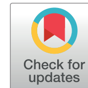

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Review
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Experimental Mouse Models and Human Lung Organoid Models for Studying Chronic Obstructive Pulmonary Disease

Young Ae Joe[1,2,*], Min Ju Lee[1,2] and Hong Seok Choi[1,2]

[1]*Department of Medical Lifescience, College of Medicine, The Catholic University of Korea, Seoul 06591,*
[2]*Department Medical Sciences, Graduate School of The Catholic University of Korea, Seoul 06591, Republic of Korea*

Abstract

Chronic obstructive pulmonary disease (COPD), a leading cause of morbidity and mortality throughout the world, is a highly complicated disease that includes chronic airway inflammation, airway remodeling, emphysema, and mucus hypersecretion. For respiratory function, an intact lung structure is required for efficient air flow through conducting airways and gas exchange in alveoli. Structural changes in small airways and inflammation are major features of COPD. At present, mechanisms involved in the genesis and development of COPD are poorly understood. Currently, there are no effective treatments for COPD. To develop better treatment strategies, it is necessary to study mechanisms of COPD using proper experimental models that can recapitulate distinctive features of human COPD. Therefore, this review will discuss representative established mouse models to investigate inflammatory processes and basic mechanisms of COPD. In addition, human COPD-mimicking human lung organoid models are introduced to help researchers overcome limits of mouse COPD models.

Key Words: Chronic obstructive pulmonary disease (COPD), Mouse model, Lung organoid, Cigarette smoke, Emphysema

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide. It features airflow obstruction and respiratory symptoms. Risk factors for the development of COPD include exposure to inhaled cigarette smoke and air pollutants in combination with genetic, developmental, and social factors (Christenson *et al*., 2022). Major pathological features of COPD are poorly reversible airway obstruction due to obstructive bronchiolitis, emphysema, and mucus hypersecretion (chronic bronchitis), which can lead to air trapping and shortness of breath during physical exertion (Fig. 1A) (Barnes *et al*., 2015). COPD develops slowly with occasional exacerbation caused by inflammatory responses induced by noxious gases, bacteria, or viruses (Li *et al*., 2012). Increase of ageing population also affects COPD prevalence because of chronic exposure to particulate matter (Christenson *et al*., 2022). COPD shares several symptoms with asthma, such as shortness of breath, chronic inflammatory response, and excessive mucus production (Athanazio, 2012; Yayan and Rasche, 2016). However, asthma differs from COPD in the following aspects: 1) younger age of onset, 2) risk factors including allergies or genetic predisposition, 3) an inflammatory profile associated with eosinophils, mast cells, and CD4+ T lymphocytes, 4) reversible airway obstruction, and 5) high responsiveness to corticosteroids (Barrecheguren *et al*., 2015; de Marco *et al*., 2015). Therefore, COPD is a complex respiratory condition that requires careful differentiation from other lung diseases for accurate diagnosis and management. The primary diagnostic criterion for COPD is the presence of persistent airflow limitation, typically defined as a post-bronchodilator FEV1/FVC ratio below 0.7 or the lower limit of normal (LLN) (Kahnert *et al*., 2023). This airflow limitation is less reversible compared to asthma, which is characterized by variable expiratory airflow limitation (Barrecheguren *et al*., 2015). Better treatment strategies and public health and personalized efforts to limit risk factors are necessary to reduce the burden of COPD.

Currently, there are no effective treatments for COPD. COPD treatment options rely on minimal exposure to smoke inhalation and reducing the occurrence and severity of exacerbations (Singh *et al*., 2018). COPD is a complex condition with pathophysiological and clinical variabilities among patients (Agusti *et al*., 2010). Mechanisms underlying COPD are poorly

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***Corresponding Author**
E-mail: youngjoe@catholic.ac.kr
Tel: +82-2-3147-8406, Fax: +82-2-593-2522

