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Review

A potential *ex vivo* infection model of human induced pluripotent stem cell-3D organoids beyond coronavirus disease 2019

Hang Zhou[1], Li-Ping Liu[1], Mei Fang[1], Yu-Mei Li[1] and Yun-Wen Zheng[1,2,3,4,5]

[1]Institute of Regenerative Medicine, Affiliated Hospital of Jiangsu University, Jiangsu University, Zhenjiang, Jiangsu, China, [2]Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, [3]School of Medicine, Yokohama City University, Yokohama, Kanagawa, [4]Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan and [5]School of Biotechnology and Health Sciences, Wuyi University, Jiangmen, Guandong, P.R. China

Summary. The novel coronavirus disease 2019 (COVID-19) outbreak began in the city of Wuhan, whereupon it rapidly spread throughout China and subsequently across the world. Rapid transmission of COVID-19 has caused wide-spread panic. Many established medications have been used to treat the disease symptoms; however, no specific drugs or vaccines have been developed. Organoids derived from human induced pluripotent stem cells (iPSCs) may serve as suitable infection models for *ex vivo* mimicking of the viral life cycle and drug screening. Human iPSC-3D organoids, self-organised tissues with multiple cell environments, have a similar structure and function as real human organs; hence, these organoids allow greater viral infection efficiency, mimic the natural host-virus interactions, and are suitable for long-term experimentation. Here, we suggest the use of a functional human iPSC-organoid that could act as a reliable and feasible *ex vivo* infection model for investigation of the virus. This approach will provide much needed insight into the underlying molecular dynamics of COVID-19 for the development of novel treatment and prevention strategies.

Key words: COVID-19, Human induced pluripotent stem cells, Organoids, Disease model, 3D

Offprint requests to: Yun-Wen Zheng, PhD, University of Tsukuba, Faculty of Medicine, Tsukuba, Ibaraki 305-8575, Japan. e-mail: ywzheng@md.tsukuba.ac.jp or Yu-Mei Li, MD, PhD, The Affiliated Hospital of Jiangsu University, No.438 Jiefang Rd, Zhenjiang 212001, Jiangsu Province, China. e-mail: yumeili@ujs.edu.cn

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Introduction

Up until 8th June, 2020, the 2019 novel coronavirus (2019-nCoV) has resulted in over 6,931,000 people being infected, and more than 400,000 deaths due to novel coronavirus–infected pneumonia (NCIP). The population of the infection and death still changing, data can be tracked by the website https://www.who.int/. The pathology of infection involves direct virus attacks on cells and secondary attacks on the body after immune system activation. This means both the virus and the immune response can cause damage to the body, presenting as a cough, fever, headache, and common complications, or secondary infection may occur (Huang et al., 2020). While restrictions limiting the movement of people has been enforced to reduce the spread of the coronavirus disease 2019 (COVID-19), many people were already infected, some of whom are experiencing worsening conditions. The development of feasible and effective therapies is extremely urgent. The use of human induced pluripotent stem cell (iPSC)-developed organoids may provide a robust model for investigating viral infection and drug screening. Lung bud organoids (LBOs) using human iPSCs have been designed for respiratory syncytial virus infection research (Chen et al., 2017). Similarly, a functional liver organoid (LO) generated by human iPSCs was developed as an individualised hepatitis B virus (HBV) infection model, which could prove to be a robust and, at the same time, long-term HBV infection model for studying the disease and screening new drug therapies (Nie et al., 2018). As COVID-19 also causes renal functional damage (Chen et al., 2020), kidney organoids from iPSCs (Phipson et al.,

2018) may be a potential model for the study. The aim of this review is to provide an overview of COVID-19 and present an original idea to ultimately restrict the spread of infection.

COVID-19: Knowns and unknowns

COVID-19 has been raging for several months, leading to the development of a pandemic. Through genome sequence analysis and other methods, 2019-nCoV has been shown to be similar to some beta-coronaviruses detected in bats, which indicates that 2019-nCoV is possibly of bat origin (Lu et al., 2020; Wu et al., 2020; Zhu et al., 2020). One study indicated that the mean incubation period of COVID-19 was 5.2 days, and the population of infected patients doubled every 7.4 days in its early stages (Li et al., 2020). A transmission model was developed and the pandemic was found to follow an exponential growth in cases, and according to the epidemiological characteristics and outbreak dynamics, human-to-human transmission (Chan et al., 2020) was demonstrated. Without a complete understanding of the infection process and the development of an effective treatment for 2019-nCoV, the consequences are unimaginable. Men showed a higher infection rate than women, most of the infections were in quinquagenarians, and not all of the infected people had frequented the Huanan seafood market (Chen et al., 2020).

