Received: 15 May 2021 | Accepted: 5 July 2021

DOI: 10.1002/sctm.21-0183

PLURIPOTENT STEM CELLS

STEM CELLS TRANSLATIONAL MEDICINE

SARS-CoV-2 research using human pluripotent stem cells and organoids

Sayaka Deguchi[1] | Ángel Serrano-Aroca[2] | Murtaza M. Tambuwala[3] | Bruce D. Uhal[4] | Adam M. Brufsky[5] | Kazuo Takayama[1]

[1]Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, 606-8507, Japan

[2]Biomaterials and Bioengineering Lab, Centro de Investigación Traslacional San Alberto Magno, Universidad Católica de Valencia San Vicente Mártir, Valencia, 46001, Spain

[3]School of Pharmacy & Pharmaceutical Sciences, Ulster University, Londonderry, Northern Ireland, UK

[4]Department of Physiology, Michigan State University, East Lansing, Michigan, 48824, USA

[5]University of Pittsburgh, Magee-Women's Hospital, Pittsburgh, Pennsylvania, 15213, USA

Correspondence
Kazuo Takayama, PhD, Center for iPS Cell Research and Application, Kyoto University, Shogoin Kawaharacho 53, Sakyo-ku, Kyoto 606-8507, Japan.
Email: kazuo.takayama@cira.kyoto-u.ac.jp

Abstract

Experimental cell models are indispensable for clarifying the pathophysiology of coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and for developing therapeutic agents. To recapitulate the symptoms and drug response of COVID-19 patients in vitro, SARS-CoV-2 studies using physiologically relevant human embryonic stem (ES)/induced pluripotent stem (iPS) cell-derived somatic cells and organoids are ongoing. These cells and organoids have been used to show that SARS-CoV-2 can infect and damage various organs including the lung, heart, brain, intestinal tract, kidney, and pancreas. They are also being used to develop COVID-19 therapeutic agents, including evaluation of their antiviral efficacy and safety. The relationship between COVID-19 aggravation and human genetic backgrounds has been investigated using genetically modified ES/iPS cells and patient-derived iPS cells. This review summarizes the latest results and issues of SARS-CoV-2 research using human ES/iPS cell-derived somatic cells and organoids.

KEYWORDS
COVID-19, human ES cells, human iPS cells, organoids, SARS-CoV-2

Significance statement

COVID-19 and SARS-CoV-2 are dominating discussion in the scientific community and the news. Although many clinical trials are underway worldwide, basic research on SARS-CoV-2 entry and replication and identification of the best drug targets is lacking. Therefore, this study introduces the human pluripotent stem cell-derived cells and organoids available for research on SARS-CoV-2, with consideration to their strengths and weaknesses. This overview will help researchers select suitable human pluripotent stem cell-derived cells and organoids for SARS-CoV-2 studies. Thus, this review will provide valuable information to accelerate drug discovery for COVID-19.

1 | INTRODUCTION

As of 10 June 2021, the number of coronavirus disease 2019 (COVID-19) patients is about 173 million, and the number of deaths about 3.74 million. Although pneumonia and acute respiratory distress syndrome are widely recognized as symptoms of COVID-19, many symptoms of extrapulmonary organs are also known. These symptoms include cardiac arrhythmias, myocardial ischemia, diarrhea, stroke,

acute kidney injury, and hyperglycemia. Elucidation of the complex pathophysiology of COVID-19 and the development of therapeutic agents are essential for stopping this pandemic. While several COVID-19 vaccines are now available, progress in therapeutic agents has been slower. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of COVID-19, uses its spike (S) protein to enter host cells. Because the viral S protein binds to angiotensin converting enzyme 2 (ACE2) on the host cell surface, cells expressing ACE2 are susceptible to SARS-CoV-2 infection. Accordingly, SARS-CoV-2 has high infectivity in primates, including humans, rhesus monkeys, and cynomolgus monkeys, but low infectivity in wild-type mice,[1–4] limiting the animal species that can be used for experiments. Moreover, it is ethically difficult to use these animals in large quantities. Therefore, physiologically relevant human embryonic stem (ES)/induced pluripotent stem (iPS) cell-derived somatic cells and organoids are being developed as cell models for SARS-CoV-2 infection. Organoids are three-dimensional structures that can be generated from somatic stem cells[5] or human ES/iPS cells.[6] The derived somatic cells and organoids have closer cellular and organ functions to primary cells than other cell lines commonly used in in vitro SARS-CoV-2 research, such as Vero, Calu-3, and Caco-2, suggesting that they more accurately reproduce the pathophysiology of COVID-19 and drug effects.[7] Although the infection efficiency of SARS-CoV-2 in Vero, Calu-3, and Caco-2 cells, it is difficult to reproduce the cellular and organ responses due to SARS-CoV-2 infection. This review introduces the latest findings and issues of SARS-CoV-2 research using human ES/iPS cell-derived somatic cells and organoids.

