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Review Article

Organoids in COVID-19: can we break the glass ceiling?

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Abstract

COVID-19 emerged in September 2020 as a disease caused by the virus SARS-CoV-2. The disease presented as pneumonia at first but later was shown to cause multisystem infections and long-term complications. Many efforts have been put into discovering the exact pathogenesis of the disease. In this review, we aim to discuss an emerging tool in disease modeling, organoids, in the investigation of COVID-19. This review will introduce some methods and breakthroughs achieved by organoids and the limitations of this system.

Keywords: COVID-19, SARS-CoV-2, disease modeling, organoids

1. COVID-19 and SARS-CoV-2

COVID-19 is a disease caused by SARS-CoV-2, a novel betacoronavirus first isolated in patients with idiopathic pneumonia in Wuhan 2020.[1] This coronavirus, along with the better-known SARS-CoV and MERS-CoV, belongs to betacoronavirus, although phylogenetic study shows that SARS-CoV-2 is more distant to these 2 strains.[2]

Coronavirus is a positive single-stranded RNA enveloped virus with characteristic spike glycoproteins on the envelope, hence the name coronavirus. It is made of 4 structural proteins: the spike glycoprotein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N). The RNA genome with the 5′ cap and a poly-A tail is protected inside the nucleocapsid. The 5′ cap frameshift between 2 Orfs, Orf1a and Orf1b, allows production of 2 polyproteins, which are then further cleaved to produce 16 nonstructural proteins. The 3′ end encodes the 4 structural proteins.[3]

2. Multisystemic pathology in COVID-19

The disease COVID-19, originally discovered as a pneumonia in Wuhan, shares similar characteristics to MERS-CoV and SARS-CoV and presents pneumonia-like symptoms.[4] ACE2, like the SARS coronaviruses, was identified as the major receptor mediating virus entry.[5] However, as new variants emerge and the infected population grows, pathologies in the circulatory system, kidneys, brain, and so on have been reported.[6] Postinfection complications like brain fog and persistent pulmonary fibrosis are also new pathologies grouped together as "long COVID" (beautifully summarized by Michelen et al.[7] in a systematic review). These presentations are shown in Fig. 1A–D.

There are many theories for how long COVID develops. The major directions proposed include autoimmunity, immune dysregulation, microbiota dysbiosis, vascular and hemodynamic abnormality, and dysfunctional nervous system.[8] Autoimmunity may be caused by bystander activation, viral persistence, or viral mimicry.[9] Immune dysregulation is also related to an imbalance of lymphocytes, particularly the dysregulated proinflammatory macrophage activation, leading to increased sensitivity toward stimuli or release of cytokines such as tumor necrosis factor (TNF)–α.[8,10–13] Microbiota dysbiosis is a new theory that has gained attention but has little evidence on its cause or effect.[14,15] Vascular damages after infection may be explained by regenerated senescent or dysfunctional endothelium, leading to a prothrombotic environment and vasoconstriction.[16–18] Dysfunctional nervous system may be a result of injury by direct infection or autoimmune inflammation.[19–22]

ACE2 expression was found to be very limited in tissues by transcriptomics[23] and could not fully explain the multisystemic manifestations. New evidence suggests that structural proteins may activate pattern recognizing receptors (PRRs),[24,25] and nonstructural proteins may be responsible for the cytokine storm.[26] More receptors have also been identified.[27] To better understand the pathophysiology and tropism of SARS-CoV-2, scientists have employed a new model, organoids.

2.1 Organoids

Organoids make use of stem cells in 3-dimensional (3D) aggregates, when given appropriate growth factors and nutrition, and they differentiate and self-assemble into a cluster of cells with functional cell types, mimicking the structure and function of an organ in vivo. The idea of in vitro organism regeneration was first raised in 1907 by Henry Van Peters Wilson.[28] Efforts have since been made to generate organs in vitro from dissociated embryonic cells. Now, many more organoids have been developed, such as lung, kidney, brain, tonsil, gut tube, liver, heart, vascular, and cancer organoids.[29–39] The commonly agreed-on criteria for defining an organoid is a 3D self-organizing and expandable tissue recapitulating the structure and development of in vivo organs. Hence, organoids are typically derived from stem cells, which are embryonic stem cells, induced pluripotent stem cells, or multipotent stem cells. Organoids are also generated from differentiated niche cells.[40,41]

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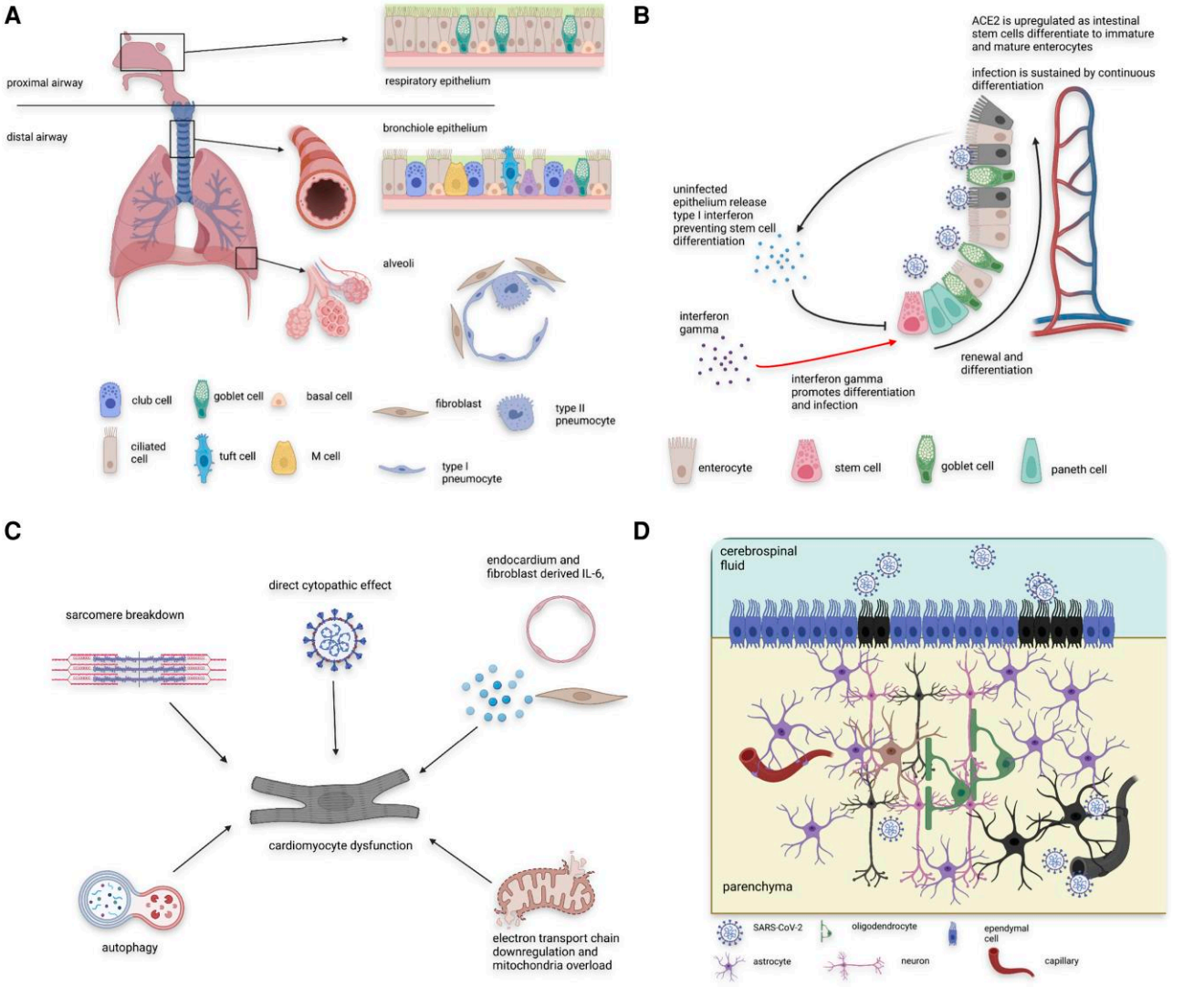

Fig. 1. SARS-CoV-2 immune responses in each organ. (A) Cell types, location, and structure of the respiratory tract. (B) Sustained infection in intestinal epithelium by dynamics of interferons. Intestinal stem cells are naturally immune to SARS-CoV-2 infection due to lack of ACE2 expression. As the cells differentiate into enterocytes, ACE2 is expressed and cells become permissive to infection. Type I interferon reduces infection by preventing differentiation of intestinal stem cells while interferon gamma promotes differentiation, thus leading to sustained infection in the intestine. Gray cells represent infected enterocytes. Figure created with biorender.com. (C) Proposed mechanisms for systolic and diastolic dysfunction. Cardiomyocyte dysfunction is proposed to be contributed by cytopathic effect, sarcomere breakdown, autophagy, and mitochondria overload during infection. Endocardium (endothelium) and fibroblasts also contribute to dysfunction of uninfected cells by producing cytokines such as IL-6 and TNF. Figure created with biorender.com. (D) Modes of infection in the central nervous system. Different models have proposed direct or indirect killing of neurons, infection and death of astrocytes, and infection of endothelium and choroid plexus epithelium (ependymal cells) and gain entry via these 2 methods. Figure created with biorender.com

In the field of neural organoids, scientists assembled 2 organoids to model an interconnection of neurons, hence named "assembloids."[42] The idea of assembloids can be extended to describe in vitro tissues that are assembled from organoids with another organoid, immune cell, or any kind of cell.

Organoids leverage both self-organization of tissue patterning during development and interactions of heterologous cell types in a 3D context.[43,44]

2.2 Modeling COVID-19 with organoids

With complex pathophysiology and multisystemic consequences, the COVID-19 disease is difficult to model with simply cell lines. Therefore, more organoids are being used in the study of this disease than other diseases in the past. Organoids are broadly classified into adult stem cell–derived or patient-derived organoids and also pluripotent stem cell (PSC)–derived organoids.

Adult stem cell–derived organoids are easy to generate and hence most widely employed. The protocol involves sampling primary tissue, dissociate and reaggregation into spheroids. The spheroids are then embedded in extracellular matrices such as Matrigel and cultured with growth factors to support in vitro expansion.[44] The organoid generated from patient-derived adult stem cells avoids the troublesome differentiation of tissue-specific cells from PSCs. However, the genetic traits between different patients makes this type of organoid difficult to standardize, and the modeling of disease may be biased significantly by the population of patients used to generate organoids. At the same time, the specific genetic makeup of patient-derived organoids makes this an ideal platform for personalized cancer drug screening.[45]

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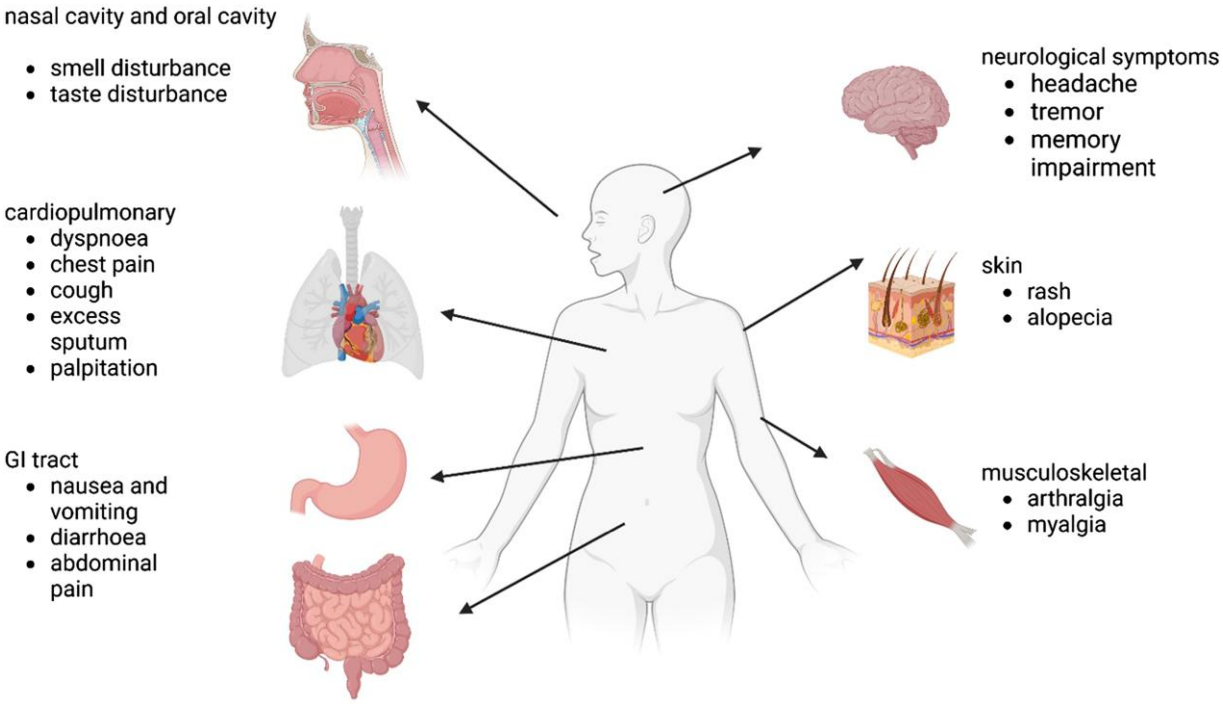

Fig. 2. Immune responses to SARS-CoV-2 infection. Shared cellular responses of all organoids reported in this review. Tissue epithelial cells expressing ACE2 (lung, intestine, pancreas) are shown. The pathways or phenomenon reproduced in organoids are highlighted by a red box. While organoids can produce a higher level of complexity, this also limits details on activation of pathways except by scRNA-seq. Even so, the protein interactions are not characterized. The major pathways reproduced and observed in organoids include membrane serine protease-mediated virus fusion with cell, cathepsin-dependent endosomal entry, dampened interferon-stimulated genes (ISG) transcription, and type I/III interferon response and NF-κB activation. Figure created with biorender.com.

