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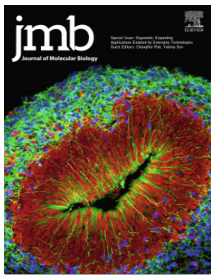
Review Article



An Adverse Outcomes Approach to Study the Effects of SARS-CoV-2 in 3D Organoid Models

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Abstract

The novel SARS-CoV-2 virus outbreak is the major cause of a respiratory disease known as COVID-19. It has caused a global pandemic and has resulted in mortality in millions. The primary mode of infection is respiratory ailments, however, due to multi-organ complications, COVID-19 patients displays a greater mortality numbers. Due to the 3Rs Principle (Refine, Reduce, Replacement), the scientific community has shifted its focus to 3D organoid models rather than testing animal models. 3D organoid models provide a better physiological architecture as it mimics the real tissue microenvironment and is the best platform to recapitulate organs in a dish. Hence, the organoid approach provides a more realistic drug response in comparison to the traditional 2D cellular models, which lack key physiological relevance due to the absence of proper surface topography and cellular interactions. Furthermore, an adverse outcome pathway (AOPs) provides a best fit model to identify various molecular and cellular events during the exposure of SARS-CoV-2. Hence, 3D organoid research provides information related to gene expression, cell behavior, antiviral studies and ACE2 expression in various organs. In this review, we discuss state-of-the-art lung, liver and kidney 3D organoid system utilizing the AOPs to study SARS-CoV-2 molecular pathogenesis. Furthermore, current challenges are discussed for future application of 3D organoid systems for various disease states.

Introduction

Coronaviruses are a large group of enveloped single-stranded RNA virus known to infect different mammals, avian species, and human.[1] There are 6 known coronavirus species infectinghuman where severity ranges from common cold such as 229E, OC43, NL63, and HKU1 to more severe such as Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV).[2,3]

At the end of 2019, an outbreak of the novel coronavirus strain SARS-CoV-2 in Wuhan, Hubei Province of China was reported and now turned into a devastating ongoing global pandemic[4] with more than 3 million deaths reported as of April 2021.[5] SARS-CoV-2 is suggested to be one of the leading cause of respiratory failure and acute respiratory distress syndrome (ARDS).[6,7] Coronavirus represents a spike-like s-glycoprotein that helps in a receptor recognition and infects several mammals and avian species.[8] The spike protein is composed

of S1 and S2 subunits, wherein the S1 subunit recognizes and binds to the angiotensin-converting enzyme 2 (ACE2) receptor of the host's cell. The S2 subunit focuses on the viral cell membrane fusion by forming a six helical bundle.[9] Upon binding onto the ACE2 receptor, virus replicates its genomic RNA intracellularly and incorporates it into a newly produced viral particle.[10,11] The ACE2 recognition and fusion into the host cells are considered critical steps for viral infections and a crucial determinant of cross-species spillover events.[12]

Besides the rapid explosion of knowledge regarding SARS-CoV-2, there is still a lack in the SARS-CoV-2 pathogenic mechanism. Many infectious disease studies employ a 2D monolayer cell culture system as it is the least time-consuming and inexpensive path for a preclinical stage of anti-viral drugs and vaccine development[13]; however, that comes with many shortcomings. The traditional 2D model using various cell lines cannot accurately measure the effects of the relevant environment as 2D cell cultures lose their *in vivo* resemblance; therefore, they are unable to accurately predict the *in vivo* aspect of virus-host interactions.[13–15]

Hence, 2D does not suffice a physiologically relevant model, and 3D organoid models compensate the conventional cell culture model, as it can simulate the native cellular microenvironments.[13,16,17] Furthermore, the 3Rs principles aim to implement the methods that can Replace, Reduce, and Refine the employment of the animals for the preclinical studies.[18,19] Thus, it has shifted the focus to an alternative model that can simulate the *in vivo* counterpart, and 3D organoid models fit in with the 3Rs framework for the research.

