin in human liver ductal organoids. The picture was taken at 24 h post SARS-CoV-2 infection

and confirmed the direct attack of SARS-CoV-2 on human liver (Zhao et al. 2020). In their work, by performing single-cell RNA sequencing (scRNA-seq) assay to interrogate the transcriptome of cholangiocytes, a population of human-specific ACE2⁺/TMPRSS2⁺ cells were identified from liver ductal organoids, which turned out to be the potential target of SARS-CoV-2 (Fig. 3). Their pioneering work provided strong experimental evidences to support the speculation that coronavirus could directly infect tissues and organs involved in non-respiratory system (Zhao et al. 2020). A succession of studies of co-culturing various human organoid with SARS-CoV-2 were subsequently reported and indicated that coronavirus directly targeted different parts of human body, echoing the initial report by Zhao et al.

Using infected liver ductal organoid model, researchers explored the exact liver injury mechanism under the virus attack. They found that the impairment of the barrier and bile acid transporting functions of cholangiocytes was achieved by the dysregulated genes involved in tight junction formation and bile acid transportation, and the direct viral cytopathogenic effect on target cells expressing ACE2 and TMPRSS2 most possibly led such change. Results demonstrated that great importance should be attached to viral infection-induced liver damage in the treatment of COVID-19 patients (Zhao et al. 2020).

As the biggest organ in the whole body, intestine presents the largest and most vulnerable surface to the outside world, making it an ideal place for virus infection and replication. The appeared gastrointestinal symptoms and the presence of viral RNA in rectal swabs and feces confirmed the infection even for some patients got negative test of nasopharyngeal swabs. However, the isolation of infective live virus from human stool could be detected in only a few studies, whereas the stool viral RNA was mainly detected using quantitative reverse transcription polymerase chain reaction (qRT-PCR) in most research work which could not be a necessary reflection of the existence of viable virus (Kipkorir et al. 2020;

Wolfel et al. 2020). Therefore, the possibility for fecal transmission of SARS-CoV-2 still remains unclear, although multiple health organizations worldwide have emphasized this possibility.

Similar to lung organoids, two types of intestinal organoids have been developed: PSCs-derived intestinal organoids which contain epithelial cells and mesenchymal cells, and ASCs derived intestinal organoids which comprise epithelial cells only (Dedhia et al. 2016). The culture system of murine epithelial organoids established from Lgr5⁺ intestinal stem cells in 2009 has laid foundation for the initiation and development of a series of organoid-associated scientific research, followed by the generation of human intestinal organoids (Sato et al. 2009). Intestinal organoids are so far the most well-developed organoids (Yi et al. 2019; Sato et al. 2011; Barker et al. 2010). The nomenclature for in vitro cultures of the small and large intestine was proposed by the intestinal stem cell consortium in 2012. Human jejunal organoids and human colon organoids are used henceforth to describe relevant 3D cultures (Stelzner et al. 2012).

The expression of ACE2 and TMPRSS2 in intestinal tract was studied in Krüger's work. These entry factors were most prominent in the epithelial lining of duodenum, gallbladder, and colon (Kruger et al. 2020), and the highest expression of ACE2 could be detected within brush border of intestinal enterocytes in the human body (Mahalingam et al. 2021) (Fig. 2B). Lamers et al. demonstrated that SARS-CoV-2 could infect enterocytes in human small intestinal organoids derived from primary tissues (Lamers et al. 2020), and such viral infection could also be detected in bat small intestinal organoids (Zhou et al. 2020). Intriguingly, researchers explored new mechanism of SARS-CoV-2 infection using intestinal organoids. In Zhang's studies, using the tissue-derived organoids, two mucosa-specific serine proteases TMPRSS2 and TMPRSS4 were confirmed to play the roles of facilitating S protein mediated cell-cell infusion through the induction of S cleavage, and assisting SARS-CoV-2 to enter host cells. They also found that viruses released into the intestinal lumen were inactivated by simulated human colonic fluid, and no infectious virus was recovered from the stool feces of COVID-19 patients (Zang et al. 2020). Although it needs to be further verified, the highly debated and confusing concern about the possibility of fecal-oral transmission seems to have the answer. Stanifer *et al.* proved the SARS-CoV-2 infection and viral replication in intestinal epithelial cells using colon-derived organoids, and highlighted the critical role of type III IFN response in controlling virus infection (Stanifer et al. 2020).

