Review Article

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Understanding the cellular pathogenesis of COVID-19 symptoms using organoid technology

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Patients with coronavirus disease 2019 (COVID-19), which has recently caused a pandemic, have reported symptoms of coronavirus infection that are not well understood by the medical community in general. After severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, several symptoms, including acute clinical signs and possible sequelae, manifest in multiple organs. It is necessary to precisely identify the cells susceptible to SARS-CoV-2 infection in order to comprehend the mechanism of symptom occurrence, identify molecular targets for therapeutic development, and prevent current or future threats. Following the use of cell lines, animal models, and stem cell-derived symptom-relevant cells, recent research on the pathophysiology of human diseases has utilized organoid models. This article provides a summary of recent research on the tissue- or organ-specific cellular targets of SARS-CoV-2 aiming to understand the pathophysiology of COVID-19.

Keywords: COVID-19; SARS-CoV-2; Organoids; Pathogenesis; Communicable diseases

Introduction

Amid the increasing risk of emerging infectious diseases, we are struggling with coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); this virus appeared after SARS-CoV in the 2000s and Middle East respiratory syndrome (MERS)–related coronavirus in the 2010s [1]. The recent COVID-19 epidemic has had far more fatal consequences for humans than those in the past [2]. Despite sufficient vaccine supplies and advances in medical technology [3–7], more than 5 million people have already died worldwide, and infections continue to spread [8]. The global burden of COVID-19 is rapidly increasing due to the growing incidence of various diseases directly or indirectly related to the ongoing COVID-19 pandemic [9,10]. However, effective strategies for prevention and treatment have not yet been proposed.

Coronavirus infections generally cause respiratory symptoms, but patients with COVID-19 have reported additional unconventional symptoms as well as major symptoms in the respiratory tract. Unexpected symptoms described as being caused by a hyperimmune response referred to as the "cytokine storm" have been observed in various organs [11–14]. Severe respiratory symptoms and inflammation of various organs in the circulatory, digestive, nervous, and endocrine systems are affected [15–18]. Notably, more than 40% of patients experience neurological symptoms (e.g., chemosensory impairment or brain fog) that have not been reported in previous coronavirus infections [19,20]. These symptoms often remain even after the respiratory symptoms caused by viral infection are over, developing into sequelae called "long COVID" [21]. To understand the expression of unusual symptoms and devel-

op effective therapeutics, it is required to identify the precise pathogenesis of COVID-19.

To identify the mechanisms through which the symptoms caused by COVID-19 manifest, studies were conducted using cell-based *in vitro* or animal-based *in vivo* models [22,23]. It has been found that angiotensin-converting enzyme 2 (ACE2), a cell membrane protein, contributes to the introduction of SARS-CoV-2 into the cell [23]. Multiple tissues and organs were validated as infection targets of SARS-CoV-2 [11,12,24–26], and a genetically modified mouse model expressing human ACE2 has been established to recapitulate the infectivity and pathophysiology of the virus [27,28]. However, inaccuracies in the expression pattern and the activity of ACE2 across the variety of tissues in the transgenic animal model still cause confusion regarding the pathology of SARS-CoV-2 infection, make it difficult to explain the mechanisms of patients' symptoms, and have been pointed out as an obstacle to the development of appropriate therapeutics [29]. Debate continues regarding the target cell type of SARS-CoV-2 infection in animal or human olfactory tissues, and accordingly, the route of viral infection leading to neurological manifestation remains to be explained [26,30]. Although a recent study employing human pluripotent stem cell (hPSC)-derived olfactory neurons has provided evidence of neuronal infection of SARS-CoV-2 that had not been obtained from animal studies [31], it remains challenging to determine the mechanisms of viral transmission and interrelations between cells and tissues using cell-based models. In order to overcome the limitation of utilizing cell models and animal models, it is necessary to employ improved research models representing the characteristics of human cells, tissues, or organs [31]. The recently developed organoid technology is considered an alternative option that provides appropriate research models for recapitulating the *in vivo* conditions of the human body [32–34]. This review summarizes current studies on the utilization of organoids to understand COVID-19 pathogenesis.

Ethics statement: This study was a literature review of previously published studies and was therefore exempt from institutional review board approval.