2 | SARS-CoV-2-TARGET CELLS AND ORGANS

The main symptoms of COVID-19 are manifested in the respiratory system, but many cases of multiple organ failure have been reported,[8–10] indicating many organs are affected by SARS-CoV-2 infection. Accordingly, human ES/iPS cell-derived somatic cells and organoids have been used to study the infection in several of these organs (Table 1).

Because respiratory failure is one of the most critical symptoms of SARS-CoV-2 infection, experiments using bronchial and alveolar models are especially being studied. We generated human bronchial organoids from cryopreserved human bronchial epithelial cells to reproduce the infection of SARS-CoV-2 in the bronchi.[22] These bronchial organoids have cellular constituents resembling basal, ciliated, goblet, and club cells, and we confirmed that some basal cells can be infected with SARS-CoV-2. Pei et al also performed SARS-CoV-2 infection experiments using airway organoids generated from human ES cells, finding ciliated and club cells are susceptible to infection.[14] Huang et al performed SARS-CoV-2 infection experiments using human iPS cell-derived alveolar epithelial type 2-like cells.[12] The expression of surfactant protein C, a critical component of lung surfactant that is expressed only in type II alveolar epithelial cells of the lung, was decreased and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signal was activated after SARS-CoV-2 infection.

Because myocardial damage, neuropathy, and diarrhea are frequently observed in COVID-19 patients,[36-38] SARS-CoV-2 infection experiments using myocardial, brain, and intestinal tract models have also been performed. Perez-Bermejo et al found that human iPS cell-derived cardiomyocytes, but not cardiac fibroblasts or endothelial cells, can be infected by SARS-CoV-2.[24] Approximately 20% of the cardiomyocytes infected with SARS-CoV-2 showed myofibrillar fragmentation. Pellegrini et al conducted SARS-CoV-2 infection experiments using human ES cell-derived cerebral organoids.[34] The virus infected choroid plexus epithelial cells but hardly infected neurons or glial cells. In addition, in human choroid plexus organoids infected with SARS-CoV-2, apolipoprotein J, which is a cerebrospinal fluid (CSF) component, leaked from the inside of the organoid into the medium, recapitulating destruction of the blood-CSF barrier. Krüger et al conducted SARS-CoV-2 infection experiments using human ES cell-derived intestinal organoids.[29] They found that SARS-CoV-2 infects enterocytes, enteroendocrine cells, and paneth cells, but hardly goblet cells. SARS-CoV-2 infection experiments using kidney and pancreas models have also been conducted.[28,32] Pancreatic alpha and beta cells differentiated from human ES cells express ACE2 and TMPRSS2 and are permissive to SARS-CoV-2.[28] Proximal tubular cells and podocytes differentiated from human ES cells express ACE2 and are permissive to SARS-CoV-2.[32]

Overall, these experiments have clarified which cells are vulnerable to infection and the resulting cellular and organ damage. By utilizing multiple organ organoids, it is anticipated that the mechanisms of the multiple organ failure due to COVID-19 will be elucidated. Furthermore, by performing long-term culture of infected human ES/iPS cell-derived somatic cells and organoids, it will be possible to investigate the state of organs after the virus elimination.

3 | COVID-19 DRUG DEVELOPMENT

RNA-dependent RNA polymerase inhibitors, such as Remdesivir[39] and EIDD-2801,[40] are currently being used to inhibit the intracellular genome replication of SARS-CoV-2. Remdesivir has already been approved in many countries and administered to many COVID-19 patients. Huang et al reported that the amount of intracellular viral genome decreased five magnitudes (10^5) in human iPS cell-derived AT2-like cells treated with Remdesivir.[12] Perez-Bermejo et al showed that pretreating human iPS cell-derived cardiomyocytes with Remdesivir also reduced the intracellular viral genome five magnitudes.[24] Remdesivir was shown to have an antiviral effect on human ES cell-derived intestinal organoids, with the intracellular viral genome level reduced four magnitudes.[29]

To inhibit the infection of SARS-CoV-2, a soluble recombinant protein of human ACE2, which is a SARS-CoV-2 receptor, has been used. Monteil et al showed that treating human iPS cell-derived capillary organoids and human ES cell-derived kidney organoids with human recombinant soluble ACE2 (hrsACE2) can inhibit SARS-CoV-2 infection.[32] Bojkova et al also reported that intracellular viral genome levels in human iPS cell-derived cardiomyocytes are reduced by recombinant

