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Application of Human Induced Pluripotent Stem Cell-Derived Cellular and Organoid Models for COVID-19 Research

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The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its rapid international spread has caused the coronavirus disease 2019 (COVID-19) pandemics, which is a global public health crisis. Thus, there is an urgent need to establish biological models to study the pathology of SARS-CoV-2 infection, which not only involves respiratory failure, but also includes dysregulation of other organs and systems, including the brain, heart, liver, intestines, pancreas, kidneys, eyes, and so on. Cellular and organoid models derived from human induced pluripotent stem cells (iPSCs) are ideal tools for *in vitro* simulation of viral life cycles and drug screening to prevent the reemergence of coronavirus. These iPSC-derived models could recapitulate the functions and physiology of various human cell types and assemble the complex microenvironments similar with those in the human organs; therefore, they can improve the study efficiency of viral infection mechanisms, mimic the natural host-virus interaction, and be suited for long-term experiments. In this review, we focus on the application of *in vitro* iPSC-derived cellular and organoid models in COVID-19 studies.

Keywords: induced pluripotent stem cell, organoid, cellular model, COVID-19, SARS-CoV-2

INTRODUCTION

Since its outbreak in 2019, the coronavirus disease (COVID-19) pandemics have infected more than 190 million people and caused more than 4 million deaths[1]. COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an enveloped positive-sense single-stranded RNA virus. It enters the host cells using angiotensin-converting enzyme 2 (ACE2) as the cell surface receptor and transmembrane serine protease 2 (TMPRSS2) as the effector to cleave its spike protein (Hoffmann et al., 2020). SARS-CoV-2 spreads mainly through the respiratory

[1]www.worldometers.info/coronavirus

tract (Lu et al., 2020). Respiratory failure is the most common cause of death in COVID-19 patients; meanwhile, severe fatal manifestations are also observed in other organs, such as the brain, heart, liver, intestines, and pancreas (Puelles et al., 2020). Therefore, it is of particular importance to find models that can imitate the natural host-virus interactions of SARS-CoV-2 in a variety of human cell types and organs, thus improving the study efficiency for identifying key molecular regulators and the underlying mechanisms of virus infection and disease progression.

There have been both animal models (e.g., transgenic mice expressing human ACE2 and non-human primates) and cell line models (e.g., African green monkey Vero E6 cells and human cancer cell lines) available for COVID-19 research (Takayama, 2020). However, animal models are quite costly and display very different physiological characteristics from human, and cell line models have limitations in reproducing the viral life cycle and the pathology of COVID-19 in different human organs and tissues that contain a variety of cell types (Liu et al., 2011). For example, the entry routes of SARS-CoV-2 vary between cell lines and human tissues, as do immune responses and host-virus interactions (Milewska et al., 2020). In addition, human cancer cells carry numerous tumor-associated mutations, such as P53 mutations, which could interfere the SARS-CoV-2 infection (Ma-Lauer et al., 2016). Therefore, there is an urgent need to establish more cost-efficient and human-relevant models for COVID-19 research.

The emergence of human induced pluripotent stem cells (iPSCs) has enabled derivation of functional human cells or organoids to model human diseases, including infectious diseases, to develop new therapeutic approaches and to promote drug discovery (Yu et al., 2007; Luo et al., 2015a, 2018), without ethical issues like the human embryonic stem cells (Luo et al., 2014). For example, functional liver organoids generated by human iPSCs have been developed as personalized models of hepatitis B virus (HBV) infection, which is a powerful long-term platform for both research and drug screening of HBV (Nie et al., 2018). Recently, iPSC-derived cellular and organoid models have been utilized to simulate SARS-CoV-2 infections in multiple organs, not only the lung, but also the heart, brain, liver, intestines, and pancreas (Jacob et al., 2020; Yu et al., 2020) (**Figure 1**). These studies have demonstrated that SARS-CoV-2 can infect and propagate in a variety of cell types, leading to transcriptional alternations that indicate inflammatory responses and changes in cell function (Marshall, 2020). The purpose of this review is to describe the usefulness of these iPSC-derived cellular and organoid models in simulating human cellular physiology and tissue microenvironment, and enabling the study of host-virus interaction and drug screening for the COVID-19 disease, thus leading to a more comprehensive understanding of the SARS-CoV-2 pathogenesis.