The clinical manifestations of COVID-19 include fever, cough, dyspnoea, myalgia or fatigue, confusion, headache, sore throat, sputum, rhinorrhoea, haemoptysis, chest pain, diarrhoea, nausea, vomiting, and other common complications (Chan et al., 2020; Chen et al., 2020; Huang et al., 2020; Wang et al., 2020). Common complications include acute respiratory distress syndrome (ARDS), RNAaemia, acute cardiac injury, and secondary infection. A minority of patients developed ARDS and septic shock, and some of these patients worsened in a short period of time and died of multiple organ failure or persistent hypoxemia. According to CT examination, most imaging showed bilateral pneumonia, some patients showed multiple mottling and ground-glass opacity, and a few had pneumothorax (Huang et al., 2020). Biochemical examination (Chan et al., 2020, Chen et al., 2020, Huang et al., 2020, Wang et al., 2020a,b) revealed that some patients had abnormal platelet counts, liver functional damage, myocardial zymogram abnormalities, and renal functional damage as observed by blood urea nitrogen or serum creatinine elevation. C-reactive protein levels in most patients were above the normal range. All of these complicated symptoms and auxiliary inspection results render the pathway of 2019-nCoV infection and progression more obscure; additionally, underlying diseases (Chen et al., 2020) further interfere with the current understanding of disease progression.

Lymphocyte levels were reduced in most patients, which indicated that 2019-nCoV might mainly affect these cells, especially T lymphocytes (Chen et al., 2020). Plasma IL1B, IL1RA, IL7, IL8, IL9, IL10, basic FGF, GCSF, GMCSF, IFNγ, IP10, MCP1, MIP1A, MIP1B, PDGF, TNFα, and VEGF concentration levels were elevated in infected patients (Huang et al., 2020). The difference between non- intensive care unit (ICU) patients and ICU patients was that the latter had higher plasma levels of IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNFα. All of these detection results signify the immune response triggered in infected patients. The induction of immunological reactions can cause indirect damage to cells, including antibody-mediated immune damage, cell-mediated immune damage, autoimmune disease, cytokine-mediated inflammation, and immunological inhibition. The precise immune activation process is still not clear, and the beneficial and harmful processes remain to be elucidated.

The angiotensin converting enzyme 2 (ACE2) receptor is widely distributed in the lungs (Oarhe et al., 2015; Zhang et al., 2018), bile ducts (Paizis et al., 2005), myocardium (Ramchand et al., 2020), kidneys, brain, and other tissues (Santos et al., 2018). Some studies have reported ACE2 as the cell entry receptor of 2019-nCoV (Lu et al., 2020; Tian et al., 2020; Zhou et al., 2020). Based on the sequence of 2019-nCoV, the receptor-binding motif (RBM) indicates ACE2 as its potential receptor, and some residues in the 2019-nCoV RBM (particularly Gln493) have effectively combined with human ACE2, supporting this hypothesis. The affinity (KD) calculated between the 2019-nCoV receptor-binding domain (RBD) and human ACE2 was 15.2 nM, which is similar to severe acute respiratory syndrome (SARS)-CoV RBD (15 nM). Furthermore, CR3022, a SARS-CoV-specific antibody, was found to have the ability to bind with 2019-nCoV RBD (Tian et al., 2020). Therefore, tissues or organs with ACE2 may have the potential to be infected, and CR3022 might have the potential to be used for 2019-nCoV vaccine research. To confirm these hypotheses, an efficient model is indispensable.

Treatment of COVID-19 includes antiviral drugs, antibiotics, steroid hormones, oxygen support (e.g., nasal cannula and invasive ventilator to assist ventilation), and extracorporeal membrane oxygenation (Chen et al., 2020). While all of these approaches are established therapies, none of them are directed against COVID-19, and large gaps in the treatment and prevention of this disease remain.