Han et al. 2021; Duan et al. developed human pluripotent stem cell-derived colonic organoids (hPSC-COs) using human embryonic stem cell (hESC) line HUES8. As indicated, all cell types within hPSC-COs were permissive to SARS-CoV-2 virus infection, including KRT20⁺

enterocytes, MUC2⁺ goblet cells, EPHB2⁺ transit-amplifying (TA) cells, CHGA⁺ enteroendocrine (EE) cells, Lgr5⁺ and Bmi1⁺ stem cells. KRT20⁺ enterocytes, which express high level of ACE2 and TMPRSS2, act as the major viral targets among those cell types. Drugs like imatinib, mycophenolic acid and QNHC that have been tested in lung organoids also prove efficiency in hPSC-COs (Han et al. 2021). Duan et al. generated human pluripotent stem cell-derived colonic organoids, and screened out two anti-coronavirus candidates, mycophenolic acid (MPA) and QNHC, using the organoid modeling system (Duan et al. 2020). Krüger and his colleagues confirmed the drug-screening results using the human intestinal organoids (HIOs) derived from the cell line HUES8, and this model was also applied to validate drug efficacy for COVID-19 patients in urgent needs. Data indicated that a low micromolar concentration of remdesivir could effectively control SARS-CoV-2 infection and rescue HIO morphology (Kruger et al. 2020). Using both intestinal and lung organoids, Bozzo et al. identified interferon-induced transmembrane proteins (IFITMs) as an inhibitor of viral entry and replication (Bozzo et al. 2020). Various intestinal organoids offer a valuable platform to explore SARS-CoV-2 infection mechanisms and to validate drug efficacy.

The Nervous System

Neurological involvement has been reported in COVID-19 patients, which include various neurological symptoms: headache, anosmia, ageusia, confusion, seizure and encephalopathy (Mao et al. 2020; MaL et al. 2020). Moreover, viral RNA transcripts are identified from the cerebrospinal fluid (CSF) according to the results of human autopsy studies (Moriguchi et al. 2020). These clinical data implicate the invasive potential of SARS-CoV-2 in the human brain. However, it remains unclear about the direct cause to result in symptoms like that. Are they caused by direct neural infection, para-infectious, post-infectious immune-mediated disease, or sequelae of systemic disease (Jacob et al. 2020)? To address these concerns, detailed experimental evidences are of urgent need to show how the SARS-CoV-2 destroys the brain.

Brain organoids derived from hPSCs, including hiPSCs and hESCs, form an organized architecture comprised of progenitor, neuronal and glial cell types through self-assembling, which resemble the fetal human brain and present a good model to mimic human brain development and function (Qian et al. 2019). They are applied in modeling cerebral cortex development, brain evolution and nervous disorders, including autism spectrum disorders (ASDs), Alzheimer's disease, and Zika virus (ZIKV) infection. They are also reported as a drug-screening system for COVID-19 and

helped identify sofosbuvir as an inhibitor of SARS-CoV-2 (Mesci et al. 2020).

