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ORIGINAL ARTICLE

Human-induced pluripotent stem cell-derived hepatocyte platform in modeling of SARS-CoV-2 infection

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Key words
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Abstract

Background and Aim: Currently, SARS-CoV-2 is still spreading rapidly and globally. A large proportion of patients with COVID-19 developed liver injuries. The human-induced pluripotent stem cell (iPSC)-derived hepatocytes recapitulate primary human hepatocytes and have been widely used in studies of liver diseases.
Methods: To explore the susceptibility of hepatocytes to SARS-CoV-2, we differentiated iPSCs to functional hepatocytes and tried infecting them with different MOI (1, 0.1, 0.01) of SARS-CoV-2.
Results: The iPSC-derived hepatocytes are highly susceptible to virus infection, even at 0.01 MOI. Other than the ancestral strain, iHeps also support the replication of SARS-CoV-2 variants including alpha, beta, theta, and delta. More interestingly, the ACE2 expression significantly upregulated after infection, suggesting a vicious cycle between virus infection and liver injury.
Conclusions: The iPSC-derived hepatocytes can support the replication of SARS-CoV-2, and this platform could be used to investigate the SARS-CoV-2 hepatotropism and hepatic pathogenic mechanisms.

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Introduction

The ongoing pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses a serious threat to human health. Although the virus primarily affects the respiratory system, liver injury symptoms have been commonly observed. Although the impact of COVID-19 on the liver remains poorly characterized, a significant proportion of patients with liver enzyme elevations have been reported. In the hospitalized COVID-19 patients, emerging data suggest that abnormal liver functions were observed in around 30%–60% of COVID patients,[1–4] and the liver injuries were proven to be independently associated with adverse clinical outcomes in patients with COVID-19.[5] Although COVID-19 may trigger acute liver injury in patients with chronic liver diseases, many COVID-19 patients had normal liver functions without underlying liver disorders.[5] Furthermore, there have been case series reporting SARS-CoV-2-related fulminant liver failure.[6–8]

The influence of liver injury on the hepatotropism of SARS-CoV-2 still remains unclear, so it is of clinical importance to elucidate the underlying pathogenic mechanisms. The human-induced pluripotent stem cells (hiPSCs) and their hepatic derivatives offer conveniences to study hepatic disease *in vitro*. The iPSC-derived hepatocytes (iHeps) have been widely used in recapitulating human hepatocytes.[9–13] In this study, we exploited our previously established iPSC hepatic differentiation platform to study the susceptibility of iHeps to SARS-CoV-2 infection.

Methods

Cell culture. The human iPSC line used in this study was a gift from the University of Hong Kong and was reprogrammed from a healthy individual.[12] iPSCs were cultured using mTeSR1 medium (Stem Cell) on Matrigel (Corning)-coated surface in a 37°C incubator with 5% CO_2. The medium was changed every day.

Differentiation of iPSCs to hepatocytes. A classic three-step hepatic differentiation method was used as described in our previous studies.[12,13] Briefly, single-cell iPSCs were seeded at a density of 0.025 million cells/cm^2 on surfaces coated with GFR-reduced Matrigel (Corning). When iPSCs reached 60–70% confluency, we changed the medium to RPMI1640 supplemented with 1X B27, 100 ng/ml Activin A, and 3 μM CHIR99021 for 24 h. In the following 2 days, CHIR99021 was withdrawn from the stage 1 medium, and the medium was changed daily. From day 3 to day 11, the medium was changed to KO-DMEM containing 20% Knockout Serum Replacement, 1% NEAA, 1% L-GlutaMax, 1% DMSO, and 0.1 mM β-Mecaptoethonal. Finally, from day 11 to day 18, iHeps were further matured by culturing in hepatocyte culture medium (LONZA) supplemented with 20 ng/ml HGF (Peprotech) and 20 ng/ml Oncostatin M (R&D).

Low-density lipoprotein (LDL) uptake assay, periodic acid–Schiff (PAS) staining, and oil red O staining. The LDL uptake assay, periodic acid–Schiff (PAS) staining, and oil red O staining were used for iHeps characterization, and the procedures are described previously.[13]

Immunofluorescence staining. iHeps were differentiated on Matrigel-coated coverslips. On day 18 of differentiation, iHeps were washed twice with PBS and fixed with 4% paraformaldehyde at room temperature for 20 min and incubated in PBS containing 10% donkey serum and 1% Triton X-100 at room temperature for 1 h for blocking and permeabilization. The primary antibodies were diluted in the blocking buffer at different dilution rates listed in Supplemental Table S1. Then, the iHeps were incubated with primary antibodies at 4°C overnight, and then with secondary antibodies (1:1000 diluted in PBS) at room temperature for 1 h. The nuclei were stained using DAPI (Sigma-Aldrich, 1: 10000 dilution) for 5 min at room temperature. Finally, the coverslips were mounted using FlourSave Reagent (Millipore) and observed under a confocal microscope (Carl Zeiss, LSM900).