Organoids can be defined as 3D structures which originate from embryonic stem cells or induced pluripotent stem cells and contain organ specific cell types that develop due to self-organization *via* cell sorting and spatially restricted lineage.[20] Previously, virology research has shown 3D studies to be more practical in producing a more relevant approach against various viruses such as Hepatitis B virus (HPB),[21] Zika virus,[22] Camelpox virus,[23] and Cowpox virus.[24] Spheroids when infected, expressed HSV-1, adenovirus, and cytomegalovirus antigens at a much earlier phase than 2D cultures[25] and has enabled the study of non-cultivable norovirus, which has been difficult to study in regular 2D cell culture.[26] Various cell lines, organoids and animal models have been used to study the detailed pathophysiology and identify the best drug targets for SARS-CoV-2.[27] Additionally, a recent study showed that only 7 cell lines have a cytopathogenic effects from complete lysis of cells from a total of 34 cell lines via 4 locally isolated SARS-CoV-2.[28] Therefore, organoids are establishing to be the versatile tools and sensitive cell model for studying the SARS-CoV-2 pathophysiology and other treatment methods. Researchers are using organoids to mimic the *in vivo* condition at both cellular and molecular levels to study the invasion and transmission of SARS-CoV-2 *via* the adverse outcome pathway. An Adverse Outcome Pathway (AOP) is an organized model that identifies and sequences the molecular and cellular events to evaluate the toxicity of any organism when it is exposed to that specific substance.[14,17,20,21,29–31] In this review, an AOP starts with SAR-CoV-2 infection in the human and is categorized as a molecular initiating event which further elicits the key events that occurs at the cellular, tissue and organ level leading to the adverse outcome at the organism level.[32] It provides detailed information regarding the toxicity mechanisms (cytokine storm) and biological interaction (ACE2 receptors) *via* various biochemical tests to develop strategies for the toxicity. Due to the lack of proper research models, virus transmission and effects on tissue studies have not been established. Mostly, clinical trials, bioinformatics and autopsy results have been studied for the mechanism and pathogenesis.[33] The widespread ACE2 expression in different cell types and tissues was studied extensively in 3D organoids.[34–37] However, there is a rise of organoid studies in the virology field since the surge of the pandemic. Therefore, we reviewed human 3D organoid studies on SAR-CoV-2 in AOPs format. We describe different forms of lung, liver and kidney organoids employed to study different cell type tropism, inflammation and anti-viral drugs testing.

Lung organoid

The emergence of the ongoing COVID-19 pandemic caused by the SARS-CoV-2 virus causes severe respiratory disorder, leading to high mortality rates worldwide. The widely known mechanism of SARS-CoV-2 that leads to severe respiratory failure and ultimately mortality is hyper inflammation which is the production of cytokines, mainly cytokine storm in the lungs.[38] In the research setting, studying the molecular mechanism of SARS-CoV-2 infection *in vitro* becomes extremely difficult as lung cells lose their phenotype, requiring multiple factors such as serums and feeder cells to maintain or utilize transformed cell lines.[26,39–40] Therefore, to overcome such limitations, studies are developing and employing organoids as the platform to dissect the mechanism of SARS-CoV-2 pathogenesis and screening for anti-SARS-CoV-2 drugs.[41–43]

Molecular initiating event

The mode of viral transmission in humans is mainly by respiratory droplets, primarily transmitted through contact transmission, which occurs by inhalation of virus from respiratory droplets and aerosol particles, deposition of a

virus on exposed mucous membranes, and touching mucous membrane with soiled hands contaminated with the virus (cdc.gov). Post transmission, SARS-CoV-2 rapidly infects epithelial cells in the nasal cavity and conducting airways of respiratory tracts in 80% of the cases, leading to the innate immune response and manifesting COVID-19 disease. However, about 20% of the infected population develop severe coronavirus cases, in the stretch of the pandemic. In this stage, patients have severe pneumonia, high concentration of cytokines in the plasma of critically ill patients,[44] leading to acute respiratory distress syndrome (ARDS), and ultimately respiratory failure.[45,46] Severe respiratory failure occurs when infection infiltrates the lower respiratory region, which is the gas exchange unit of the lungs, and attacks alveolar epithelial type-2 (AT-2) cells,[46–48] mainly expressing ACE2 receptors.[49–51] An AOP based on the current lung organoid mechanistic study is presented in this review (Figure 1).

Key event

A study by Katsura et al. generated a feeder-free 3D cell expansion of human primary AT2 cells into the alveolosphere.[44] The study confirmed the expression of ACE2 in the alveolosphere organoid using single-cell RNA sequencing and immunostaining assay. The organoid was infected with GFP tagged SARS-CoV-2 for easy quantification of viral infection and further downstream analysis. A genome-wide transcriptomic analysis confirmed that the SARS-CoV-2 virus elicits a cytokine storm by triggering an immune response mediated by interferons (IFN), mainly IFN-I/III and multiple molecules of cytokines and chemokines such as CXCL10, 11, and 17 in response to the infection. AT2 cells in the organoid also had an upregulated apoptotic cell death signaling and a dramatic loss of surfactant production, which also plays a key role in protecting against the viruses and microbial infection.[45–47] These findings were validated by comparing publicly available RNA sequencing data from bronchoalveolar lavage fluid of six severe COVID-19 patients, further confirming that AT2derived organoid responds to SARS-CoV-2 similar to human lungs after the infection. To find therapeutics to protect against SARS-CoV-2, a study found that pre-treatment with a low dose of interferon protected against the infection by eliciting prophylactic effect; however, blocking interferon receptors before the infection led to severe propagation of SARS-CoV-2 in the alveolospshere.[44]