Direct pathogenicity between viruses and host cells have many patterns, including cytocidal infection, steady state infection, apoptosis, cell proliferation and transformation, gene integration, and the formation of inclusion bodies. If it is a cytocidal infection, as in the case of adenoviruses, viruses mature within host cells and release a large number of progeny viruses in a short time, leading to cell lysis and death (Huang et al., 2020). Viral infection could disturb nucleic acid and protein metabolism. Steady state infection, as in the case of the herpes virus, would not lead to cell lysis, but would

result in host cell fusion and cause new antigens to be presented on the surface (Liu et al., 2019). Apoptosis is a positive progression, it is a protective mechanism that limits the replication of viruses, but it damages organ function. For cell proliferation and transformation, some viruses promote cell proliferation and cause tumours (Rotondo et al., 2019). As for gene integration, as in the case of the papillomavirus, viruses integrate their genes into the host cell genome, with unpredictable consequences (Drolet et al., 2019; Massarelli et al., 2019). The formation of inclusion bodies could assist in the diagnosis of some virus diseases (Ringel et al., 2019). The pathogenic process of the 2019-nCoV remains to be determined.

Human iPSC-organoids

While COVID-19 has already spread globally, the infection pathway and pathogenic mechanisms of 2019-nCoV *in vivo* are still uncertain. To study the pathophysiological features of virus infection or drug screening, an excellent disease model is necessary. Two-dimensional (2D)-models (Xu et al., 2016) and animal disease models (Bian et al., 2017; Grosenbach et al., 2018) are frequently used in viral infection studies. However, 2D-models cannot simulate an *in vivo* microenvironment, which limits the reliability of the research. The large gap between different species, even between primates and humans, limits the effectiveness of animal models (Boni et al., 2018); moreover, the restricted use of primates is another obstacle. Airway organoids derived from broncho-alveolar resections or lavage material are able to be maintained for over 1 year and are amenable to drug screening (Sachs et al., 2019); however, these tissues are often not freely available, and do not comprise suitable cells for mass amplification. Human iPSC-3D organoids may be an ideal tool to help investigate the remaining uncertainties surrounding COVID-19.

Relatively perfect structures and functions could be an advantage for a disease model. LOs derived from human iPSCs, cultured in three-dimensional microwells, showed promising potential in investigating the precise roles of HBV and evaluating drug toxicity (Nie et al., 2018). Human iPSC-derived endodermal, mesenchymal, and endothelial cells are contained in the system, which highly simulate the human liver. Intercellular interactions like tight junctions are essential for a stable structure, and have been observed in the LO. Further, human iPSC-LOs not only have very similar structures to microvilli, lipid droplets, and bile capillaries between hepatic cells but also have almost perfect function, such as protein secretion and metabolism. Expression of specific hepatic functional genes in differentiated LOs was significantly higher than that in human iPSC-derived hepatic-like cells. In another study, human Embryonic Stem Cells- and iPSCs-LOs expressed cytochrome P450 proteins (CYP) families at a higher level than in 2D-differentiated mature hepatocytes (Mun et al., 2019).

Susceptibility is a precondition for viral infection no matter whether *in vivo* or *in vitro*. Human pluripotent stem cell (hPSC)-derived 3D LBOs express the lung markers EPCAM, KRT8, NKX2.1, FOXA1, FOXJ1, CC10, mucins, and P63 (Chen et al., 2017). These LBOs demonstrated swelling, detachment, and shedding, which is similar to human lungs when infected with respiratory syncytial virus. Compared to primary human hepatocytes, Na^+-taurocholate co-transporting polypeptide, an HBV entry receptor, was higher in human iPSC-LOs (Nie et al., 2018), which indicated a high susceptibility to HBV infection. There are also factors that enhance infection efficiency, such as GPC5, PPARA, and CEBPA, which were higher in human iPSC-Los. After infection with HBV, pg RNA, intercellular vDNA, cccDNA, and supernatant vDNA were found at a higher level in human iPSC-LOs compared to human iPSC-hepatic-like cells.

