Disease modeling in organoid cultures: a new tool for studying viruses

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Introduction

In vitro experiments have been widely used for more than a century to elucidate molecular mechanisms in cells and pathogen-host interactions, as well as for drug screening. Cell lines have been modified to reflect researchers’ specific purposes, and in vitro experiments have become fundamental for biological studies, with an ability to replace in vivo experiments. However, immortalized cell lines and cancer-derived cell lines have the limitation of losing their inherent properties, potentially resulting in changes in signaling pathways and cell metabolism. These limitations have made it necessary for researchers to find a novel way to overcome the limitations of cell lines. In recent years, organoids, which are 3-dimensional multicellular in vitro tissue constructs that fundamentally imitate in vivo organs, have been developed as alternative systems to study various aspects of organs. Herein, we review recent studies on the application of organoids in disease modeling, with a focus on intestine, lung, and tonsil organoids. These 3 organoids have been of utmost interest to researchers since their initial development. Most importantly, organoids are novel experimental models that can simulate in vivo organs and can therefore replace or support existing in vitro and in vivo models.

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single cell type and do not represent the structure, complexity, and function of a tissue. Thus, classical studies using cell lines have been replaced by other methods, such as tissue cultures and model organisms, although these methods also do not fully represent the in vivo environment.

Organoids are 3-dimensional (3D) multicellular in vitro tissue constructs that fundamentally imitate the corresponding in vivo organs in a culture dish [7]. For this reason, organoids have recently been used as alternative systems to study certain aspects of organs. Organoids can be derived from embryonic stem cells, pluripotent stem cells (PSCs), and adult stem cells from diverse organs [8]. An organoid can comprise complex systems based on the developmental potential of the stem cells. Therefore, organoid systems have advantages in human physiological studies, disease simulation studies, and preclinical studies. Furthermore, organoids can mature and differentiate in different directions in a 3D culture system, which could be useful for visualizing the growth of organoids and their genetic diversity [9,10]. For this purpose, several growth factors are used in the culture of organoids to control the proliferation and differentiation signaling pathways of organoids in a tissue-specific manner [11].

In the current review, we discuss several tissue organoid systems, with a particular focus on lung, intestine, and tonsil organoids, which can be adopted for research on drug screening and modes of action. This review deals with intestine, lung, and tonsil organoids because these organoids are similar to organs that respond quickly to viruses. In previous decades, sufficient in vitro assays were developed to study viral mechanisms, and in vivo assays could provide further support to reveal the mechanisms of viral infections. However, there are sometimes discrepancies between mechanisms identified in vitro and in vivo, due to many potential factors [12]. One possible explanation for these discrepancies could be the limitation of cell lines that they lose their original functions. This limitation has prompted the use of organoids as a novel experiment tool. Researchers expect that studies using organoids could reveal the mechanisms of viruses or bacteria without the limitation of cell lines. In this review, we discuss the viruses that infect each organoid and list the latest papers.

Disease modeling using tissue-specific organoids

1. Intestinal organoids

The gastrointestinal tract is a complex organ of the digestive system. It is composed of various polarized epithelial layers containing diverse cell types. The small intestine, including the duodenum, jejunum, and ileum, and the large intestine, including the proximal and distal colon, have distinct functions and interact with various symbiotic bacteria or pathogens [13]. The intestine contains multiple types of cells, including enterocytes, goblet cells, Paneth cells, and stem cells. The first intestinal organoid cultures were successfully established in 2009 from Lgr5+ murine epithelial intestinal stem cells [14]. Similarly, organoids derived from human PSCs and biopsy samples were developed. Two types of intestinal stem cells have been used: PSCs from epithelial cell culture with or without mesenchyme cells [15,16].

