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Review Article

New Developments In Organoid Models Of The Nose And Lung For Respiratory Viral Studies.

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Abstract

Historically, immortalized cells and animal models have been used to study human respiratory virus infections and pathogenesis. The intricate structure of the human airway and the entire range of illness symptoms seen in people, however, cannot be fully replicated by these models. Lung and nose organoids have recently transformed culture complexity in infection biology and shown promise for studying human respiratory viral infections. We discuss how developments in human nose and lung organoid models, which can express all respiratory epithelia cell types, including club, basal, goblet, and ciliated cells, have shed new light on the pathophysiology, age-dependent susceptibility, and viral attenuation signature.and defense mechanisms against respiratory viruses, including influenza, respiratory syncytial virus, and SARS-CoV-2. The models have also shown promise for researching human viruses that have not yet been cultivated and for assisting with zoonotic risk research.

Keywords : *respiratory virus, human, lung, nose, stem cells, and organoids.*

INTRODUCTION

The majority of newly discovered illnesses are caused by respiratory viruses, which nevertheless represent a serious risk to human health. We still don't fully understand how it spreads and adapts to humans, but [1]. Despite the tremendous advancements in epidemiology and virology, a major problem in forecasting and evaluating respiratory virus adaptation and spillover risk is the lack of biologically relevant models of assessment [2]. Although the identification of novel viruses can aid in determining the animal source of zoonoses, these findings are not very useful for predicting the likelihood of a pandemic or spillover [3]. Important details regarding the evolution of the severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) have been revealed by sequence analysis. While animal studies are critical for understanding the pathogenesis of emerging viruses, they often do not recapitulate the full spectrum of disease observed in humans. For example, none of the SARS-CoV-2 animal models involve acute respiratory distress symptoms [5]. Thus, non-transformed cell model systems that recapitulate the human tissue and cellular environment are necessary for understanding the transmission and adaption mechanisms of respiratory viruses [6]. Over the last few years, human stem cell-derived respiratory organoids have emerged as powerful tools for bridging the gap between transformed cell lines and in vivo human conditions. These airway organoid models are also included in the WHO's list of tools for assessment of the pandemic risk of influenza [7,8].

These organoid models have been shown in numerous studies to be extremely physiologically comparable human disease models for viral respiratory infections [9, 10]. Recent developments employing these models have improved our knowledge of human respiratory viral infections [6,11] and may contribute to a deeper comprehension of the pathophysiology of these viruses [9,12,13]. Here, we highlight significant recent research on the use of human nose and lung organoid models to evaluate respiratory virus infections and the methodology's ability to improve knowledge of viral attenuation, pathogenesis, and age-dependent susceptibility. Organoids are stem cell-derived, self-organizing, three-dimensional (3D) in vitro culture systems that mimic the architecture found in vivo.the original tissues' genetic signature, functioning, and state [9]. Either pluripotent stem cells (PSCs), such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), which are somatic cells that have been reprogrammed into a pluripotent state, or somatic adult stem cells can be used to create organoids

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[10]. Because they are derived from embryos, ESCs are a contentious ethical issue. Although the ethical issues with ESCs are resolved by converting somatic cells into iPSCs, the production of organoids from iPSCs necessitates stringent progressive differentiation techniques. Adult stem cells, on the other hand, can be extracted straight from tissues or organ washes and used to create organoids that closely mimic the original tissue. After 3–10 days of incubation, respiratory adult stem cells can produce cystic organoids. More recently, bronchial and alveolar organoids have been cultivated from adult stem cells in bronchoalveolar lavage (BAL) fluid, removing the need for tissue samples [11]. A non-invasive technique for obtaining nasal adult stem cells has also been disclosed [12]. Adult stem cell-derived organoids have a mature, tissue-specific phenotype that makes them valuable for researching how each person reacts to viral infections. Organoids need a specific culture medium to preserve their stem cell characteristics while being cultivated in 3D Matrigel. They can be frozen, grown, or differentiated into 3D tissue structures or two-dimensional (2D) air-liquid interface (ALI) cultures. The apical surface of ALI cultures is exposed to air, simulating in vivo circumstances.