Immunity is an inherent characteristic of the human body, except in immunodeficient patients. Organoids can provide space and facilitation for immune activities. Immune activation was observed in human iPSC-LOs that controls replication of HBV but increased additional hepatic injury, which closely mimicked the *in vivo* process (Nie et al., 2018). Lung stem cell-derived human airway organoids were infected with influenza A H1N1 subtype (H1N1) and avian influenza A H7N9 viruses; these organoids were similar to airway tissues in composition, cell diversity, and the organisation of p63a+ basal cells, acetyl-α-tubulin+ ciliated cells, MUC5AC+ secretory goblet cells, and SCGB1A1+/CC10+ secretory club cells. After viral infection, T-cell and related cytokines were increased in the airway organoid (Hui et al., 2018).

To observe the complete infection process, virus life cycle, and fully study pharmacological actions, a long-term model is essential. Human iPSC-LOs were able to maintain HBV propagation in the system for a minimum of 20 days (Nie et al., 2018), which is superior to the time scale of cell culture experiments. LOs have been maintained for more than 3 months after *in vitro* culture (Mun et al., 2019). In addition, there are other long-term alveolar organoids (Yamamoto et al., 2017) and LOs (Hu et al., 2018) that may be maintained over a relatively long time. There are also iPSC-derived cardiac organoids (Mills et al., 2019; Schulze et al., 2019) and kidney organoids (Phipson et al., 2018) that have suitable cellular density, microarchitecture and functioning, which can be similarly used for viral infection and drug screening research.

Expectations of an *ex vivo* COVID-19 infection model

Many treatments have been employed to treat the symptoms of COVID-19, including anti-infection agents and oxygen support, but none of these therapeutic methods is specific for this disease. The need for medication designed for COVID-19 has become more

and more critical, especially for severe cases. Human airway epithelium was cultured to simulate 2019-nCoV-infection and observed over 6 days; 96 hours after inoculation cytopathic effects appeared on the surface layers of the human airway epithelial cells and there was an observed absence of cilia beating, but specific cytopathic effects were not observed in the comparative Vero E6 and Huh-7 cell lines until the last day (Zhu et al., 2020) – the difference between Vero E6 cells and human airway epithelial cells was obvious. Another study evaluated the antiviral efficiency of several drugs against 2019-nCoV in Vero E6 cells (ATCC-1586), and found that remdesivir and chloroquine are highly efficacious (Wang et al., 2020a,b). While the results of this study are promising, the fact that Vero E6 cells were used means the reliability of the results must be considered, as per the study by Zhu et al. (2020). Research models that mimic 2019-nCoV life cycle, infection routes, pathophysiological process, and potential replication inhibition strategies are limited.

Previous research has shown that human iPSCs have natural advantages allowing them to propagate indefinitely *in vitro* and differentiate into multiple cell types. Human iPSC-developed organoids have similar structures and functions as human organs, which makes them suitable for COVID-19 modelling (Fig. 1). After viral infection, the organoids can simulate an immune response, which happens during all kinds of infection. From the known COVID-19 clinical symptoms, complications, biochemical analyses, and imaging results, it is apparent that the lungs, heart, liver, kidneys, and other organs are involved in disease progression. ACE2 has been identified as the cell entry receptor of 2019-nCoV, and these receptors have been found in bile duct, lung, kidney, heart, brain, and other tissues. It is likely that the aforementioned organs or tissues are directly affected by COVID-19, but the connection between them still needs to be corroborated. Thus, human iPSC-developed organoids could serve as an ideal model to examine viral infection and perform drug screening; furthermore, the effects of both infection and host immune response in different cell types can be observed in a single system.