Another study by Han et al. utilized human induced pluripotent stem cells (hiPSCs) to generate AT2 derived 3D organoids for drug screenings.[48] Upon SARS-CoV-2 infection, hiPSCs-derived alveolar had a robust viral replication and infection, confirmed by immunostaining, RT-qPCR, and RNA-seq analysis. Additionally, viral infection was confirmed by employing luciferase-based vesicular stomatitis virus pseudo typed with SARS-CoV-2 spiked protein, which allows detection of viral infection *via* luciferase activity. Furthermore,

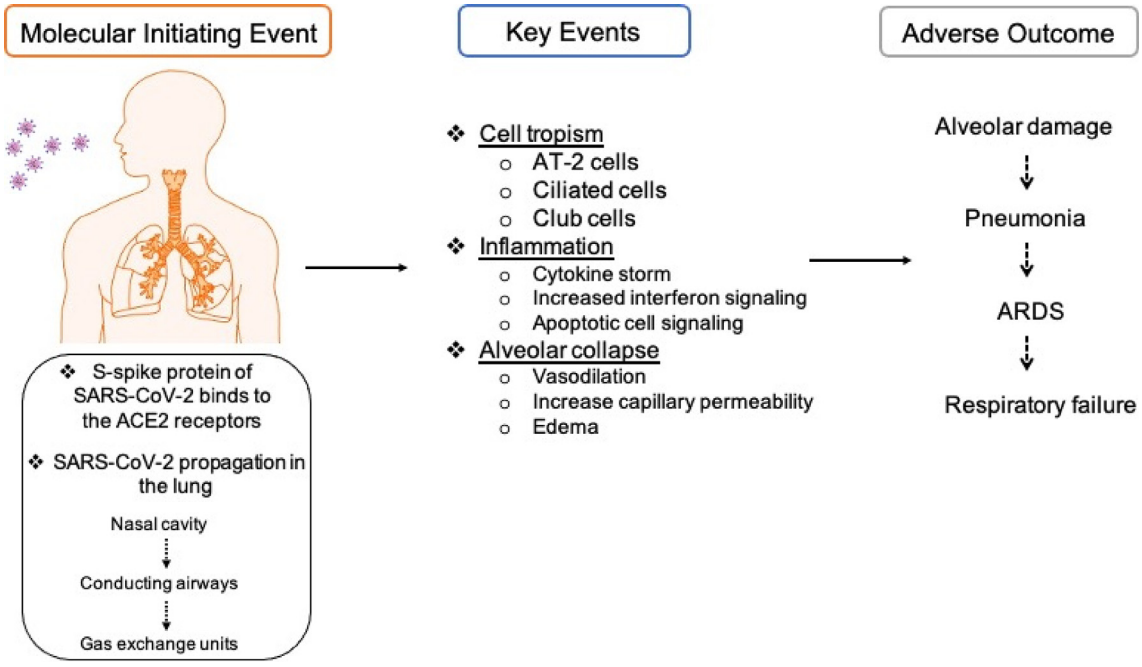

Figure 1. A schematic diagram of adverse outcome pathway describing an effect of SARS-CoV-2 infection in the lungs.

xenograft implantation of the alveolar organoid into immunodeficient NCG mice was conducted to test AT2 cell tropism further validating the findings that SARS-CoV-2 readily infects AT2 cells in the alveoli *in vivo*. Interestingly, a study by Huang et al. derived AT2 cells from hiPSCs and were grown in an air–liquid interface (ALI).[49] ALI is a method that mimics the airway *in vivo* condition where the apical side of the cell is exposed to the air known as airlifting and the basal side of the cells exposed to cell culture media.[50,51] The group also found AT2 cells permissive to SARS-CoV-2 virus infection where immunofluorescent, flow cytometry, and transmission electron microscopy analysis were utilized to detect viral infection.[49] Also, an increase in multiplicities of infection was detected over time, indicating viral propagation post-infection.

Both studies of the AT2 derived 3D organoid[48] and ALI model[9] confirmed that infecting organoids with the SARS-CoV-2 virus led to the induction of chemokine transcripts. In the AT2 derived 3Dorganoid,[48] RNA seq analysis confirmed interleukin-17 signaling, and cytokine-cytokine receptor interaction was detected upon viral infection similar to other findings.[48,52–53] A similar pathway is upregulated in the lung autopsy tissues of COVID-19 patients,[48] therefore, confirming the alveolar organoid model to study the mechanism of the pathogenesis. Furthermore, the study identified several drugs using organoids such as imatinib, mycophenolic acid (MPA), quinacrinedihydrochloride (QNHC) that led to virus blockade from an infection. Similarly, in the ALI model of AT2 cells,[9] SARS-CoV-2 infection led to the secretion of chemokines encoded by NF kappa-B target genes and an induction in IFN signaling leading to a rapid shift towards inflammatory phenotypes and an increase in cell death pathways.