PSC-derived organoids are differentiated from established cell lines (e.g. induced PSCs or embryonic stem cells). These organoids require strict control of the culture condition to generate an organoid. These models can give a more reproducible result, making the study more valid. Cell lines can also be modified with various gene editing techniques (e.g. CRISPR or lentivirus) to study the effect of certain genes' roles in the disease.[46]

There are many organoids currently employed to model COVID-19, and these models will be discussed below.

2.3 Pathophysiology study using organoids

A lot is still not known about the pathophysiology of COVID-19. Much effort has been put into using organoids to model the disease. Commonly used techniques with organoids include transcriptome analysis, histology, genetic modification, proteomics, and metabolomics.[47,48] Together with postmortem analysis, an in vivo mice model, and tissue biopsy, organoids can offer concrete explanations to many questions. Scientists have discovered a number of pathways used by SARS-CoV-2, many of which can be observed or reproduced in vitro with organoids. Organoids have been used to study host entry factors and inflammatory reactions in cells. Figures 2 and 3 show a simplified diagram of various discovered pathways and highlight the findings of organoids (detailed signaling pathways regarding Toll-like receptors and retinoic acid inducible gene I in diseases and COVID-19 can be found in studies and reviews by Yamada et al.,[49] Khanmohammadi and Rezaei,[25] and Mabrey et al.[50]). It has been shown that delayed type I interferon response is associated with severe clinical outcomes such as the cytokine storm.[51,52] The earliest interferon and TNF responses come from the mucosa, where Toll-like receptors activate transcription of these genes.[53,54] As immune cells are critical mediators of the cytokine storm syndrome, the current challenge of organoid modeling is how to endow immune cells. Either coculture with peripheral blood mononuclear cells or codevelopment of immune cell in organoids will be taken. The details of findings in each type of organoid will be discussed below.

2.4 Respiratory tract organoids

Respiratory tract organoids are the first organoids used to study COVID-19 due to pneumonia being the most ubiquitous presentation in the early stage of pandemic. The use of respiratory organoids in disease modeling includes airway organoids with respiratory epithelium, alveolar organoids, bronchioalveolar organoids, lung organoids, and bronchial organoids (reviewed by Tindle et al.,[30]).

The generation of respiratory organoids from pluripotent stem cells generally starts from differentiation into the endoderm. Pluripotent stem cells are programmed into the definitive endoderm and anterior foregut by manipulation of various pathways, including bone morphogenetic protein (BMP), wingless/INT (Wnt), activin/nodal, hedgehog, fibroblast growth factor (FGF), and retinoic acid. The final step is to differentiate into lung progenitor cells (LPCs). Then the LPCs can be further differentiated into respiratory epithelium by xenograft in a severe combined immunodeficiency mice renal capsule. Alternatively, in vitro culture as spheroids will produce a distal airway such as alveoli. Such organoids generally consist of an epithelium supported by either Matrigel or a small number of stromal cells, including mesenchymal cells.[55,56] Some researchers have shown that by creating an air–liquid interface, the cells can be polarized and differentiate into respiratory epithelium resembling the upper respiratory

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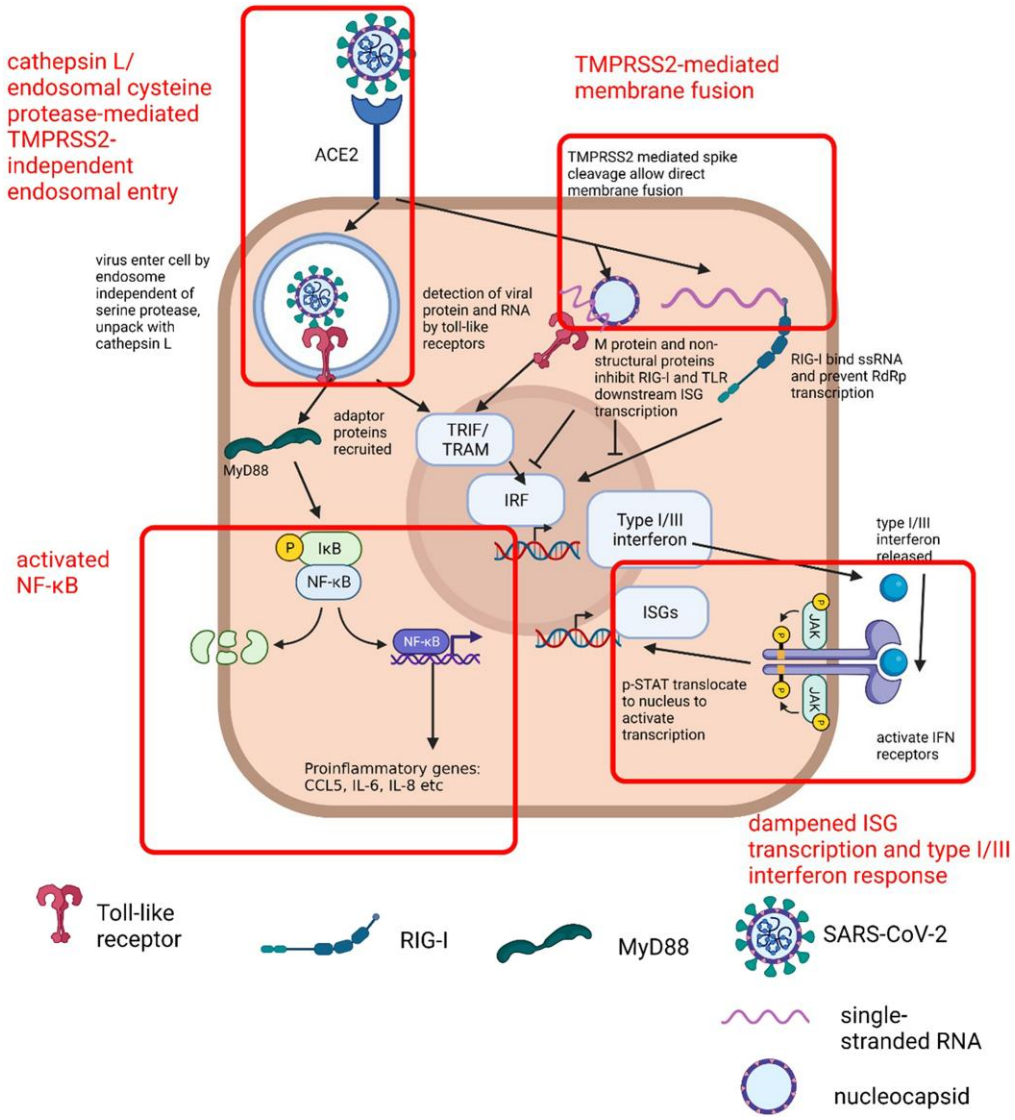

Fig. 3. Consequences of SARS-CoV-2 immune responses. Tissue-level inflammatory response as observed in organoids. Tissue epithelial cells expressing ACE2 (lung, intestine, pancreas) are shown. Cell death of infected cell may be caused by disturbed metabolism such as downregulation of electron transport chain genes or inhibition of autophagy, caspase activation by proinflammatory genes, or dysfunctional stromal cells. Adjacent cells activate apoptosis pathways under stimulation by cytokines or due to a disturbed tissue environment. Figure created with biorender.com.

tract. Respiratory organoids are reviewed more deeply by Yang et al.[57]

Using respiratory organoids, scientists can model SARS-CoV-2 infections in vitro and study tropism of the virus along the respiratory tract (Fig. 4). Ekanger et al.[58] used airway organoids and alveolar organoids developed from a surgical resection specimen to study viral tropism of SARS-CoV-2 and influenza A. They confirmed that sialic acid, ACE2, and TMPRSS2 were expressed in the organoids and studied viral tropism in both bronchial and alveolar epithelia by infection with a clinical isolate of SARS-CoV-2 and pseudotyped influenza A viruses. Infection was quantified by quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) or a reporter gene. From hematoxylin and eosin–stained sections, they observed organoid cytopathology, which resembled that from patient samples. Using similar strategies with organoids, scientists have repeatedly confirmed that ACE2 and TMPRSS2 are expressed in the respiratory tract, and the presence of both proteins is required for infection.[30,59–64]

Single-cell analysis on organoids identified alveolar type 2 pneumocytes (AT2) and club cells as targets of viral tropism in distal airway organoids.[30,58,62,64,65] Although ACE2 is ubiquitously expressed on the apical side of the respiratory epithelium and alveoli, variable TMPRSS2 expression limits tropism of the virus in the respiratory tract as only cells colabeled with ACE2 and TMPRSS2 are infected.[64] Mykytyn et al.[66] took a step further by using airway organoids to investigate the effect of multibasic cleavage motif in spike protein (i.e. unique for SARS-CoV-2). They found that a deletion in the motif results in a lower infection rate, while serine protease inhibitor but not cathepsin 3 inhibitor could reduce the virus entry rate, suggesting that TMPRSS2 is predominantly used in airway infection.

The role of the proximal airway for sustaining viral infection has been established by Tindle et al.,[30] who found that organoids with only proximal or distal airway cell types could not mount an overzealous host response. Ciliated cells have been identified as targets for infection by Sano et al.[59] with bronchial organoids. Chiu et al.[61]

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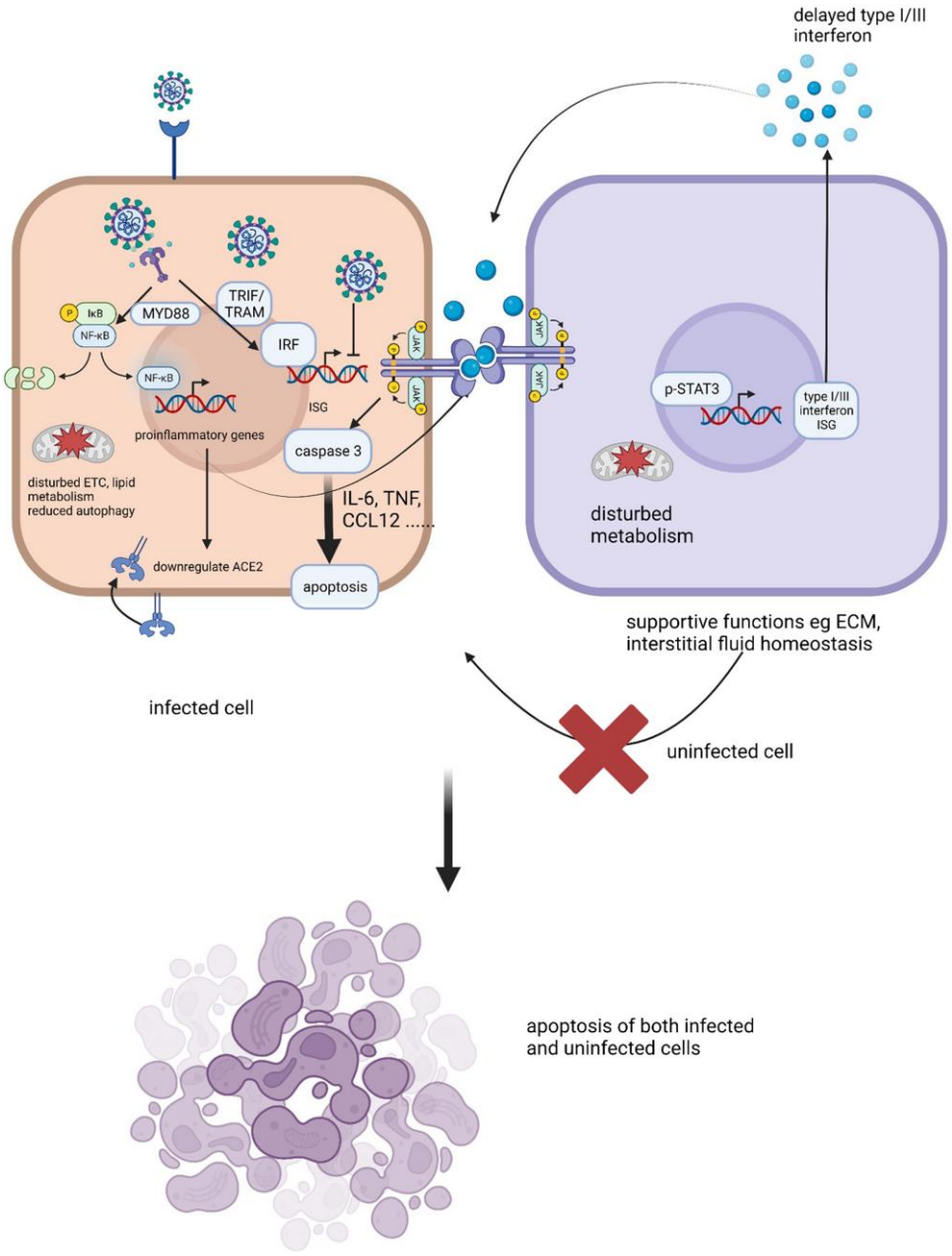

Fig. 4. Long COVID symptoms. Authors' selected list of long COVID symptoms reported with low bias from a systematic review by Michelen et al.[7] COVID-19 is a systemic disease. While infections of the nervous system, gastrointestinal tract, heart, respiratory tract, and skin have been reported, scientists have not been able to demonstrate the pathologic mechanisms of long COVID after resolution of primary disease. Other reports of long COVID symptoms include many more symptoms or manifestations such as fatigue and autoimmune disease flares like type 1 diabetes mellitus. However, due to the rarity of such events or lack of unbiased studies, we cannot say for sure what can be attributed to long COVID, and there is no consensus of what should be included as long COVID yet. Figure created with biorender.com.

demonstrated the loss of intercellular tight junctions in nasal organoids after infection by estimating epithelial integrity with transepithelial electrical resistance. They also found that the omicron variant could infect nasal organoids more easily than the previous delta or wild-type virus, which is explained by Meng et al.,[67] who demonstrated lower dependence of omicron on TMPRSS2 for infection by using various cell lines. Hence, Chiu et al.[61] suggested that infection and breakdown of the nasal epithelium may contribute to a higher transmission rate for the omicron variant.