Conclusion

Despite COVID-19 being so prevalent, knowledge of the mechanism of infection, pathological processes, and

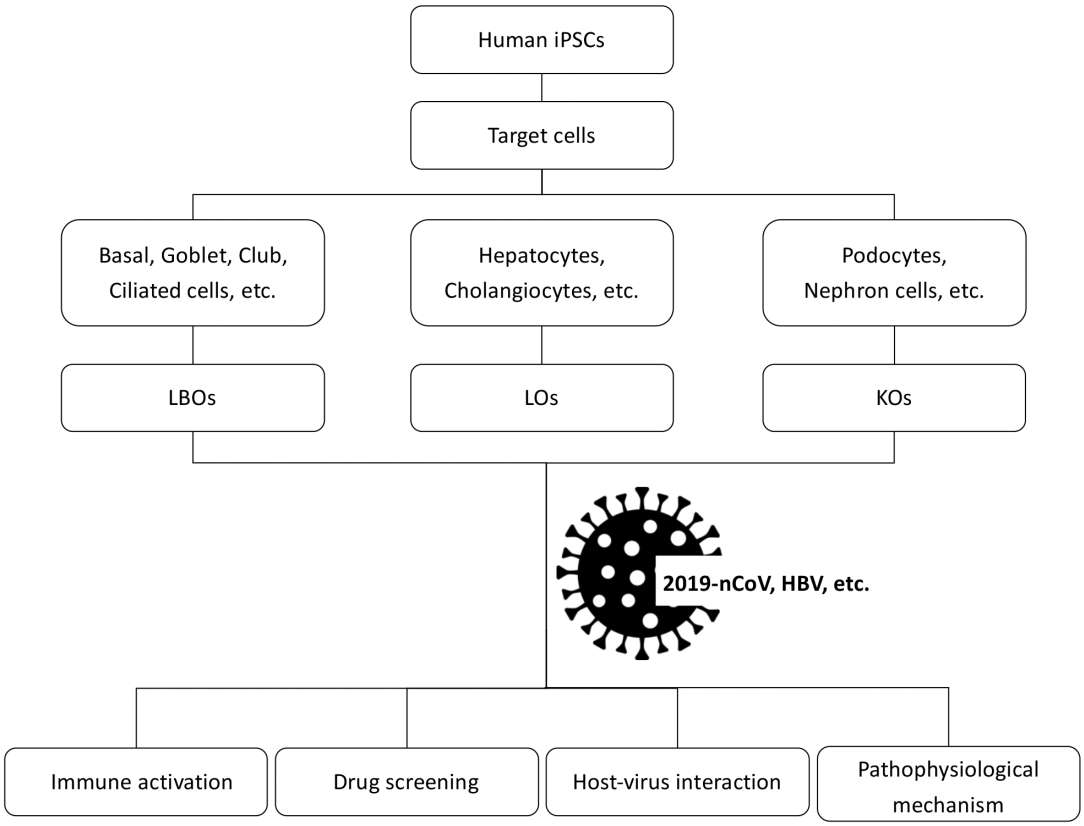

Fig. 1. Self-organization and potential application of iPSC-3D organoids. Lung bud organoids (LBOs), liver organoids (LOs) and kidney organoids (KOs) are developed from human iPSCs, these iPSC-3D organoids can be used for virus researches (e.g. respiratory syncytial virus, hepatitis B virus), they may be potential models for COVID-19.

treatment strategies are still limited. Human iPSC-3D organoids provide an ideal system to explore COVID-19 pathogenesis, with suitable structures and functioning, a high susceptibility to viruses, immune system response capabilities, and the potential for long-term maintenance. Moreover, this system could serve as a potential *ex vivo* infection model for viruses other than 2019-nCoV.

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Conflict of interest. The authors declare no potential conflict of interest.

References

Bian Y., Zhang Z., Sun Z., Zhao J., Zhu D., Wang Y., Fu S., Guo J., Liu L., Su L., Wang F.-S., Fu Y.-X. and Peng H. (2017). Vaccines targeting preS1 domain overcome immune tolerance in hepatitis B virus carrier mice. Hepatology 66, 1067-1082.

Boni C., Vecchi A., Rossi M., Laccabue D., Giuberti T., Alfieri A., Lampertico P., Grossi G., Facchetti F., Brunetto M. R., Coco B., Cavallone D., Mangia A., Santoro R., Piazzolla V., Lau A., Gaggar A., Subramanian G. M. and Ferrari C. (2018). TLR7 agonist increases responses of hepatitis b virus-specific t cells and natural killer cells in patients with chronic hepatitis B treated with nucleos(T)ide analogues. Gastroenterology 154, 1764-1777. e1767.