Further extensive study on SARS-CoV-2 infection was done by Pei et al., employing human embryonic stem cell (hESC) derived AT2 cells positive lung alveolar organoid and lung airway organoid, which contains ciliated and club cells from the upper/middle airway.[54] Similar to lung organoid generated *via* different approach[44,48,49] hESC-derived lung organoids have a high expression of ACE2 receptors. Upon SARS-CoV-2 infection, alveolar AT2 and ciliated cells were highly permissive to the infection along with the club cells, and no AT-1 cells were infected. These findings employed immunofluorescent imaging of viral nucleoprotein and transmission electron microscopy.[54] Similarly, apoptotic cell death was observed in both organoids and prominently in alveolar organoids. A similar phenomena which were observed in the human primary AT2 cell-derived alveolosphere.[44]

Several immune response genes were upregulated, such as NF-kappa B-related mRNA, interferon-stimulated genes.[55] Upregulation of several cytokines and chemokines genes such as interleukin-6, tumor necrosis factor, and CXCL8, 2, 3, 10, and 11 was observed. Interestingly, the authors also revealed a decrease in gene expression relating to lipid metabolism in airways and alveolar organoids. Furthermore, a small molecule remdesivir and human neutralizing antibody CB6 inhibited SARS-CoV-2 replication complementing the works done on non-human primate rhesus macaques with SARS-CoV-2 infection.[55] Thus the study further implicates the role of the human organoid system as a key platform to study the underlying mechanism of pathogenesis of the SARS-CoV-2 virus and to test anti-viral drug discoveries.[55] However, these distal lung organoids can be generated not only from ESC but also generated from adult human AT2 cells and adult lung basal stem cells. A study by Salahudeen et al. generated distal lung organoids from human lung tissues.[56] The organoids were positive for AT2, ciliated, and club cells population, confirmed by RNA-seq analysis.

All key major hallmarks from these studies of a variety of lung organoids on SARS-CoV-2 infection are summarized in Table 1.

Adverse outcome pathways

From these studies, it is apparent that immune response is triggered by AT2 cells. Other studies in COVID-19 patients have shown an increased inflammatory mediator such as cytokines and chemokines in the blood as well in the lung autopsies,[38,44] suggesting that COVID-19fatalities are caused by cytokine storm and potentially leading to ARDS in the patients.

Liver organoid

The COVID-19 outbreak has affected the majority of people with a prior form of liver disease and has increased the overall severity of the disease and forms a major deal for the complexity of acute respiratory distress syndrome (ARDS).[57–59] The various prospective pathophysiological mechanisms for hepatic tropism of SARS-CoV-2 liver injury would be iatrogenic causes entailing drugs and ventilation or hypoxic changes and systemic inflammation.[60] Studies from various countries like China, Singapore and others have shown to report elevated levels of liver enzymes nearly 20–50% and severe cases would result in more drastic levels on liver enzymes[61] but there was an exception of an elderly patient with COVID-19 infection with acute liver failure without any history of any liver disease.[62,63] An interesting paper presented by Mathieu Vienken shows a construct of the adverse outcome pathway induced by SARS-CoV-2, which results in liver injury *via* various direct or indirect methods from the state of hypoxia to systemic inflammation in patients.[64]

Molecular initiating event

It has been observed that 15–65% of SARS-CoV-2 infected patients have abnormalities associated

Table 1 SARS-CoV-2 effect on 3D lungs, liver and kidney organoids.