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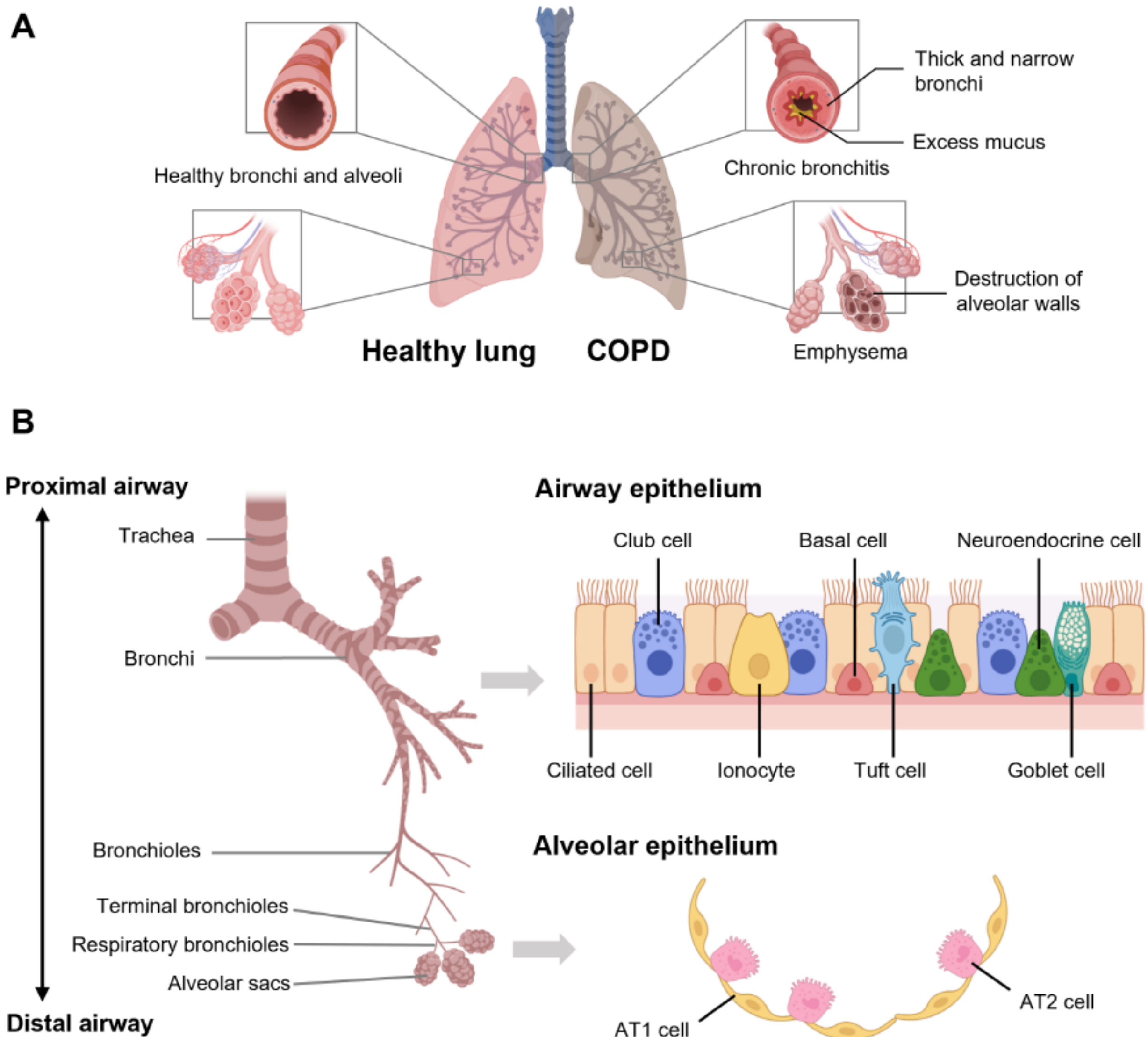

Fig. 1. Schematic diagrams of chronic obstructive pulmonary disease and lung structure. (A) For respiratory function, an intact lung structure is required for efficient air flow through conducting airways and gas exchange in alveoli. Structural changes in small airways and alveoli and inflammation are major features of COPD. The airway obstruction in COPD may occur due to a combination of small airway narrowing, airway wall inflammation, and emphysema (destruction of alveolar wall and loss of elastic recoil), and mucus hypersecretion. (B) The structure and epithelial cell composition of the lung are shown. Created in BioRender. Choi, H. (2024) BioRender.com/a53r504.

understood at molecular and cellular levels. To develop better treatment strategies, it is necessary to study mechanisms of COPD using proper experimental models that can recapitulate distinctive features of human COPD. Therefore, the present review will introduce easily applicable mouse models for investigating inflammatory processes and basic mechanisms of COPD. In addition, human COPD-mimicking human lung organoid models are introduced to help researchers overcome limits of mouse COPD models.