Research on infectious diseases often requires complex conditions for pathogen culture. As a result of host specificity for a particular pathogen, it is sometimes the case that only humans can be infected as a host, while experimental animals or cell lines cannot be infected with a pathogen [17]. In addition, experiments using cell lines are generally not suitable considering the life cycle of a pathogen. However, organoid cultures, including both 3D and monolayer culture systems, could be used to study viruses that only infect human cells, such as norovirus, rotavirus, enteroviruses, and adenoviruses [18,19] (Fig. 1). Organoid cultures can also be adopted for functional research on human gastrointestinal pathology under infectious conditions [20]. Globally, norovirus, along with rotavirus, is one of the main causes of aseptic gastroenteritis and is known to be a major cause of diarrhea or benign convulsions in children [21]. Attempts to cultivate human norovirus using various cell lines or animal models have continued for about 40 years since norovirus was discovered in 1968 [22]. Most experiments have failed, while very few have succeeded under highly limited conditions. These issues have hindered researchers from accurately understanding the mechanisms of human norovirus infections. However, human norovirus has been successfully cultured in monolayer organoids or enteroids, suggesting the possibility of culturing non-cultivable human pathogens using organoids [23,24]. It has been shown that human norovirus can proliferate in enteroids, and bile acids aid in the proliferation of some norovirus strains [25,26]. The replication of norovirus strains is based on the expression of the human norovirus histo-blood group antigen (HBGA) [27]. HBGA biosynthesis is regulated by genetically encoded glycosyltransferases such as fucosyltransferase 2 [27]. This implies that organoids are very useful systems with a high level of biological relevance in culturing human pathogens and understanding host-virus interactions.

Rotavirus infections in enteroendocrine cells have been reported in cell lines and mice [28]. However, it is not known
Gastric organoids to study the host specificity of a particular pathogen. It is widely recognized that some pathogens are difficult to culture because they require specific conditions depending on their life cycle. In particular, attempts have been made to culture norovirus, rotavirus, and enterovirus in various cell lines and animal models. However, most of those trials failed, except in gastric organoids, in which researchers have observed that the infection exhibited similar characteristics as observed in patients’ organs. In the near future, pathogens that do not easily infect cell lines or animal models could be studied using 3-dimensional organoid systems to develop novel treatments and further understand the mechanisms of pathogens’ pathophysiology. MPE, malignant pleural effusion.

whether human rotavirus strains infect enteroendocrine cells in the human body [29]. Organoid studies have demonstrated that rotavirus only infects differentiated enterocytes and enteroendocrine cells [28]. It was suggested that the differentiation of organoids is important for virus replication. Inclusion bodies and lipid droplets, which are known to be the main features of rotavirus replication, were observed in differentiated organoids [30,31]. In addition, when rotavirus infects organoids, it induces unique physiological responses such as lumen expansion and fluid secretion through the action of viral enterotoxin [32]. Before the adoption of organoids as experimental systems, it was shown that rotavirus infection in colon carcinoma cells increased the transcription and translation of type I interferon (IFN) expression [33]. Based on this, we can presume that type I IFN may be critical for suppressing viral replication in vivo, although both type III and type I IFN responses are primary responses of antiviral activity [31]. However, it is not yet clear which route is dominant. Therefore, further research using organoids is needed.

Globally, enteroviruses, which include poliovirus, coxsackievirus, echovirus, and enterovirus A71, are serious causes of human infections [34]. Researchers’ understanding of enteroviral infections in the gastrointestinal tract is also entering a new phase through the development of systems that can induce immune responses in organoids, especially in enteroid models [28]. Previous studies on enteroviral infections were limited to murine models, in which the host immune cells were ablated and the infection was delivered via intraperitoneal injection [35]. Infection of enteroviruses in colon carcinoma cell lines showed no induction of strong antiviral responses, suggesting that the colon carcinoma cell lines had attenuated host innate immunity [32]. Diverse enteroviruses, including coxsackievirus B, echovirus 11, and enterovirus, have been used to infect enteroids and induce virus-specific antiviral responses, as well as inflammatory signaling pathways [36,37]. Echovirus 11 infection in enteroids induced the differential expression of 350 transcripts, while coxsackievirus B induced the differential expression of 13 transcripts [37]. In these studies, new discoveries on the cell-specificity of infections were reported in organoids with echovirus 11 infections. Infections were observed in enterocytes and enteroendocrine cells, but not in goblet cells [38]. Furthermore, bacteria including Clostridium difficile, Salmonella, and Escherichia coli, as well as parasites such as Cryptosporidium, were reported to be available for the development of infection models using organoids [39,40].