Virus preparation. SARS-CoV-2 virus strains, including ancestral strain (GISAID accession number: EPI_ISL_434571), alpha (B.1.1.7), beta (B.1.351), theta (P.3), and delta (B.617.2) variants, were propagated in Vero-E6 TMPRSS2 cells (JCRB Cell Bank Catalogue no. JCRB1819), which were maintained in MEM with 1% FBS at 37°C and 5% CO_2, and then was harvested and stored at −80°C.

Inoculation of SARS-CoV-2 to iHeps. iHeps were inoculated with ancestral strain at different multiplicity of infection (MOI) (0.01, 0.1, 1) in triplicate. After 1-h infection, cells were washed twice with medium. Then fresh hepatocyte culture medium was added to the cells and incubated at a 37°C incubator. We harvested the supernatant every 24 h, and collected the cells every 24 and 72 h. The cell morphologies were recorded daily.

Susceptibility of iHeps to different SARS-CoV-2 strains. iHeps on 24-well plates were incubated with different

SARS-CoV-2 strains at 0.01 MOI in triplicate for 1 h. Then, virus was removed from plates followed by washing cells twice with medium. Finally, iHeps were kept in fresh hepatocyte culture medium and incubated at 37°C and 5% CO_2. The supernatant was harvested at 24, 48, and 72 h after infection, and cells were fixed with 4% PFA at 72 h after infection.

Viral load measurement. The supernatant was lysed for viral load analysis. Total nucleic acid (TNA) was extracted from the supernatant of infected cells by easyMAG (Biomerieux, France). Then, real-time reverse transcription-quantitative polymerase chain reaction assay (RT-qPCR) was performed on Light Cycler 96 (Roche, Switzerland) to determine the viral load. The primers sequences were 5′- CGCATACAGTCTTRCAGGCT-3′, 5′-GTGTGATGTTGAWATGACATGGTC-3′, and the probe (5′ FAMTTAAGATGTGGTGCTTGCATACGTAGAC-IABkFQ 3′) was targeting RdRp/Hel region of SARS-CoV-2. The viral copies were calculated according to the standard curve.

Virus titration. The virus in supernatant harvested from infected iHeps was titrated with 50% tissue culture infectious dose (TDIC50) assay. 100 µL of a serial 10-fold diluted virus was added to VeroE6 TMPRSS2 cells on 96-well plates and incubated at 37°C. On day 3 post-infection, cytopathic effect (CPE) was observed under microscope.

RNA isolation and reverse transcription-quantitative PCR (RT-qPCR) analysis. Total RNA was extracted using TRIzol (ThermoFisher) following the manufacturer's protocol. The concentration of total RNA was measured using NanoDrop Microvolume Spectrophotometers (ThermoFisher). Then the extracted RNAs were reverse transcripted to cDNA using PrimeScript RT Master Mix Kit (Takara). The RT-qPCR was performed using the StepOne Real-Time PCR System (Applied Biosystems). GAPDH was used for normalization. Each sample was measured in duplicate. Primers used in this study include human ACE2 (forward: 5′-TCCATTGGTCTTCTGTCACCCG-3′; reverse: 5′-AGACCATCCACCTCCACTTCTC-3′), and human GAPDH (forward: 5′-AGGGCTGCTTTTAACTCTGGT-3′; reverse: 5′-CCCCACTTGATTTTGGAGGGA-3′).

Results

Differentiation of iPSCs to functional hepatocytes. We employed a classic three-step hepatic differentiation protocol[9,14] to differentiate iPSCs into definitive endoderm cells, hepatoblast, and hepatocytes stepwise (Fig. 1a). During 18 days of hepatic differentiation, the undifferentiated iPSCs, which are tightly packed colonies with large nuclei and less cytoplasm, were morphologically changed to well-arranged polygonal hepatocyte-like cells with clear cell margins (Fig. 1b). The differentiated iHeps expressed hepatocyte-specific markers including albumin (ALB), alpha-1 antitrypsin (AAT), hepatocyte nuclear factor 4A (HNF4A), and asialoglycoprotein receptor-1 (ASGR1) (Fig. 1c–f). In addition, a small proportion of iHeps expressed ACE2, the receptor for SARS-CoV-2 (Fig. 1g). We also observed a strong LDL uptake capability in most iHeps by incubating them with fluorescently labeled (FL-LDL) (Fig. 1h). Other essential hepatocyte functions were also observed in these stem cell differentiated iHeps, such as glycogen storage, indicated by PAS staining (Fig. 1i), and lipid storage, shown by oil red O staining (Fig. 1j).

iHeps are susceptible to SARS-CoV-2 infection. To study the susceptibility of iHeps to SARS-CoV-2 infection, we inoculated the cells with a wide range of MOI (from 0.01 to 1) and collected samples until 72-h post-infection. After the infection at all MOIs, there was an increasing number of dead cells that could be seen under the microscope (Fig. 2a), and the remaining cell numbers (indicated by total RNA yield) decreased significantly at 72-h post-infection (Fig. 2b). Immunofluorescent staining for SARS-CoV-2 nucleocapsid protein (SARS-CoV-2 NP) revealed that the viruses had spread extensively in iHeps (Fig. 2c). To further ascertain active viral replication, the supernatant was collected for measuring the viral load of SARS-CoV-2 by RT-qPCR. A progressive increase in SARS-CoV-2 RNA copies in the supernatant was observed, even though at a very low MOI (0.01), suggesting an active SARS-CoV-2 replication in iHeps (Fig. 2d).