Chan J. F.-W., Yuan S., Kok K.-H., To K. K.-W., Chu H., Yang J., Xing F., Liu J., Yip C.C.-Y., Poon R.W.-S., Tsoi H.-W., Lo S.K.-F., Chan K.-H., Poon V.K.-M., Chan W.-M., Ip J.D., Cai J.-P., Cheng V.C.-C., Chen H., Hui C.K.-M. and Yuen K.-Y. (2020). A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 395, 514-523.

Chen N., Zhou M., Dong X., Qu J., Gong F., Han Y., Qiu Y., Wang J., Liu Y., Wei Y., Xia J.A., Yu T., Zhang X. and Zhang L. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 395, 507-513.

Chen Y.W., Huang S.X., de Carvalho A., Ho S.H., Islam M.N., Volpi S., Notarangelo L.D., Ciancanelli M., Casanova J.L., Bhattacharya J., Liang A.F., Palermo L.M., Porotto M., Moscona A. and Snoeck H.W. (2017). A three-dimensional model of human lung development and disease from pluripotent stem cells. Nat. Cell Biol. 19, 542-549.

Drolet M., Bénard É., Pérez N., Brisson M. and HPV Vaccination Impact Study Group (2019). Population-level impact and herd effects following the introduction of human papillomavirus vaccination programmes: updated systematic review and meta-analysis. Lancet 394, 497-509.

Grosenbach D.W., Honeychurch K., Rose E.A., Chinsangaram J., Frimm A., Maiti B., Lovejoy C., Meara I., Long P. and Hruby D.E. (2018). Oral tecovirimat for the treatment of smallpox. N. Engl. J. Med. 379, 44-53.

Hu H., Gehart H., Artegiani B., López-Iglesias C., Dekkers F., Basak O., van Es J., Chuva de Sousa Lopes S.M., Begthel H., Korving J., van den Born M., Zou C., Quirk C., Chiriboga L., Rice C.M., Ma S., Rios A., Peters P.J., de Jong Y.P. and Clevers H. (2018). Long-term expansion of functional mouse and human hepatocytes as 3D organoids. Cell. 175, 1591-1606.e1519.

Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y., Zhang L., Fan G., Xu J., Gu X., Cheng Z., Yu T., Xia J., Wei Y., Wu W., Xie X., Yin W., Li H., Liu M., Xiao Y., Gao H., Guo L., Xie J., Wang G., Jiang R., Gao Z., Jin Q., Wang J. and Cao B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497-506.

Hui K.P.Y., Ching R.H.H., Chan S.K.H., Nicholls J.M., Sachs N., Clevers H., Peiris J.S.M. and Chan M.C.W. (2018). Tropism, replication competence, and innate immune responses of influenza virus: an analysis of human airway organoids and ex-vivo bronchus cultures. Lancet Respir. Med. 6, 846-854.

Li Q., Guan X., Wu P., Wang X., Zhou L., Tong Y., Ren R., Leung K.S.M., Lau E.H.Y., Wong J.Y., Xing X., Xiang N., Wu Y., Li C., Chen Q., Li D., Liu T., Zhao J., Liu M., Tu W., Chen C., Jin L., Yang R., Wang Q., Zhou S., Wang R., Liu H., Luo Y., Liu Y., Shao G., Li H., Tao Z., Yang Y., Deng Z., Liu B., Ma Z., Zhang Y., Shi G., Lam T.T.Y., Wu J.T., Gao G.F., Cowling B.J., Yang B., Leung G.M. and Feng Z. (2020). Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N. Engl. J. Med. 382, 1199-1207.

Liu Y.-T., Jih J., Dai X., Bi G.-Q. and Zhou Z.H. (2019). Cryo-EM structures of herpes simplex virus type 1 portal vertex and packaged genome. Nature 570, 257-261.

Lu R., Zhao X., Li J., Niu P., Yang B., Wu H., Wang W., Song H., Huang B., Zhu N., Bi Y., Ma X., Zhan F., Wang L., Hu T., Zhou H., Hu Z., Zhou W., Zhao L., Chen J., Meng Y., Wang J., Lin Y., Yuan J., Xie Z., Ma J., Liu W.J., Wang D., Xu W., Holmes E.C., Gao G.F., Wu G., Chen W., Shi W. and Tan W. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395, 565-574.