LUNG ORGANOIDS

Human iPSC-derived airway and alveolar organoids have been developed and used for studying the processes of SARS-CoV-2 infection and transmission in the lungs (Pei et al., 2020). With these organoid models, the researchers have determined the cellular tropism of the virus. Ciliated cells, club cells, and alveolar type II cells (AT2) cells, which are arranged from the proximal to the distal airway and terminal alveoli in sequence, have been confirmed as SARS-CoV-2-targeted cells in the study (Mulay et al., 2021). Moreover, the viral infection downregulates metabolic processes, particularly the lipid metabolism, which, together with the already known upregulation of immune responses, is another molecular feature of SARS-CoV-2-infected cells (Pei et al., 2020). On the other hand, infected SARS-CoV-2 can decrease the level of its target ACE2 on the host cell surface through a variety of mechanisms. A comprehensive analysis of these information might enable better understanding about the viral pathogenesis and discovery therapeutic targets for the treatment of COVID-19 (Pei et al., 2020).

In addition, the early immune responses to viral infections were investigated using iPSC-derived AT2 (iAT2) cells (Huang J. et al., 2020). The results showed that AT2 cells are a central component of the inflammatory signaling that responds to SARS-CoV-2 infection within the first 24 h, with NF-κB signaling predominating this response (Huang J. et al., 2020). These findings are consistent with those in newly purified primary AT2 cells infected with SARS-CoV-2 (Mulay et al., 2021). They also observed cellular stress, toxicity, iAT cell death, and significant loss of surfactant genes expression in their model (Huang J. et al., 2020). These findings may be clinically relevant, as similar results were found in lung autopsies of multiple individuals who died of COVID-19 (Bradley et al., 2020; Hou et al., 2020). Other researchers have previously shown that the primary AT2 cells can be infected with SARS-CoV in the body (Qian et al., 2013); it has also been recently shown that AT2 cells may help promote lung regeneration in COVID-19 survivors (Chen J. et al., 2020). The correlation between AT2 cells and SARS-CoV-2 infection was further emphasized.

Furthermore, the researchers have demonstrated that human iPSC-derived lung cells and organoids could serve as powerful platforms for discovering and testing anti-SARS-CoV-2 drugs (Huang J. et al., 2020; Pei et al., 2020). They have demonstrated that Remdesivir, a predrug nucleotide analog that inhibits virus replication (Eastman et al., 2020), CB6, a human neutralizing antibody (Shi R. et al., 2020), as well as TMPRSS2 protease inhibition could effectively inhibit the replication of SARS-CoV-2 in the iPSC-derived models. These results are consistent with those in fundamental studies using primary cell models and in clinical trials (Beigel et al., 2020; Wang M. et al., 2020). Therefore, iPSC-derived *in vitro* human models could be employed to identify and test therapeutic entities for the treatment of COVID-19.

Taken together, the above results demonstrated that lung cells and organoids derived from human iPSCs can be utilized as pathophysiological models to study the potential mechanisms of SARS-CoV-2 transmission and to identify and test COVID-19 therapeutic agents (Pei et al., 2020).