A pathophysiology study using organoids also gives us many hints to the pathogenesis. Strikoudis and colleagues modeled lung fibrosis with human PSC-derived organoids. They defined that IL-11 mediates fibrogenesis and a potential therapeutic target.[68] Kim and colleagues used lung fibrosis organoids for drug discovery and identified ERK signaling could be a potential therapeutic target.[69]

2.5 Gut, pancreatic, liver, and gastric organoids

Reports of patients with COVID-19 started to increase in 2020 when major outbreaks started.[70] At the time, there was a hypothesis of SARS-CoV-2 infecting the gut due to high ACE2 transcripts in the intestine.[23,71] Therefore, researchers turned to study the interaction of SARS-CoV-2 with the gut. Although 2-dimensional

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culture of a single type of cells could offer many insights, the benefit of organoids is its capacity in modeling the interaction of heterologous cell types in a 3D context. Gut organoids follow a similar differentiation protocol to lung organoids since both are endoderm derived. In fact, lung organoid generation protocols are built on modification of gut organoids. PSCs are first differentiated into definitive endoderm by activin A. Then, tissue-specific inhibition and activation of FGF and Wnt are provided in addition to hormones such as gastrin or hepatocyte growth factor, which specify the fate of the endoderm-derived organoid.[72] Finally, the organoid is maintained in a medium consisting of 4 factors discovered by Sato et al.[73]: Wnt (in the form of R-spondin), epidermal growth factor, and Notch (Noggin) signaling and Matrigel to support Lgr5 + universal gut stem cell. Gut organoids are reviewed in depth by Date and Sato.[74]

As expected, infection of the intestine is demonstrated in intestinal organoids using the methods illustrated in sections above by different researchers.[75–80] As expected, both distal and proximal intestines were found to be permissive to infection,[75] and ACE2 expression was confirmed in intestinal organoids.[77–80] To identify the tropism range, single-cell analysis and confocal microscopy were used. Lamers et al.,[78] using organoids, identified infection in proliferating and nonproliferating enterocyte lineage cells but not in enteroendocrine cells by confocal immunofluorescent. Meanwhile, Triana et al.[79] found that less than 10% of cells marked by confocal microscopy were genuinely infected, as confirmed by RT-qPCR. Nevertheless, they identified immature enterocytes as primary target of the virus. However, a discrepancy between a different organoid model on tropism and receptor theory has been observed. Lamers et al.[78] observed in PSC-derived organoids that enterocyte precursors and differentiated enterocytes have a similar infection rate despite a 1,000-fold higher ACE2 transcript in the latter. Their conclusion is reproduced by Triana et al.,[79] who used PSC-derived organoids and identified TMPRSS2 as the major limiting factor for entry since ACE2 is highly expressed in all cell types. Meanwhile, Jang et al.[77] could positively correlate patient-derived organoid virus titer with ACE2 and TMPRSS2 transcript level.

Further work on investigation of tropism reveals common dynamics with respiratory infections (Fig. 4). Heuberger et al.,[76] using colon organoids, demonstrated that interferon (IFN) gamma promoted enterocyte infections by promoting differentiation. As a result, the ACE2 + cell population increased and supported robust virus replication, which is similar to the results of Tindle et al.,[30] who suggested that AT2 differentiation into AT1 increased the ACE2 + cell population and supported viral infections. Meanwhile, ACE2 has been downregulated in infected cells,[79] which is similar to their counterparts in lung.[81] Using intestinal organoids, the tropism of different virus variants was tested. Similar to results from lung organoids,[67] omicron has been observed to depend more on endosomal entry instead of membrane fusion in contrast to delta and wild type, thus decreasing its reliance on TMPRSS2 expression and gain wider tropism.[77]

The presence of inflammation in the gut also has been investigated. Inflammation is significant in intestinal organoids. Infected intestinal organoids upregulate interferon-related genes, including IL-2, IL-6, and JAK/STAT, which are common also in infected lung or other epithelial cell types,[75,79] suggesting that all epithelial pathophysiology may be similar and contribute to multisystemic inflammation in the same way. This is further illustrated by Triana et al.,[79] who used patient-derived organoids to demonstrate the same finding in addition to ACE2 downregulation and proinflammatory gene upregulation. They also described that while a strong NF-κB/TNF-mediated response is elicited in infected immature enterocytes, only bystander cells activate interferon-mediated responses, with a higher interferon-stimulated genes (ISG) transcription level in ileum organoids than colonic organoids. They further produced IRF3 knock-out enterocytes and infected them with SARS-CoV-2 and astrovirus, showing ISG upregulation in the latter only, suggesting IFN signaling pathway suppression by SARS-CoV-2, which is in good agreement with results of other studies.[82] Jang et al.[77] stratified patient-derived organoids into high-infection and low-infection groups. They observed insignificant changes in gene expression between uninfected and infected groups before 72 h and observed more pronounced ISG upregulation in the high-infection group 72 h postinfection, suggesting that type I IFN response is delayed (delayed IFN response by viral nonstructural proteins is reviewed by Znaidia et al.[83]).

Metabolic derangement is also observed using intestinal organoids. Jang et al.[77] showed that genes associated with zinc and copper homeostasis were upregulated in high-infection groups, suggesting these might be a stress response from infection. Gassen et al.[84] observed decreased protein breakdown in infected cells by metabolomics and proved that autophagy is blocked in cell lines and a primary tissue sample. In primary intestinal organoids, they could upregulate autophagy and reduce virus titer by supplementation of polyamine to reactivate autophagy, suggesting that autophagy is suppressed to prevent virus degradation and shunt more resources to virus production.

Liver organoids showed that both hepatocytes and cholangiocytes are permissive to infection and supported viral replication.[85,86] Yang et al.[85] observed a direct cytopathic effect, which may cause liver injury, while an upregulated chemokine response also has been observed by others, hinting at inflammation-mediated liver injury.

Pancreatic organoids also shed light on pancreatic complications in patients with COVID-19. Pancreatic injury is observed in patients as flares of type 1 diabetes mellitus or acute necrotic pancreatitis.[87,88] Using pancreatic organoids, it is shown that both endocrine and exocrine pancreas could be infected and release CXCL12, which is a well-known driver of pancreatic inflammation leading to acute pancreatitis.[89]

Gastric organoids also have shown interesting viral tropism patterns. The stomach is composed of epithelial cells, epithelial progenitors, enteroendocrine cells, and other secretory cells. While results illustrated above show no infection of enteroendocrine cells and only enterocytes are infected, it raises the question of whether the stomach can be infected. Giobbe et al.[90] used primary gastric organoids sampled from different developmental stages to study infection in the stomach. They reported high TMPRSS2 across all organoids while ACE2 expression varied across different patients and developmental stages. Nevertheless, all organoids were permissive to infection. They observed apoptosis of gastric cells indicated by activated caspase 3, and genes related to antiviral pathways were upregulated. Interestingly, IFN transcripts were not upregulated. Single-cell analysis reveals that >15% of somatostatin-secreting delta cells were infected while less than 5% of goblet cells were infected, suggesting a different role of enteroendocrine cells in infection along the gut tube.

2.6 Cardiac organoids

Cardiac organoids refer to a 3D culture of cardiomyocytes with neurons, endothelial cells, fibroblasts, and sometimes resident macrophages. Unlike gut and respiratory organoids mentioned,

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cardiac organoids take many more forms than just spheroids, tubes, or hollow spheres as a physiologic heart cannot be represented by an epithelium alone. Simple cardiac organoids are usually spheroids while complex organoids typically use bioprinting or molded extracellular matrix (ECM) as a scaffold, and cells are seeded inside to mimic the structure and function of heart in vitro (reviewed by Zhu et al.[33]).

There is no common or widely adopted protocol for the generation of cardiomyocytes. Unlike gut or intestinal organoids, there are no primary cell-derived organoids as cardiac stem cells do not exist. Thus, generation of the organoid must start with PSCs. PSCs are first differentiated into mesoderm by BMP, FGF, nodal, and TGF signaling.[91] Cardiomyocyte differentiation is often spontaneous in an embryoid body (EB)[33] but can be enhanced by ascorbic acid, careful manipulation of Wnt signals, or GSK3 inhibition.[92] Cardiac organoids for a long time had been produced by reaggregation of mature cardiomyocytes with other cell types, which were not exactly organoids by definition. It was not until 2021 when Hofbauer et al.[93] produced the first self-organizing "cardioids" from PSCs that cardiac organoids truly existed.

For this reason, as of 29 December 2022, no research has been published on COVID-19 modeling with cardiac organoids that met the definition of self-organizing. Nevertheless, other published studies of mock cardiac organoids offer much insight for us to understand cardiovascular pathologies in COVID-19.

Bailey et al.[94] constructed an engineered heart tissue capable of spontaneous contraction by bioprinting of PSC-derived cardiomyocytes, cardiac fibroblasts, and macrophages in a mold. The organoid was permissive to infection, and infection was not present when cardiomyocytes were removed from the system. They detected ACE2 transcripts and protein in cardiomyocytes and suggested that tropism was ACE2 dependent. However, low TMPRSS2 expression and success in reducing infection by endosomal cysteine protease inhibitor but not serine protease inhibitor suggested viral entry utilized the endosomal pathway. Then they proceeded to investigate the cause for clinically observed left ventricular systolic dysfunction. Histology of the organoid showed recruitment of macrophage that resembled myocarditis. Reduced chronotropy, inotropy, and slow relaxation of the infected organoid successfully modeled LV systolic dysfunction. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining revealed apoptosis of cardiomyocytes. Single-cell RNA sequencing revealed downregulation of electron transport chain, metabolic enzymes, and pathways related to muscle contraction, which may account for the cardiomyocyte dysfunction. Sarcomere breakdown was also observed. The team proceeded to investigate the role of inflammation in cardiac pathology. Bailey et al.[94] hence concluded damage to cardiomyocytes was caused by direct infection.

However, the effect of inflammation is not to be overlooked. Mills et al.[95] used cardiac organoids reaggregated with PSC-derived cardiomyocytes, endothelial cells, and fibroblasts to study cardiac dysfunction due to inflammation. First, they identified that cytokine receptors for TNF-α, IL-6, and so on were highly expressed in epicardial cells and fibroblasts. By culturing the organoids with a recipe of cytokines that they identified from patient serum, they observed systolic and diastolic dysfunction in organoids by TNF-α and IFN-γ, IL-1β, and poly(I:C), respectively. They identified increased phosphorylation of STAT1 and BRD4, the former being a signal transducer of type 1 interferon pathways[96] and the latter identified to mediate mitochondrial homeostasis in cardiomyocytes.[97] Bromodomain extraterminal inhibitors were tested in the organoids, and they attenuated diastolic dysfunction and downregulated some inflammatory genes, which were stimulated by infection, proving BRD4's involvement in cardiac dysfunction during the cytokine storm. In short, these 2 studies revealed that both direct infection and cytokines could possibly cause cardiac dysfunction (Fig. 4).