2. Lung organoids
The lung is a highly complex organ. The bronchial airway is con-
Connected to a single tube, and at the distal end, the bronchial airway narrows and extends to the bronchial tubes, which branch into smaller tubes called bronchioles [41]. Each bronchiole is lined with alveolar ducts and terminates with alveolar sacs and alveoli [42]. The resident cells of lung tissue include all the cells in 3 compartments: cells that make up the bronchial tubes, alveolar unit cells, and pulmonary vascular cells, which are further divided into about 40 different cell types. In the first trial for the development of lung organoids, which was carried out in 1987, researchers could culture alveolar epithelial type 2 cells in vitro [43]. The daughter cells of hemorrhoid epithelial type 2 cells showed the characteristics of the original cells. Subsequently, in 1991, lung cancer cells were cultured using a specific system that used a gas-medium interface and showed reorganized and differentiated structures similar to the original tissues with typical histological characteristics [44,45]. When mouse and human cells were cultured as organoid cultures, they could self-renew and develop into the original tissue. Alveolar epithelial type 2 cells were also cultured into a 3D co-culture system with niche factors [46]. In 2014, human alveolar epithelial progenitor cells were cultured into spheroids, and in the following year, human lung organoids were induced from human PSCs to convert them into branching airways and early alveolar structures containing the mesoderm and pulmonary endoderm [47,48] (Fig. 2). Lung organoid systems are an emerging research topic, as well as a useful tool for the modeling of respiratory diseases, such as lung cancer or idiopathic pulmonary fibrosis [49].

Respiratory diseases result in high morbidity and mortality in humans. Idiopathic pulmonary fibrosis is a chronic disease that causes lung function to decline and results in the progression of fibrosis [50]. Although idiopathic pulmonary fibrosis has traditionally been considered a rare disease, the number of idiopathic pulmonary fibrosis patients has consistently increased in recent years. Smoking and viral or bacterial respiratory infections are considered to be the major risk factors for this disease [51,52]. The etiology of idiopathic pulmonary fibrosis is unknown, although it is assumed to be a progressive form of pneumonia. Pulmonary hypertension (PH) is commonly observed in idiopathic pulmonary fibrosis patients and increases with the severity of idiopathic pulmonary fibrosis [53]. No appropriate treatment currently exists for PH associated with idiopathic pulmonary fibrosis [54]. Targeted drug treatment for PH is rare, and clinical trials have yet to report evidence for improvements in symptoms or other outcomes in idiopathic pulmonary fibrosis patients [55]. Thus, studies based on lung organoids might help to identify drug candidates for the treatment of PH associated with idiopathic pulmonary fibrosis, as well as ameliorating idiopathic pulmonary fibrosis.

Fig. 2. Using organoids to model a disease that is difficult to mimic in vitro or in vivo. The lung is a highly complex organ that contains 3 tissue types. Lung organoids were developed decades ago, and researchers cultured alveolar epithelial type 2 cells in vitro. Since their development, lung organoids have been used to model respiratory diseases such as lung cancer, idiopathic pulmonary fibrosis, and chronic obstructive pulmonary disease. A human lung bud tip organoid was recently developed to be used for disease modeling and drug screening.
Among respiratory diseases, chronic obstructive pulmonary disease (COPD) is the next leading cause of death after idiopathic pulmonary fibrosis. COPD is characterized by the loss of parenchymal lung tissue function and the loss of gas exchange in alveoli [56]. It is an irreversible and progressive disease that leads to shortness of breath, chronic cough, and catarh production, and it is characterized by 2 defined pathological patterns: chronic obstructive bronchitis and emphysema of the lung [57,58]. It also results in narrowing of the airways due to alveolar thickening [59]. Lifestyle habits such as smoking reduce epithelial integrity and cause dramatic epithelial remodeling, contributing to the development of COPD and lung cancer [60,61]. Reactive oxygen species (ROS) are considered a mediator of COPD due to their involvement in apoptosis, mitochondrial damage, and inflammation of AT2 cells [62]. It is generally accepted that alveolar macrophages play an important role in the occurrence of COPD. In patients with COPD, the number of alveolar macrophages increases noticeably in the airways, lung parenchymal tissues, bronchoalveolar lavage fluid, and sputum [63]. Patients with obstructive pulmonary disease have a large number of macrophages, and alveoli are destroyed as a consequence of the release of matrix metalloproteinase (MMP)-2, MMP-9, and cathepsin K [64]. Damage to alveolar macrophages leads to bacterial clustering and exacerbation of the condition by respiratory viruses or bacteria [65].