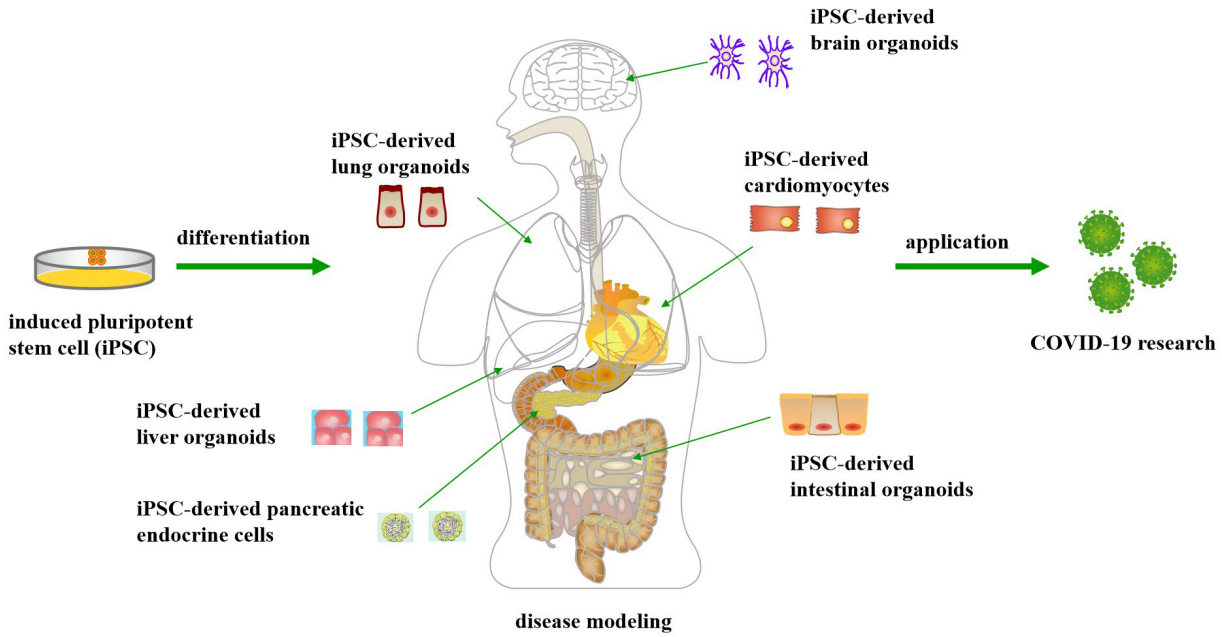

FIGURE 1 | Application of human induced pluripotent stem cell-derived cellular and organoid models for COVID-19 research.

CARDIOMYOCYTES

There is growing evidence that patients with COVID-19 exhibit severe heart complications, elevated biomarkers of heart damage, and cardiac function deterioration, including cardiovascular complications such as cardiomyopathy, acute myocardial infarction, arrhythmia, and heart failure, greatly increasing the risk of death (Aggarwal et al., 2020a; Bansal, 2020; Long et al., 2020; Madjid et al., 2020; Shi S. et al., 2020). Several compounds and antibodies, such as Remdesivir, Olumiant + Remdesivir, Casirivimab + Imdevimab, Bamlanivimab + Etesevimab, Sotrovimab and Tocilizumab are currently licensed drug for treatment of COVID-19 patients under emergency use authorization[2]. However, there is limited safety information on these recommended drugs, especially since heart toxicity caused by the drug can lead to lethal complications, including myocardial ischemia, arrhythmias, and heart failure. Therefore, it is critical to evaluate any potential adverse effects on the cardiovascular system associated with current COVID-19 medications to avoid fatal side effects (Aggarwal et al., 2020b).

Human iPSC-derived cardiomyocytes (CMs) can be utilized to recapitulate cardiac pathophysiology and are considered as one of the most promising sources for cardic disease modeling, heart repair and cardiac toxicology screening (Mitcheson et al., 1998; Lan et al., 2013; Moreno and Pearson, 2013; Sharma et al., 2014, 2017; Burridge et al., 2016). In this context, iPSC-CMs are recommended as a reliable method of heart toxicity examination in the comprehensive *in vitro* proarrhythmia assay (CiPA), as a non-clinical safety pharmacological paradigm, to circumvent the limitations of existing methods used in preclinical safety assessment of drugs (Gintant et al., 2016; Goineau and Castagné, 2017; Sala et al., 2017). Choi et al. (2020) have shown that iPSC-CMs acutely treated with Remdesivir show a risk of arrhythmia and changes in the electrophysiological properties of myocardial cells in a dose-dependent manner, indicating that overdose or drug accumulation may lead to noteworthy adverse heart reactions, such as prolonged QT interstitial periods. In addition, they have demonstrated that iPSC-CMs not only allow SARS-CoV-2 infection, but also support the propagation of infectious viral particles (Choi et al., 2020).