LUNG DEVELOPMENT AND LUNG STRUCTURE FOR GAS EXCHANGE

Lung development in both mouse and human progresses in five phases through successive branching: embryonic phase, pseudoglandular phase, canalicular phase, saccular phase, and alveolar phase (Rackley and Stripp, 2012). Patterning and differentiation are regulated via FGF, EGF, TGF-β/BMP, WNT, retinoid acid, Hedgehog, and Notch signaling pathways. The epithelium is initially composed of multipotent progenitor cells that can proliferate and differentiate during development processes, thereby forming more restricted, differentiated progeny to make up the developed lung epithelium. Embryonic endoderm progressively develops epithelial progenitor cells with increasingly restricted developmental potential. During the pseudoglandular phase, epithelial cells show differentiated characteristics. Ciliated cells and neuroendocrine cells show a proximal-to-distal wave of differentiation. Secretory cells and basal cells of conducting airways and alveolar type (AT) 1 and

2 epithelial cells of the alveolar epithelium appear during later canalicular and saccular periods. Finally, maturation of alveoli occurs to provide a delicate alveolar structure needed for efficient gas exchange across the blood-air barrier (BAB) through septation of primitive alveoli and continuing expansion of the microcapillary network during the alveolar phase. Alveologenesis occurs in mouse from birth to postnatal 20 days and in human from week 36 before birth to 3 years after birth.

The lung is composed of airways and parenchyma (Fig. 1A). Cartilaginous bronchi, membranous bronchioles, and gas exchange ducts are three major intrapulmonary airways. Proximal airway with no gas change is >2 mm in diameter. It includes trachea, bronchi, and bronchioles that conduct air, whereas distal airway refers to smaller airway (<2 mm in diameter) that includes small membranous, terminal and respiratory bronchioles, and alveolar ducts (Jain and Sznajder, 2007). Respiratory bronchioles and alveolar ducts both conduct and exchange gas, whereas small membranous and terminal bronchioles only conduct air. Alveoli are saclike structures (~200 μm in diameter) to carry out gas exchange. They can evaginate from respiratory bronchioles, alveolar ducts, and alveolar sacs. Airways consist of various epithelial cells including ciliated cell, goblet cell, club cell, tuft cell, neuroendocrine cell, and basal cells (Fig. 1B).

The structure of a lung is optimized to carry out its main function: gas exchange. Gas exchange takes place in the alveolar region (parenchyma). For an efficient gas exchange, air and blood are brought in close proximity over large surface, constituting an alveolar BAB (Knudsen and Ochs, 2018). The BAB consists of a continuous epithelium (AT1 and AT2), a continuous specialized endothelium (aerocyte), and a connective tissue layer between these two layers. The respiratory function of a lung is highly correlated with the structure of airways and alveolar BAB (Weibel, 2017). The thinness of the barrier is essential for efficient flux of oxygen by passive diffusion and the strength of the barrier is crucial for maintaining structural integrity against mechanical stress (Maina and West, 2005). The extracellular matrix and particularly type IV collagen in the basement membrane are assumed to be the main stress-bearing components of the thin and strong BAB.

MOUSE MODELS FOR COPD

Since one of the main pathological features of COPD is inflammation, *in vivo* immune competent mouse models are necessary for assessing effects of drugs on COPD progress. Infiltration of inflammatory cells such as macrophages, neutrophils, T and B-lymphocytes appears in the airway wall of COPD (Barnes, 2008). Airway obstruction and emphysema are other main symptoms of COPD. The structural integrity of lung airways and alveoli is highly correlated with lung function (Maina and West, 2005; Christenson *et al*., 2022). The airway obstruction of COPD may occur by a combination of small airway narrowing, airway wall inflammation, and emphysema-related loss of elastic recoil (Penman *et al*., 1970; Colebatch *et al*., 1973). Thus, *in vivo* mouse model for testing lung function is required for assessing COPD severity. There are several approaches to initiate COPD in mouse models, including exposing mice to cigarette smoke, inflammation stimuli, proteolytic enzymes, and genetic modification (Fig. 2A) (Ghorani *et al*., 2017). Here, some examples of mouse models for COPD will be introduced according to inducers of COPD (Table 1).