In a previous study, a 2-dimensional in vitro COPD model was used using cigarette smoke extract [66]. Researchers observed that cigarette smoke extract induced inflammation and ROS production [59]. They also suggested that high expression of RAGE by DAMP-Nrf signaling in COPD-like conditions damaged AT2 cells in vitro and in vivo [67]. In contrast, treatment with a RAGE antagonist (FPS-ZM1) prevented damage by blocking RAGE-mediated DAMP-Nrf2 signaling [68]. It is important to determine the mechanism of disease occurrence or infection in in vitro models that are similar to the human lung environment. The obvious limitations of animal models desperately need to be supplemented by applying human alveolar cell compartments. In another study, human lung bud tip organoids were grown and differentiated into alveolar-like cells under special conditions [69]. Furthermore, human lung bud tip organoids that expressed ACE2 and TMPRSS2 were infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [69]. Therefore, organoid systems are expected to be used for disease modeling and drug screening. Furthermore, organoid models could also be used in preclinical research for drug discovery for human COPD and SARS-CoV-2 infection.

3. Tonsil organoids

The tonsils (a term that usually refers to the palatine tonsils) are a pair of soft tissue masses located on the rear sides of the human throat. They are lymphoid organs located at the junction of the respiratory and alimentary canals and play an important role in the immune system by receiving antigens from the bloodstream. The palatine tonsils and the adenoid tonsils are organs consisting of lymphoepithelial tissue located near the oropharynx and nasopharynx [70]. The tonsils develop after birth and decrease in size to some extent after adolescence. The pharyngeal tonsils develop extensively at a young age, becoming adenoids that could be subjected to removal if necessary [71]. The palatine tonsils predominantly contain B cells, with a few myeloid cells [72]. A peculiarity of tonsils is that, unlike the normal peripheral lymph nodes, they have no lymphatic vessels. In the tonsils, antigen-presenting cells such as dendritic cells and B cells are considered to be a starting point for the recognition of antigens [73]. When B cells recognize antigens, they start to produce a neutralizing antibody within the germinal centers (GCs) and extrafollicular regions in the lymphoid organs [74]. T follicular helper cells and some of the hematopoietic and non-hematopoietic cells transduce signals that are required for survival, proliferation, antibody affinity maturation, class-switch recombination, and differentiation to GC B cells [75].

In a previous study, researchers reported that thymus cells separated from a murine model could be cultured and re-associated in vitro to reenact a major role in selecting T cells [76]. In another recent study, researchers applied a similar approach to culture human tonsil organoids. Cells obtained from the tonsil were plated at high density into trans-wells and were treated with the antigen of interest in the presence of B-cell activating factor [77]. Several days later, tonsil cell culture showed re-aggregated regions of clustered cells, as well as GC-like structures with B cells and T cells. In tonsil organoids, an organization of a light zone and dark zone was observed, which is a characteristic of GCs. Affinity maturation was also observed to have occurred in tonsil organoids, which is one of the key functions of GCs [78]. Within these tonsil organoids, SARS-CoV-2 is potently amplified through multiple rounds of infections, resulting in abundant secretion of the viral particles [79]. These studies suggest that tonsil organoids could mimic various aspects of the human immune responses, especially the interaction of the innate immune response with the adaptive immune response, providing a novel tool for the production of human antibodies against specific antigens of interest (Fig. 3). For example, research on viral infections could use liver organoids, intestine organoids, or lung organoids and even brain organoids as a tool, and tonsil
organoids could be used in research on the viral response.

**Conclusion**

Organoids are an experimental model similar to cell lines that have been used for decades to study cellular mechanisms and to develop novel treatments for various diseases. Although *in vitro* assay systems using cell lines have several advantages, they cannot directly mimic *in vivo* systems; instead, organoids could be used as alternatives to mimic the actual situation in organs. Organoid systems also have several advantages over cell lines, since there is no loss of function due to immortality, as is the case *in vivo*, and they are easy to manipulate and observe. Moreover, organoids can reproduce the cell layers and tissue structures that can be seen in organs. This suggests that organoids can be cultured for longer periods, frozen like cell lines, and used to study physiological phenomena more realistically than is possible with cell lines using next-generation *in vitro* analysis methods.