Respiratory organoids

SARS-CoV-2, which reaches the lungs through the respiratory tract, causes pulmonary pathology. Respiratory failure is the most common symptom of COVID-19 and is primarily responsible for death [35]. To understand pulmonary pathology in COVID-19 patients and establish clinical concepts for prevention and treatment, it is necessary to reveal the target cell type of SARS-CoV-2 infection in lung tissues. However, the lungs contain a variety of cell types, including alveolar type 1 cells (AT1), AT2 cells, vascular endothelial cells, and immune cells, and thus SARS-CoV-2 infection in the lungs induces complex symptoms, including difficulty breathing and inflammation [36,37]. The coexistence of multiple cell types and complex interactions between different cell types give rise difficulties in identifying the primary target cell type of SARS-CoV-2 and tracking the pathological sequences within lung tissues. The alveolar epithelium mainly consists of ACE1-expressing AT1 cells and ACE2-expressing AT2 cells [38]. A recent study using cancer cell lines taken from respiratory tissue-derived tumors revealed the viral tropism of SARS-CoV-2 for an adenocarcinoma cell line H522, but failed to explain the cell line-specific ACE2-independent infection that requires a mutation in the spike protein of the virus [39]. Although it was confirmed that SARS-CoV-2 replicated within cells in pulmonary epithelia-originating cell lines, abnormal cellular conditions including cancer pathology and ACE2 deficiency still limited the interpretation of the pathophysiological observations.

As an advanced experimental model, hPSC-derived lung organoids containing ACE2-expressing AT2 cells were utilized to determine the infectivity of SARS-CoV-2 and the molecular mechanisms employed in the viral entry process [40]. AT2 cells, the only population expressing ACE2 in lung tissues, were the sole infection targets of SARS-CoV-2 in the lung organoid model [41]. Studies using alveolar organoids supported the proposal that AT2 cells are the major target of SARS-CoV-2 infection in the respiratory tract [42]. However, a study utilizing an airway organoid demonstrated that 90% of infected cells were ciliated cells, 10% were club cells, and other cells such as goblet cells (GCs) and basal cells were uninfected. While multiple cell types were observed as viral infection targets in animal model studies [43], organoid studies reproduced the physiological environment *in vitro* and effectively presented the primary target cells of viral infection, yielding a better understanding of the pathological sequence, which is important for preventing the inter-tissue spread of SARS-CoV-2 in the early stage of infection [44]. Although AT2 cells, club cells, TUBA-positive ciliated cell-like cells, and MUC5AC-positive GC-like cells were presented in lung organoids as infection targets of SARS-CoV-2 [45,46], debate continues on the primary cellular target and infection mechanisms [47]. Interestingly, contrary to the ubiquitous expression of transmembrane serine protease 2 (TMPRSS2)

through various cell types, not all ACE2-expressing cells were permissive for SARS-CoV-2 infection in organoid models, even though infected cells expressed ACE2 [42]. These findings suggested that ACE2 and/or TMRPSS2 expression are insufficient to adequately explain SARS-CoV-2 infection of cells, and that integrative research using cell lines and animal models with organoids will be necessary to provide an unbiased interpretation.

Gastrointestinal organoids

As is the case with other coronavirus infections [48,49], some COVID-19 patients report gastrointestinal symptoms including diarrhea, vomiting, and abdominal pain [50,51]. In addition, SARS-CoV-2 RNA particles were detected in the excrement of COVID-19 patients [52], confirming the presence of gastrointestinal symptoms and suggesting that SARS-CoV-2 may be spread by the fecal-oral transmission route [53]. In the gastrointestinal tract, it has been reported that gastric epithelial cells, intestinal endothelial and epithelial cells, rectal epithelial cells, and colonocytes express ACE2, and the population of intestinal enterocytes (ECs) that form the brush border has been validated as having the highest level of ACE2 expression in the human body [54].

Intestinal organoids, including ECs, GCs, and enteroendocrine cells (EECs), have been used as *in vitro* models to confirm the infectivity of viruses [47,55]. However, SARS-CoV-2–infected enterocyte precursors (ECPs), ECs, GCs, and EECs were not infected. Notably, despite the thousand-fold higher *ACE2* mRNA expression in differentiated ECs than in ECPs, SARS-CoV-2 infected both populations with comparable efficiency. Meanwhile, TMPRSS2 and TMPRSS4 were found to facilitate the fusogenic activity of the SARS-CoV-2 spike, promoting virus entry into mature ACE2-expressing ECs [56]. These results suggest that ACE2, which has minimal expression levels, in the intestinal environment determines the target cell selectivity of SARS-CoV-2, while the expression pattern of TMPRSS contributes to the level of the virus entry and the severity of symptom expression. Another study found that chromogranin A-positive EECs and lysozyme-positive Paneth cells expressed ACE2 and were infected with SARS-CoV-2, but not mucin 2-positive GCs [56–58]. Dysregulation of these cells is associated with a mechanism of tissue damage that leads to severe necrosis with immune responses and digestive dysfunction in COVID-19 patients [59,60].