2.7 Brain organoids

Brain organoids consists of neurons, glial cells, and, in some models, endothelium or choroid plexus generated from 3D differentiation of human pluripotent stem cells. The organoid is derived from neuroectoderm self-aggregation and maturation. The differentiation begins with EBs, and neuroectoderm differentiation is enhanced by using Neural Basal Medium with B27 supplements and maintenance of neural stem cells with insulin or 2-mercaptoethanol.[98] Alternatively, PSCs can be directed to neural stem cells by dual SMAD inhibition.[99] Dual SMAD inhibition uses 2 small molecules, Noggin and SB431542, to inhibit the SMAD family. Noggin is a BMP inhibitor and SB431542 inhibits the Lefty/Activin/TGF-β pathways by blocking the phosphorylation of ALK4, ALK5, and ALK7 receptors. In addition, Wnt inhibition can be used to enhance cortical neural differentiation.[100] Then brain organoids are cultured typically for more than 200 d for maturation. The resulting organoids, depending on method used, may resemble structures such as ventricles, cortical layers, blood-brain barrier, or choroid plexus. Brain organoids are reviewed in depth by Koo et al.[101]

Brain organoids have provided us with valuable evidence to explain some presentations of long COVID such as brain fog. However, some evidence presented by brain organoids is conflicting and illustrates the inconsistency of organoids. Here we will briefly summarize some of the findings and conflicts.

The tropism of SARS-CoV-2 in the brain has always been debated (Fig. 4). A study showed expression of ACE2 and TMPRSS2 in organoids, especially neurons, and suggested that neurons were directly infected with very limited glial cell involvement.[64,102–104] However, other studies showed that neurons were not infected directly.[105–107] Instead, astrocytes and choroid plexus endothelium are suggested to be infected by ACE2 and alternative entry receptors such as BSG, DPP4, and NRP-1.[105,107] Of all glial cells, astrocytes are most often suggested to be permissive to infection. Even then, there is discrepancy in the expression level of ACE2, DPP4, BSG, and NRP-1 and which receptor is used for entry.[105,106] A study by Pellegrini et al.[107] identified the choroid plexus epithelium as the only infected cells in their cerebral organoid, disagreeing with most other studies. For infectivity among variants, Kong et al.[106] showed that omicron and delta are less infectious than the wild type, which is in partial agreement with animal models.[21]

As tropisms identified by different organoid models were different, the resulting pathophysiology is also different. Nevertheless, there is still common ground.

Inflammatory genes, including IL-6 and type 1 interferon-related genes, like all other organoid models, were identified to be activated in infected cells, whether astrocytes[105,106] or neurons,[64] while antiapoptotic genes were downregulated. For studies that identified astrocyte infections but not neurons, indirect neuron killing was observed, as reflected by uninfected apoptotic neurons by TUNEL or cell count.[104–106] Kong et al.[106] reported NOD2 upregulation activated NF-κB transcription and mitogen-activated protein kinase (MAPK), leading to proinflammatory chemokine and cytokine release. In addition, they observed downregulation of genes involved in cell–cell communication, cell

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junction organization, ion and fatty acid homeostasis, and solute carrier–mediated transmembrane transport in infected astrocytes, implying metabolic derangement. Meanwhile, hypermethylation of genes involved in neurogenesis, axonogenesis, and synaptic transmission was observed in neurons, suggesting impaired synapse function. In good agreement with Kong et al.,[106] Andrews et al.[105] reported upregulation of proinflammatory genes, microglial activation pathway, and downregulated cholesterol synthesis with altered calcium transport, cell size, and morphology in infected astrocytes. Interestingly, Wang et al.,[103] who observed low-grade infection of both neurons and astrocytes, also observed increased ApoE4 in apoptotic astrocytes and proved that increased ApoE4 relative to ApoE3 increased susceptibility. Although exact mechanisms were not proposed in both pathways, we may speculate involvement of lipid metabolism in infection of astrocytes by the virus.

On the other hand, studies that proposed direct neuron infections identified both direct and indirect killing. Ramani et al.[102] observed colocalization of the virus staining with TUNEL + signals. Confocal microscopy showed altered Tau localization and aberrant phosphorylation of Tau, hinting that Tau phosphorylation may be induced by viral infection stress, thus inducing apoptosis. However, Mesci et al.[104] suggested infection of neurons but proposed indirect killing as they observed that TUNEL + cells were not all stained positive for N protein of the virus and vice versa. (From our point of view, this may be due to attachment of viral particles, as suggested by Triana et al.[79] in their study.) They proceeded to study physiology of infected neurons. It was found that presynaptic proteins vGLUT1 and synapsin 1 were reduced by 70% upon infection while postsynaptic protein PSD95 expression did not differ significantly.

To conclude, both theories suggested indirect killing of neurons and altered neuron physiology despite different models of tropism, and they potentially give a basis for explaining neurologic disorders observed in clinics.

2.8 Kidney organoids

Kidney functionally can be divided into 3 parts: glomeruli, tubules, and urothelium and endothelium. A protocol developed by Takasato et al.[108] can produce endothelium, glomeruli, and tubules in 1 single PSC aggregate. The process involves manipulation of Wnt, BMP, FGF, and nodal signals. The rationale is to induce a primitive streak, an intermediate mesoderm, and finally aggregation to spontaneously differentiate into kidney organoids. Following the same differentiation path, many similar protocols have been developed (reviewed by Khoshdel-Rad et al.[109]). However, organoids produced in this manner lack urothelium. Aggregation with a ureteric bud organoid was found to be useful for generating kidney organoids with a collecting duct tree.[110]

Kidney has received much attention since the pandemic began as the first entry receptor identified ACE2 as a key enzyme in the renin–angiotensin–aldosterone system (RAAS). There were speculations of complications developing from dysfunction of RAAS and the possibility of targeting viral entry by existing drugs such as ACE inihbitor or angiotensin receptor blocker.[111] Acute kidney injury has been observed in patients, and pathology showed inflammation, tubular injury, and glomerulopathy despite inconsistent infections.[112,113]

Using renal organoids, scientists have unsurprisingly identified ACE2 for mediating infection in almost all cell types in the kidney. Loop of Henle progenitor, proximal tubule, podocytes, and mesenchymal cells were all found to be infected,[114–117] while serine protease inhibitor reduced infections, suggesting the use of a cell fusion pathway.[117] Inflammation patterns similar to other organs were observed. MAPK, TNF-α, NF-κB, interferon signaling pathways, and JAK/STAT were upregulated in infected cells.[117]

To address different pathologies observed clinically, scRNA-seq was used. It was shown that inflammation was the main driver of acute kidney injury. Nystrom et al.[118] showed expression of APOL1 induced by cytokines resulted in podocyte loss and nephropathy. Use of JAK inhibitors could block APOL1 expression, suggesting that the process is mediated by JAK/STAT signaling by cytokines. Another interesting study aiming to describe kidney fibrosis with organoids has shown a novel mechanism for fibrosis.[117] Jansen et al.[117] observed upregulated antiapoptotic and proinflammatory pathways in infected podocytes, proximal tubular cells, and mesenchymal cells. In addition, TGF-β, PI3K/Akt, MAPK, and WNT signaling in proximal tubular cells and mesenchymal clusters was upregulated, which is known to mediate fibrosis. Gene ontogeny enrichment showed upregulation of collagen-related ECM genes, and pathway analysis revealed increased MAPK, NF-κB, TNF-α, WNT, and TGF-β activity, which was reasoned to cause fibrosis. Other upregulated terms include cellular stress and injury response and cytoskeleton rearrangement. Using ligand–receptor analysis, they also observed upregulated ligands from infected epithelium, which potentially promoted mesenchyme fibrosis. Their work illustrates the crosstalk between tissues in mediating fibrosis, which is insightful to many other organs.

Finally, some studies also modeled kidney infections with comorbidities. Infection of a diabetes-modeling organoid by long-term coculture in high glucose showed increased viral load and increased ACE2 expression,[114] while polycystic kidney organoids generated from $PKD^{-/-}$ cells showed infection of the cyst epithelium and cytopathic effect.[116] Although clinical implications are ambiguous, these studies demonstrate the potential of investigating effects of various comorbidities in COVID-19.

2.9 Drug screening and testing using organoids

Organoids resembling the function and structure of human organs can be used for antiviral drug screening. The current approach to drug screening starts with a short hairpin RNA (shRNA) or small-molecule library that is then applied to different organoids and infected by viruses. The virus may be clinically isolated strains, engineered clones with reporter gene, or pseudoviruses each with different methods for tracking infection. Then organoids that show different tropism or cytopathic effect are analyzed by transcriptomics, proteomics, and metabolomics to uncover the underlying over- or underexpressed pathways and hence suggest new therapeutic options or pathogenesis.

Duan et al.[119] employed a small-molecule library in infected airway organoids and identified that GW6471 blocks infection. RNA sequencing (RNA-seq) showed that the molecule inhibited HIF-1α pathways. As chemokine or interferon pathways were not affected, the team turned to metabolic profiling and revealed decreased levels of fatty acids, including oleic acid, palmitic acid, palmitoleic acid, and amino acids, as well as D-glucose 6-phosphate and citric acid, which agreed with the downregulation of glycolysis genes as discovered by RNA-seq. Inhibition of glycolysis by shRNA also produced the same effect, and the authors suggested that reduced lipid metabolism disrupted replication of viruses, for which the effect is observed in many other viruses[120,121] and reproduced by Chu et al.[122] by treating cell lines with fatty acid synthase inhibitor.

Similarly, Han et al.,[80] using colonic and lung organoids, identified imatinib, mycophenolic acid, and quinacrine dihydrochloride

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as viral entry inhibitors at the physiologic level from a library of drugs approved by the US Food and Drug Administration.

While drug screening makes use of a library, drug testing is simply applying a drug to an organoid. Candidates that were identified to reduce infection or damage in organoids include type 1/3 interferon,[60,62,81] remdesivir,[81,84,103] polyclonal antibodies,[81,116,123] atorvastatin,[124] sofosbuvir,[104] and EIDD-2801, camostat, and cycloheximide in combination with remdesivir.[81]

Still, not many studies were published utilizing organoids for large-scale drug screening for COVID-19. This is in part due to the difficulty of handling contagious biosafety level 3 virus in laboratories. However some greater constraints would be scalability, consistency, and difficult readouts. Since organoids are heterogeneous, a bigger sample size is needed for valid conclusions. In addition, the tissue-level response can only be observed through histology, immunohistochemistry, or spatial transcriptomics, which are labor intensive or expensive, rendering large-scale drug library screening impractical at institutional laboratories. Furthermore, the biggest pitfall of using organoid infection models for drug screening is that immune cells are excluded from the model, which are in fact the most important drug targets against the COVID-19 cytokine storm.

2.10 How organoids could contribute to COVID-19 study

The above organoid models have given us many insights into molecular pathways but do not push organoids to their fullest potential. The advantage of organoids over cell lines is the cell-type diversity and spatial organization. This allows the study of intercell communication and the effect of spatial organization on tissue or molecular pathway heterogeneity. For example, many immunology components involve transsignaling. IL6R can be cleaved by membrane-bound protease or matrix metalloproteinases to form soluble IL6R, which may bind to IL-6 and signal on cells that do not express IL6R but gp130 to activate JAK/STAT signaling.[125] Moreover, as mentioned previously, the entry of virus by ACE2 requires another protease cleavage.[126,127] Such proteases may be coagulation factors, ADAM17, or the most well known, TMPRSS2.[128,129] Whether these proteases are expressed on the membrane surface, released by other adjacent cells, or upregulated during inflammation is not studied in details. For example, thrombin and factor Xa are found to cleave ACE2 and promote virus particle entry, which may be speculated to extend the tropism of virus in blood vessels.[128] ADAM17 is a secreted protease that cleaves membrane-bound pro-TNF protein to release the functional TNF-α and is known to be regulated positively and negatively during inflammation.[130,131] Such proteases are reported to have great significance in studies conducted with patient samples but are yet to be investigated in organoids.[132,133]

Existing modeling data sets are yet to be explored in the direction of searching for heterogeneity and intercell communications.

2.11 Limitations and future directions to in vitro models

Although organoids recapitulate the function and structure of organs, they are often a layer of simple epithelium resting on synthetic or animal-derived ECM such as Matrigel. The lack of connective tissue limits the growth and size of the organoids, lowering their physiologic relevance to real organs due to the lack of blood flow and tissue-specific stroma. For example, lung organoids lack perfusion by capillaries and their own ECM, and thus scientists are unable to reproduce fibrosis in vitro or assess the tissue remodeling during a cytokine storm.