In general, two different types of methodologies can be used to generate brain organoids: unguided methods and guided methods (Qian et al. 2019). The unguided methods which is also known as cerebral organoid depend on promoting self-organization and self-patterning within hPSC aggregates. The first attempt to develop 3D mini-brain in vitro was accomplished by Knoblich group in 2013 (Lancaster et al. 2013). Based on this original method, unguided approaches progressed to generate organoids resembling the whole human brain. Using this method, large and heterogeneous cerebral tissue could be obtained, which display discrete brain regions (forebrain, midbrain, and hindbrain) and subregions (various cortical lobes, choroid plexus, and retina) (Lancaster and Knoblich 2014). By contrast, guided organoid methods require additional external patterning factors to induce hPSCs to differentiate directly towards diversified cell lineages to form independent brain region-specific organoids, such as the cerebral cortex, hippocampus and midbrain organoid (Qian et al. 2019; Jo et al. 2016; Mariani et al. 2015; Pasca et al. 2015; Sakaguchi et al. 2015; Yoon et al. 2019). TGF- β , Shh, BMP, and Wnt pathways play very important roles in the process of cell fate determination. For example, the inhibition of Wnt, BMP and Shh pathway can derive hPSCs into forebrain region tissue (Wataya et al. 2008; Eiraku et al. 2011; Nakano et al. 2012; Germain et al. 2013; Kadoshima et al. 2013; Liu et al. 2013). Mid-brain organoids can be generated by inhibition of TGF- β , BMP, activation of Wnt and Shh, and FGF8 treatment (Jo et al. 2016; Kim et al. 2019). Interestingly, region-specific organoids can be fused to each other to model regional-interactions, such as cellular migration and long-range connectivity (Bagley et al. 2017; Birey et al. 2017; Mich et al. 2017; Xiang et al. 2017, 2018, 2019). These two types of organoids have been used in the studies of the damage that SARS-CoV-2 caused to brain.

Zhang *et al.* used hPSCs-derived human neural progenitor cells (hNPCs), neurospheres, and three-dimensional (3D) brain organoids to evaluate SARS-CoV-2 infection and found that SARS-CoV-2 could replicate in hNPCs and neurospheres. They also discovered that the novel virus could productively infect cortical neurons and NPCs in 3D brain organoids (XaJ and WeiLin 2020; Zhang et al. 2020). Bullen *et al.* employed a hPSC-derived brainsphere model, which consists of different types of neurons, astrocytes and oligodendrocytes, to incubate with SARS-CoV-2. Infected neuronal cells were indicated as accumulation of virus particles within the brain spheres, accompanying with the increased viral RNA level (Bullen et al. 2020).

Song et al. observed a clear phenomenon of SARS-CoV-2 infection that provided exact evidence of metabolic changes in the infected and neighboring neurons. However,

no evidence for the type I interferon responses was detected as compared to lung organoid. These results are consistent with the discoveries of Ramani and his colleagues (Ramani et al. 2020), in which they observed more detailed evidence for SARS-CoV-2 direct targeting neurons of 3D human brain organoids. Neurons at the cortical area invaded by SARS-CoV-2 displayed altered distribution of Tau, Tau hyperphosphorylation, and apparent neuronal death (Ramani et al. 2020; Song et al. 2020). Some scientists have explored the effect of SARS-CoV-2 on region-specific brain organoids using guided methods what we have mentioned above. Jacob and his colleagues developed monolayer cultures of hPSCs-derived region-specific brain organoids, and investigated the susceptibility of brain cells to SARS-CoV-2 (Jacob et al. 2020). Very limited tropism for neurons and astrocytes of multiple brain regions were observed. The choroid plexus epithelial cells were found to be more susceptible in SARS-CoV-2 infection process. Active replication of SARS-CoV-2 was detected in hPSCs-derived choroid plexus (ChP) organoids, which was associated with increased cell death and dysregulated transcripts of inflammatory response and cellular function. One limitation of Jacob's works was that an intact blood–brain-barrier (BBB) or blood-CSF-barrier (B-CSF-B), which modulate accessible route of SARS-CoV-2 to the brain, was of lack in their developed brain organoid models. In the study by Pellegrini and his colleagues (Pellegrini et al. 2020), a choroid plexus (ChP) organoid model was generated with an optimized protocol, recapitulating the epithelial polarization of ChP cells and the formation of a tight barrier that separates the surrounding media from the CSF-like fluid secreted by ChP (Pellegrini et al. 2020). The model could mimic B-CSF-B that formed by a single epithelial lining of ChP cells which separate the fenestrated, leaky capillaries of the stroma from the CSF. The infected models proved high infectivity for ChP cells, whereas little infection was detected in neurons or glia. SARS-CoV-2 damages choroid plexus epithelium by causing the leakage to allow pathogens, immune cells and cytokines cross the important barrier which normally prevent entry of them into cerebrospinal fluid and the brain. This might be able to explain the appearance of some clinical symptoms likewise (Pellegrini et al. 2020). hPSCs-derived brain organoids provide a feasible tool to probe the neurotropism of SARS-CoV-2, to understand the virus-induced mechanisms of brain dysfunction, and to develop therapeutic strategies.