Cigarette smoking is the major risk factor for COPD. It is used as the most common COPD-inducer in *in vivo* animal models (Sopori, 2002; Barnes *et al*., 2003). Constituents of cigarette smoke used for exposure, delivery system (whole body vs nose-only), and the dose of smoke exposed to animals should be controlled. Different inbred mouse strains show differences in alveolar sizes that might have significant influence on lung disease models such as development of emphysema (Soutiere *et al*., 2004). Different stains of mice also show various levels of sensitivity to cigarette smoke exposure (Wright *et al*., 2008; Ghorani *et al*., 2017). Cigarette smoke can induce many features of COPD in mice, including pulmonary inflammation, airway fibrosis, emphysema, and reduced lung function (Churg *et al*., 2006; Wright *et al*., 2008; Beckett *et al*., 2013). Cigarette smoke-induced mouse model of COPD can produce pathological alterations similar to humans. However, this model does not produce a severe disabling disease shown in humans. In addition, it requires several months of exposure.

Lipopolysaccharide (LPS) is a major proinflammatory glycolipid component of Gram-negative bacteria cell walls. It exists as a contaminant in air pollution, organic dusts, and cigarette smoke (Vernooy *et al*., 2002). LPS alone or combined with cigarette smoke can induce acute COPD exacerbations (Wright *et al*., 2008). Chronic instillation of LPS to mice can also induce pathological features of COPD, including pulmonary inflammation and pathological structural changes in the lung (Vernooy *et al*., 2002). LPS may be important for developing COPD as it can mimic bacterial infection-induced exacerbations of COPD.

Based on the idea that emphysema develops as a result of cigarette-smoke-mediated influx of inflammatory cells that can release proteases known to destroy the parenchymal matrix, elastase, a proteolytic enzyme released by activated neutrophils in the lung, has been used successfully as an emphysema inducer among various proteolytic enzymes (Janoff, 1985; Wright *et al*., 2008). Only enzymes that could degrade intact elastin are known to produce emphysema, whereas collagenases are ineffective (Lieberman, 1976). This model can induce the disease easily only by a single instillation of the enzyme in the lung. In addition, it can control disease severity by adjusting the amount of the enzyme (Ghorani *et al*., 2017). The disadvantage of this model is that the function of elastase in COPD emphysema depends on several complicated pathophysiological mechanisms. Elastase emphysema mouse models by intratracheal or oropharyngeal instillation of elastase have been characterized (Vidal *et al*., 2012; Hisata *et al*., 2021).

Although cigarette smoke is the leading cause of COPD, 25-45% of patients with COPD are never smokers (Salvi and Barnes, 2009). Risk factors for COPD in never-smokers include air pollution, asthma, occupational exposures, and infections (Salvi and Barnes, 2009; Yang *et al*., 2022). Fine particulate matter (FPM) is a component of air pollutants that comprises aerodynamic particles smaller than 2.5 μm. These particles are found in a variety of sources, including biomass smoke, engine soot, and factory fumes. The impact of FPM on human health is significant as it can cause COPD by triggering inflammatory cascades and lung tissue damage (Zhang *et al*., 2015). Accordingly, in a mouse model where atmospheric FPM was collected and injected intratracheally for 3 months,