The physiologic relevance of organoids is also a limitation. Organoids developed from PSCs are programmed to differentiate by a combination of growth factors and small molecules. However, exogenous signals may create cell phenotypes in organoids that may not resemble that of human tissue. The discrepancy between organoids and human body is amplified by the lack of systems. Our body is maintained by a cooperation of many systems, while organoids solely interact with the culture medium and limited types of stromal cells. Evidence has shown that entry of viral particles in non-ACE2-expressing cells can be achieved by a complex of spike, ACE2, and vasopressin, which allows endocytosis of virus by AT1 and AVPR1B.[134] Meanwhile, organoid studies have shown that viral particle entry depends heavily on ACE2 expression on the cell surface and demonstrated that lung organoid infections are limited to ACE2-expressing cell types,[63] contradictory to findings of Yeung et al.[134] Yet, evidence of recombinant ACE2 rescuing infection[135,136] in lung or preferential infection in glomerular cells were reported in autopsy studies.[137]

In addition, the resolution of pathways in organoids is limited to the transcriptome. As demonstrated above, most inflammation pathways studied in organoids are confined to immunofluorescent staining of NF-κB, caspase 3/7, and transcriptome analysis by scRNA-seq. Meanwhile, studies with cell lines have provided us with immense value of the molecular mechanisms of viral immune evasion.[49,51,82,138,139] Current methods for proteomics analysis include Western blot, mass spectrometry, cytometry time of flight or enzyme-linked immunosorbent assay, and so on. While subcellular spatial proteomic techniques exist (reviewed by Lundberg and Borner[140]), the spatial information provided is lost during single-cell dissociation or extraction. Therefore, for organoids to provide us with tissue-level proteomics, a method for tissue-level spatial proteomics is needed. New technology such as hydrogel expanded tissue for microscopy,[141] machine learning,[142] laser microdissection, robotics, or a combination of all[143] will help to build a platform for organoid proteomics.

The lack of innate immunity or tissue-resident immune cells also poses a great challenge for modeling diseases with organoids, especially in the case of infections and inflammation. The major pathologies of COVID-19 are found to be related to activation of macrophages and immune dysregulation while the actual cytopathic effect is limited.[144–149] Hence, the immune system is a key component for modeling disease pathology with in vitro platforms. Mucosal immunity is often overlooked in COVID-19 and consists of lymphoid cells, dendritic cells, antimicrobial peptides, and so on (reviewed by Russell et al.[150]). The response of mucosal and PRR-mediated innate immunity is also found to correlate with constant microbial challenges (reviewed by McDermott and Huffnagle[151] and Shi et al.[152]). In the case of organoids in vitro, lack of stromal immune cells impairs the 2 types of immune responses and hence limits its use in modeling COVID-19, where many pathologic presentations are mediated by dysfunction in interferon pathways or the cytokine storm. The current approach to including immune cells is by coculture with an immune cell line such stem cell–derived macrophages.[153]

However, owing to dependence of organoids on growth factors, the immune reaction is often altered as organoid maintenance medium may consist of anti-inflammatory cytokines or hormones such as the TGF family and cortisol.[154] The lack of a proper microbial challenge to cells results in a physiologically different immune response. For example, antigen presentation by endothelium is diminished in vitro but plays an important role in vivo for lymphocyte activation.[155] Hence, the validity of immune reactions observed in organoid coculture models is

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compromised. The organoid medium and lack of tissue-resident immune cells also prevent scientists in studying tissue remodeling.

To overcome these limitations, a new in vitro platform, organ-on-chip (OOC), can be adopted.[156] OOC is a microfluidics chamber lined by cells inside channels. By precise bioprinting, hemodynamics can be simulated by controlling the channel size, structure, and flow rate to create an environment that supports cell line organization (reviewed by Sosa-Hernández et al.[157]). Despite lower resemblance to human bodies than organoids due to incorporation of exogenous materials, OOCs were shown to model physiology accurately (reviewed by Yum et al.[158]). OOCs are superior to organoids because they are able to use somatic cell lines instead of stem cells, thus removing the need for strict control of medium growth factors and allowing greater flexibility in culture medium. The platform is also more consistent as well-established cell lines can be used, thus reducing variation between protocols and cell behaviors due to different stem cell lines. A major advantage of OOCs over organoids is that multiorgan modeling is possible by microfluidic channels, hence expanding the scope of study from a single organ to multiple systems.[159] The new direction will be to combine organoids with OOCs, leveraging both advantages in tissue complexity and multiorgan modeling. The further direction could be incorporation of microbiota.

3. Conclusion

Organoid-based approaches allows convenient investigation of pathophysiology in COVID-19. Organoids also allow high-throughput screening of drugs or small molecules due to their physiologic relevance and animals not being used. Supplying stroma and immune cells would offer modeling systemic pathologies. At the same time, technology advancement is needed to fully utilize organoids for the study of protein interactions. On the other hand, microfluidics-based OOC has successfully overcome some of these challenges, albeit with lower physiologic resemblance and complexity. We can foresee that microfluidics with organoids will be the next step for in vitro disease modeling.

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References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, *et al*. Brief report: a novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020:382(8):727. https://doi.org/10.1056/NEJMoa2001017
2. Li T, Liu D, Yang Y, Guo J, Feng Y, Zhang X, Cheng S, Feng J, *et al*. Phylogenetic supertree reveals detailed evolution of SARS-CoV-2. *Sci Rep*. 2020:10(1):1–9. https://doi.org/10.1038/s41598-020-79484-8
3. Mariano G, Farthing RJ, Lale-Farjat SLM, Bergeron JRC. Structural characterization of SARS-CoV-2: where we are, and where we need to be. *Front Mol Biosci*. 2020:7:605236. https://doi.org/10.3389/fmolb.2020.605236
4. Ganesh B, Rajakumar T, Malathi M, Manikandan N, Nagaraj J, Santhakumar A, Elangovan A, Malik YS. Epidemiology and pathobiology of SARS-CoV-2 (COVID-19) in comparison with SARS, MERS: an updated overview of current knowledge and future perspectives. *Clin Epidemiol Glob Health*. 2021:10:100694. https://doi.org/10.1016/j.cegh.2020.100694
5. Scialo F, Daniele A, Amato F, Pastore L, Matera MG, Cazzola M, Castaldo G, Bianco A, *et al*. ACE2: the Major cell entry receptor for SARS-CoV-2. *Lung*. 2020:198(6):867. https://doi.org/10.1007/s00408-020-00408-4
6. Synowiec A, Szczepański A, Barreto-Duran E, Lie LK, Pyrc K. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): a systemic infection. *Clin Microbiol Rev*. 2021:34(2):e00133-20. https://doi.org/10.1128/CMR.00133-20
7. Michelen M, Manoharan L, Elkheir N, Cheng V, Dagens A, Hastie C, O'Hara M, Suett J, Dahmash D, Bugaeva P, *et al*. Characterising long COVID: a living systematic review. *BMJ Glob Health*. 2021:6(9):e005427. https://doi.org/10.1136/bmjgh-2021-005427
8. Davis HE, McCorkell L, Vogel JM, Topol EJ. Long COVID: major findings, mechanisms and recommendations. *Nat Rev Microbiol*. 2023:21(3):133–146. https://doi.org/10.1038/s41579-022-00846-2
9. Fujinami RS, von Herrath MG, Christen U, Whitton JL. Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. *Clin Microbiol Rev*. 2006:19(1):80–94. https://doi.org/10.1128/CMR.19.1.80-94.2006
10. Bohnacker S, Hartung F, Henkel F, Quaranta A, Kolmert J, Priller A, Ud-Dean M, Giglberger J, Kugler LM, Pechtold L, *et al*. Mild COVID-19 imprints a long-term inflammatory eicosanoid- and chemokine memory in monocyte-derived macrophages. *Mucosal Immunol*. 2022:15(3):515–524. https://doi.org/10.1038/s41385-021-00482-8
11. Phetsouphanh C, Darley DR, Wilson DB, Howe A, Munier CML, Patel SK, Juno JA, Burrell LM, Kent SJ, Dore GJ, *et al*. Immunological dysfunction persists for 8 mo following initial mild-to-moderate SARS-CoV-2 infection. *Nat Immunol*. 2022:23(2):210–216. https://doi.org/10.1038/s41590-021-01113-x
12. Mehandru S, Merad M. Pathological sequelae of long-haul COVID. *Nat Immunol*. 2022:23(2):194–202. https://doi.org/10.1038/s41590-021-01104-y
13. Glynne P, Tahmasebi N, Gant V, Gupta R. Long COVID following mild SARS-CoV-2 infection: characteristic T cell alterations and response to antihistamines. *J Investig Med*. 2022:70(1):61–67. https://doi.org/10.1136/jim-2021-002051
14. Liu Q, Mak JWY, Su Q, Yeoh YK, Lui GC-Y, Ng SSS, Zhang F, Li AYL, Lu W, Hui DS-C, *et al*. Gut microbiota dynamics in a prospective cohort of patients with post-acute COVID-19 syndrome. *Gut*. 2022:71(3):544–552. https://doi.org/10.1136/gutjnl-2021-325989
15. Yeoh YK, Zuo T, Lui GC-Y, Zhang F, Liu Q, Li AY, Chung AC, Cheung CP, Tso EY, Fung KS, *et al*. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. *Gut*. 2021:70(4):698–706. https://doi.org/10.1136/gutjnl-2020-323020
16. Haffke M, Freitag H, Rudolf G, Seifert M, Doehner W, Scherbakov N, Hanitsch L, Wittke K, Bauer S, Konietschke F, *et al*. Endothelial dysfunction and altered endothelial biomarkers in patients with post-COVID-19 syndrome and chronic fatigue syndrome (ME/CFS). *J Transl Med*. 2022:20(1):138. https://doi.org/10.1186/s12967-022-03346-2
17. Charfeddine S, Amor HIH, Jdidi J, Torjmen S, Kraiem S, Hammami R, Bahloul A, Kallel N, Moussa N, Touil I, *et al*. Long COVID 19 syndrome: is it related to microcirculation and endothelial dysfunction? Insights from TUN-EndCOV study. *Front Cardiovasc Med*. 2021:8:745758. https://doi.org/10.3389/fcvm.2021.745758