INTESTINAL ORGANOIDS

Up to 50% of COVID-19 patients develop gastrointestinal symptoms associated with longer duration and increased severity of the disease (Luo et al., 2020; Wang F. et al., 2020; Wei et al., 2020; Xiao et al., 2020). However, it remains debatable whether the virus found in the intestines is contagious, as few studies have examined infectious viruses in feces (Zang et al., 2020). In cell culture, primary intestinal cells are highly susceptible to SARS-CoV-2 and can produce infectious viral particles. Intestinal organoids can quickly grow from adult stem cells derived from cells of large intestine and small intestine biopsy tissue (Sato et al., 2011). Stem cell-derived intestinal organoids have similar characteristics with primary intestinal cells and have been widely used to study viral infection (Sato et al., 2011; Ettayebi et al., 2016).

Studies have employed human iPSC-derived intestinal organoids to study SARS-CoV-2 tropisms in different intestinal cell types. In both *in vivo* and iPSC-derived organoid models,

[2]www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#coviddrugs

ACE2 is strongly expressed in the small intestine, as well as TMPRSS2. In contrast, colon organoids have lower ACE2 expression (Zang et al., 2020). In intestinal organs, TMPRSS4 performs the same functions as TMPRSS2 to support virus entry (Zang et al., 2020). Experiments have shown that two groups of small intestinal organoid models can be infected with SARS-CoV-2 (Lamers et al., 2020; Zang et al., 2020). In these organoid models, SARS-CoV-2, like its closest relative SARS-CoV, mainly infects mature enterocytes and dividing cells (Lamers et al., 2020; Zang et al., 2020). On the other hand, studies have shown that when the SARS-CoV-2 viruses are cultured in gastric fluid in the large intestine and small intestine, they quickly lose their infectious power (Simoneau and Ott, 2020; Zang et al., 2020). Therefore, even though viral particles were found in the feces occasionally, it might not be the primary pathway for virus transmission.

Furthermore, human iPSC-derived intestinal organoids generate valuable pathological models for studying the underlying mechanisms of intestinal SARS-CoV-2 infection. It is reported that SARS-CoV-2 actively infects both proximally and distally patterned intestinal organoids, thus resulting in production of infectious viral particles and significant transcriptional alterations, such as upregulation of the interferon-related genes, in multiple epithelial cell types (Mithal et al., 2021). Another organoid study shows that SARS-CoV-2 can infect all intestinal cell types investigated except goblet cells, and disrupt intestinal integrity, which might be the cause of diarrhea and other gastrointestinal symptoms associated with COVID-19 (Krüger et al., 2021).

More importantly, these organoids can serve as a potential platform for organ-specific drug testing and drug screening. For example, the researchers found that Remdesivir therapy inhibits SARS-CoV-2 viral replication in the intestinal organoids (Krüger et al., 2021). Therefore, clinical treatment with this drug may prevent intestinal damage caused by SARS-CoV-2 and relieve intestinal symptoms.

BRAIN ORGANOIDS

Approximately 36.4% of the COVID-19 patients develop a variety of neurological complications, ranging from loss of smell, nausea, dizziness, and headache to encephalopathy and stroke (Mao et al., 2020). RNA of SARS-COV-2 was found in the brains of some patients (Helms et al., 2020; Moriguchi et al., 2020). The mechanisms of SARS-COV-2 disrupting the brain-blood barrier and infecting the central nervous system (CNS) draw great concerns (Li et al., 2020). Studies have shown that CNS infections may lead to the pathophysiological and clinical manifestations associated with COVID-19 (Steardo et al., 2020). Therefore, it is necessary to establish a suitable *in vitro* model to study nerve infection by SARS-CoV-2.