Ocular symptoms that mainly focused on conjunctivitis comprised of sore eyes, itching, foreign body sensation, tearing, redness, dry eyes, eye secretions and floaters, have been reported in COVID-19 patients, although with a low prevalence (Ling et al. 2020). Makovoz developed infected eye organoids with SARS-CoV-2, and observed virus replication in conjunctiva and proto-corneal cells (Makovoz et al. 2020). These data suggested that SARS-CoV-2 could

directly infect the human eyes, and eye protection should be required against COVID-19.

Other Systems

According to a single-cell RNA sequencing analysis for SARS-CoV-2 entry receptors of a wide range of human organoids, ACE2 is found abundant in all except for prostate and brain organoids, while TMPRSS2 is omnipresent in all tissues. Therefore, it is theoretically possible for SARS-CoV-2 to attack most of the human organs (Mahalingam et al. 2021). Appropriate experimental evidences are needed for validating this speculation before drawing conclusion.

Yang et al. reported a hPSCs-based platform which could be used to generate a variety of cells and organoids, including pancreatic endocrine cells, cardiomyocytes, dopaminergic neurons and liver organoid. These cells or organoid cultures could be infected by a spike-enabled pseudo-entry virus, accompanying with striking expression of cytokines. Of note, pancreatic beta cells and liver organoids are most susceptible to SARS-CoV-2 infection as indicated, using adult primary human islets and adult hepatocyte and cholangiocyte organoids (Yang et al. 2020b).

People suffering with severe cases of COVID-19 are showing signs of kidney damage, even for those who had no underlying kidney problems before infected with the coronavirus. Early reports said that up to 30% of patients hospitalized with COVID-19 in China and New York developed moderate or severe kidney injury. Many patients with severe COVID-19 are those with co-existing, chronic conditions, including high blood pressure and diabetes, that increase the risk of kidney disease (Sperati 2020).

To date, there are several reported methods that have been developed by researchers to generate kidney organoids. Mae and colleagues successfully induced hPSCs to intermediate mesoderm (IM), identified by an earliest marker for IM, OSR1 gene in 2013 (Mae et al. 2013). Takasato et al. generated the self-organizing 3D kidney structures from hPSCs and showed that the involvement of Wnt signaling in determining the anterior or posterior fate of IM (Takasato et al. 2015). In 2020, Tsujimoto et al. generated three kinds of mesodermal tissues (paraxial and lateral plate mesoderm and IM) within an inclusive differentiation system through manipulating BMP signaling (Tsujimoto et al. 2020). Chronic kidney disease (CKD) organoids which can also be generated with hPSCs were derived from CKD patients (Morizane and Bonventre 2017; Little and McMahon 2012). Therefore, it might be possible to use kidney organoids to study personalized disease mechanisms and perform in vitro drug screening (Freedman et al. 2015).

In addition, during COVID-19 pandemic, more and more patients are diagnosed as unusual rashes, blood clots and

strokes, which could all be linked to damaged blood vessels. Chilblains, also known as COVID toes, are being seen with increasing frequency in children and young adults (Colmenero et al. 2020; Lopez-Robles et al. 2020). As compared with patients who died from flu, the lung tissue of coronavirus patients had nine times as many small blood clots as those who died of the H1N1 flu. Importantly, the viral RNA can be detected in the blood vessel organoids, and it will increase from day3 to day6 after infection (Monteil et al. 2020).

Perspectives

As SARS-CoV-2 exhibits strong species-specific, tissue-specific or cell-type-specific tropisms, it is difficult to investigate the pathogenic mechanism of SARS-CoV-2 using transformed cell lines or animal models. To date, human organoids have provided an exclusive human tissue or organ model for direct SARS-CoV-2 infection *ex vivo*, which greatly facilitate the discovery of viral tropisms and potential therapeutics of SARS-CoV-2.