All of the main cell types identified in vivo are present in the complex, stratified epithelium that develops from nasal and bronchial organoids (Figure 1). By secreting the anti-inflammatory protein uteroglobin and promoting tissue repair through the generation of new cell types, secretory club cells are essential for immune protection [13]. Goblet cells release antibacterial mucus that supports mucociliary defense, whereas ciliated cells contribute in mucociliary clearance by capturing and expelling germs. With the ability to self-renew, basal progenitor cells can develop into a variety of cell types. Like their in vivo counterparts, alveolar organoids express alveolar type I (AT1) and type II (AT2) cells [14]. In addition to secreting surfactants, AT2 cells can self-renew and develop into AT1 cells [14].

The primary cell types seen in respiratory tract and organoid air-liquid interface cultures are depicted in Figure 1. The primary cell types seen in the nose's upper respiratory epithelia include ciliated cells, Club cells, goblet cells, basal cells, and uncommon pneumoendocrine (PNEC) cells. These cell types are comparable to those found in the bronchial epithelia. Along the respiratory tract, the content, subtype, and shape may vary despite the epithelia's similarities. Alveolar type I (AT1) and type II (AT2) cells are found in the alveolar cells, which are where gas exchange takes place.cells. Organoids from the nose and various lung regions can now be cultured thanks to recent developments in organoid culture, which can produce epithelia that mimic those found in vivo. Adult stem cells, bronchial and alveolar organoid models, and induced pluripotent stem cells (iPSCs) can all be used to create all three models. The apical portions of the epithelia are exposed to air by the use of air-liquid interface (ALI) culture, which accurately replicates the in vivo environment. Individual-level and physiologically relevant studies of human respiratory airways are made possible by ALI-cultures of nasal epithelia (A), bronchial epithelia (B), and alveolar epithelia (C). BioRender-generated figure, adapted from [14].

SARS-COV-2

Measurement of viral fitness in the nasal epithelial cells can serve as a stand-in test for possible human infection and transmission because these cells constitute the respiratory tract's first barrier and the point of entry for respiratory infections. Accordingly, Wu et al. [15] examined how SARS-CoV-2 penetrated the mucus-based airway barrier using nasal epithelial organoids. They discovered that SARS-CoV-2 travels through the mucus layer and binds to motile cilia through the angiotensinconverting enzyme 2 (ACE2) receptor; the Omicron form demonstrates a greater capacity for cilia-dependent penetration through the airway mucin barrier. Therefore, it was discovered that SARS-CoV-2 entrance was inhibited by cilia depletion.

Robinot et al. [16] used a primary human bronchial epithelial model, which closely resembles the normal lining of the airways, to study the structural and functional effects of SARSCoV-2 infection. They found that ciliated cells were the primary target of SARS-CoV-2, resulting in severe damage and compromised motile activity. An animal model was used to further validate these findings. Morrison et al. [17] used a similar approach and discovered that SARSCoV-2 inhibited IL-13, which affected the entrance, replication, and dissemination of the virus. They also observed abnormal ciliary structure and shedding, which may indicate that airway clearance may be hampered by the virus's affinity for ciliated cells and the ensuing epithelial damage. These results are consistent with pathological findings from COVID-19 lung autopsy, which showed widespread cell shedding and epithelial degradation, exposing basal cells and causing airway blockage [17]. One important finding was that only 5% of infected cells were goblet cells, whereas SARS-CoV-2 showed a high tropism for ciliated cells [17]. Choi et al. [18] created adult human lung air-liquid interface organoids that preserved epithelial and stromal architecture in addition to lung-resident immune cells, such as T, B, NK, and myeloid cells, whereas primary human epithelial organoid cultures normally lack immune cells. These organoids showed an adaptive, virus-specific T cell response to SARS-CoV-2 infection.