IPSC-derived brain organoids are valuable tools for investigating the biological properties of SARS-CoV-2 in the CNS (Bullen et al., 2020; Jacob et al., 2020). Studies using iPSC-derive brain organoids find that choroid plexus epithelial cells are the main target of SARS-CoV-2 infection in the CNS; meanwhile, neurons, and astrocytes are sparsely infected (Jacob et al., 2020; Pellegrini et al., 2020). This finding is consistent with the discovery that the choroid plexus region is one of the hotspots of ACE2 expression in the CNS under inflammatory status, and it is more susceptible to SARS-CoV-2 infection than other regions (Chen R. et al., 2020). After SARS-CoV-2 infection, increased cellular remodeling and inflammatory responses were observed in choroid plexus epithelial cells (Chen R. et al., 2020). This finding provides an evidence that SARS-CoV-2 infection of the choroid plexus leads to disruptions in blood-cerebrospinal fluid barrier (BCB) integrity. Researchers have proposed that BCB decomposition can promote entry of the virus as well as immune cells expressing cytokines into the cerebrospinal fluid and brain tissue, potentially causing nerve inflammation (Pellegrini et al., 2020).

Whether SARS-CoV-2 propagates in the CNS remains controversial. Some studies have reported successful SARS-CoV-2 replication in brain organoids (Zhang et al., 2020), while the others suggest that the viral replication and proliferation are less efficient in the brain organoids (Ramani et al., 2020). These opposite results may be due to differences in the methods of establishing brain organoid models and the multiplicities of infection (MOI) used in these studies. In the former study, the neural progenitor cell (NPC) population is also found to be a target of SARS-CoV-2 (Ramani et al., 2020). This is an important finding as NPCs are responsible for repairing brain lesions caused by degenerative diseases or malignancies (Zhu et al., 2013, 2014; Luo and Zhu, 2014; Luo et al., 2015b). The impaired NPC population might be the reason for late or incomplete recovery of neurological manifestations in COVID-19 patients. On the other hand, it should be noted that although human brain organoids represent valuable models for *in vitro* research on SARS-CoV-2 infection, they merely have simplified structures (e.g., vein systems and blood-brain barriers) like the developing fetal brain, and lack mature cells, particularly asteroid cells and astrocytes (Jacob et al., 2020; Ramani et al., 2020).

PANCREATIC ENDOCRINE CELLS

Single-cell RNA-seq analysis of primary human islets has indicated that both alpha cells and beta cells are positive for ACE2 and TMPRSS2 (Yang et al., 2020). Further validation experiments in humanized mouse model established by human iPSC-derived pancreatic endocrine cells confirm that both alpha cells and beta cells are susceptible to SARS-CoV-2 (Yang et al., 2020). Infected pancreatic endocrine cells display higher expression of pathways associated with apoptosis and viral infection, and lower expression of pathways associated with the normal functions of alpha cells and beta cells, thus leading to increased cell death and loss of cell identities (Yang et al., 2020). The infected cells are also expressing higher levels of chemokines, including CCL2, CXCL5, and CXCL6, and other degenerative factors and cytokines, which is similar with cells found in autopsies from COVID-19 patients (Blanco-Melo et al., 2020).

LIVER ORGANOIDS

More than 50% of COVID-19 patients have symptoms of viral hepatitis (Ong et al., 2020). Particularly, the proportion of patients with liver damage in patients with severe symptoms is much higher than that in patients with mild symptoms (Huang C. et al., 2020). However, due to the lack of suitable research models, it was unclear whether the liver damages were caused by a direct viral infection or by systemic dysfunctions, such as cytokine storms.