The bio-similarity of organoids makes it easier to proceed with the sophisticated modeling of complex diseases, such as infectious diseases, genetic diseases, and cancer. The modeling of these diseases is not restricted to discovering new treatments for the diseases, but could also yield insights into the mechanism of disease onset. One of the advantages of organoid models over cell lines is their ability to reproduce complex interactions between organs, which could otherwise only be investigated in animal experiments, to evaluate the efficacy and toxicity of drugs.

However, organoids are not yet reliable enough to completely replace experiments using cell lines or animal models. Organoid culture systems have developed rapidly since they were first introduced and are still developing, but organoid cultures are expensive and have difficulty replicating the scale of massive cell-line culture systems. The external substrates or scaffolds used for 3D culture are expensive and difficult to handle, which imposes a limitation on culture. In addition, imbalances in the distribution of nutrients, growth and differentiation factors, and the number of the stem cells obtained from the organ may occur during the culture, preventing the quantification of growing organoids. Moreover, one of the main obstacles in current organoid systems is that the histological structure and functions of organoids are still limited in comparison to actual organs. However, the most important aspect of organoids is that they are *in vitro* experimental models that can simulate *in vivo* phenomena, and due to this advantage, we believe that organoids will replace or support existing models as a higher-level experimental model.
Notes

Conflict of interest
Hyun-Jeong Ko has been an editor of Organoid since 2021. No other potential conflict of interest relevant to this article was reported.

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References

1. Yao T, Asayama Y. Animal-cell culture media: History, characteristics, and current issues. Reprod Med Biol 2017;16:99–117.
2. Skloot R. The immortal life of Henrietta Lacks. New York: Broadway Paperbacks; 2011.
3. Earle WR. Production of malignancy in vitro. J Natl Cancer Inst 1943;4:165–212.
4. Jenkinson SE, Chung GW, van Loon E, Bakar NS, Dalzell AM, Brown CD. The limitations of renal epithelial cell line HK-2 as a model of drug transporter expression and function in the proximal tubule. Pflugers Arch 2012;464:601–11.
5. Gumbleton M, Audus KL. Progress and limitations of screening assays to assess engineered nanoparticle toxicity in a human cell line. Toxicol Appl Pharmacol 2009;234:222–35.
6. Monteiro-Riviere NA, Inman AO, Zhang LW. Limitations and relative utility of screening assays to assess engineered nanoparticles in vitro. J Pharmacol Exp Ther 2010;332:1083–91.
7. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2010;468:111–5.
8. Hofer M, Lutolf MP. Engineering organoids. Nat Rev Mater 2021;6:402–20.
9. Nikolić MZ, Carigt O, Jeng Q, Johnson JA, Sun D, Howell KJ, et al. Human embryonic lung epithelial tips are multipotent progenitors that can be expanded in vitro as long-term self-renewing organoids. Elife 2017;6:e26575.
10. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 2011;470:105–9.
11. Gjorevski N, Ranga A, Lutolf MP. Bioengineering approaches to guide stem cell-based organogenesis. Development 2014;141:1794–804.
12. Lorand V. Differences between in vitro and in vivo studies. Antimicrob Agents Chemother 1988;32:1600–1.
13. Sobala GM, Crabtree JE, Dixon MF, Schorah CJ, Taylor JD, Rathbone BJ, et al. Acute Helicobacter pylori infection: clinical features, local and systemic immune response, gastric mucosal histology, and gastric juice ascorbic acid concentrations. Gut 1991;32:1415–8.
14. Sato T, Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. Science 2013;340:1190–4.
15. Gargett CE, Schwab KE, Zillwood RM, Nguyen HP, Wu D. Isolation and culture of epithelial progenitors and mesenchymal stem cells from human endometrium. Biol Reprod 2009;80:1136–45.
16. Mithal A, Capilla A, Heinzle D, Berical A, Villacorta-Martin C, Vedaie M, et al. Generation of mesenchyme free intestinal organoids from human induced pluripotent stem cells. Nat Commun 2020;11:215.
17. Bäumler A, Fang FC. Host specificity of bacterial pathogens. Cold Spring Harb Perspect Med 2013;3:a010041.
18. Fong TT, Lipp EK. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. Microbiol Mol Biol Rev 2005;69:357–71.
19. Holly MK, Smith JG. Adenovirus infection of human enteroids reveals interferon sensitivity and preferential infection of goblet cells. J Virol 2018;92:e00250.
20. Blutt SE, Crawford SE, Ramani S, Zhou WY, Estes MK. Engineered human gastrointestinal cultures to study the microbiome and infectious diseases. Cell Mol Gastroenterol Hepatol 2017;5:241–51.
21. Chen SY, Tsai CN, Lai MW, Chen CY, Lin KL, Lin TY, et al. Norovirus infection as a cause of diarrhea-associated benign infantile seizures. Clin Infect Dis 2009;48:849–55.
22. Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR,
23. Papafragkou E, Hewitt J, Park GW, Greening G, Vinjé J. Challenges of culturing human norovirus in three-dimensional organoid intestinal cell culture models. PLoS One 2013;8:e63485.