Omicron quickly overtook Delta as the predominant SARS-CoV-2 strain in 2022 [19]. The Omicron variant's enhanced transmissibility and infectivity could suggest an upper respiratory tract replication advantage. Specifically, Ozono et al. [19] discovered that, maybe as a result of improved receptor

contact, the Omicron variations penetrate and multiply more effectively in the nasal epithelium than the D614G and Delta variants. Similarly, utilizing nasal organoids, Chiu et al. [20] discovered that clinical SARS-CoV-2 samples from various individuals showed varying replication capacity and that the Omicron variant had better infectivity and replicative fitness than previous variations. Specifically, the wild-type strain (Wuhan) had the lowest infection rate, whereas the Omicron variant was more contagious than the Delta variant. Tanneti et al. [11] examined the differences between SARS-CoV-2 variants in the human nasal model in a related investigation. They discovered that the Delta variation was more cytopathic than the Omicron version, which favored replication.

Li et al. [21] examined the replicative fitness of BA.5 and previous variants in human nasal organoids, which corroborated the aforementioned findings. They discovered that the BA.5 subvariant had much higher replicative fitness and infectivity compared to the earlier wildtype and B.1.1.529 variant. The EG.5.1 and XBB.1.9.1 mutations reproduced more robustly in nasal organoids obtained from a younger adult than in organoids derived from an older adult, according to Zhang et al. [22], who examined host-dependent differences. All of these results point to different age-dependent pathogenesis mechanisms in infected persons as well as functional variations among variants of concern at the cellular level. Crucially, these results showed that nasal organoids might be useful for estimating the probability of future variations.Although immortalized cells were shown to have limited potential for coronavirus medication treatment investigations, Beumer et al. [23] conducted another elegant study that used CRISPR/Cas9-engineered organoids to identify important host factors for coronaviruses.

According to Breugem et al. [24], camelid nasal organoids are extremely vulnerable to infection with the Middle East respiratory syndrome coronavirus (MERS-CoV), but not with other SARS-CoV-2 variants (614G, BA.1, or EG.5.1.1). According to this study, camelids' upper respiratory tracts do not express ACE2, which is linked to a higher risk of contracting SARS-CoV-2 [24].

RESPIRATORY SYNCYTIAL VIRUS (RSV)

RSV employs nucleolin as an entrance coreceptor and targets ciliated cells in the human bronchial epithelium specifically [26]. Griffiths et al.'s research with human bronchial epithelial cells [27] showed that protein kinase C zeta is activated by the interaction between the pre-fusion RSV-F glycoprotein and the insulin-like growth factor-1 receptor (IGF1R). These results point to a method of viral entry where a coreceptor is recruited to viral particles at the cell surface by receptor interaction and subsequent signal transduction. Before spreading to the lower respiratory system, an RSV infection usually starts in the upper respiratory tract. Even while it doesn't always lead to a lower respiratory infection, In nasal, bronchial, and small-airway tissue cultures, the replicative fitness and innate responses of RSV A and B were compared. It was discovered that although both subgroups could replicate in the upper and lower airways, subgroup A had a replicative advantage, especially in nasal and bronchial cultures. They came to the conclusion that airway organoids are a useful model for researching RSV and can be utilized in the future to investigate variables that affect the severity of the disease. How age affects RSV interactions with the bronchial epithelium and why infections in young children are more severe than in older children are important questions. Zhao and associates. [29] used lung and ALI cultures from human donors who were both infants and adults to study this. According to their findings, RSV caused a considerable amount of apoptotic cell death and disseminated widely throughout the newborn bronchial epithelium. On the other hand, there was no barrier damage and the adult bronchial epithelium showed just a minor RSV infection. Similarly, in a sophisticated work, Aloisio et al. [30] used the power of human nasal organoids to explore why RSV infection in children is more severe than in healthy adults. They discovered notable distinctions between the responses of the adult and baby epithelium to RSV infection. Compared to adult nasal organoids, infant-derived human nasal organoids showed more mucous production, a stronger cytokine response, and more cellular damage, making them more vulnerable to RSV replication.

They concluded from their research that newborns may be more susceptible to a more severe RSV infection than adults due to dysregulated innate epithelial immune responses brought on by their epithelial cellular responses [30]. In a different investigation, Rajan et al. [31] compared the treatment results and pathogenic characteristics of SARS-CoV-2 and RSV using an organoid model of the human nose. SARS-CoV-2 caused low mucus secretion, no interferon I response, and significant damage to the cilia and epithelium. On the other hand, RSV caused ciliary injury, a strong interferon-λ response, and excessive mucus secretion [31].