Organs	Organoid Model	SARS CoV-2 sample	Major Hallmarks	Reference
LUNGS	Alveolosphere: 3-D alveolar culture of primary human epithelial AT2 cells	SARS-CoV-2 USA-WAI1/2020, GFP tagged SARS-CoV-2	Inflammatory response mediated by interferon and AT-2 organoids had cytokine storms of immune molecules and apoptotic signaling in response to SARS-CoV-2. Pre-treatment with INF protected against the infection however blocking INF led to severe infection.	44
	3-D Alveolar Organoid using human induced pluripotent stem cell	SARS-CoV-2 USA-WAI1/2020 (NR52281), SARS-CoV-2-entry virus: VSV G system	Permissive to SARS-CoV-2 infection and robust induction of chemokines and interleukin 17 signaling. Anti-SARS-CoV-2 drug screening	48
	Air-liquid interface (ALI) approach of iPSC derived AT-2 cell study	SARS-CoV-2 USA-WAI1/2020	iPSC derived AT-2 cells were subjected to ALI. SARS-CoV-2 infections led to a rapid shift to inflammatory phenotype by upregulation of NF-kappa B mediated Interferon signaling and cell death.	54
	Human embryonic stem cell derived lung airway and alveolar organoid	SARS-CoV-2 (WIV04)	SARS-CoV-2 infects mostly ciliated, club, and AT-2 cells. Apoptosis was observed in both organoids and prominently in the alveolar organoid. Downregulate metabolic processes such as lipid metabolism and upregulated cytokines response.	56
	Human adult stem cell generated alveolar and adult basal stem cell organoid	SARS-CoV-2 USA-WAI1/2020	Distal lung organoid population: AT-2, ciliated and club cells.	49
LIVER	Liver Bile duct derived progenitor cells grown on Matrigel; liver ductal organoids	COVID-19 patient in Shanghai, isolated and plague purified SARS-CoV-2	– SARS-CoV-2 infection initiates cell death of host cholangiocytes – liver damage due to cholangiocyte injury and bile acid accumulation due to infection	75
	Adult liver organoids: Human hepatocytes and nonparenchymal fractions obtained from Liver Tissue Cell Distribution System (Pittsburgh, Pennsylvania) grown on matrigel.	SARS-CoV-2, isolate USA-WA1/2020 (NR-52281) deposited by the Center for Disease Control and Prevention obtained through BEI Resources, NIAID, NIH	– Adult hepatocyte andcholangiocyte organoids displayed permissive to SARS-CoV-2 infection. – High Luciferase activity and increased viral sgRNA transcripts of the replicating viral RNA.	119
	Pediatric liver tissue: Non-tumor margin of hepatoblastoma (HB).	SARS-CoV-2 (BetaCoV/Hong Kong/VM20001061/2020, SCoV2) isolated from a COVID-19 patient in Hong Kong in 2020 SARS-CoV (strain HK39849, SCoV) isolated from a hospitalized SARS patient in Hong Kong in 2003	– Mechanism of SARS-CoV-2 liver injury attributed to direct cytopathic viral damage. – Cholangiocytes support extensive replication of SARS-CoV-2.	120
KIDNEY	Human embryonic stem cells into 3D suspensionculture	SARS-CoV-2isolated from a nasopharyngealsample of a patient in Sweden with confirmedCOVID-19	– SARS-CoV-2 can infect such human kidney organoids, resultingin infectious viral load, inhibited by hrsACE2. – Human ACE2 can reduce SARS-CoV-2 infection in cells and multiple human organoid models.	110
	H9 human embryonic stem cells were used to generate human kidney organoids	SARS-CoV-2 (nCoV/Wasington1/2020; supplied by the Bational Biocontainment Laboratory, Galveston, TX)	– Kidney organoids showed tightly packed tubularclusters filled with tubular cells. – ACE2 was expressed in tubular cells. – Human kidney organoids were usedto isolate cytosolic, nuclear, and membranefractions for assess-mentof ACE2 activitysimultaneously.	118

with their liver biochemistry results.[65,66] The novel SARS-CoV-2usually invades the human cells *via* the receptor angiotensin converting enzyme II (ACE2). An interesting study showed the most susceptible organs for SARS-CoV-2 infection was done by creating a risk map of various organs *via* cell RNA sequencing.[67] Cholangiocytes represented around 60% of the ACE2 cells and hepatocytes comprise around 3% while it is absent in the Kupffer cells.[68,69] Cholangiocytes are the epithelial cells lining the intrahepatic and extrahepatic bile ducts, which have the main function of bile production wherein, the bile duct cells express ACE2 cells higher than any other liver cells and it also suffices the function of maintaining homeostasis which may otherwise result in liver injury.[70]

Key events

COVID-19 patients exhibited elevated serum liver biochemistry results which portrayed abnormal ALT, AST and total bilirubin levels.[71] Elevated biomarkers were observed LDH: 35.1%, AST: 21.6%, ALT: 18.2%, ALP: 4.1%, in a retrospective single-center study of 14 COVID-19 patients.[72] It was also seen that 37.2% of patients had an abnormal liver function and furthermore, patients with abnormal liver function have a longer stay in the hospital (15.09 + 4.79 days).[72] A single-center cohort study was conducted where 176 patients were studied among which 109 had a liver injury and 67 controls. There was a significant increase observed in the inflammatory markers and IL-6 (121 vs 71.8 pg/mL) in the liver injury group which resulted in increasing the number of days in the hospital. Though, there was no elevation observed in IL-8, TNF-a and IL-1b but due to the cytokine storm, increased levels of LDH, ferritin and IL-6 was observed.[73] The infection with SARS-CoV-2 cause systemic inflammation which results in elevated biochemistry levels due to the cytokine storm.[74] In a study done by Zhao et al., liver bile duct cells were embedded in matrigel to produce liver ductal 3D organoids, which retained the tissue of origin and also displayed genetic stability during self-renewing and they conducted scRNA-seq (Single Cell sequencing) to see the presence of chloangiocytes which expressed ACE2 and TMPRSS2 *via* the epithelial cell adhesion molecules and keratin 9 and they found uniformly expressed in all clusters. Further studies were conducted which inoculated with SARS-CoV-2 showed the infected cholangiocytes went through membrane fusion, formed synctia and helped extensively to be considered as a model for studying viral pathogenesis and for testing of antiviral agents.[75]

Adverse outcome pathway

There are predominantly two types of liver injury, where the first type comprises mild liver abnormalities and specifically inclined towards general inflammation, while the second type alters the biochemical labs linked to liver function and should be attended by healthcare professionals.[76] It was observed that there was an elevated level of alanine aminotransferase (ALT) in 29–39% while an increase of aspartate aminotransferase (AST) in 38–63% of patients.[77,78] Studies done by Garrido et. al., showed that liver injury could be due to the direct viral effect, systemic inflammation, or toxicity from drugs.[71] Patients with cirrhosis, hepatocellular carcinoma, non-alcoholic fatty liver, autoimmune, or liver transplant were at a major risk for COVID-19 in comparison to patients with chronic liver disease (CLD).[71,79] An adverse pathway of SARS-CoV-2 infection on the liver has been briefly described with the major hallmarks and end result in Figure 2 and Table 1.