24. Estes MK, Ettayebi K, Tenge VR, Murakami K, Karandikar U, Lin SC, et al. Human norovirus cultivation in nontransformed stem cell-derived human intestinal enteroid cultures: success and challenges. Viruses 2019;11:638.

25. Costantini V, Morantz EK, Browne H, Ettayebi K, Zeng XL, Atmar RL, et al. Human norovirus replication in human intestinal enteroids as model to evaluate virus inactivation. Emerg Infect Dis 2018;24:1453–64.

26. Kilic T, Koromyslova A, Hansman GS. Structural basis for human norovirus capsid binding to bile acids. J Virol 2019;93:e01581.

27. Nordgren J, Svensson L. Genetic susceptibility to human norovirus infection: an update. Viruses 2019;11:226.

28. Saxena K, Blutt SE, Ettayebi K, Zeng XL, Broughman JR, Crawford SE, et al. Human intestinal enteroids: a new model to study human rotavirus infection, host restriction, and pathophysiology. J Virol 2015;90:43–56.

29. Crawford SE, Ramani S, Tate JE, Parashar UD, Svensson L, Haghom M, et al. Rotavirus infection. Nat Rev Dis Primers 2017;3:17083.

30. Cheung W, Gill M, Esposito A, Kaminski CF, Courrousse N, Chwetzoff S, et al. Rotaviruses associate with cellular lipid droplet components to replicate in viroplasms, and compounds disrupting or blocking lipid droplets inhibit viroplasm formation and viral replication. J Virol 2010;84:6782–98.

31. Yin Y, Bijvelds M, Dang W, Xu L, van der Eijk AA, Knipping K, et al. Modeling rotavirus infection and antiviral therapy using primary intestinal organoids. Antiviral Res 2015;123:120–31.

32. Crawford SE, Ramani S, Blutt SE, Estes MK. Organoids to dissect gastrointestinal virus-host interactions: what have we learned? Viruses 2021;13:999.

33. Sen A, Rott L, Phan N, Mukherjee G, Greenberg HB. Rotavirus NSP1 protein inhibits interferon-mediated STAT1 activation. J Virol 2014;88:41–53.

34. Lugo D, Krogstad P. Enteroviruses in the early 21st century: new manifestations and challenges. Curr Opin Pediatr 2016;28:107–13.

35. Gai X, Zhang Q, Lu H, Yang Z, Zhu L, Li X, et al. A neonatal murine model for evaluation of enterovirus E HY12 virus infection and pathogenicity. PLoS One 2018;13:e0193155.

36. Hornby PJ, Cooper PR, Kliwinski C, Ragwan E, Mabus JR, Harman B, et al. Human and non-human primate intestinal FcRn expression and immunoglobulin G transcytosis. Pharm Res 2014;31:908–22.

37. Drummond CG, Bolock AM, Ma C, Luke CJ, Good M, Coyne CB. Enteroviruses infect human enteroids and induce antiviral signaling in a cell lineage-specific manner. Proc Natl Acad Sci U S A 2017;114:1672–7.