ACE2 upregulated in iHeps after SARS-CoV-2 infection. As ACE2 is the cellular entry receptor for SARS-CoV-2, its expression level primarily affects SARS-CoV-2 infection. Therefore, we next measured the changes in ACE2 expression in iHeps post-infection. Previous studies showed that ACE2 downregulated after SARS-CoV-2 infection in stem cell-derived cardiomyocytes[15] and some commonly used cell lines including Vero-E6, HeLa, and HEK293T.[16] In contrast, the iHeps displayed a significant upregulation of ACE2. We observed an obvious increase of ACE2 positive iHeps at 72-h post-infection in all MOIs (Fig. 3a). The qPCR analysis also confirmed over threefold ACE2 upregulation in Heps at 72-h post-infection compared with iHeps without infection (Mock) (Fig. 3b). This virus infection-induced ACE2 upregulation indicates a vicious cycle between SARS-CoV-2 infection and hepatocyte injury.

iHeps supports replication of different variants of SARS-nCoV-2 infection. Other than the ancestral strain, we would like to know whether there were any differences in the susceptibility of iHeps to SARS-CoV-2 variants. We then inoculated iHeps with alpha, beta, theta, and delta variants at 0.01 MOI. After 72 h of infection, high percentages of SARS-CoV-2 NP positive cells were observed in iHeps infected with all types of variants (Fig. 4a). Viral load also increased in supernatant within 72 h of infection (Fig. 4b), suggesting that all types of virus variants were good replication inside the iHeps. At 24-h post-infection, the ancestral strain and alpha variant showed comparable viral load, and with higher viral load than beta, delta, and theta variants. However, no significant differences of viral load were observed between different variants at 48-h and 72-h post-infection.

Discussion

In this study, we employed human iPSC-derived hepatocytes as a cell model to study the susceptibility of human liver cells to SARS-CoV-2 infection. Obvious cell death and cytopathogenic