Kidney organoid

Angiotensin-converting-enzyme 2 (ACE2) is a monocarboxypeptidase present on the surface of various kinds of cells in lungs, heart, blood vessels, liver.[80,81] ACE2 is also found in the kidneys and mostly present in endothelial, tubular and glomerular epithelial cells. The ACE2 expression may be altered in diabetic kidneys as ACE2 increases in the tubular level while decreasing at the glomerular level.[82]

Molecular initiating event

Initially, ACE2 has been studied for its properties as a monocarboxypeptidase, which cleaves Angiotensin (Ang) II to form Ang 1–7.[83,84] ACE2 also cleaves numerous substrates of interest in kidney and cardiovasculardiseases, namely apelins 13 and 36 and des 9Arg bradykinin.[85–97] Several researchers have shown ACE2 expression in multiple tissues such as the heart, kidneys, blood vessels, and intestine,[98–103] indicating that SARS-CoV- 2 might also infect these tissues. The ACE2 tissue distribution in these organs can elucidate the multi-organ dysfunction observed in patients.[65,104]

Key events

ACE2 plays a very important role in the biochemical pathway of modulating the activity of angiotensin II (ANG II) protein, which is exclusively responsible for increasing blood pressure and inflammation.[105] ANG II has quite an important role as it can cause the death of cells, which brings oxygen into the body.[106] Usually, when SARS-CoV-2 binds to ACE2, it disturbs the normal functioning of regulating ANG II, which results in injuring mostly lung and heart tissues and causing severe pneumonia in certain cases.[107,108] Though ACE2 has been associated with the pathogenesis of any chronic kidney diseases (CKD) but transmembrane protease, serine 2 (TMPRSS2) is responsible for the attachment of the virus, TMPRSS2 expression was quite similar in tubulointerstitium but lower in glomeruli of CKD patients.[109] It was reported that clinical-grade human recombinant soluble ACE2 (hrsACE2) can minimize viral growth in Vero E6 cells by a factor of 1000–5000. Also, human blood vessel organoids and kidney organoids might be infected, which results in significant inhibitionby hrsACE2 at the primary stage of infection.[110,111] In Figure 3, SARS-CoV-2 expression and an overview of AOP of SARS-CoV-2, which leads to multi-organ dysfunction are shown. In Table 1, all key hallmarks of kidney organoids on SARS-CoV-2 infection are summarized.

Adverse outcome pathway

COVID-19 disease is categorized by multi-organ dysfunction, which specifies viral spread to various