TABLE 1 SARS-CoV-2 infection experiments using human embryonic stem/induced pluripotent stem (ES/iPS) cell-derived somatic cells and organoids

	Organ model	Original cell or tissue	Cell composition	Permissiveness to SARS-CoV-2 infection	Effective drugs or compounds	Ref.
Respiratory	Human alveolar epithelial type 2 alveolospheres	Human lung tissues	Alveolar epithelial type 2 cells	+	IFN-α, IFN-γ	11
	Human alveolar epithelial type 2 alveolospheres[a]	Human iPS cells	Alveolar epithelial type 2 cells	+	Remdesivir, Camostat	12
	Human airway epithelial cells[a]	Human lung tissues	Ciliated cells	+	−	13
			Goblet cells	+		
	Human lung alveolospheres	Human lung tissues	Alveolar epithelial type 2 cells	+	Remdesivir, IFN-β, Hydroxychloroquine	
			Alveolar epithelial type 1 cells	Unknown		
	Human airway organoids	Human ES cells	Ciliated cells	+	Remdesivir, Camostat, SARS-CoV-2 antibody CB6	14
			Basal cells	−		
			Club cells	+		
			Goblet cells	−		
	Human alveolar organoids	Human ES cells	Alveolar epithelial type 2 cells	+	Remdesivir, SARS-CoV-2 antibody CB6	
			Alveolar epithelial type 1 cells	−		
	Human airway organoids[a]	Human lung tissues	Ciliated cells	+	−	15
	Human alveolar epithelial type 2 cells	Human iPS cells	Alveolar epithelial type 2 cells	+	−	16
	Human airway epithelial cells	Human iPS cells	Ciliated cells	+[b]	Remdesivir	17
			Goblet cells			
			Club cells			
			Basal cells			
			Pulmonary neuroendocrine cells			
	Human small airway organoids[a]	Human lung tissue	Ciliated cells	+	−	18
			Club cells	+		
			Goblet cells	−		
	Human organoid-derived bronchioalveolar model[a]	Human lung tissue	Alveolar epithelial type 2 cells	+	IFN-λ1	
			Alveolar epithelial type 1 cells	+		
			Basal cells	−		
			Pulmonary neuroendocrine cells	−		
			Tuft cells	Unknown		
	ACE2-overexpressed human lung organoids	Human lung tissue	−	+	Camostat	19
	Human 3D alveolar type 2 cell cultures (h3ACs)	Human lung tissue	Alveolar epithelial type 2 cells	+	Camostat	20

(Continues)

TABLE 1 (Continued)

	Organ model	Original cell or tissue	Cell composition	Permissiveness to SARS-CoV-2 infection	Effective drugs or compounds	Ref.
	3D cultures of human bronchial cells (h3BCs)	Human lung tissue	Airway cells including basal cells and secretory cells	+[b]	-	
	Human lung organoids	Human ES cells	Alveolar epithelial type 2 cells	+	Imatinib, Mycophenolic acid, Quinacrine dihydrochloride, Chloroquine	21
			Alveolar epithelial type 1 cells	Unknown		
			Stromal cells			
			Pulmonary neuroendocrine cells			
			Airway epithelial cells			
			Proliferating cells			
			Fibroblasts			
	Human bronchial organoids[a]	Cryopreserved adult human bronchial epithelial cells	Basal cells	+	Camostat	22
			Ciliated cells	Unknown		
			Goblet cells			
			Club cells			
Heart	Human cardiomyocytes	Human iPS cells	Cardiomyocytes	+	Remdesivir, *N*-acetyl-L-leucyl-L-leucyl-L-methionine, recombinant human ACE2 protein, ACE2 neutralizing antibody	23
	Human cardiospheres	Human iPS cells	Cardiomyocytes	+	–	
	Human cardiac cells	Human iPS cells	Cardiomyocytes	+	ACE2 neutralizing antibody, Aloxistatin, Remdesivir, IFN-β, Apilimod, Bafilomycin, Z-FY(tBu)-DMK	24
			Cardiac fibroblasts	–	–	
			Endothelial cells	–	–	
	Human cardiomyocytes	Human iPS cells	Cardiomyocytes	+	Berzosertib, Remdesivir	25
	Human cardiomyocytes	Human iPS cells	Cardiomyocytes	+	–	16
	Human cardiac cells	Human ES and iPS cells	Cardiomyocytes	+	–	26
			Smooth muscle cells	–	–	
	Human cardiac organoids	Human ES and iPS cells	Cardiomyocytes	+[b]	INCB054329 (BET inhibitor)	27
			Epicardial cells			
			Fibroblasts/pericytes			
			Endothelial cells			
	Human cardiomyocytes	Human ES cells	Cardiomyocytes	+	–	28
Intestine	Human intestinal organoids	Human ES cells	Enterocytes	+	Remdesivir, EK1	29
			Enteroendocrine cells	+		