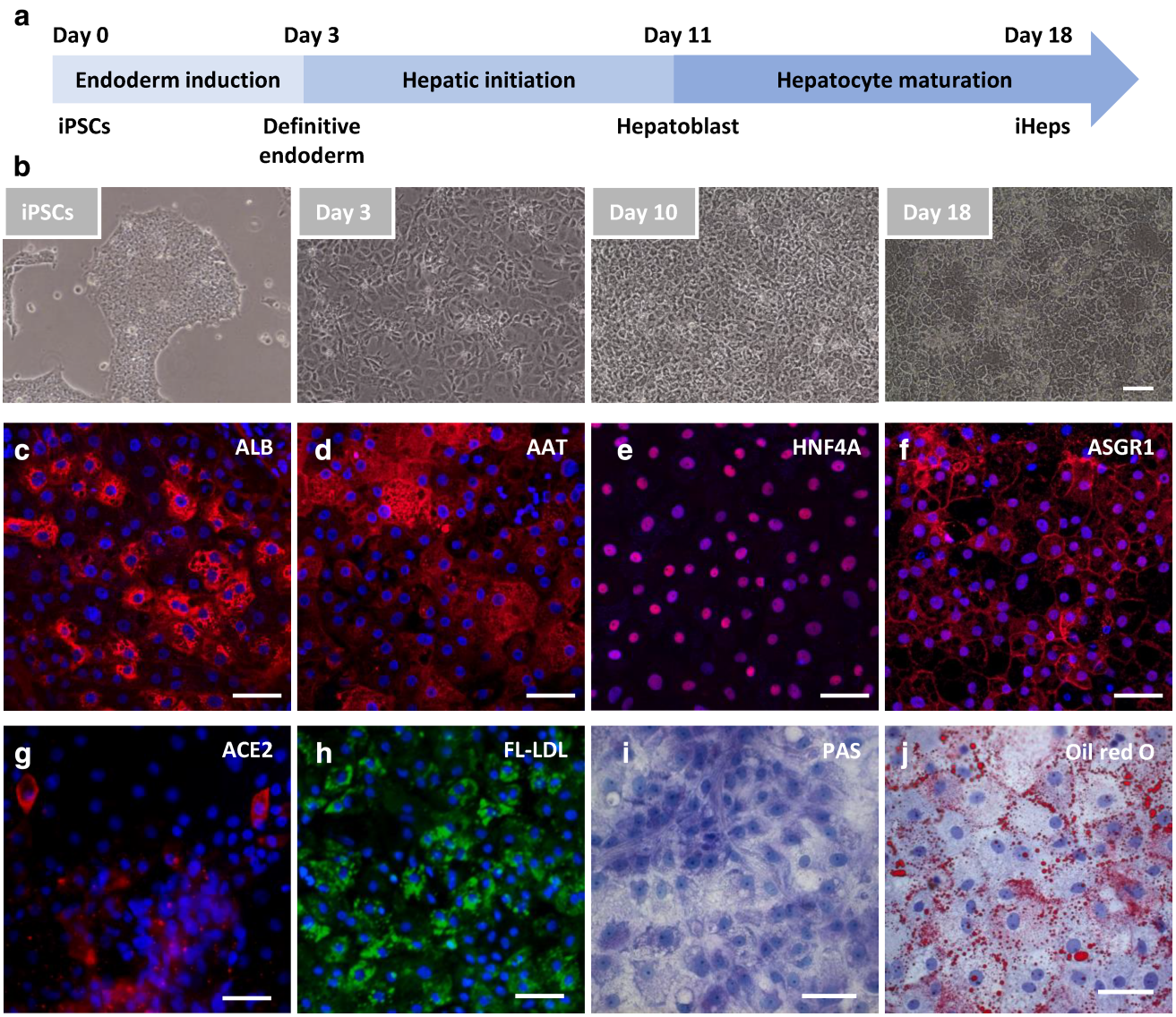

Figure 1 Differentiation of iHeps from iPSCs. (a) Schematic of the three-step hepatic differentiation procedure. (b) Typical morphologies of iPSCs, definitive endoderm cells, hepatoblast, and iHeps. Scale bar represents 200 μm. (c–g) Immunofluorescence staining for hepatic markers (ALB, AAT, HNF4A, ASGR1) and ACE2 in iHeps. Scale bars represent 50 μm. (h) LDL uptake assay using FL-LDL. Scale bar represents 50 μm. (i,j) PAS and oil red O staining show glycogen and lipid storage, respectively. Scale bar represents 50 μm.

effects caused by virus infection and replication were observed, accompanied by a significant upregulation of ACE2 expression.

Previous studies have reported that SARS-CoV-2 is capable of infecting and replicating in human hepatocellular carcinoma-derived cell lines and primary human hepatocytes.[16–19] Theoretically, direct characterization of primary human hepatocytes with SARS-CoV-2-related liver injury could provide the much needed insight, but the use of primary hepatocytes is limited by donor organ scarcity, technical difficulties in the *in vitro* culture, as well as ethical concerns. iHeps differentiated from iPSCs, which cannot be infected by SARS-CoV-2,[20,21] are an unlimited cell source and easier to be cultured *in vitro*, which makes them a good substitutional cell model for primary human hepatocytes. Importantly, iHeps can support infection and replication of SARS-CoV-2 very well, and a $10^{4.4}$-fold increase of viral load was observed at day 3 after infected with virus at 0.1 MOI, when compared it at baseline (Fig. 2d). The respiratory system, especially lung, is targeted by SARS-CoV-2. *In vitro*, Calu-3 and A549 cells, which are alveolar epithelial cells, support the SARS-CoV-2 replication. However, after inoculated with SARS-CoV-2 wild-type strain at 0.1 MOI, the viral load on Calu-3 and A549 increase around 10-fold and 10^2-fold, respectively, which is lower than that on iHep.[22] Therefore, iHep cells could be a good model to investigate SARS-CoV-2.

Interestingly, researchers have shown that in many human cell lines (HeLa, Vero-E6, HEK293T), in the context of both SARS-CoV[23] and SARS-CoV-2[16] infection, the spike proteins