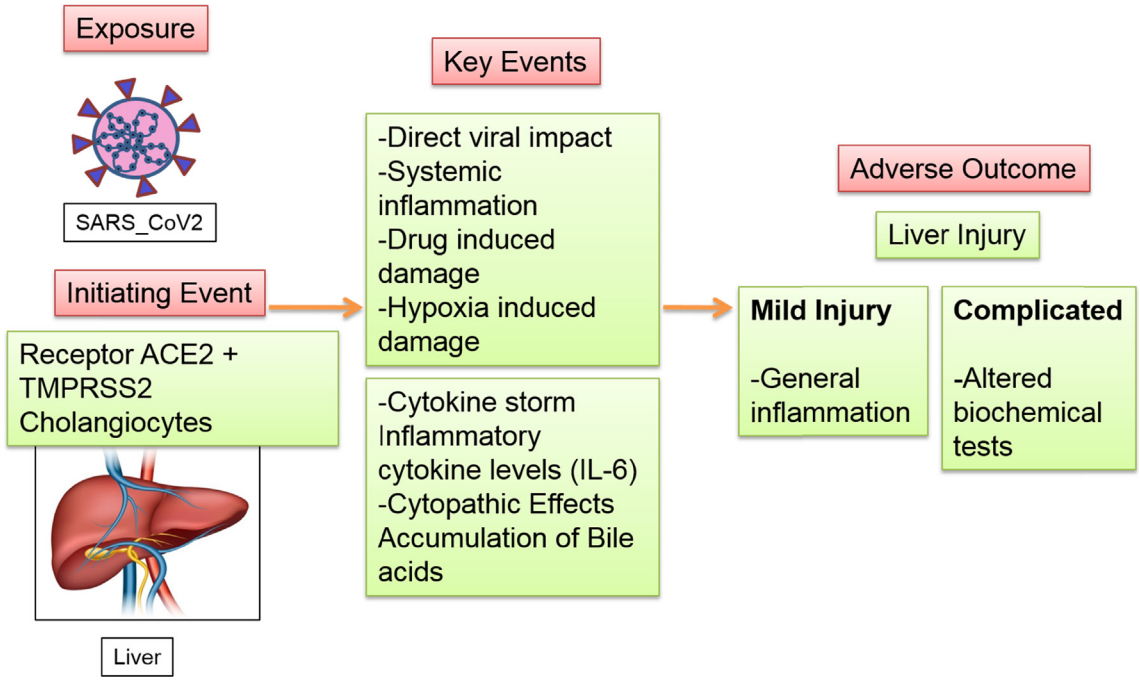

Figure 2. Adverse outcome pathway of SARS-Cov-2 on liver starting with the exposure to the initiating event followed by key events and adverse outcome.

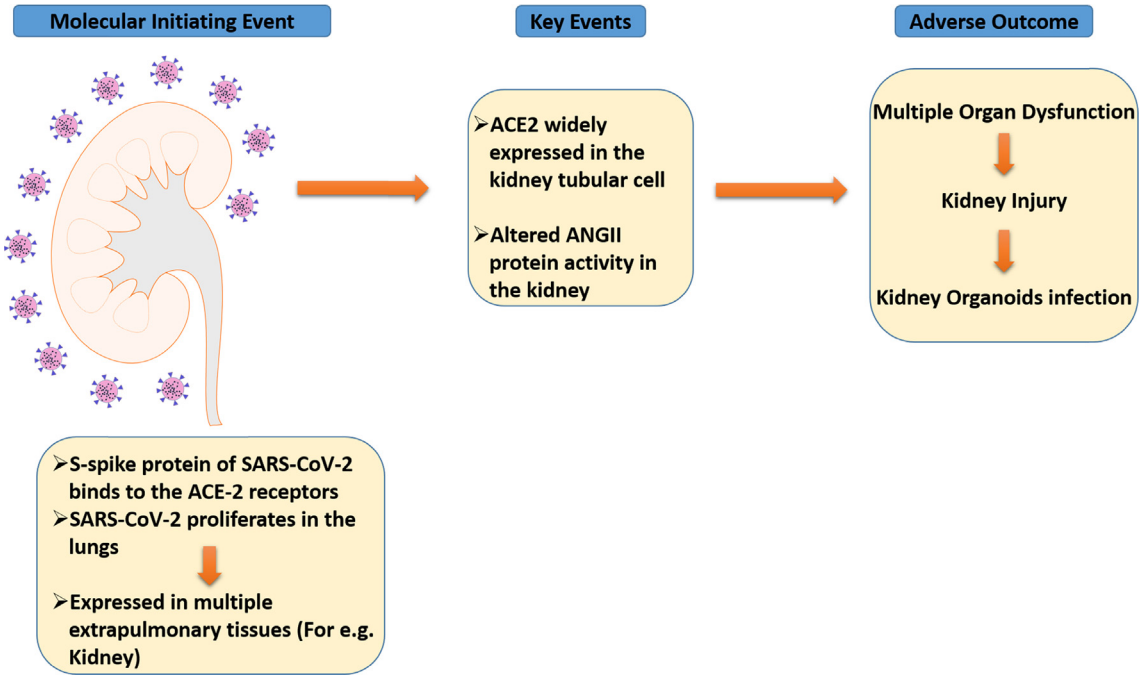

Figure 3. SARS-CoV-2 expression, key events and adverse outcomes lead to multiple organ dysfunction.

tissues. Particularly, kidney organoids are known to express ACE2, and might encourage scientists in studying the universal impacts of the disease.[112,113] However, except lung, the role of epithelial tissues in the pathology and transmission of SARS-CoV-2 is still need to be studied. Recently, Monteil et al. generated 3D kidney organoids along with the blood vessels that were then infected with SARS-CoV-2 and monitored 6 days post infection.[110] They found that SARS-CoV-2 simulated in

kidney organoids and produced infectious viral progeny, which results in hrsACE2 inhibition. It was also found that human recombinant soluble ACE2 significantly reduced SARS-CoV-2 infection of human kidney organoids. These organoids accommodate endothelial cells, mesenchymal cells, podocytes, and epithelial cells of the proximal and distal tubules. Previously, it was publicized that ACE2 over expressed in kidney tubules.[102,110] Furthermore, it has been proved that human tubular kidney cells can directly be infected by SARS-CoV-2.[114] It was demonstrated that SARS-CoV-2 present in urine[114] andinseveralCOVID-19 patients with cardiovascular and renal dysfunctions.[104,115–117] In a study, Wysocki et al. examined the neutralization effect of ACE2 on SARS-CoV-2 infection in human kidney organoids, during its long duration of action in *in vivo* experiments resulted in efficacy improvement to prevent viral escape.[118]