TABLE 1 (Continued)

	Organ model	Original cell or tissue	Cell composition	Permissiveness to SARS-CoV-2 infection	Effective drugs or compounds	Ref.
			Paneth cells	+		
			Goblet cells	−		
	Human colonic organoids	Human ES cells	Enterocytes	+	Imatinib, Mycophenolic acid, Quinacrine dihydrochloride	21
			Goblet cells	+		
			Transit-amplifying cells	+		
			Enteroendocrine cells	+		
			Intestinal stem cells	+		
	Human intestinal organoids	Human iPS cells	Enterocytes	+[b]	−	30
			Paneth cells			
Kidney	Human kidney organoids	Human ES cells	Proximal tubules	+	Human recombinant soluble ACE2 protein	31
	Human kidney organoids	Human ES cells	Proximal tubular epithelial cells	+[b]	Human recombinant soluble ACE2	32
			Podocytes			
Blood vessels	Human blood vessel organoids	Human iPS cells	Endothelial cells	+[b]	Human recombinant soluble ACE2	32
			Pericytes			
	Human endothelial cells	Human ES cells	Endothelial cells	−	−	28
Brain	Human dopaminergic neurons	Human ES cells	Dopaminergic neurons	+	−	28
	Human cortical neurons	Human ES cells	Cortical neurons	−	−	28
	Human brain organoids	Human iPS cells	Neural progenitors/outer radial glia	+[b]	Anti-ACE2 antibodies, cerebrospinal fluid from a COVID-19 patient	33
			Intermediate progenitor/ interneurons			
			Neurons			
			Cortical neurons			
	Human cerebral organoids[a]	Human ES cells	Choroid plexus epithelial cells	+	−	34
			Neuronal progenitor cells	−	−	
			Neurons	−	−	
			Glial cells	−	−	
	Human brain cells	Human iPS cells	Neurons	−	−	35
			Astrocytes	−	−	
			Microglia	−	−	
	Human cortical organoids		−	−	−	
	Human hippocampal organoids		−	−	−	

(Continues)

TABLE 1 (Continued)

	Organ model	Original cell or tissue	Cell composition	Permissiveness to SARS-CoV-2 infection	Effective drugs or compounds	Ref.
	Human hypothalamic organoids		−	−	−	
	Human midbrain organoids		−	−	−	
	Human choroid plexus organoids		Choroid plexus epithelial cells	+	−	
	Human microglia	Human ES cells	Microglial cells	−	−	28
Pancreas	Human pancreatic endocrine cells	Human ES cells	Alpha cells	+	−	28
			Beta cells	+	−	
			Delta cells	−	−	
	Primary human islets	Human pancreatic organs	Beta cells	+	−	
			Alpha cells	+	−	
			Acinar cells	Unknown	−	
			Ductal cells		−	
			Mesenchymal cells		−	
			Poly-peptide cells		−	
			Delta cells		−	
			Endothelial cells		−	
			Immune cells		−	
Liver	Human liver organoids	Human iPS cells	−	+	−	28
	Human adult hepatocyte organoids	Human liver tissue	Hepatocytes	+	−	
	Human adult cholangiocyte organoids	Human liver tissue	Cholangiocytes	+	−	
Blood	Human macrophages	Human ES cells	Macrophages	−	−	28

[a]Air-liquid interface (ALI) culture.
[b]Did not analyze each component cell type separately.