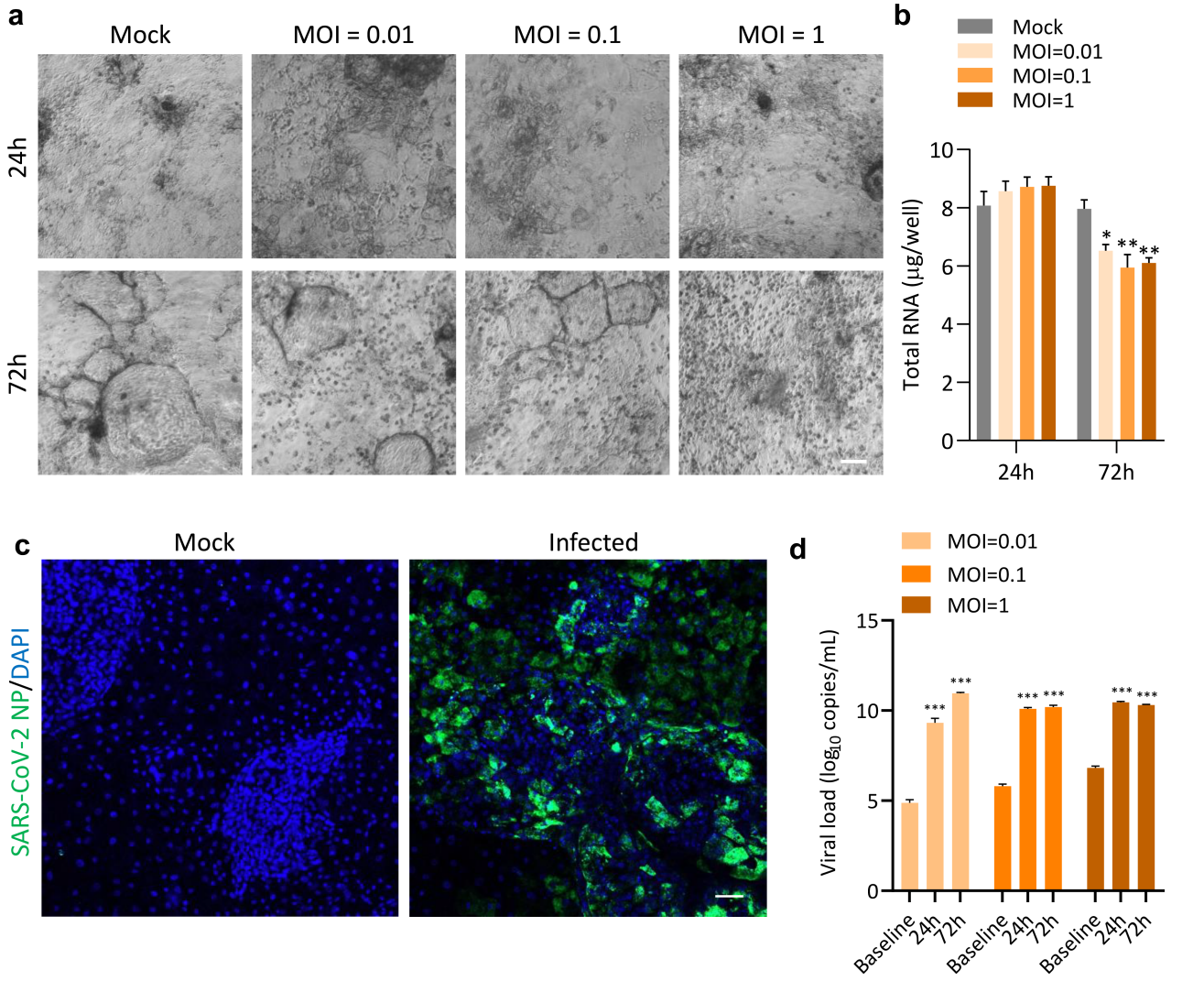

Figure 2 Infection of iHeps with SARS-CoV-2. (a) Morphological changes of iHeps after being infected with different MOI of SARS-CoV-2. Scale bar represents 50 μm. (b) Measurement of total RNA suggested cell numbers after virus infection. $n = 3$ in each group. (c) IF staining for SARS-CoV-2 NP in iHeps with or without virus infection. Scale bar represents 50 μm. (d) Viral load changes in iHeps supernatant during 72-h post-infection. $n = 3$ in each group. For all measurements, P values were obtained using one-way ANOVA adjusted with Dunnett's multiple comparison; error bars indicate SEM. $^{**}P < 0.01$,$^{***}P < 0.001$ *versus* Mock.

triggered translocation of ACE2 and led to downregulation of ACE2 transcription. Similarly, a recent study using stem cell-derived cardiomyocytes also found that ACE2 was down-regulated following SARS-CoV-2 infection. In contrast, in this study, we observed a significant upregulation of ACE2 expression after virus infection. This indicated a vicious cycle—once liver cells got infected, they may become more and more susceptible. It has been found that there is ACE2 expression in human liver organ, and liver injury in COVID-19 patients with inflammation response and elevated AST and ALT.[24] In animal model, liver injury is associated with upregulation of ACE2 expression in liver.[25] More research is needed to investigate the correlation between SARS-CoV-2 infection and ACE 2 in the liver of COVID-19 patients.

Other than ancestral strain, we showed that iHeps are also susceptible to other SARS-CoV-2 variants. Furthermore, ancestral strain and alpha variant showed comparable viral load with ancestral strain, and higher viral load than beta, delta, and theta variants at 24-h post-infection. The reason for the difference in viral load could be that viral load is correlated to virulence, and with the evolution, SARS-CoV-2 will have lower virulence to the host.[26] Shuai et al. reported similar results that in Vero E6 cells, ancestral strain and alpha variants showed faster replication than beta, delta, and omicron variants at 24-h post-infection. Furthermore, ancestral strain and alpha variants induced higher mortality than the other three variants in hACE2 transgenic mice.[27]

In addition to direct cytopathogenic effects, the liver injuries in COVID-19 patients could be resulted from viral