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18. Katsoularis I, Fonseca-Rodríguez O, Farrington P, Jerndal H, Lundevaller EH, Sund M, Lindmark K, Connolly A-MF, *et al.* Risks of deep vein thrombosis, pulmonary embolism, and bleeding after covid-19: nationwide self-controlled cases series and matched cohort study. *BMJ*. 2022:377:e069590. https://doi.org/10.1136/bmj-2021-069590
19. Guerrero JI, Barragán LA, Martínez JD, Montoya JP, Peña A, Sobrino FE, *et al.* Central and peripheral nervous system involvement by COVID-19: a systematic review of the pathophysiology, clinical manifestations, neuropathology, neuroimaging, electrophysiology, and cerebrospinal fluid findings. *BMC Infect Dis*. 2021:21(1):515. https://doi.org/10.1186/s12879-021-06185-6
20. Soung AL, Vanderheiden A, Nordvig AS, Sissoko CA, Canoll P, Mariani MB, Jiang X, Bricker T, Rosoklija GB, Arango V, *et al.* COVID-19 induces CNS cytokine expression and loss of hippocampal neurogenesis. *Brain*. 2022:145(12):4193–4201. https://doi.org/10.1093/brain/awac270
21. Song E, Zhang C, Israelow B, Lu-Culligan A, Vieites Prado A, Skriabine S, Lu P, Weizman O-E, Liu F, Dai Y, *et al.* Neuroinvasion of SARS-CoV-2 in human and mouse brain. *J Exp Med*. 2021:218(3):e20202135. https://doi.org/10.1101/2020.06.25.169946
22. Li Q, Dang C, Wang LH. Neuroinflammation in mild respiratory COVID-19: insights into cognitive impairment in milder cases. *Mil Med Res*. 2022:9(1):1–3. https://doi.org/10.1186/s40779-021-00361-0
23. Hikmet F, Méar L, Edvinsson Å, Micke P, Uhlén M, Lindskog C. The protein expression profile of ACE2 in human tissues. *Mol Syst Biol*. 2020:16(7):e9610. https://doi.org/10.15252/msb.20209610
24. Ma Z, Li X, Fan RLY, Yang KY, Ng CSH, Lau RWH, Wong RHL, Ng KK, Wang CC, Ye P, *et al.* A human pluripotent stem cell-based model of SARS-CoV-2 infection reveals an ACE2-independent inflammatory activation of vascular endothelial cells through TLR4. *Stem Cell Rep*. 2022:17(3):538–555. https://doi.org/10.1016/j.stemcr.2022.01.015
25. Khanmohammadi S, Rezaei N. Role of toll-like receptors in the pathogenesis of COVID-19. *J Med Virol*. 2021:93(5):2735–2739. https://doi.org/10.1002/jmv.26826
26. Song P, Li W, Xie J, Hou Y, You C. Cytokine storm induced by SARS-CoV-2. *Clin Chim Acta Int J Clin Chem*. 2020:509:280. https://doi.org/10.1016/j.cca.2020.06.017
27. Masre SF, Jufri NF, Ibrahim FW, Raub SHA. Classical and alternative receptors for SARS-CoV-2 therapeutic strategy. *Rev Med Virol*. 2021:31(5):1–9. https://doi.org/10.1002/rmv.2207
28. Hv W. A new method by which sponges may be artificially reared. *Science*. 1907:25(649):912–915. https://doi.org/10.1126/science.25.649.912
29. Nugraha B, Buono MF, Boehmer L, Hoerstrup SP, Emmert MY. Human cardiac organoids for disease modeling. *Clin Pharmacol Ther*. 2019:105(1):79–85. https://doi.org/10.1002/cpt.1286
30. Tindle C, Fuller M, Fonseca A, Taheri S, Ibeawuchi S-R, Beutler N, Katkar GD, Claire A, Castillo V, Hernandez M, *et al.* Adult stem cell-derived complete lung organoid models emulate lung disease in COVID-19. *eLife*. 2021:10::e66417. https://doi.org/10.7554/eLife.66417
31. Gunti S, Hoke ATK, Vu KP, London NR. Organoid and spheroid tumor models: techniques and applications. *Cancers (Basel)*. 2021:13(4):874. https://doi.org/10.3390/cancers13040874
32. Harrison SP, Baumgarten SF, Verma R, Lunov O, Dejneka A, Sullivan GJ. Liver organoids: recent developments, limitations and potential. *Front Med*. 2021:8:574047. https://doi.org/10.3389/fmed.2021.574047
33. Zhu L, Liu K, Feng Q, Liao Y. Cardiac organoids: a 3D technology for modeling heart development and disease. *Stem Cell Rev Rep*. 2022:18(8):2593–2605. https://doi.org/10.1007/s12015-022-10385-1
34. Wagar LE, Salahudeen A, Constantz CM, Wendel BS, Lyons MM, Mallajosyula V, Jatt LP, Adamska JZ, Blum LK, Gupta N, *et al.* Modeling human adaptive immune responses with tonsil organoids. *Nat Med*. 2021:27(1):125–135. https://doi.org/10.1038/s41591-020-01145-0
35. Bleijs M, Wetering M, Clevers H, Drost J. Xenograft and organoid model systems in cancer research. *EMBO J*. 2019:38(15):e101654. https://doi.org/10.15252/embj.2019101654
36. Qian X, Song H, Ming GL. Brain organoids: advances, applications and challenges. *Dev Camb Engl*. 2019:146(8):dev166074. http://doi.org/10.1242/dev.166074
37. Schmidt S, Alt Y, Deoghare N, Krüger S, Kern A, Rockel AF, Wagner N, Ergün S, Wörsdörfer P, *et al.* A blood vessel organoid model recapitulating aspects of vasculogenesis, angiogenesis and vessel wall maturation. *Organoids*. 2022:1(1):41–53. https://doi.org/10.3390/organoids1010005
38. Tsuruta S, Uchida H, Akutsu H. Intestinal organoids generated from human pluripotent stem cells. *JMA J*. 2020:3(1):9. https://doi.org/10.31662/jmaj.2019-0027
39. Vsw L. Modelling intestinal inflammation and infection using "mini-gut" organoids. *Nat Rev Gastroenterol Hepatol*. 2021:18(2):89–90. https://doi.org/10.1038/s41575-020-00391-4
40. Khan AO, Rodriguez-Romera A, Reyat JS, Olijnik A-A, Colombo M, Wang G, Wen WX, Sousos N, Murphy LC, Grygielska B, *et al.* Human bone marrow organoids for disease modeling, discovery, and validation of therapeutic targets in hematologic malignancies. *Cancer Discov*. 2023:13(2):364–385. https://doi.org/10.1158/2159-8290.CD-22-0199
41. Ma C, Witkowski MT, Harris J, Dolgalev I, Sreeram S, Qian W, Tong J, Chen X, Aifantis I, Chen W. Leukemia-on-a-chip: dissecting the chemoresistance mechanisms in B cell acute lymphoblastic leukemia bone marrow niche. *Sci Adv*. 2020:6(44):eaba5536. https://doi.org/10.1126/sciadv.aba5536
42. Paşca SP, Arlotta P, Bateup HS, Camp JG, Cappello S, Gage FH, Knoblich JA, Kriegstein AR, Lancaster MA, Ming G-L, *et al.* A nomenclature consensus for nervous system organoids and assembloids. *Nature*. 2022:609(7929):907–910. https://doi.org/10.1038/s41586-022-05219-6
43. Corrò C, Novellasdemunt L, Li VSW. A brief history of organoids. *Am J Physiol Cell Physiol*. 2020:319(1):C151–C165. https://doi.org/10.1152/ajpcell.00120.2020
44. Kim J, Koo BK, Knoblich JA. Human organoids: model systems for human biology and medicine. *Nat Rev Mol Cell Biol*. 2020:21(10):571–584. https://doi.org/10.1038/s41580-020-0259-3
45. Driehuis E, Kretzschmar K, Clevers H. Establishment of patient-derived cancer organoids for drug-screening applications. *Nat Protoc*. 2020:15(10):3380–3409. https://doi.org/10.1038/s41596-020-0379-4
46. Beumer J, Geurts MH, Lamers MM, Puschhof J, Zhang J, van der Vaart J, Mykytyn AZ, Breugem TI, Riesebosch S, Schipper D, *et al.* A CRISPR/cas9 genetically engineered organoid biobank reveals essential host factors for coronaviruses. *Nat Commun*. 2021:12(1):5498. https://doi.org/10.1038/s41467-021-25729-7
47. Dutta D, Heo I, Clevers H. Disease modeling in stem cell-derived 3D organoid systems. *Trends Mol Med*. 2017:23(5):393–410. https://doi.org/10.1016/j.molmed.2017.02.007
48. Clevers H. . Modeling development and disease with organoids. *Cell*. 2016:165(7):1586–1597. https://doi.org/10.1016/j.cell.2016.05.082

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49. Yamada T, Sato S, Sotoyama Y, Orba Y, Sawa H, Yamauchi H, Sasaki M, Takaoka A, *et al.* RIG-I triggers a signaling-abortive anti-SARS-CoV-2 defense in human lung cells. *Nat Immunol*. 2021:22(7):820–828. https://doi.org/10.1038/s41590-021-00942-0
50. Mabrey FL, Morrell ED, Wurfel MM. TLRs in COVID-19: how they drive immunopathology and the rationale for modulation. *Innate Immun*. 2021:27:503–513. https://doi.org/10.1177/17534259211051364
51. Xia H, Cao Z, Xie X, Zhang X, Chen JYC, Wang H, Menachery VD, Rajsbaum R, Shi P-Y, *et al.* Evasion of type I interferon by SARS-CoV-2. *Cell Rep*. 2020:33(1):108234. https://doi.org/10.1016/j.celrep.2020.108234
52. Acharya D, Liu G, Gack MU. Dysregulation of type I interferon responses in COVID-19. *Nat Rev Immunol*. 2020:20(7):397–398. https://doi.org/10.1038/s41577-020-0346-x
53. Mangan NE, Fung KY. Type I interferons in regulation of mucosal immunity. *Immunol Cell Biol*. 2012:90(5):510–519. https://doi.org/10.1038/icb.2012.13
54. Seo SH, Webster RG. Tumor necrosis factor alpha exerts powerful anti-influenza virus effects in lung epithelial cells. *J Virol*. 2002:76:1071–1076. https://doi.org/10.1128/JVI.76.3.1071-1076.2002
55. Bosáková V, De Zuani M, Sládková L, Garlíková Z, Jose SS, Zelante T, Hortová Kohoutková M, Frič J. Lung organoids—the ultimate tool to dissect pulmonary diseases? *Front Cell Dev Biol*. 2022:10:899368. https://doi.org/10.3389/fcell.2022.899368
56. Kong J, Wen S, Cao W, Yue P, Xu X, Zhang Y, Luo L, Chen T, Li L, Wang F, *et al.* Lung organoids, useful tools for investigating epithelial repair after lung injury. *Stem Cell Res Ther*. 2021:12(1):1–13. https://doi.org/10.1186/s13287-021-02172-5
57. Yang W, Li Y, Shi F, Liu H. Human lung organoid: models for respiratory biology and diseases. *Dev Biol*. 2022:494:26–34. https://doi.org/10.1016/j.ydbio.2022.12.001
58. Ekanger CT, Zhou F, Bohan D, Lotsberg ML, Ramnefjell M, Hoareau L, Røsland GV, Lu N, Aanerud M, Gärtner F, *et al.* Human organotypic airway and lung organoid cells of bronchiolar and alveolar differentiation are permissive to infection by influenza and SARS-CoV-2 respiratory virus. *Front Cell Infect Microbiol*. 2022:12:841447. https://doi.org/10.3389/fcimb.2022.841447
59. Sano E, Suzuki T, Hashimoto R, Itoh Y, Sakamoto A, Sakai Y, Saito A, Okuzaki D, Motooka D, Muramoto Y, *et al.* Cell response analysis in SARS-CoV-2 infected bronchial organoids. *Commun Biol*. 2022:5(1):516. https://doi.org/10.1038/s42003-022-03499-2
60. Katsura H, Sontake V, Tata A, Kobayashi Y, Edwards CE, Heaton BE, Konkimalla A, Asakura T, Mikami Y, Fritch EJ, *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.e8. https://doi.org/10.1016/j.stem.2020.10.005
61. Chiu MC, Li C, Liu X, Song W, Wan Z, Yu Y, Huang J, Xiao D, Chu H, Cai J-P, *et al.* Human nasal organoids model SARS-CoV-2 upper respiratory infection and recapitulate the differential infectivity of emerging variants. *mBio*. 2022:13(4):e0194422. https://doi.org/10.1128/mbio.01944-22
62. Lamers MM, van der Vaart J, Knoops K, Riesebosch S, Breugem TI, Mykytyn AZ, Beumer J, Schipper D, Bezstarosti K, Koopman CD, *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. https://doi.org/10.15252/embj.2020105912
63. Pei R, Feng J, Zhang Y, Sun H, Li L, Yang X, He J, Xiao S, Xiong J, Lin Y, *et al.* Host metabolism dysregulation and cell tropism identification in human airway and alveolar organoids upon SARS-CoV-2 infection. *Protein Cell*. 2021:12(9):717–733. https://doi.org/10.1007/s13238-020-00811-w
64. Tiwari SK, Wang S, Smith D, Carlin AF, Rana TM. Revealing tissue-specific SARS-CoV-2 infection and host responses using human stem cell-derived lung and cerebral organoids. *Stem Cell Rep*. 2021:16(3):437–445. https://doi.org/10.1016/j.stemcr.2021.02.005
65. Salahudeen AA, Choi SS, Rustagi A, Zhu J, van Unen V, de la O SM, Flynn RA, Margalef-Català M, Santos AJM, Ju J, *et al.* Progenitor identification and SARS-CoV-2 infection in human distal lung organoids. *Nature*. 2020:588(7839):670–675. https://doi.org/10.1038/s41586-020-3014-1
66. Mykytyn AZ, Breugem TI, Riesebosch S, Schipper D, van den Doel PB, Rottier RJ, Lamers MM, Haagmans BL, *et al.* SARS-CoV-2 entry into human airway organoids is serine protease-mediated and facilitated by the multibasic cleavage site. *eLife*. 2021:10:e64508. https://doi.org/10.7554/eLife.64508
67. Meng B, Abdullahi A, Ferreira IATM, Goonawardane N, Saito A, Kimura I, Yamasoba D, Gerber PP, Fatihi S, Rathore S, *et al.* Altered TMPRSS2 usage by SARS-CoV-2 omicron impacts infectivity and fusogenicity. *Nature*. 2022:603(7902):706–714. https://doi.org/10.1038/s41586-022-04474-x
68. Strikoudis A, Cieślak A, Loffredo L, Chen Y-W, Patel N, Saqi A, Lederer DJ, Snoeck H-W. Modeling of fibrotic lung disease using 3D organoids derived from human pluripotent stem cells. *Cell Rep*. 2019:27(12):3709–3723.e5. https://doi.org/10.1016/j.celrep.2019.05.077
69. Kim J-H, An GH, Kim J-Y, Rasaei R, Kim WJ, Jin X, Woo D-H, Han C, Yang S-R, Kim J-H, *et al.* Human pluripotent stem cell-derived alveolar organoids for modeling pulmonary fibrosis and drug testing. *Cell Death Discov*. 2021:7(1):48. https://doi.org/10.1038/s41420-021-00439-7
70. D'Amico F, Baumgart DC, Danese S, Peyrin-Biroulet L. Diarrhea during COVID-19 infection: pathogenesis, epidemiology, prevention, and management. *Clin Gastroenterol Hepatol*. 2020:18(8):1663–1672. https://doi.org/10.1016/j.cgh.2020.04.001
71. Zhang H, Kang Z, Gong H, Xu D, Wang J, Li Z, Li Z, Cui X, Xiao J, Zhan J, *et al.* Digestive system is a potential route of COVID-19: an analysis of single-cell coexpression pattern of key proteins in viral entry process. *Gut*. 2020:69(6):1010–1018. https://doi.org/10.1136/gutjnl-2020-320953
72. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, Hoskins EE, Kalinichenko VV, Wells SI, Zorn AM, *et al.* Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature*. 2011:470(7332):105–109. https://doi.org/10.1038/nature09691
73. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, *et al.* Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*. 2009:459(7244):262–265. https://doi.org/10.1038/nature07935
74. Date S, Sato T. Mini-Gut organoids: reconstitution of the stem cell niche. *Annu Rev Cell Dev Biol*. 2015:31:269–289. https://doi.org/10.1146/annurev-cellbio-100814-125218
75. Mithal A, Hume AJ, Lindstrom-Vautrin J, Villacorta-Martin C, Olejnik J, Bullitt E, Hinds A, Mühlberger E, Mostoslavsky G, *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. https://doi.org/10.1016/j.stemcr.2021.02.019
76. Heuberger J, Trimpert J, Vladimirova D, Goosmann C, Lin M, Schmuck R, Mollenkopf H-J, Brinkmann V, Tacke F,