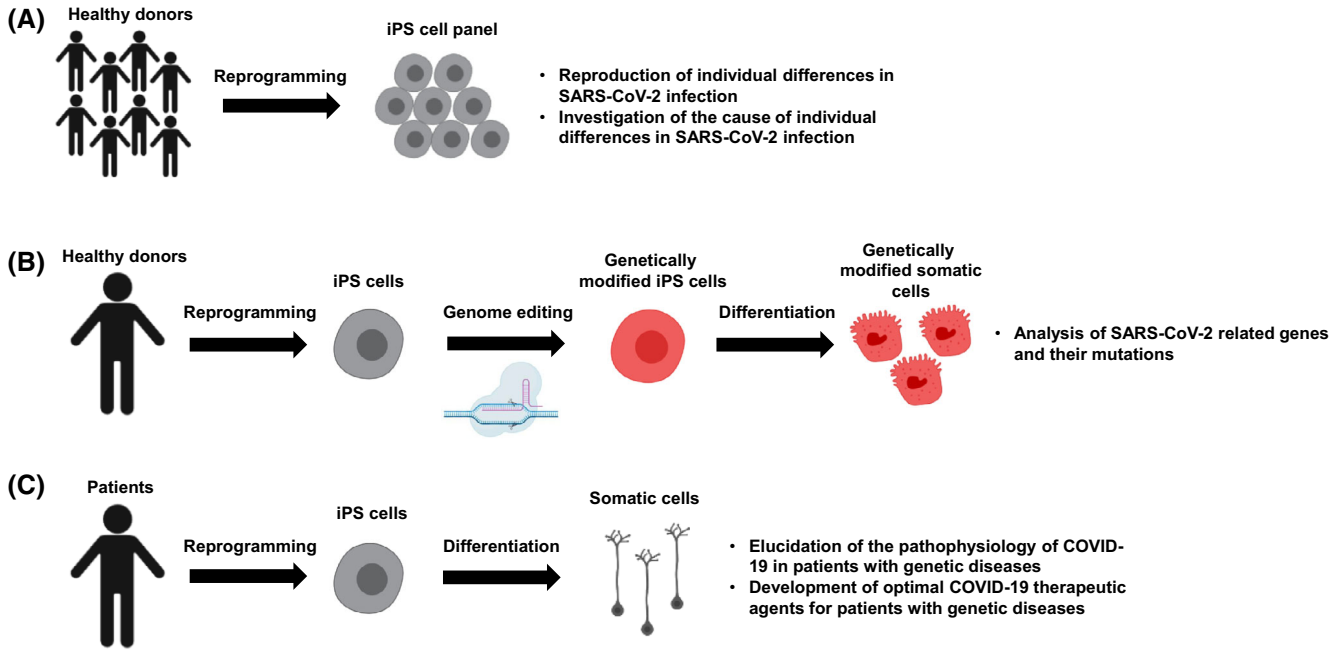

FIGURE 1 Search for genetic factors that increase the risk of COVID-19 aggravation. A, An induced pluripotent stem (iPS) cell panel can be used to recapitulate individual differences in SARS-CoV-2 infection. B, Genetically modified iPS cells and their derivatives can be used to analyze SARS-CoV-2-related genes. C, Patient-derived iPS cells can be used to elucidate the pathophysiology and select effective drugs at the individual level. This figure was created using BioRender (https://biorender.com/)

ACE2 treatment.[23] Following these findings, Aperion Biologics is conducting a phase 2 trial on human recombinant soluble ACE2.

Camostat and Nafamostat can inhibit type II transmembrane serine protease (TMPRSS2) to prevent SARS-CoV-2 entry.[41] We reported that Camostat treatment reduced the number of viral genome copies found in cell supernatants derived from cryopreserved human bronchial epithelial cell-derived bronchial organoids to approximately one-twentieth that in the untreated group.[22] Li et al also reported that Camostat pretreatment reduced the number of viral genome copies found in cell supernatants in human lung organoids derived from lung tissues to approximately one-tenth that of the untreated group.[19] TMPRSS2 expression is regulated by androgen receptors in human prostate cancer-derived LNCaP cells. Accordingly, treating LNCaP cells with an antiandrogen agent, enzalutamide, inhibits SARS-CoV-2 infection. However, since TMPRSS2 expression is not regulated by androgen receptors in lung organoids, the inhibitory effect of enzalutamide on SARS-CoV-2 infection has not been confirmed.

Other SARS-CoV-2 entry inhibitors have been discovered in drug screenings using organoids. Han et al screened for FDA-approved drugs in human ES cell-derived lung organoids and colonic organoids and found that Imatinib, Mycophenolic acid, and Quinacrine dihydrochloride each inhibit SARS-CoV-2 entry.[21] These drugs also showed antiviral effects in humanized mice with human ES cell-derived lung xenografts.

These studies show the benefits of human ES/iPS cell-derived somatic cells and organoids in the search for anti-SARS-CoV-2 drugs. In addition, they are expected to assist in searches for anti-inflammatory drugs. Furthermore, they can be used to evaluate the toxicity and safety of COVID-19 therapeutic agents.

4 | SEARCH FOR GENETIC FACTORS THAT INCREASE THE RISK OF COVID-19 AGGRAVATION

About 80% of COVID-19 patients are asymptomatic or mild, but the other 20% become severe. Various factors, such as aging, medical history, racial differences, and genetics, are predicted in COVID-19 aggravation.[42-45] Genome-wide association studies performed on mild and severe COVID-19 patients found differences in genetic backgrounds.[46] Because human iPS cells can be established from individuals of any genetic background, they make an attractive model to study the relationship between genetic factors and COVID-19 severity.