Massarelli E., William W., Johnson F., Kies M., Ferrarotto R., Guo M., Feng L., Lee J.J., Tran H., Kim Y. U., Haymaker C., Bernatchez C., Curran M., Zecchini Barrese T., Rodriguez Canales J., Wistuba I., Li L., Wang J., van der Burg S.H., Melief C.J. and Glisson B. (2019). Combining Immune Checkpoint Blockade and Tumor-Specific Vaccine for Patients With Incurable Human Papillomavirus 16-Related Cancer: A phase 2 clinical trial. JAMA Oncol. 5, 67-73.

Mills R.J., Parker B.L., Quaife-Ryan G.A., Voges H.K., Needham E.J., Bornot A., Ding M., Andersson H., Polla M., Elliott D.A., Drowley L., Clausen M., Plowright A.T., Barrett I.P., Wang Q.-D., James D.E., Porrello E.R. and Hudson J.E. (2019). Drug screening in human PSC-cardiac organoids identifies pro-proliferative compounds acting via the mevalonate pathway. Cell Stem Cell 24, 895-907.e896.

Mun S.J., Ryu J.S., Lee M. O., Son Y.S., Oh S.J., Cho H.S., Son M.Y., Kim D.S., Kim S.J., Yoo H.J., Lee H.J., Kim J., Jung C.R., Chung K.S. and Son M.J. (2019). Generation of expandable human pluripotent stem cell-derived hepatocyte-like liver organoids. J. Hepatol. 71, 970-985.

Nie Y.Z., Zheng Y.W., Miyakawa K., Murata S., Zhang R.R., Sekine K., Ueno Y., Takebe T., Wakita T., Ryo A. and Taniguchi H. (2018). Recapitulation of hepatitis B virus-host interactions in liver organoids from human induced pluripotent stem cells. EBioMedicine 35, 114-123.

Oarhe C.I., Dang V., Dang M., Nguyen H., Gopallawa I., Gewolb I.H. and Uhal B.D. (2015). Hyperoxia downregulates angiotensin-converting enzyme-2 in human fetal lung fibroblasts. Pediatr. Res. 77, 656-662.

Paizis G., Tikellis C., Cooper M.E., Schembri J.M., Lew R.A., Smith A.I., Shaw T., Warner F.J., Zuilli A., Burrell L.M. and Angus P.W. (2005). Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. Gut 54, 1790-1796.

Phipson B., Er P.X., Combes A.N., Forbes T.A., Howden S.E., Zappia L., Yen H.-J., Lawlor K.T., Hale L. J., Sun J., Wolvetang E., Takasato M., Oshlack A. and Little M.H. (2018). Evaluation of variability in human kidney organoids. Nature Methods 16, 79-87.

Ramchand J., Patel S.K., Kearney L.G., Matalanis G., Farouque O., Srivastava P.M. and Burrell L.M. (2020). Plasma ACE2 activity predicts mortality in aortic stenosis and is associated with severe myocardial fibrosis. JACC Cardiovasc. Imaging. 13, 655-664.

Ringel M., Heiner A., Behner L., Halwe S., Sauerhering L., Becker N., Dietzel E., Sawatsky B., Kolesnikova L. and Maisner A. (2019). Nipah virus induces two inclusion body populations: Identification of novel inclusions at the plasma membrane. PLoS Pathog. 15, e1007733.

Rotondo J.C., Mazzoni E., Bononi I., Tognon M. and Martini F. (2019). Association between simian virus 40 and human tumors. Front Oncol. 9, 670-670.

Sachs N., Papaspyropoulos A., Zomer-van Ommen D. D., Heo I., Bottinger L., Klay D., Weeber F., Huelsz-Prince G., Iakobachvili N., Amatngalim G.D., de Ligt J., van Hoeck A., Proost N., Viveen M.C., Lyubimova A., Teeven L., Derakhshan S., Korving J., Begthel H., Dekkers J.F., Kumawat K., Ramos E., van Oosterhout M.F., Offerhaus G.J., Wiener D.J., Olimpio E.P., Dijkstra K.K., Smit E.F., van der Linden M., Jaksani S., van de Ven M., Jonkers J., Rios A.C., Voest E.E., van Moorsel C.H., van der Ent C.K., Cuppen E., van Oudenaarden A., Coenjaerts F.E., Meyaard L., Bont L.J., Peters P.J., Tans S.J., van Zon J.S., Boj S.F., Vries R.G., Beekman J.M. and Clevers H. (2019). Long-term expanding human airway organoids for disease modeling. EMBO J. 38,