Conclusion

The surge of SARS-CoV-2 is ongoing in many parts of the world and our understanding of the viral mechanism is still limited. Since the pandemic, research on SARS-CoV-2 has increased tremendously boosting the platform to conduct SARS-CoV-2 research. The 3D organoid system which is closer to the human physiological condition has further validated the expression of ACE2 in multiple cell types of different organs such as AT2, ciliated and club cells in the lung, colangiocytes in the liver and kidney tubular cells. Variety of lung organoid systems are generated from primary cells from the tissue to stem cells and all of these lung organoids showed AT2 cells to be widely cell tropism and further ciliated and club cells permissiveness was also displayed along with a widespread increase in inflammation markers. These studies mimic what is seen in the COVID-19 patients with an increase in serum inflammatory markers validating the study furthermore.[121,122]

Liver injury has been reported in patients with COVID-19, which could be the direct or indirect adverse outcome of the infection. Studies have shown changes in the biochemistry profile such as significant elevation of ALT, AST and GGT andcholangiocytes to be the target of SARS-CoV-2 infection. Patients with abnormal liver function were mostly male and had elevated levels of procalcitonin and C-reactive protein and they also received lopinavir and ritonavir which has to be monitored quite closely.[72] It was also observed that patients with an underlying problem of cirrhosis were at a significantly higher risk of hepatic decompensation which could result in liver failure and eventually death by SARS-CoV-2 infection.[123]

In the kidney, tubular cells have an extensive expression of ACE2andhuman tubular kidney cells can be directly infected by SARS-CoV-2. Specifically, the infected kidney organoids can be significantly inhibited by hrsACE2 at the initial stage of infection.[110] Volker M. Lauschke and colleagues had a comprehensive summary of SARS-CoV-2 infection on 3D models which emphasize new pharmacological tools to prevent further infection.[124] Combining complementary *in vivo* animal models with *ex-vivo* human studies such as CRISPR systems, genetically modified mouse models and adenoviral systems will be required to understand the disease in a better perspective and to formulate better treatment models.[125] At present there are very few studies reporting the *ex vivo* 3D organoid model system to study mechanisms of the COVID-19 effect and which can be further used for drug efficacy against the virus infectivity. Emphasis is laid on tissue specific cell derived organoid system developed to study the effects of COVID-19, compared with various in vitro and in vivo and other animal models to show the advantage and disadvantage of each model.[126] SARS-CoV-2 infection was conducted in organotypic 3D models of pulmonary and extrapulmonary tissues to study the entry of the virus and the virus-host cell interaction.[124] An extensive review compared various models such as pulmonary organoid, intestinal organoid and neuronal organoids and showed how stem cell research can be an accepted model for the purpose of research and more complex organoids and organ-on-a-chip studies would be required to conclude the same.[127]

Future perspective

The COVID-19 pandemic will likely remain a significant cause of mortality in many parts of the world for several years. Therefore, dissecting the SARS-CoV-2 pathogenic mechanism employing human 3D organoids can mimic *in vivo* setting, helping us find effective new therapeutics faster.

The established 3D organoid system can be employed to study mechanistic studies and screen for drugs against the SARS-CoV-2 virus. Studies should focus on an early stage of infection to explain how anti-viral drugs can stop the admission of SARS-CoV-2 infections in host cells. However, research will be required to study the effect of drugs in later stages of disease progression. Future studies will be required to explain the consequence of drugs at advanced stages of infection in *in vitro* and *in vivo* models to address the above issues. However, further research needs to understand the crosstalk between human organs *via* a 3D organoid system. Hence, findings can then be validated in further clinical studies.

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lung 3D organoids;
liver 3D organoids;
kidney 3D organoids;
adverse outcome pathway (AOP);
angiotensin-converting enzyme 2 (ACE2)

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Abbreviations:
ACE2, angiotensin-converting enzyme 2; AOP, Adverse Outcome Pathway; COVID-19, Coronavirus disease 2019; CLD, Chronic Liver Disease; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane serine protease 2; IL-6, Interleukin 6,; LDH, Lactate Dehydrogenase; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; ARDS, Acute Respiratory Distress Syndrome; AT2, Alveolar Type-2; hiPSCs, human induced Pluripotent Stem cells; hESC, Human Embryonic Stem Cell; ALI, Air-Liquid Interface; ANG II, Angiotensin II; CKD, Chronic Kidney Disease; hrsACE2, human recombinant soluble angiotensin-converting enzyme 2,; MERS, Middle East Respiratory Syndrome; SARS-CoV, Severe Acute Respiratoy Syndrome; 2D, 2-Dimensional; 3D, 3-Dimensional; GFP, Green fluorescent Protein; IFN, Interferon; MPA, Mycophenolic Acid; QNHC, Quinacrine dihydrochloride; ESC, Embryonic stem cells; TNF-a, Tumor Necrosis Factor- a

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