The mortality rate of COVID-19 has been reported to be higher in men than in women.[42] Therefore, we examined whether the gender differences in SARS-CoV-2 infection efficiency can be reproduced using human ES/iPS cells[47] (Figure 1A). Because human ES/iPS cells do not express ACE2, the gene was overexpressed. As a result, the copy number of the viral genome in the cell supernatant of male-derived ES/iPS cells was higher than that of females. Furthermore, male-derived ES/iPS cells tended to show higher TMPRSS2 expression levels than their female counterparts, suggesting that this difference may contribute to gender differences in SARS-CoV-2 infection efficiency.

Since genome editing in human ES/iPS cells is relatively efficient, functional analyses of gene mutations related to SARS-CoV-2 have also been performed. Dobrindt et al investigated SARS-CoV-2 infection in human iPS cells with a single nucleotide polymorphism (SNP) present in the FURIN gene[48] (Figure 1B). They used a CRISPR/Cas9-based allelic conversion system to generate isogenic human iPS

cells that have an SNP at the FURIN locus (rs4702) and confirmed that the expression level of FURIN and the amount of intracellular viral genome was low in human iPS cell-derived alveolospheres and neurons with SNP rs4702. Wang et al investigated how isoforms of the apolipoprotein E (ApoE) gene affect COVID-19 aggravation[49] (Figure 1C). The ApoE4 isoform is associated with an increased risk for Alzheimer's disease (AD), but the ApoE3 isoform is not. They generated iPS cells from an AD patient with the ApoE4 isoform and then modified the isoform to ApoE3 using CRISPR/Cas9 technology, finding that neurons derived from unmodified iPS cells were more easily infected than otherwise. In addition, SARS-CoV-2 infection significantly shortened the neurite length of ApoE4 iPS cell-derived neurons, and a large number of fragmented nuclei were observed in astrocytes derived from the same iPS cells. It was also confirmed that the ApoE4 isoforms did not affect the antiviral effect of Remdesivir.

Overall, human ES/iPS cells are being used as models to study individual differences in COVID-19 severity. Because iPS cells have been established from various populations, the study of individual differences will be accelerated by utilizing established human iPS cell panels and assist in clarifying the risks associated with the severity. Furthermore, by conducting SARS-CoV-2 infection experiments using iPS cells for genetic disorders, such as AD, it will be possible to elucidate the pathophysiology and select an appropriate treatment method for different genetic disorders.

5 | CONCLUSION AND FUTURE PERSPECTIVES

Human ES/iPS cell-derived somatic cells and organoids have helped identify how SARS-CoV-2 infects cells and causes organ failure. In addition, they have contributed to the development of many therapeutic agents, including Remdesivir and human recombinant soluble ACE2. By comparing the results obtained using these somatic cells and organoids with the results of clinical trials of COVID-19 therapeutic agents, the clinical predictability of these models can be clarified. Future work can use these somatic cells and organoids to study the SARS-CoV-2 life cycle and COVID-19 pathology and to develop safe and effective therapeutic agents. However, it is still difficult to reproduce the complex pathophysiology of COVID-19, including cytokine storms, using these models. Immune cells, especially T cells, are known to play an important role in cytokine storms. Coculturing T cells with non-immune cells, such as alveolar epithelial cells and vascular endothelial cells, may capture cytokine storms in a dish. Although this review introduced the application of these models to COVID-19 drug discovery, the models are also expected to contribute to regenerative medicine for COVID-19 patients. In particular, they can be used to generate cells that are less susceptible to SARS-CoV-2 infection for transplantation. With further development, human ES/iPS cell-derived somatic cells and organoids will contribute to the eradication of COVID-19.

ACKNOWLEDGMENTS

We thank Dr. Peter Karagiannis (Kyoto University) for critical reading of the manuscript and Dr. Misaki Ouchida (Kyoto University) for drawing the graphical abstract.