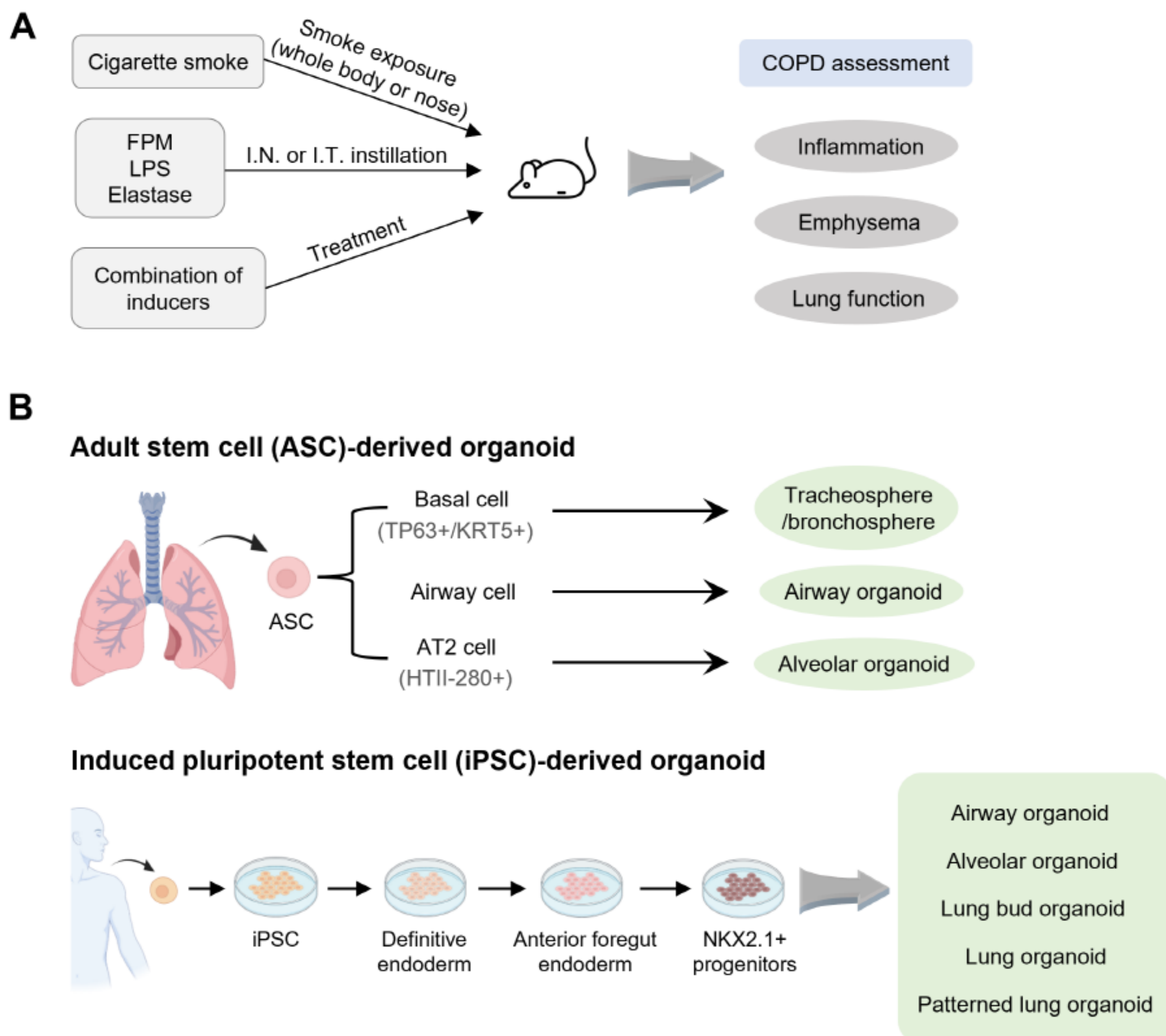

Fig. 2. Overview of experimental mouse models and human lung organoid models for modeling chronic obstructive pulmonary disease. (A) Cigarette smoke, fine particulate matter (FPM), lipopolysaccharide (LPS), elastase, or combination of inducers are used for development of COPD in mice. COPD assessment is conducted by measurement of inflammation, emphysema, and lung function. (B) Human lung organoids can be derived from both adult stem cells (ASCs) and induced pluripotent stem cells (iPSCs). Human ASCs-derived lung organoids can be generated from basal cells, airway cells, and AT2 cells derived from patients' lung specimen. NKX2.1+ progenitors derived from human iPSCs serve as the stem cell source for culture of human lung organoids. Both ASCs- and iPSCs-derived lung organoids can be applied for modeling various lung diseases including COPD. I.N., intranasal; I.T., intratracheal. Created in BioRender. Choi, H. (2024) BioRender.com/k37d254.

mice developed COPD by showing impaired lung function, emphysematous lesions, induced pulmonary inflammation, and airway wall remodeling (Li *et al*., 2020), suggesting that FPM alone could cause COPD in mice.