Santos R.A.S., Sampaio W.O., Alzamora A.C., Motta-Santos D., Alenina N., Bader M. and Campagnole-Santos M.J. (2018). The ACE2/angiotensin-(1-7)/MAS axis of the renin-angiotensin system: Focus on angiotensin-(1-7). Physiol. Rev. 98, 505-553.

Schulze M.L., Lemoine M.D., Fischer A.W., Scherschel K., David R., Riecken K., Hansen A., Eschenhagen T. and Ulmer B.M. (2019). Dissecting hiPSC-CM pacemaker function in a cardiac organoid model. Biomaterials 206, 133-145.

Tian X., Li C., Huang A., Xia S., Lu S., Shi Z., Lu L., Jiang S., Yang Z., Wu Y. and Ying T. (2020). Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. Emerg. Microbes Infect. 9, 382-385.

Wang D., Hu B., Hu C., Zhu F., Liu X., Zhang J., Wang B., Xiang H., Cheng Z., Xiong Y., Zhao Y., Li Y., Wang X. and Peng Z. (2020a). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA.

Wang M., Cao R., Zhang L., Yang X., Liu J., Xu M., Shi Z., Hu Z., Zhong W. and Xiao G. (2020b). Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) *in vitro*. Cell Res. 30, 269-271.

Wu A., Peng Y., Huang B., Ding X., Wang X., Niu P., Meng J., Zhu Z., Zhang Z., Wang J., Sheng J., Quan L., Xia Z., Tan W., Cheng G. and Jiang T. (2020). Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe. 27, 325-328.

Xu M., Lee E.M., Wen Z., Cheng Y., Huang W.-K., Qian X., Tcw J., Kouznetsova J., Ogden S.C., Hammack C., Jacob F., Nguyen H.N., Itkin M., Hanna C., Shinn P., Allen C., Michael S.G., Simeonov A., Huang W., Christian K.M., Goate A., Brennand K.J., Huang R., Xia M., Ming G.-L., Zheng W., Song H. and Tang H. (2016). Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. Nat. Med. 22, 1101-1107.

Yamamoto Y., Gotoh S., Korogi Y., Seki M., Konishi S., Ikeo S., Sone N., Nagasaki T., Matsumoto H., Muro S., Ito I., Hirai T., Kohno T., Suzuki Y. and Mishima M. (2017). Long-term expansion of alveolar stem cells derived from human iPS cells in organoids. Nature Methods 14, 1097-1106.

Zhang J., Dong J., Martin M., He M., Gongol B., Marin T.L., Chen L., Shi X., Yin Y., Shang F., Wu Y., Huang H. Y., Zhang J., Zhang Y., Kang J., Moya E.A., Huang H.D., Powell F.L., Chen Z., Thistlethwaite P.A., Yuan Z.Y. and Shyy J.Y. (2018). AMP-activated protein kinase phosphorylation of angiotensin-converting enzyme 2 in endothelium mitigates pulmonary hypertension. Am. J. Respir. Crit. Care Med. 198, 509-520.

Zhou P., Yang X.L., Wang X.G., Hu B., Zhang L., Zhang W., Si H.R., Zhu Y., Li B., Huang C.L., Chen H. D., Chen J., Luo Y., Guo H., Jiang R.D., Liu M.Q., Chen Y., Shen X.R., Wang X., Zheng X. S., Zhao K., Chen Q.J., Deng F., Liu L.L., Yan B., Zhan F.X., Wang Y.Y., Xiao G.F. and Shi Z.L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 579, 270-273.

Zhu N., Zhang D., Wang W., Li X., Yang B., Song J., Zhao X., Huang B., Shi W., Lu R., Niu P., Zhan F., Ma X., Wang D., Xu W., Wu G., Gao G.F., Tan W., China Novel Coronavirus I. and Research T. (2020). A novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J. Med. 382, 727-733.

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