CONFLICT OF INTEREST

A. M. Brufsky declared Advisory role with Agendia, Eisai, Novartis, Genentech, Seattle Genetics, Pfizer, Tyme, Gliead. The other authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

S.D.: conception and design, manuscript writing; Á.S.-A., M.M.T., B.D.U., A.M.B.: manuscript writing; K.T.: conception and design, manuscript writing, final approval of manuscript.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

ORCID

Ángel Serrano-Aroca https://orcid.org/0000-0002-9953-3848
Kazuo Takayama https://orcid.org/0000-0002-1132-2457

REFERENCES

1. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579(7798):270-273.
2. Jiang R-D, Liu M-Q, Chen Y, et al. Pathogenesis of SARS-CoV-2 in transgenic mice expressing human angiotensin-converting enzyme 2. *Cell*. 2020;182(1):50-58.e58.
3. Winkler ES, Bailey AL, Kafai NM, et al. SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function. *Nat Immunol*. 2020;21(11):1327-1335.
4. Muñoz-Fontela C, Dowling WE, Funnell SG, et al. Animal models for COVID-19. *Nature*. 2020;586(7830):509-515.
5. Artegiani B, Clevers H. Use and application of 3D-organoid technology. *Hum Mol Genet*. 2018;27(R2):R99-R107.
6. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131(5):861-872.
7. Takayama K. In vitro and animal models for SARS-CoV-2 research. *Trends Pharmacol Sci*. 2020;41(8):513-517.
8. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *New Eng J Med*. 2020;382(8):727-733.
9. Guan W-j, Ni Z-Y, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *New Eng J Med*. 2020;382(18):1708-1720.
10. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061-1069.
11. Katsura H, Sontake V, Tata A, et al. Human lung stem cell-based alveolospheres provide insights into SARS-CoV-2-mediated interferon responses and pneumocyte dysfunction. *Cell Stem Cell*. 2020;27(6):890-904.e898.
12. Huang J, Hume AJ, Abo KM, et al. SARS-CoV-2 infection of pluripotent stem cell-derived human lung alveolar type 2 cells elicits a rapid epithelial-intrinsic inflammatory response. *Cell Stem Cell*. 2020;27(6):962-973.e967.
13. Mulay A, Konda B, Garcia G Jr, et al. SARS-CoV-2 infection of primary human lung epithelium for COVID-19 modeling and drug discovery. *Cell Rep*. 2021;35(5):109055.