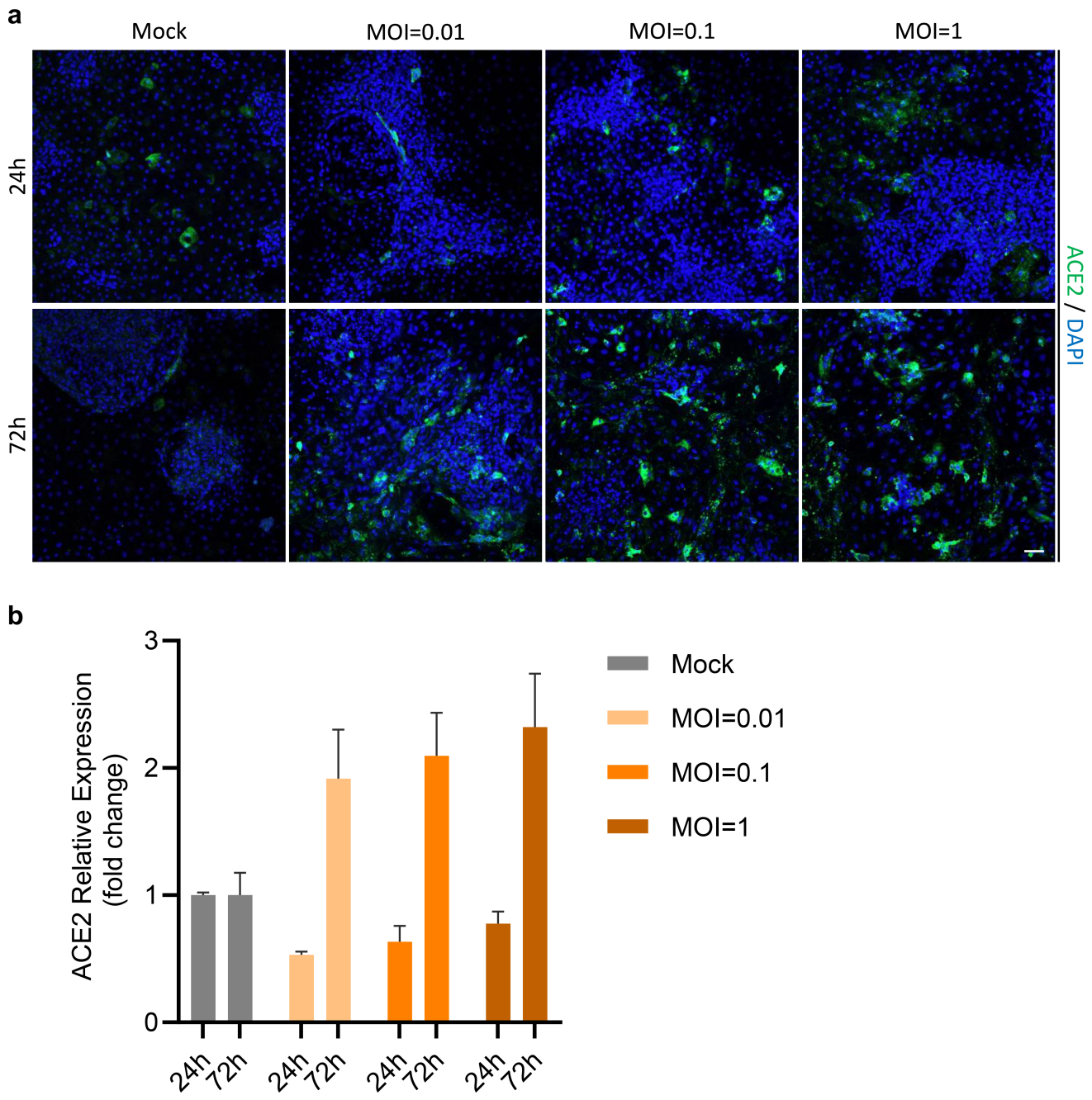

Figure 3 SARS-CoV-2 infection induced ACE2 upregulation in iHeps. (a) Immunofluorescence staining for ACE2 protein in iHeps at 24-h and 72-h post-infection. Scale bar represents 50 μm. (b) qPCR analysis for ACE2 gene expression at certain time points. n = 3 in each group. *P* values were obtained using one-way ANOVA adjusted with Dunnett's multiple comparison; error bars indicate SEM.

infection-induced systematic immune response,[28] or the use of anti-COVID-19 drugs.[5] On one hand, SARS-CoV-2 infection in other nonhepatic organs, most commonly the lungs, may cause systematic inflammation that activates immune cells (e.g., monocytes, macrophages, dendritic cells, NK cells) to produce pro-inflammatory cytokines, chemokines, lipid messengers, and reactive oxygen species that contribute to the apoptotic or necrotic demise of hepatocytes.[29] On the other hand, the commonly used anti-COVID-19 drugs, especially remdesivir[30–32] and lopinavir/ritonavir,[14] have been reported to be associated with abnormal hepatic enzymes including AST, ALT, and GGT in COVID-19 patients. In future studies, more experiments can be conducted to explore the role of host immune response, and the use of anti-virus drugs in COVID-19 patient liver injuries.