A comparative study of proximal or colonic intestinal organoids found that the differentially expressed genes (DEGs) increased rapidly by SARS-CoV-2 infection more sensitively in the proximal region than at the colonic site, but DEGs in both regions reached similar levels over time [61]. The later-increasing DEGs in the colonic region displayed the upregulation of general pro-inflammatory factors and downregulation of canonical interferon-stimulated genes. Taken together, SARS-CoV-2 infects cells involved in immune function in the gastrointestinal tract, altering the mechanisms associated with type I or type III interferon responses [47,61,62]. These changes are believed to cause an imbalance in intestinal immune homeostasis or mobilization of the systemic immune system, leading to gastrointestinal symptoms and further tissue necrosis.

Central nervous system organoids

Neurological manifestations in coronavirus diseases are generally unusual, but neurological symptoms are common in COVID-19 patients [63]. Patients frequently report headaches, dizziness, and nausea caused by SARS-CoV-2 infection, as well as a symptom known as brain fog, which includes memory loss and dazed feelings, even after the treatment of the infection is completed. It is challenging to reveal the mechanism by which coronavirus infections cause symptoms in the brain. Recent studies have suggested that SARS-CoV-2 may be detected in the brain [63–65]. Autopsy data of COVID-19 deaths confirmed the presence of viral genomes and particles in various regions of the brain [63,64], and experiments using hPSC-derived brain organoids recapitulated the brain infection of SARS-CoV-2 *in vitro* [65]. In particular, in hPSC-derived brain organoids with a cerebral cortex-like structure, antigens were detected not only in the peripheral region of the brain but also in the deeper region when they were infected with SARS-CoV-2, proving that the virus directly penetrated into the deep brain [66]. However, the mechanism of the spread of the virus in affected patients' brains remains a topic of debate.

In recent reports, cerebral edema and acute necrotizing encephalopathy associated with cytokine storm and blood-brain-barrier breakdown were observed in COVID-19 patients [67–69]. A study using advanced brain organoids found that epithelial cells of the choroid plexus (ChP) are more vulnerable targets to SARS-CoV-2 tropism than neurons and astrocytes [70]. The difference in viral infectivity is induced by higher expression of ACE2 and TMPRSS2 in epithelial cells of the ChP than in other cell types in the brain, resulting in more abundant SARS-CoV-2 infection and increased cell-cell fusion promoting the spread of the virus. Indeed, a single cell containing 12 nuclei due to cell membrane fusion by SARS-CoV-2 spikes was observed, and the virus actively replicated in the

brain organoid [66,70]. In addition, cerebrovascular pericytes have been suggested as a cell type with susceptibility to SARS-CoV-2, and pericyte-like cells served as a "viral replication hub" in integrated conditions with cortical organoids, contributing to viral spread and the type I interferon response [71,72]. These findings support that cells in the ChP play a gateway role in the invasion of SARS-CoV-2 into the brain.

Simultaneously, cell death in the ChP, which increases due to viral infection, seems to induce a cytokine storm and impaired cellular function by provoking the upregulation of pro-inflammatory cytokines and downregulation of cerebrospinal fluid secretion [70,73].

Another hypothesis refers to synaptic transmission that passes through olfactory neurons [74–76]. Depending on the research conditions, 30% to 70% of patients complain of chemosensory impairment of the olfactory or gustatory system, and this is the most common neurological symptom in COVID-19 patients [19,77]. SARS-CoV-2 has been found to infect olfactory neurons, and brain infections have been reported along with damage to olfactory neurons in COVID-19 patients [26,78–80]. Although a transgenic mouse model expressing human ACE2 indicated that SARS-CoV-2 infection occurred only in epithelial cells, not olfactory neurons [30], the results of the direct infection of hPSC-derived peripheral neurons with SARS-CoV-2 [31], along with autopsy data [26,79,80], still presents a potential nerve track for viral spread through synaptic transmission. In order to overcome the limitations of animal or cell-based research, studies using mature brain organoids with the concept of a neuronal assembloid are required in the near future [71,81,82].