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Osterrieder N, *et al*. Epithelial response to IFN-γ promotes SARS-CoV-2 infection. *EMBO Mol Med*. 2021:13(4):e13191. https://doi.org/10.15252/emmm.202013191

77. Jang KK, Kaczmarek ME, Dallari S, Chen Y-H, Tada T, Axelrad J, Landau NR, Stapleford KA, Cadwell K, *et al*. Variable susceptibility of intestinal organoid–derived monolayers to SARS-CoV-2 infection. *PLOS Biol*. 2022:20(3):e3001592. https://doi.org/10.1371/journal.pbio.3001592
78. Lamers MM, Beumer J, van der Vaart J, Knoops K, Puschhof J, Breugem TI, Ravelli RBG, van Schayck JP, Mykytyn AZ, Duimel HQ, *et al*. SARS-CoV-2 productively infects human gut enterocytes. *Science*. 2020:369(6499):50–54. https://doi.org/10.1126/science.abc1669
79. Triana S, Metz-Zumaran C, Ramirez C, Kee C, Doldan P, Shahraz M, Schraivogel D, Gschwind AR, Sharma AK, Steinmetz LM, *et al*. Single-cell analyses reveal SARS-CoV-2 interference with intrinsic immune response in the human gut. *Mol Syst Biol*. 2021:17(4):e10232. https://doi.org/10.15252/msb.202110232
80. Han Y, Duan X, Yang L, Nilsson-Payant BE, Wang P, Duan F, Tang X, Yaron TM, Zhang T, Uhl S, *et al*. Identification of SARS-CoV-2 inhibitors using lung and colonic organoids. *Nature*. 2021:589(7841):270–275. https://doi.org/10.1038/s41586-020-2901-9
81. Ianevski A, Yao R, Zusinaite E, Lello LS, Wang S, Jo E, Yang J, Ravlo E, Wang W, Lysvand H, *et al*. Synergistic interferon-alpha-based combinations for treatment of SARS-CoV-2 and other viral infections. *Viruses*. 2021:13(12):2489. https://doi.org/10.3390/v13122489
82. Shemesh M, Aktepe TE, Deerain JM, McAuley JL, Audsley MD, David CT, Purcell DFJ, Urin V, Hartmann R, Moseley GW, *et al*. SARS-CoV-2 suppresses IFNβ production mediated by NSP1, 5, 6, 15, ORF6 and ORF7b but does not suppress the effects of added interferon. *PLOS Pathog*. 2021:17(8):e1009800. https://doi.org/10.1371/journal.ppat.1009800
83. Znaidia M, Demeret C, van der Werf S, Komarova AV. Characterization of SARS-CoV-2 evasion: interferon pathway and therapeutic options. *Viruses*. 2022:14(6):1247. https://doi.org/10.3390/v14061247
84. Gassen NC, Papies J, Bajaj T, Emanuel J, Dethloff F, Chua RL, Trimpert J, Heinemann N, Niemeyer C, Weege F, *et al*. SARS-CoV-2-mediated dysregulation of metabolism and autophagy uncovers host-targeting antivirals. *Nat Commun*. 2021:12(1):3818. https://doi.org/10.1038/s41467-021-24007-w
85. Yang L, Han Y, Nilsson-Payant BE, Gupta V, Wang P, Duan X, Tang X, Zhu J, Zhao Z, Jaffré F, *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. https://doi.org/10.1016/j.stem.2020.06.015
86. Lui VC, Hui KP, Babu RO, Yue H, Chung PH, Tam PK, Chan MC, Wong KK, *et al*. Human liver organoid derived intra-hepatic bile duct cells support SARS-CoV-2 infection and replication. *Sci Rep*. 2022:12(1):5375. https://doi.org/10.1038/s41598-022-09306-6
87. Kumaran NK, Karmakar BK, Taylor OM. Case report: coronavirus disease-19 (COVID-19) associated with acute necrotising pancreatitis (ANP). *BMJ Case Rep*. 2020:13(9):e237903. https://doi.org/10.1136/bcr-2020-237903
88. Liu F, Long X, Zhang B, Zhang W, Chen X, Zhang Z. ACE2 Expression in pancreas may cause pancreatic damage after SARS-CoV-2 infection. *Clin Gastroenterol Hepatol*. 2020:18(9):2128–2130.e2. https://doi.org/10.1016/j.cgh.2020.04.040
89. Shaharuddin SH, Wang V, Santos RS, Gross A, Wang Y, Jawanda H, Zhang Y, Hasan W, Garcia G, Arumugaswami V, *et al*. Deleterious effects of SARS-CoV-2 infection on human pancreatic cells. *Front Cell Infect Microbiol*. 2021:11:678482. https://doi.org/10.3389/fcimb.2021.678482
90. Giobbe GG, Bonfante F, Jones BC, Gagliano O, Luni C, Zambaiti E, Perin S, Laterza C, Busslinger G, Stuart H, *et al*. SARS-CoV-2 infection and replication in human gastric organoids. *Nat Commun*. 2021:12(1):6610. https://doi.org/10.1038/s41467-021-26762-2
91. Batalov I, Feinberg AW. Differentiation of cardiomyocytes from human pluripotent stem cells using monolayer culture. *Biomark Insights*. 2015:10(Suppl 1):71. https://doi.org/10.4137/BMI.S20050
92. Lian X, Zhang J, Azarin SM, Zhu K, Hazeltine LB, Bao X, Hsiao C, Kamp TJ, Palecek SP, *et al*. Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating wnt/β-catenin signaling under fully defined conditions. *Nat Protoc*. 2013:8(1):162–175. https://doi.org/10.1038/nprot.2012.150
93. Hofbauer P, Jahnel SM, Papai N, Giesshammer M, Deyett A, Schmidt C, Penc M, Tavernini K, Grdseloff N, Meledeth C, *et al*. Cardioids reveal self-organizing principles of human cardiogenesis. *Cell*. 2021:184(12):3299–3317.e22. https://doi.org/10.1016/j.cell.2021.04.034
94. Bailey AL, Dmytrenko O, Greenberg L, Bredemeyer AL, Ma P, Liu J, Penna V, Winkler ES, Sviben S, Brooks E, *et al*. SARS-CoV-2 infects human engineered heart tissues and models COVID-19 myocarditis. *Basic Transl Sci*. 2021:6(4):331–345.
95. Mills RJ, Humphrey SJ, Fortuna PR, Lor M, Foster SR, Quaife-Ryan GA, Johnston RL, Dumenil T, Bishop C, Rudraraju R, *et al*. BET Inhibition blocks inflammation-induced cardiac dysfunction and SARS-CoV-2 infection. *Cell*. 2021:184(8):2167–2182. https://doi.org/10.1016/j.cell.2021.03.026
96. Jung SR, Ashhurst TM, West PK, Viengkhou B, King NJ, Campbell IL, Hofer MJ, *et al*. Contribution of STAT1 to innate and adaptive immunity during type I interferon-mediated lethal virus infection. *PLoS Pathog*. 2020:16(4):e1008525. https://doi.org/10.1371/journal.ppat.1008525
97. Padmanabhan A, Alexanian M, Linares-Saldana R, González-Terán B, Andreoletti G, Huang Y, Connolly AJ, Kim W, Hsu A, Duan Q, *et al*. BRD4 (bromodomain-containing protein 4) interacts with GATA4 (GATA binding protein 4) to govern mitochondrial homeostasis in adult cardiomyocytes. *Circulation*. 2020:142(24)):2338–2355. https://doi.org/10.1161/CIRCULATIONAHA.120.047753
98. Lancaster MA, Knoblich JA. Generation of cerebral organoids from human pluripotent stem cells. *Nat Protoc*. 2014:9(10):2329–2340. https://doi.org/10.1038/nprot.2014.158
99. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol*. 2009:27(3):275. https://doi.org/10.1038/nbt.1529
100. Rosebrock D, Arora S, Mutukula N, Volkman R, Gralinska E, Balaskas A, Aragonés Hernández A, Buschow R, Brändl B, Müller FJ, *et al*. Enhanced cortical neural stem cell identity through short SMAD and WNT inhibition in human cerebral organoids facilitates emergence of outer radial glial cells. *Nat Cell Biol*. 2022:24(6):981–995. https://doi.org/10.1038/s41556-022-00929-5
101. Koo B, Choi B, Park H, Yoon KJ. Past, present, and future of brain organoid technology. *Mol Cells*. 2019:42(9):617. https://doi.org/10.14348/molcells.2019.0162
102. Ramani A, Müller L, Ostermann PN, Gabriel E, Abida-Islam P, Müller-Schiffmann A, Mariappan A, Goureau O, Gruell H, Walker A, *et al*. SARS-CoV-2 targets neurons of 3D human brain