14. Pei R, Feng J, Zhang Y, et al. Host metabolism dysregulation and cell tropism identification in human airway and alveolar organoids upon SARS-CoV-2 infection. *Protein Cell*. 2020.
15. Lamers MM, Mykytyn AZ, Breugem TI, et al. Human airway cells prevent SARS-CoV-2 multibasic cleavage site cell culture adaptation. *Elife*. 2021;10:e66815.
16. Li Y, Renner DM, Comar CE, et al. SARS-CoV-2 induces double-stranded RNA-mediated innate immune responses in respiratory epithelial-derived cells and cardiomyocytes. *Proc Natl Acad Sci*. 2021;118(16):e2022643118.
17. Yin X, Riva L, Pu Y, et al. MDA5 governs the innate immune response to SARS-CoV-2 in lung epithelial cells. *Cell Rep*. 2021;34(2):108628.
18. Lamers MM, van der Vaart J, Knoops K, et al. An organoid-derived bronchioalveolar model for SARS-CoV-2 infection of human alveolar type II-like cells. *EMBO J*. 2021;40(5):e105912.
19. Li F, Han M, Dai P, et al. Distinct mechanisms for TMPRSS2 expression explain organ-specific inhibition of SARS-CoV-2 infection by enzalutamide. *Nat Commun*. 2021;12(1):1-14.
20. Youk J, Kim T, Evans KV, et al. Three-dimensional human alveolar stem cell culture models reveal infection response to SARS-CoV-2. *Cell Stem Cell*. 2020;27(6):905-919.e910.
21. Han Y, Duan X, Yang L, et al. Identification of SARS-CoV-2 inhibitors using lung and colonic organoids. *Nature*. 2021;589(7841):270-275.
22. Suzuki T, Ito Y, Sakai Y et al. Generation of human bronchial organoids for SARS-CoV-2 research. *BioRxiv* 2020.
23. Bojkova D, Wagner JU, Shumliakivska M, et al. SARS-CoV-2 infects and induces cytotoxic effects in human cardiomyocytes. *Cardiovasc Res*. 2020;116(14):2207-2215.
24. Perez-Bermejo JA, Kang S, Rockwood SJ, et al. SARS-CoV-2 infection of human iPSC-derived cardiac cells reflects cytopathic features in hearts of patients with COVID-19. *Sci Transl Med*. 2021;13:eabf7872.
25. Garcia G Jr, Sharma A, Ramaiah A, et al. Antiviral drug screen identifies DNA-damage response inhibitor as potent blocker of SARS-CoV-2 replication. *Cell Rep*. 2021;35(1):108940.
26. Marchiano S, Hsiang T-Y, Khanna A, et al. SARS-CoV-2 infects human pluripotent stem cell-derived cardiomyocytes, impairing electrical and mechanical function. *Stem Cell Rep*. 2021;16(3):478-492.
27. Mills RJ, Humphrey SJ, Fortuna PR, et al. BET inhibition blocks inflammation-induced cardiac dysfunction and SARS-CoV-2 infection. *Cell*. 2021;184(8):2167-2182.e2122.
28. Yang L, Han Y, Nilsson-Payant BE, et al. A human pluripotent stem cell-based platform to study SARS-CoV-2 tropism and model virus infection in human cells and organoids. *Cell Stem Cell*. 2020;27(1):125-136.e127.
29. Krüger J, Groß R, Conzelmann C, et al. Drug inhibition of SARS-CoV-2 replication in human pluripotent stem cell–derived intestinal organoids. *Cell Mol Gastroenterol Hepatol*. 2021;11(4):935-948.
30. Mithal A, Hume AJ, Lindstrom-Vautrin J, et al. Human pluripotent stem cell-derived intestinal organoids model SARS-CoV-2 infection revealing a common epithelial inflammatory response. *Stem Cell Rep*. 2021;16(4):940-953.
31. Wysocki J, Ye M, Hassler L, et al. A novel soluble ACE2 variant with prolonged duration of action neutralizes SARS-CoV-2 infection in human kidney organoids. *J Am Soc Nephrol*. 2021;32(4):795-803.
32. Monteil V, Kwon H, Prado P, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell*. 2020;181(4):905-913.e907.
33. Song E, Zhang C, Israelow B, et al. Neuroinvasion of SARS-CoV-2 in human and mouse brain. *J Exp Med*. 2021;218(3):e20202135.
34. Pellegrini L, Albecka A, Mallery DL, et al. SARS-CoV-2 infects the brain choroid plexus and disrupts the blood-CSF barrier in human brain organoids. *Cell Stem Cell*. 2020;27(6):951-961.e955.
35. Jacob F, Pather SR, Huang W-K, et al. Human pluripotent stem cell-derived neural cells and brain organoids reveal SARS-CoV-2 neurotropism predominates in choroid plexus epithelium. *Cell Stem Cell*. 2020;27(6):937-950.e939.
36. Shi S, Qin M, Shen B, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol*. 2020;5(7):802-810.
37. Mao L, Jin H, Wang M, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol*. 2020;77(6):683-690.
38. Han C, Duan C, Zhang S, et al. Digestive symptoms in COVID-19 patients with mild disease severity: clinical presentation, stool viral RNA testing, and outcomes. *Am J Gastroenterol*. 2020;115:916-923.
39. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the treatment of Covid-19. *New Eng J Med*. 2020;383(19):1813-1826.
40. Cox RM, Wolf JD, Plemper RK. Therapeutically administered ribonucleoside analogue MK-4482/EIDD-2801 blocks SARS-CoV-2 transmission in ferrets. *Nat Microbiol*. 2021;6(1):11-18.
41. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 2020;181(2):271-280.e278.
42. Jin J-M, Bai P, He W, et al. Gender differences in patients with COVID-19: focus on severity and mortality. *Front Public Health*. 2020;8:152.
43. Zhu L, She Z-G, Cheng X, et al. Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes. *Cell Metab*. 2020;31(6):1068-1077.e1063.
44. Zhang P, Zhu L, Cai J, et al. Association of inpatient use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers with mortality among patients with hypertension hospitalized with COVID-19. *Circ Res*. 2020;126(12):1671-1681.
45. Millett GA, Jones AT, Benkeser D, et al. Assessing differential impacts of COVID-19 on black communities. *Ann Epidemiol*. 2020;47:37-44.
46. Severe Covid-19 GWAS Group. Genomewide association study of severe Covid-19 with respiratory failure. *New Eng J Med*. 2020;383(16):1522-1534.
47. Sano E, Deguchi S, Sakamoto A, et al. Modeling SARS-CoV-2 infection and its individual differences with ACE2-expressing human iPS cells. *iScience*. 2021;24(5):102428.
48. Dobrindt K, Hoagland DA, Seah C, et al. Common genetic variation in humans impacts in vitro susceptibility to SARS-CoV-2 infection. *Stem Cell Rep*. 2021;16(3):505-518.
49. Wang C, Zhang M, Garcia G Jr, et al. ApoE-isoform-dependent SARS-CoV-2 neurotropism and cellular response. *Cell Stem Cell*. 2021;28(2):331-342.e335.

How to cite this article: Deguchi S, Serrano-Aroca Á, Tambuwala MM, Uhal BD, Brufsky AM, Takayama K. SARS-CoV-2 research using human pluripotent stem cells and organoids. *STEM CELLS Transl Med*. 2021;1-9. https://doi.org/10.1002/sctm.21-0183