INFLUENZA VIRUS

The nasal epithelium is the main site of infection and the entry point for the influenza virus, which is mainly spread through the air. The virus primarily adheres to the surface of ciliated epithelial cells in the trachea, bronchi, and bronchioles; it also sporadically adheres to goblet cells and infrequently to bronchiolar non-ciliated cuboidal cells [5]. The presence of epithelial cell receptors, particularly glycans ended by an α2,6-linked sialic acid, which bind to human strains [6] and are present in the epithelial cells of the human nasal mucosa [7], is a crucial component in human influenza virus infection.

According to studies, influenza virus budding takes place at the human airway epithelium's microvilli tips [8].Important models for researching host responses to influenza, tropism, and replication kinetics are respiratory cultures made from human nasal and lung cells. Crucially, these cultures are part of the WHO's influenza pandemic risk assessment tools [7, 8]. In order to successfully infect human influenza viruses and low pathogenic avian influenza viruses, Zhou et al. [2] employed 2D and 3D differentiated airway organoids that expressed serine proteases. They demonstrated how the model replicated the known human infectivity of various influenza strains. Human and avian influenza A virus tropism and replication kinetics in human airway organoids were similar to those observed in ex vivo cultures of human bronchus explants, according to Hui et al. [7]. Another significant finding was that human ex vivo bronchus explants exhibit similar innate immune responses to infection of human airway organoids with various human and avian influenza virus strains. The highly pathogenic avian influenza H5N1 virus could only reproduce in non-ciliated cells and had a lower replication efficiency than the non-H5N1 viruses, according to a comparison of avian influenza strains. Additionally, compared to other influenza subtypes, organoids infected with the H5N1 virus expressed considerably more interleukin 6, RANTES, and interferon β. As a result, scientists were able to show that this model could replicate the human airway system and identify variations in H5N1's replication capacity.

RHINOVIRUS

Although airway epithelial cells are infected by all rhinoviruses (RV), RV-C uses a unique host protein called cadherin-related family member 3 (CDHR3) to promote particle uptake [33]. The cellular tropism of RV is limited because CDHR3 expression is limited to ciliated cells in the upper and lower airway epithelium. - RV-C replication is limited to ciliated cells in the human airway epithelium and is linked to the endoplasmic reticulum, causing cytopathic consequences, as shown by C. Gagliardi et al. [33].All rhinoviruses (RV) infect airway epithelial cells, but RV-C promotes particle uptake by using a special host protein known as cadherin-related family member 3 (CDHR3) [33]. Because only ciliated cells in the upper and lower airway epithelium express CDHR3, RV's cellular tropism is restricted. As demonstrated by C. Gagliardi et al. [33], RV-C replication is restricted to ciliated cells in the human airway epithelium and is connected to the endoplasmic reticulum, leading to cytopathic effects.

CONCLUSIONS AND FUTURE PERSPECTIVE

The modeling of respiratory disorders has greatly advanced as a result of studies employing respiratory organoids. Human nasal and lung airway organoids, which incorporate numerous differentiated cell types and recapitulate the physiology of the human respiratory system, more closely resemble human tissue than animal models and immortalized cell cultures, which are frequently produced from non-respiratory tissues [7]. The capacity of human organoids to record unique infection signatures is a significant benefit that could aid in the comprehension of host-specific reactions. These organoid models have been shown in numerous studies to be physiologically and biologically relevant for researching human disorders [6,9,10]. For example, influenza outcomes in human ex vivo bronchus cultures have been similar to those in human airway organoid cultures [7].Similar to this, Morrison et al. [17] discovered that SARS-CoV-2 infection causes ciliary organization to be disrupted and cilia shedding to increase. These findings are in line with pathological discoveries from COVID-19 patient autopsies. The necessity of CDHR3 on ciliated cells for rhinovirus type C (RV-C) infection in both the upper and lower airway epithelium is another illustration of host restriction, as it restricts the cellular tropism of RV-C [13,33]. Future research should include immune cells and neurons to further boost physiological complexity, even though these examples demonstrate the importance of respiratory organoids.

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