In comparison to SARS whose effects are confined to respiratory system, SARS-CoV-2 infection can not only cause dominating respiratory symptoms, but also induce various non-respiratory symptoms like digestive tract hemorrhage, anosmia and liver damage. At the very beginning, it was rather confusing that the multiple organ damage was caused whether by direct virus infection or by systematic dysfunction such as cytokine storm. Using human liver ductal organoids to model SARS-CoV-2 infection process, Zhao et al. first verified that coronavirus could directly attack and damage liver tissue (Fig. 3). This breakthrough for COVID-19 pathogenesis study has eventually led a leap in understanding the effects that COVID-19 poses on multi-organs other than lung (Zhao et al. 2020). Organoids, the 3D multi-cell cultures consisting of organ-specific lineages, has proven as a powerful versatile tool for dissecting the interaction between virus and human tissue.

The CRISPR/Cas9 genome editing technology can be used in most 3D culture systems. Several modified CRISPR-based methods for target genes screening are developed in human organoids. Pooled CRISPR/cas9 screening can allow hundreds of genes to be analyzed in parallel in both normal and disease human tissues (Michels et al. 2020). In searching for host factors of SARS-CoV-2, Zang et al. used CRISPR to create population-wide knockouts of *TMPRSS2* and *TMPRSS4*. *TMPRSS4* knockout led to declining of SARS-CoV-2 propagation, however, the *TMPRSS2* knockout did not result in notable change (Zang et al. 2020). Furthermore, genome-wide CRISPR screens (Ringel et al. 2020) could be adopted to identify unknown host factors in an unbiased manner. Besides, it can also be used for COVID-19

diagnosis, which has immense advantages in clinical effectiveness (Chekani-Azar et al. 2020; Geurts et al. 2021).

Currently, there are several types of advanced organoid systems established by researchers. Penninger group successfully generated self-organizing 3D human blood vessel organoids from hPSCs and hESCs in 2019, which exhibited morphological, functional and molecular features of human microvasculature (Wimmer et al. 2019a). Vascularized organoids create opportunities for investigation of metabolic vascular disease. In addition, since blood vessels are distinctly different in each organ, it will be meaningful to generate organotypic blood vessel organoids in the future that allow the investigation of organ-specific vessel diseases (Wimmer et al. 2019b). In fighting against COVID-19, Monteil et al. used PSCs-derived blood vessel organoids to validate that soluble ACE2 could protect tissues from being infected with SARS-CoV-2 (Monteil et al. 2020), and they also discovered in the drug-screening process that ACE2 improved the effect of remdesivir in SARS-CoV-2 infection (Monteil et al. 2021). An advanced high-throughput organoid microinjection platform is developed to study gastrointestinal physiology and the microbiome (Williamson et al. 2018), which seems promising to be applied to study the interactions between SARS-CoV-2 and human tissues.

Organoids maintain great potentials for their application in regenerative medicine. Scientists have already started using this technology to repair damaged organs (Sampaziotis et al. 2021; Grassi et al. 2019; Nakamura and Sato 2018; Hofer and Lutolf 2021). For example, Sampaziotis et al. successfully repair bile ducts by engrafting cholangiocyte organoids into injured human liver (Sampaziotis et al. 2021). Therefore, it is very possible in the future to use organoid methods to repair irreversible organ damage caused by SARS-CoV-2.

Finally, in terms of the fact that SARS-CoV-2 jumps specie to infect human, co-culturing human or bat organoids with novel coronavirus may help us understand the mechanism of cross-species infectivity. Zhou et al. successfully generated intestinal organoids from the horseshoe bat species *Rhinolophus sinicus* that were readily infected by coronavirus, offering a natural model to understand the role of bats in hosting SARS-CoV-2 (Zhou et al. 2020). Being armed with human organoids, the powerful tool for viral diseases modeling, we will be sure to gain more knowledge and wisdom to deal with such pandemic when again, by any chance, coming under attack from coronavirus in the future.

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Declarations

Conflict of Interest The authors declare no conflict.

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