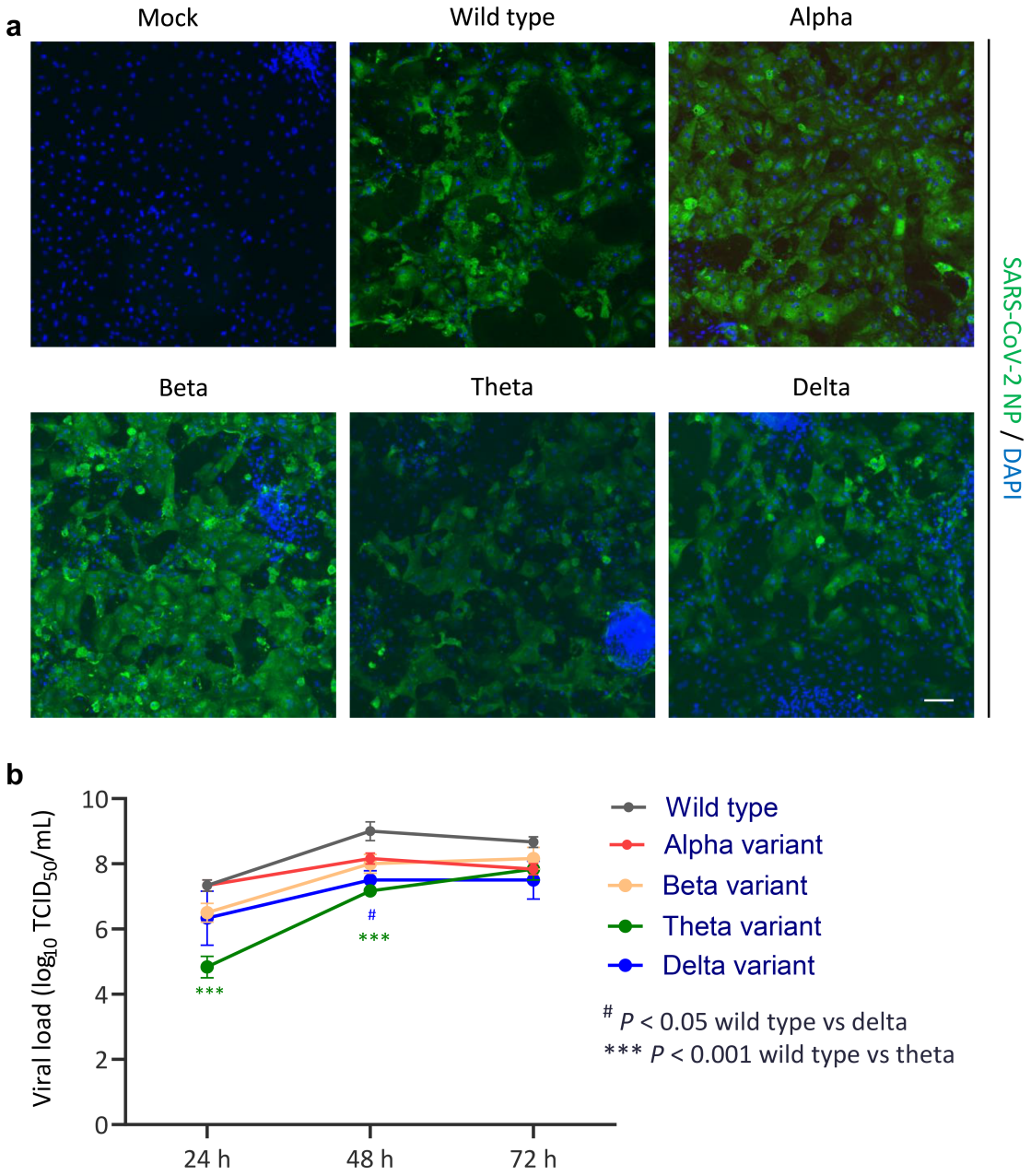

Figure 4 iHeps supports different variants of SARS-CoV-2 replication. (a) Immunofluorescence staining for SARS-CoV-2 NP in iHeps at 72-h post-infection. Scale bar represents 100 μm. (b) Measurement of viral load in iHeps supernatant within 72-h post-infection. n = 3 in each group; error bars indicate SEM.

The SARS-CoV-2 pandemic has resulted in over 580 million infections and 6 million deaths globally (until August 2022),[33] and yet there is no sign of ending. Thus, an increasing number of patients with SARS-CoV-2-related liver injuries can be anticipated. In the present study, we utilized our iHeps platform and demonstrated the iHeps susceptibility and ACE2 expression pattern change, while the underlying mechanism is still unclear. More experiments are expected to further elucidate SARS-CoV-2 hepatotropism, hepatic pathogenic mechanisms, and even to identify effective pharmacological agents.

Data availability statement. The data that support the findings of this study are available from the corresponding author [HF.T] upon reasonable request.