Relevant studies have deployed human organoids as tools to study the correlations of SARS-CoV-2 infection and liver damage at both cellular and molecular levels (Huch et al., 2015; Dutta and Clevers, 2017). Some studies have established human liver organoid models which are capable of preserving the ACE2 + /TMPRSS2 + cholangiocyte population in long-term 3-dimensional (3D) cultures (Yang et al., 2020; Zhao et al., 2020). Further, the studies have confirmed that the cholangiocytes in the human liver organoids are permissive to SARS-CoV-2 infection and supporting strong viral propagation (Yang et al., 2020; Zhao et al., 2020). Moreover, SARS-CoV-2 infection induces cell death in the host cholangiocytes. Thus, these studies supports that liver damage in COVID-19 patients might be caused by gallbladder decomposition and subsequent accumulation of bile acid due to viral infection (Yang et al., 2020; Zhao et al., 2020).

DISCUSSION AND FUTURE PERSPECTIVES

Most COVID-19 patients have mild respiratory symptoms; however, up to 20% of the patients develop severe pneumonia, leading to multi-organ failures and even death (Zhu N. et al., 2020). The development of iPSC technologies and the resulting differentiated cell models have dramatically accelerated studies of the pathogenesis of SARS-CoV-2 in various organs. Current studies have employed iPSC-derived cells and organoids, including iAT2 cells, cardiomyocytes, pancreatic endocrine cells, lung organoids, brain organoids, intestinal organoids, liver organoids, to investigate the underlying mechanisms of SARS-CoV-2 infection (Ardestani and Maedler, 2020; Bojkova et al., 2020; Huang J. et al., 2020; Jacob et al., 2020; Pei et al., 2020; Mithal et al., 2021). As COVID-19 could also cause kidney malfunctions (Chen N. et al., 2020), kidney organoids derived from iPSCs may be a potential research model as well (Phipson et al., 2019).

These iPSC-derived models are suited for leveraging the powers of the latest genetic tools, such as single-cell RNA-seq and CRISPR techniques, for COVID-19 research (Zhou et al., 2020). Single-cell RNA-seq techniques have been developed to investigate the viral tropisms and host transcriptional responses to viruses or external stimuli in complex organs and tissues (Zhu et al., 2018; Luo et al., 2019; Zhu D. et al., 2020). In the above studies, single-cell RNA-seq has been employed to screen for cell types that are positive of the SARS-CoV-2 receptor ACE2 and effector protease TMPRSS2, and to illustrate the transcriptional alternations after viral infection (Huang J. et al., 2020; Yang et al., 2020; Zang et al., 2020). Furthermore, the CRISPR system can be utilized to create genetically modified iPSC models for mechanism study of the candidate genetic factors (Luo et al., 2015c, 2016; Gkogkou et al., 2020; Kim et al., 2020; Zang et al., 2020; Yu, 2021). For example, with a CRISPR-engineered iPSC model, researchers have demonstrated that the single-nucleotide polymorphism rs4702, which is a common genetic variant located in the 3′ UTR of the protease FURIN, influences the SARS-CoV-2 permissiveness of alveolar and neuronal cells (Dobrindt et al., 2021).

On the other hand, there are a few limitations of iPSC-derived cellular and organoid platforms, such as inadequate complexity to reflect real tissue microenvironment and cell-cell interactions, and lack of real-time monitoring methods for 3D cultures. For examples, although the above studies have described the susceptibility of various cell types to SARS-CoV-2 infection with these iPSC derivatives, it is unclear whether these cell types are the primary targets for viral infection in COVID-19 without a more thorough analysis of samples from primary patients (Lamouroux et al., 2020). Besides, these iPSC-derived models are simplified ones compared to the fully functional and reacting human organs. In the future, these platforms should be exploited to produce more complex organoid models, including the immune system components that are missing from the current analysis (Yang et al., 2020).

CONCLUSION

In conclusion, human iPSC-derived cells and organoids can be used as ideal models for studying the mechanisms of viral infection and drug screening. Particularly, with the organoid models, the viral tropism and host responses of different cell types could be observed in a single system. These iPSC models help us better understand the pathogenesis of SARS-CoV-2 in different organs and systems, and provide powerful drug test and discovery platforms.

AUTHOR CONTRIBUTIONS

YL and DZ conceived the study. YL, MZ, and DZ prepared the figure. YL, MZ, YpC, YoC, and DZ wrote the manuscript. All authors read and approved the final manuscript.

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