Other organoids

SARS-CoV-2 has been reported to affect multiple organs, including the kidneys, liver, and heart, in addition to the organs expressing the aforementioned symptoms [83]. Acute kidney injury (AKI) is a severe condition associated with COVID-19; approximately 3% to 70% of COVID-19 patients were diagnosed with AKI, and severe cases require renal replacement or lead to death [84]. Proximal tubules and glomerular parietal epithelial cells have been suggested as infection targets of SARS-CoV-2. A study using kidney organoids found evidence supporting that the proximal tube and the podocyte II cell cluster express ACE2, resulting in SARS-CoV-2 infection and the production of viral progeny [85].

Hepatic dysfunction, characterized by elevated levels of aspartate aminotransferase, alanine aminotransferase, and bilirubin, in addition to hypovolemia, has been reported in 14% to 53% of deceased COVID-19 patients [56]. Since *ACE2* mRNA expression has been validated in hepatocytes and cholangiocytes of human liver tissues, it is expected that SARS-CoV-2 can directly induce acute liver injuries [54]. Long-term maintenance of liver ductal organoids derived from adult stem cells contained cholangiocytes expressing ACE2 and TMPRSS2, supporting the possibility of viral replication and subsequent cell death in response to SARS-CoV-2 infection of cholangiocytes [86]. The ablated expression of claudin-1 in cholangiocytes by SARS-CoV-2 infection is considered to disrupt the bile ductal epithelium function, causing abnormalities of bile acid collection and secretion.

The eyes are another site that can come into contact with SARS-CoV-2 by spray or droplets. There have been observations of SARS-CoV-2 viral particles in ocular tissues, such as the cornea, conjunctiva, lacrimal sac, or tears [87]. Pericytes and fibroblasts in the eye tissue express ACE2, although at lower levels than in other tissues, such as the lung and kidney [54]. Whole-eye organoids revealed that cells expressing corneal or corneal endothelium markers retained both ACE2 and TMPRSS2 [88]. Infection with SARS-CoV-2 induced ocular tissue-associated inflammatory responses [89,90].

COVID-19 patients have been reported to experience cardiac symptoms, including chest pain, palpitations, and chest pain, and myocardial edema has also been observed [91]. Single cell-RNA sequencing analysis of the human heart to examine *ACE2* expression patterns indicated that multiple cell types, including myocardial cells, mural cells, and pericytes, may be potential cellular targets of SARS-CoV-2 [92]. Recent advances in cardiac organoids generated functional modules consisting of multiple cell types, including epicardial cells, cardiomyocytes, and endocardial cells, and further formed interconnected chambers with vascular structures [93]. However, the application of cardiac organoids to COVID-19 research, is limited significantly by issues in maintaining functional and structural stability *in vitro* [94].

Conclusion and perspectives

Organoid technologies have been utilized to investigate COVID-19 from biological and pathological perspectives, but debate continues about the identity of cells subject to SARS-CoV-2 infection. First, opposite results have been reported. Differences in the susceptibility of GCs to SARS-CoV-2 were recognized in lung and gastrointestinal organoids [42,47,57]. The type I interferon response after SARS-CoV-2 infection was confirmed in pericyte-cortical organoids, but not in brain organoids [71,72]. Second, organoid models are still insufficient to

simulate the biological environment *in vitro*. While the secretion of both pro- and anti-inflammatory cytokines in the serum of COVID-19 patients was enriched, organoids with SARS-CoV-2 infection showed increases in only pro-inflammatory cytokines [95]. Brain infection of SARS-CoV-2 has been confirmed using various region-specific brain organoids, but the infection route has not been clearly identified [30,31].

Identifying the target cell types of SARS-CoV-2 is important for preventing COVID-19 infection and for developing treatments [96,97]. Research on viral infectious diseases using animal models, cell lines, and stem cell-derived symptom-relevant cells has been attempted, and the resultant data have been of great help to human health [98,99]. Although methodologies employing stem cells have been applied to obtain various appropriate cells and model diseases, there is still a need for a model that simulates the complex tissue environments of the human body. While recent organoid technologies are yielding new pathophysiological knowledge in studies on various topics, including infectious diseases [100,101], studies using organoids to simulate tissue environments remain lacking. The development of organoids with correct cell compositions and connectivity between organs will enable an accurate understanding of the mechanisms of multi-organ dysfunction and an ability to respond to persistent threats such as possible sequelae and virus mutations [102–106].

Notes

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Data availability

Please contact the corresponding author for data availability.

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