References

1 Fan Z, Chen L, Li J *et al*. Clinical features of COVID-19-related liver functional abnormality. *Clin. Gastroenterol. Hepatol.* 2020; **18**: 1561–6.
2 Phipps MM, Barraza LH, LaSota ED *et al*. Acute liver injury in COVID-19: prevalence and association with clinical outcomes in a large U.S. *Cohort. Hepatol.* 2020; **72**: 807–17.
3 Goyal P, Choi JJ, Pinheiro LC *et al*. Clinical Characteristics of Covid-19 in New York City. *N. Engl. J. Med.* 2020; **382**: 2372–4.
4 Richardson S, Hirsch JS, Narasimhan M *et al*. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. *JAMA*. 2020; **323**: 2052–9.
5 Yip TC, Lui GC, Wong VW *et al*. Liver injury is independently associated with adverse clinical outcomes in patients with COVID-19. *Gut*. 2021; **70**: 733–42.
6 Grzywa-Celińska A, Emeryk-Maksymiuk J, Szewczyk K, Krusiński A, Celiński R, Milanowski J. Acute liver injury in the course of COVID-19. *Ann. Agric. Environ. Med.* 2021; **28**: 729–32.
7 Melquist S, Estepp K, Aleksandrovich Y *et al*. COVID-19 presenting as fulminant hepatic failure: a case report. *Medicine (Baltimore)*. 2020; **99**: e22818.
8 Haji Esmaeil Memar E, Mamishi S, Sharifzadeh Ekbatani M *et al*. Fulminant hepatic failure: a rare and devastating manifestation of Coronavirus disease 2019 in an 11-year-old boy. *Arch. Pediatr.* 2019; **2020**: 502–5.
9 Kajiwara. Correction for Kajiwara *et al*. Donor-dependent variations in hepatic differentiation from human-induced pluripotent stem cells. *Proc. Natl. Acad. Sci.* 2012; **109**: 14716.
10 Cayo MA, Cai J, DeLaForest A *et al*. JD induced pluripotent stem cell-derived hepatocytes faithfully recapitulate the pathophysiology of familial hypercholesterolemia. *Hepatology*. 2012; **56**: 2163–71.
11 Rashid ST, Corbineau S, Hannan N *et al*. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. *J. Clin. Invest.* 2010; **120**: 3127–36.
12 Yang J, Wang Y, Zhou T *et al*. Generation of Human Liver Chimeric Mice with Hepatocytes from Familial Hypercholesterolemia Induced Pluripotent Stem Cells. *Stem Cell Rep.* 2017; **8**: 605–18.
13 Wei R, Yang J, Cheng C-W *et al*. CRISPR-targeted genome editing of human induced pluripotent stem cell-derived hepatocytes for the treatment of Wilson's disease. *JHEP Rep.* 2021; **4**: 100389.
14 Sodeifian F, Seyedalhosseini ZS, Kian N *et al*. Drug-induced liver injury in COVID-19 patients: a systematic review. *Front. Med. (Lausanne)*. 2021; **8**: 731436.
15 Wong CK, Luk HK, Lai WH *et al*. Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes Platform to Study SARS-CoV-2 Related Myocardial Injury. *Circ. J.* 2020; **84**: 2027–31.
16 Gao X, Zhang S, Gou J *et al*. Spike-mediated ACE2 down-regulation was involved in the pathogenesis of SARS-CoV-2 infection. *J. Infect.* 2022; **85**: 418–27.
17 Chu H, Chan JF, Yuen TT *et al*. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. *Lancet Microbe*. 2020; **1**: e14–23.
18 Mercado-Gómez M, Prieto-Fernández E, Goikoetxea-Usandizaga N *et al*. The spike of SARS-CoV-2 promotes metabolic rewiring in hepatocytes. *Commun. Biol.* 2022; **5**: 827.
19 Barreto EA, Cruz AS, Veras FP *et al*. COVID-19-related hyperglycemia is associated with infection of hepatocytes and stimulation of gluconeogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 2023; **120**: e2217119120.
20 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.
21 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**: 102428.
22 Chu H, Chan JFW, Yuen TTT *et al*. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. *Lancet Microbe*. 2020; **1**: e14–23.
23 Kuba K, Imai Y, Rao S *et al*. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.* 2005; **11**: 875–9.
24 Wanner N, Andrieux G, Badia-I-Mompel P *et al*. Molecular consequences of SARS-CoV-2 liver tropism. *Nat. Metab.* 2022; **4**: 310–9.
25 Guan GW, Gao L, Wang JW *et al*. Exploring the mechanism of liver enzyme abnormalities in patients with novel coronavirus-infected pneumonia. *Zhonghua Gan Zang Bing Za Zhi*. 2020; **28**: 100–6.
26 Huiping Shuai H, Jasper Fuk-Woo Chan JKW, Bingjie HB *et al*. Attenuated replication and pathogenicity of SARS-CoV-2 B.1.1.529 Omicron. *Nature*. 2022; **603**: 693–9.
27 Markov PV, Katzourakis A, Stilianakis NI. Antigenic evolution will lead to new SARS-CoV-2 variants with unpredictable severity. *Nat. Rev. Microbiol.* 2022; **20**: 251–2.
28 Marra F, Tacke F. Roles for chemokines in liver disease. *Gastroenterology*. 2014; **147**: 577–94 e571.
29 Brenner C, Galluzzi L, Kepp O, Kroemer G. Decoding cell death signals in liver inflammation. *J. Hepatol.* 2013; **59**: 583–94.
30 Goldman JD, Lye DCB, Hui DS *et al*. Remdesivir for 5 or 10 days in patients with severe Covid-19. *N. Engl. J. Med.* 2020; **383**: 1827–37.
31 Wang Y, Zhang D, Du G *et al*. Remdesivir in adults with severe COVID-19: a randomised, dou-ble-blind, placebo-controlled, multicentre trial. *Lancet (London, England)*. 2020; **395**: 1569–78.
32 Grein J, Ohmagari N, Shin D *et al*. Compassionate use of remdesivir for patients with severe Covid-19. *N. Engl. J. Med.* 2020; **382**: 2327–36.
33 WHO Coronavirus (COVID-19) Dashboard. cited; Available from. 2022. https://covid19.who.int/.

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Data S1. Supporting Information.