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organoids. *EMBO J*. 2020:39(20):e106230. https://doi.org/10.15252/embj.2020106230
103. Wang C, Zhang M, Garcia G, Tian E, Cui Q, Chen X, Sun G, Wang J, Arumugaswami V, Shi Y, *et al.* ApoE-Isoform-Dependent SARS-CoV-2 neurotropism and cellular response. *Cell Stem Cell*. 2021:28(2):331–342. https://doi.org/10.1016/j.stem.2020.12.018
104. Mesci P, de Souza JS, Martin-Sancho L, Macia A, Saleh A, Yin X, Snethlage C, Adams JW, Avansini SH, Herai RH, *et al.* SARS-CoV-2 infects human brain organoids causing cell death and loss of synapses that can be rescued by treatment with Sofosbuvir. *PLoS Biol*. 2022:20(11):e3001845. https://doi.org/10.1371/journal.pbio.3001845
105. Andrews MG, Mukhtar T, Eze UC, Simoneau CR, Ross J, Parikshak N, Wang S, Zhou L, Koontz M, Velmeshev D, *et al.* Tropism of SARS-CoV-2 for human cortical astrocytes. *Proc Natl Acad Sci*. 2022:119(30):e2122236119. https://doi.org/10.1073/pnas.2122236119
106. Kong W, Montano M, Corley MJ, Helmy E, Kobayashi H, Kinisu M, Suryawanshi R, Luo X, Royer LA, Roan NR, *et al.* Neuropilin-1 mediates SARS-CoV-2 infection of astrocytes in brain organoids, inducing inflammation leading to dysfunction and death of neurons. *mBio*. 2022:13(6):e02308-22. https://doi.org/10.1128/mbio.02308-22
107. Pellegrini L, Albecka A, Mallery DL, Kellner MJ, Paul D, Carter AP, James LC, Lancaster MA, *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. https://doi.org/10.1016/j.stem.2020.10.001
108. Takasato M, Er PX, Chiu HS, Little MH. Generation of kidney organoids from human pluripotent stem cells. *Nat Protoc*. 2016:11(9):1681–1692. https://doi.org/10.1038/nprot.2016.098
109. Khoshdel-Rad N, Ahmadi A, Moghadasali R. Kidney organoids: current knowledge and future directions. *Cell Tissue Res*. 2022:387(2):207–224. https://doi.org/10.1007/s00441-021-03565-x
110. Palakkan AA, Tarnick J, Waterfall M, Sallam M, Glykofrydis F, Elhendawi M, *et al.* Production of kidney organoids arranged around single ureteric bud trees, and containing endogenous blood vessels, solely from embryonic stem cells. *Sci Rep*. 2022:12(1):1–16. https://doi.org/10.1038/s41598-022-16768-1
111. Elshafei A, Khidr EG, El-Husseiny AA, Gomaa MH. ACE2 And COVID-19; a mechanistic review. *Saudi J Biol Sci*. 2021:28(11):6465–6470. https://doi.org/10.1016/j.sjbs.2021.07.003
112. Legrand M, Bell S, Forni L, Joannidis M, Koyner JL, Liu K, *et al.* Pathophysiology of COVID-19-associated acute kidney injury. *Nat Rev Nephrol*. 2021:17(11):751–764. https://doi.org/10.1038/s41581-021-00452-0
113. Sharma P, Uppal NN, Wanchoo R, Shah HH, Yang Y, Parikh R, Khanin Y, Madireddy V, Larsen CP, Jhaveri KD, *et al.* COVID-19–Associated kidney injury: a case series of kidney biopsy findings. *J Am Soc Nephrol JASN*. 2020:31(9):1948–1958. https://doi.org/10.1681/ASN.2020050699
114. Garreta E, Prado P, Stanifer ML, Monteil V, Marco A, Ullate-Agote A, Moya-Rull D, Vilas-Zornoza A, Tarantino C, Romero JP, *et al.* A diabetic milieu increases ACE2 expression and cellular susceptibility to SARS-CoV-2 infections in human kidney organoids and patient cells. *Cell Metab*. 2022:34(6):857–873. https://doi.org/10.1016/j.cmet.2022.04.009
115. Chung H, Bui-Marinos MP, Rahmani W, Corcoran JA, Chun J. Infecting kidney organoids with a cDNA reporter clone of SARS-CoV-2. *STAR Protoc*. 2022:3(3):101617. https://doi.org/10.1016/j.xpro.2022.101617
116. Helms L, Marchiano S, Stanaway IB, Hsiang T-Y, Juliar BA, Saini S, Zhao YT, Khanna A, Menon R, Alakwaa F, *et al.* Cross-validation of SARS-CoV-2 responses in kidney organoids and clinical populations. *JCI Insight*. 2021:6(24):e154882. https://doi.org/10.1172/jci.insight.154882
117. Jansen J, Reimer KC, Nagai JS, Varghese FS, Overheul GJ, de Beer M, Roverts R, Daviran D, Fermin LA, Willemsen B, *et al.* SARS-CoV-2 infects the human kidney and drives fibrosis in kidney organoids. *Cell Stem Cell*. 2022:29(2):217–231. https://doi.org/10.1016/j.stem.2021.12.010
118. Nystrom SE, Li G, Datta S, Soldano KL, Silas D, Weins A, Hall G, Thomas DB, Olabisi OA, *et al.* JAK Inhibitor blocks COVID-19 cytokine-induced JAK/STAT/APOL1 signaling in glomerular cells and podocytopathy in human kidney organoids. *JCI Insight*. 2022:7(11):e157432. https://doi.org/10.1172/jci.insight.157432
119. Duan X, Tang X, Nair MS, Zhang T, Qiu Y, Zhang W, Wang P, Huang Y, Xiang J, Wang H, *et al.* An airway organoid-based screen identifies a role for the HIF1α-glycolysis axis in SARS-CoV-2 infection. *Cell Rep*. 2021:37(6):109920. https://doi.org/10.1016/j.celrep.2021.109920
120. Martín-Acebes MA, Blázquez AB, Jiménez De Oya N, Escribano-Romero E, Saiz JC. West Nile virus replication requires fatty acid synthesis but is independent on phosphatidylinositol-4-phosphate lipids. *PloS One*. 2011:6(9):e24970. https://doi.org/10.1371/journal.pone.0024970
121. Nasheri N, Joyce M, Rouleau Y, Yang P, Yao S, Tyrrell DL, Pezacki JP, *et al.* Modulation of fatty acid synthase enzyme activity and expression during hepatitis C virus replication. *Chem Biol*. 2013:20(4):570–582. https://doi.org/10.1016/j.chembiol.2013.03.014
122. Chu J, Xing C, Du Y, Duan T, Liu S, Zhang P, Cheng C, Henley J, Liu X, Qian C, *et al.* Pharmacological inhibition of fatty acid synthesis blocks SARS-CoV-2 replication. *Nat Metab*. 2021:3(11):1466–1475. https://doi.org/10.1038/s42255-021-00479-4
123. Spitalieri P, Centofanti F, Murdocca M, Scioli MG, Latini A, Di Cesare S, Citro G, Rossi A, Orlandi A, Miersch S, *et al.* Two different therapeutic approaches for SARS-CoV-2 in hiPSCs-derived lung organoids. *Cells*. 2022:11(7):1235. https://doi.org/10.3390/cells11071235
124. Duarte RR, Copertino DC, Iñiguez LP, Marston JL, Bram Y, Han Y, Schwartz RE, Chen S, Nixon DF, Powell TR, *et al.* Identifying FDA-approved drugs with multimodal properties against COVID-19 using a data-driven approach and a lung organoid model of SARS-CoV-2 entry. *Mol Med Camb Mass*. 2021:27(1):1. https://doi.org/10.1186/s10020-020-00255-2
125. Rose-John S. IL-6 Trans-Signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. *Int J Biol Sci*. 2012:8(9):1237–1247. https://doi.org/10.7150/ijbs.4989
126. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. *Nat Rev Mol Cell Biol*. 2022:23(1):3–20. https://doi.org/10.1038/s41580-021-00418-x
127. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, *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. https://doi.org/10.1016/j.cell.2020.02.052
128. Kastenhuber ER, Mercadante M, Nilsson-Payant B, Johnson JL, Jaimes JA, Muecksch F, Weisblum Y, Bram Y, Chandar V, Whittaker GR, *et al.* Coagulation factors directly cleave SARS-CoV-2 spike and enhance viral entry. *eLife*. 2022:11:e77444. https://doi.org/10.7554/eLife.77444.sa2

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129. Jocher G, Grass V, Tschirner SK, Riepler L, Breimann S, Kaya T, Oelsner M, Hamad MS, Hofmann LI, Blobel CP, *et al.* ADAM10 And ADAM17 promote SARS-CoV-2 cell entry and spike protein-mediated lung cell fusion. *EMBO Rep*. 2022:23(6):e54305. https://doi.org/10.15252/embr.202154305
130. Scheller J, Chalaris A, Garbers C, Rose-John S. ADAM17: a molecular switch to control inflammation and tissue regeneration. *Trends Immunol*. 2011:32(8):380–387. https://doi.org/10.1016/j.it.2011.05.005
131. Bell JH, Herrera AH, Li Y, Walcheck B. Role of ADAM17 in the ectodomain shedding of TNF-alpha and its receptors by neutrophils and macrophages. *J Leukoc Biol*. 2007:82(1):173–176. https://doi.org/10.1189/jlb.0307193
132. Zipeto D, Palmeira JF, Argañaraz GA, Argañaraz ER. ACE2/ADAM17/TMPRSS2 Interplay may be the main risk factor for COVID-19. *Front Immunol*. 2020:11:576745. https://doi.org/10.3389/fimmu.2020.576745
133. Schreiber B, Patel A, Verma A. Shedding light on COVID-19: aDAM17 the missing link? *Am J Ther*. 2020:28(3):e358-60.
134. Yeung ML, Teng JLL, Jia L, Zhang C, Huang C, Cai J-P, Zhou R, Chan K-H, Zhao H, Zhu L, *et al.* Soluble ACE2-mediated cell entry of SARS-CoV-2 via interaction with proteins related to the renin-angiotensin system. *Cell*. 2021:184(8):2212–2228.e12. https://doi.org/10.1016/j.cell.2021.02.053
135. Batlle D, Monteil V, Garreta E, Hassler L, Wysocki J, Chandar V, *et al.* Evidence in favor of the essentiality of human cell membrane-bound ACE2 and against soluble ACE2 for SARS-CoV-2 infectivity. *Cell*. 2022:185(11):1837–1839. https://doi.org/10.1016/j.cell.2022.05.004
136. Monteil V, Kwon H, Prado P, Hagelkrüys A, Wimmer RA, Stahl M, Leopoldi A, Garreta E, Del Pozo CH, Prosper F, *et al.* Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell*. 2020:181(4):905–913. https://doi.org/10.1016/j.cell.2020.04.004
137. Puelles VG, Lütgehetmann M, Lindenmeyer MT, Sperhake JP, Wong MN, Allweiss L. Multiorgan and renal tropism of SARS-CoV-2. *N Engl J Med*. 2020:383:590–592. https://doi.org/10.1056/NEJMc2011400
138. Yuen C-K, Lam J-Y, Wong W-M, Mak L-F, Wang X, Chu H, Cai J-P, Jin D-Y, To KK-W, Chan JF-W, *et al.* SARS-CoV-2 nsp13, nsp14, nsp15 and orf6 function as potent interferon antagonists. *Emerg Microbes Infect*. 2020:9(1):1418–1428. https://doi.org/10.1080/22221751.2020.1780953
139. Mlcochova P, Kemp SA, Dhar MS, Papa G, Meng B, Ferreira IATM, Datir R, Collier DA, Albecka A, Singh S, *et al.* SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. *Nature*. 2021:599(7883):114. https://doi.org/10.1038/s41586-021-03944-y
140. Lundberg E, Borner GHH. Spatial proteomics: a powerful discovery tool for cell biology. *Nat Rev Mol Cell Biol*. 2019:20(5):285–302. https://doi.org/10.1038/s41580-018-0094-y
141. Li L, Sun C, Sun Y, Dong Z, Wu R, Sun X, Zhang H, Jiang W, Zhou Y, Cen X, *et al.* Spatially resolved proteomics via tissue expansion. *Nat Commun*. 2022:13(1):1–13. https://doi.org/10.1038/s41467-022-34824-2
142. Mou M, Pan Z, Lu M, Sun H, Wang Y, Luo Y, Zhu F. Application of machine learning in spatial proteomics. *J Chem Inf Model*. 2022:62(23):5875–5895. https://doi.org/10.1021/acs.jcim.2c01161
143. Bhatia HS, Brunner A-D, Öztürk F, Kapoor S, Rong Z, Mai H, Thielert M, Ali M, Al-Maskari R, Paetzold JC, *et al.* Spatial proteomics in three-dimensional intact specimens. *Cell*. 2022:185(26):5040–5058.e19. https://doi.org/10.1016/j.cell.2022.11.021
144. Gusev E, Sarapultsev A, Solomatina L, Chereshnev V. SARS-CoV-2-Specific immune response and the pathogenesis of COVID-19. *Int J Mol Sci*. 2022:23(3):1716. https://doi.org/10.3390/ijms23031716
145. Knoll R, Schultze JL, Schulte-Schrepping J. Monocytes and macrophages in COVID-19. *Front Immunol*. 2021:12:720109. https://doi.org/10.3389/fimmu.2021.720109
146. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol*. 2020:20(6):355–362. https://doi.org/10.1038/s41577-020-0331-4
147. Ragab D, Eldin HS, Taeimah M, Khattab R, Salem R. The COVID-19 cytokine storm; what we know so far. *Front Immunol*. 2020:11:1446. https://doi.org/10.3389/fimmu.2020.01446
148. Sefik E, Qu R, Junqueira C, Kaffe E, Mirza H, Zhao J, Brewer JR, Han A, Steach HR, Israelow B, *et al.* Inflammasome activation in infected macrophages drives COVID-19 pathology. *Nature*. 2022:606(7914):585. https://doi.org/10.1038/s41586-022-04802-1
149. Theobald SJ, Simonis A, Georgomanolis T, Kreer C, Zehner M, Eisfeld HS, Albert M, Chhen J, Motameny S, Erger F, *et al.* Long-lived macrophage reprogramming drives spike protein-mediated inflammasome activation in COVID-19. *EMBO Mol Med*. 2021:13(8):e14150. https://doi.org/10.15252/emmm.202114150
150. Russell MW, Moldoveanu Z, Ogra PL, Mestecky J. Mucosal immunity in COVID-19: a neglected but critical aspect of SARS-CoV-2 infection. *Front Immunol*. 2020:11:3221. https://doi.org/10.3389/fimmu.2020.611337
151. McDermott AJ, Huffnagle GB. The microbiome and regulation of mucosal immunity. *Immunology*. 2014:142(1):24. https://doi.org/10.1111/imm.12231
152. Shi N, Li N, Duan X, Niu H. Interaction between the gut microbiome and mucosal immune system. *Mil Med Res*. 2017:4(1):1–7. https://doi.org/10.1186/s40779-017-0122-9
153. Duan F, Guo L, Yang L, Han Y, Thakur A, Nilsson-Payant BE, Wang P, Zhang Z, Ma CY, Zhou X, *et al.* Modeling COVID-19 with human pluripotent stem cell-derived cells reveals synergistic effects of anti-inflammatory macrophages with ACE2 inhibition against SARS-CoV-2. *Res Sq*. 2020. https://doi.org/10.21203/rs.3.rs-62758/v1
154. Stein MC, Braun F, Krebs CF, Bunders MJ. Kidney organoid systems for studies of immune-mediated kidney diseases: challenges and opportunities. *Cell Tissue Res*. 2021:385(2):457–473. https://doi.org/10.1007/s00441-021-03499-4
155. Pober JS, Merola J, Liu R, Manes TD. Antigen presentation by vascular cells. *Front Immunol*. 2017:8:1907. https://doi.org/10.3389/fimmu.2017.01907
156. Low LA, Mummery C, Berridge BR, Austin CP, Tagle DA. Organs-on-chips: into the next decade. *Nat Rev Drug Discov*. 2021:20(5):345–361. https://doi.org/10.1038/s41573-020-0079-3
157. Sosa-Hernández JE, Villalba-Rodríguez AM, Romero-Castillo KD, Aguilar-Aguila-Isaías MA, García-Reyes IE, Hernández-Antonio A, *et al.* Organs-on-a-Chip module: a review from the development and applications perspective. *Micromachines (Basel)*. 2018:9(10):536. https://doi.org/10.3390/mi9100536
158. Yum K, Hong SG, Healy KE, Lee LP. Physiologically relevant organs on chips. *Biotechnol J*. 2014:9(1):16–27. https://doi.org/10.1002/biot.201300187
159. Ronaldson-Bouchard K, Teles D, Yeager K, Tavakol DN, Zhao Y, Chramiec A, Tagore S, Summers M, Stylianos S, Tamargo M, *et al.* A multi-organ chip with matured tissue niches linked by vascular flow. *Nat Biomed Eng*. 2022:6(4):351–371. https://doi.org/10.1038/s41551-022-00882-6

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