38. Good C, Wells AI, Coyne CB. Type III interferon signaling restricts enterovirus 71 infection of goblet cells. Sci Adv 2019;5:eaau4255.

39. Heo I, Dutta D, Schaef er DA, Iakobachvili N, Artegiani B, Sachs N, et al. Modelling Cryptosporidium infection in human small intestinal and lung organoids. Nat Microbiol 2018;3:814–23.

40. Gunasekera S, Zahedi A, O’Dea M, King B, Monis P, Thierry B, et al. Organoids and bioengineered intestinal models: potential solutions to the Cryptosporidium culturing dilemma. Microorganisms 2020;8:715.

41. Ball M, Hossain M, Padalia D. Anatomy, airway [updated 2021 Jul 31]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459258/.

42. Patwa A, Shah A. Anatomy and physiology of respiratory system relevant to anaesthesia. Indian J Anaesth 2015;59:533–41.

43. Shannon JM, Mason RJ, Jennings SD. Functional differentiation of alveolar type II epithelial cells in vitro: effects of cell shape, cell-matrix interactions and cell-cell interactions. Biochim Biophys Acta 1987;931:143–56.

44. Lohsiriwat V. Hemorrhoids: from basic pathophysiology to clinical management. World J Gastroenterol 2012;18:2009–17.

45. Kong J, Wen S, Cao W, Yue P, Xu X, Zhang Y, et al. Lung organoids, useful tools for investigating epithelial repair after lung injury. Stem Cell Res Ther 2021;12:95.

46. Fujii M, Sato T. Somatic cell-derived organoids as prototypes of human epithelial tissues and diseases. Nat Mater 2021;20:156–69.

47. Gotoh S, Ito I, Nagasaki T, Yamamoto Y, Konishi S, Korogi Y, et al. Generation of alveolar epithelial spheroids via isolated progenitor cells from human pluripotent stem cells. Stem Cell Reports 2014;3:394–403.

48. Chen YW, Huang SX, de Carvalho AL, Ho SH, Islam MN, Volpi S, et al. A three-dimensional model of human lung de-
velopment and disease from pluripotent stem cells. Nat Cell Biol 2017;19:542–9.
49. Li Y, Wu Q, Sun X, Shen J, Chen H. Organoids as a powerful model for respiratory diseases. Stem Cells Int 2020;2020:5847876.
50. Martinez FJ, Collard HR, Pardo A, Raghu G, Richeldi L, Selman M, et al. Idiopathic pulmonary fibrosis. Nat Rev Primers 2017;3:17074.
51. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011;183:788–824.
52. Ehrlich SF, Quesenberry CP Jr, Van Den Eeden SK, Shan J, Ferrara A. Patients diagnosed with diabetes are at increased risk for asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, and pneumonia but not lung cancer. Diabetes Care 2010;33:55–60.
53. Raghu G, Amatto VC, Behr J, Stowasser S. Comorbidities in idiopathic pulmonary fibrosis patients: a systematic literature review. Eur Respir J 2015;46:1113–30.
54. Collum SD, Amione-Guerra J, Cruz-Solbes AS, DiFrancesco A, Hernandez AM, Hammandu A, et al. Pulmonary hypertension associated with idiopathic pulmonary fibrosis: current and future perspectives. Can Respir J 2017;2017:1430350.
55. Barberà JA, Román A, Gómez-Sánchez MÁ, Blanco I, Otero R, López-Reyes R, et al. Guidelines on the diagnosis and treatment of pulmonary hypertension: summary of recommendations. Arch Bronconeumol (Engl Ed) 2018;54:205–15.
56. Singh D, Agusti A, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease: the GOLD science committee report 2019. Eur Respir J 2019;53:1900164.
57. Anzueto A, Miravitlles M. Pathophysiology of dyspnea in COPD. Postgrad Med 2017;129:366–74.
58. Miravitlles M. Cough and sputum production as risk factors for poor outcomes in patients with COPD. Respir Med 2011;105:1118–28.
59. Eurlings IM, Dentener MA, Cleutjens JP, Peutz CJ, Rohde GG, Wouters EF, et al. Similar matrix alterations in alveolar and small airway walls of COPD patients. BMC Pulm Med 2014;14:90.
60. Lebowitz MD, Burrows B. Quantitative relationships between cigarette smoking and chronic productive cough. Int J
61. Barkauskas CE, Cronce MJ, Rackley CR, Bowie EJ, Keene DR, Stripp BR, et al. Type 2 alveolar cells are stem cells in adult lung. J Clin Invest 2013;123:3025–36.
62. Kim SJ, Cheresh P, Jablonski RP, Morales-Nebreda L, Cheng Y, Hogan E, et al. Mitochondrial catalase overexpressed transgenic mice are protected against lung fibrosis in part via preventing alveolar epithelial cell mitochondrial DNA damage. Free Radic Biol Med 2016;101:482–90.
63. Vlahos R, Bozinovski S. Role of alveolar macrophages in chronic obstructive pulmonary disease. Front Immunol 2014;5:435.
64. Osttridge K, Williams N, Kim V, Bennett M, Harden S, Welch L, et al. Relationship between pulmonary matrix metalloproteinases and quantitative CT markers of small airways disease and emphysema in COPD. Thorax 2016;71:126–32.
65. Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. Eur Respir J 2003;22:672–88.
66. Gindele JA, Kiechle T, Benediktus K, Birk G, Brendel M, Heinemann F, et al. Intermittent exposure to whole cigarette smoke alters the differentiation of primary small airway epithelial cells in the air-liquid interface culture. Sci Rep 2020;10:6257.
67. Lee H, Park JR, Kim WJ, Sundar IK, Rahman I, Park SM, et al. Blockade of RAGE ameliorates elastase-induced emphysema development and progression via RAGE-DAMP signaling. FASEB J 2017;31:2076–89.
68. Lee H, Lee J, Hong SH, Rahman I, Yang SR. Inhibition of RAGE attenuates cigarette smoke-induced lung epithelial cell damage via RAGE-mediated Nrf2/DAMP signaling. Front Pharmacol 2018;9:684.
69. Lamers MM, van der Vaart J, Knoop K, Riesebosch S, Breugem TJ, Mykytyn AZ, et al. An organoid-derived bronchio-alveolar model for SARS-CoV-2 infection of human alveolar type II-like cells. EMBO J 2021;40:e105912.
70. Perry M, Whyte A. Immunology of the tonsils. Immunol Today 1998;19:414–21.
71. Jia MY, Zou SZ, Li JR. Study on the relationship between adenoid and tonsil hypertrophy and obesity in children. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2020;55:760–3.
72. Harabuchi Y, Takahara M. Recent advances in the immunological understanding of association between tonsil and immunoglobulin A nephropathy as a tonsil-induced autoimmune/inflammatory syndrome. Immun Inflamm Dis 2019;7:86–93.
73. Popi AF, Longo-Maugéri IM, Mariano M. An overview of

Organoid 2022;2:e15 • https://doi.org/10.51335/organoid.2022.2.e15
74. Krishnaswamy JK, Alsén S, Yrlid U, Eisenbarth SC, Williams A. Determination of T follicular helper cell fate by dendritic cells. Front Immunol 2018;9:2169.

75. Song W, Craft J. T follicular helper cell heterogeneity: time, space, and function. Immunol Rev 2019;288:85–96.

76. Tajima A, Pradhan I, Trucco M, Fan Y. Restoration of thymus function with bioengineered thymus organoids. Curr Stem Cell Rep 2016;2:128–39.

77. Fan Y, Tajima A, Goh SK, Geng X, Gualtierotti G, Grupillo M, et al. Bioengineering thymus organoids to restore thymic function and induce donor-specific immune tolerance to allografts. Mol Ther 2015;23:1262–77.

78. Wagar LE, Salahudeen A, Constantz CM, Wendel BS, Lyons MM, Mallajosyula V, et al. Modeling human adaptive immune responses with tonsil organoids. Nat Med 2021;27:125–35.

79. Kim HK, Kim H, Lee MK, Choi WH, Jang Y, Shin JS, et al. Generation of tonsil organoids as an ex vivo model for SARS-CoV-2 infection [Preprint]. Posted 2020 Aug 7. bioRxiv 2020.08.06.239574. https://doi.org/10.1101/2020.08.06.239574.