More complex mouse models have also been tried to recapitulate human COPD pathogenesis by combining COPD inducers. Cigarette smoke and LPS-induced COPD models have been used for studying basic mechanisms or complications of COPD, focusing on inflammation aspects of COPD (Hardaker *et al*., 2012; Pelgrim *et al*., 2022). Elastin is overexpressed in lung tissues of COPD patients. It has been reported that elastin peptides derived lung tissues can stimulate T helper cells, resulting in Th1- and Th17-polarized immune responses (Lee *et al*., 2007; Deslee *et al*., 2009). Zhou and colleagues have developed a novel mouse model presenting a cigarette smoke-induced autoimmune processes of COPD (Zhou *et al*., 2020). This model was achieved by cigarette smoke exposure and intratracheal instillation of elastin in mice. They demonstrated that MMP12-generated elastin fragments served as a self-antigen and drove cigarette smoke-induced autoimmune processes in mice. Intranasal exposure of elastase and LPS can also induce COPD-like inflammation (Sohn *et al*., 2013).

As genetically modified animal models, mice with emphy-

Table 1. Mouse models for chronic obstructive pulmonary disease

Inducer	Strain	Dose and duration	Treatment route	Measured parameters			Reference
				Inflammation	Emphysema	Lung function	
Cigarette smoke (CS)	BALB/c, C57BL/6, *mMCP-6*$^{-/-}$	2×75 min/day, 5 day/week, for 1-12 weeks	Smoke exposure (nose only)	+	+	+	Beckett *et al*., 2013
	BALB/c, *Rag2*$^{-/-}$	150 ± 15 mg/m^3 CS of TSP, 4 h/day, 5 day/week, for 13 weeks	Smoke exposure (whole body)	+	+	N.A.	Motz *et al*., 2010
	C57BL/6J, DBA/2, ICR, C57BL/6J *pa/pa* (pallid)	3 Cig (12 mg of tar and 0.9 mg of nicotine) for 90 min/day, 5 day/week, for 4 or 7 months	Smoke exposure (whole body)	N.A.	+	N.A.	Cavarra *et al*., 2001
Lipopolysaccharide (LPS)	BALB/c, *Trpm2*$^{-/-}$	0.3 mg/kg of LPS, animals were killed 3 h or 24 h after the challenge to LPS	Intra-nasal administration	+	N.A.	N.A.	Hardaker *et al*., 2012
	BALB/c	0.5 mg/kg; 100 µL of LPS	Intra-pulmonary instillation	+	N.A.	N.A.	Al Faraj *et al*., 2014
Elastase	C57BL/6	2 U of PPE/100 g body wt in 100 µL saline	Intra-tracheal instillation	N.A.	+	+	Vidal *et al*., 2012
	C57BL/6J, *Lrg1*$^{i\Delta EC}$	0.25 IU of PPE in 50 µL saline	Oropharyngeal instillation	N.A.	+	+	Hisata *et al*., 2021
Fine particulate matter (FPM)	C57BL/6J	200 µL (0.5914944 mg/mouse) $PM_{2.5}$ suspension, once/week for 2 or 3 months	Intra-tracheal instillation	+	+	+	Li *et al*., 2020
	C57BL/6	Single exposure of 5 or 50 µg of DEPs/mouse, single exposure of 1 or 5 µg of 1-NP/mouse	Intra-tracheal instillation	+	+	N.A.	Li *et al*., 2017
	BALB/c nude	120 µg /mL of PM2.5 for 14 days	Intra-tracheal instillation	+	N.A.	N.A.	Jia *et al*., 2021
Combination	BALB/c, *Trpm2*$^{-/-}$	750 µg /L of TWPM, 2×30 min/day, 3 consecutive days and LPS (0.3 mg/kg)	Smoke exposure (whole body) Intra-nasal administration (LPS)	+	N.A.	N.A.	Hardaker *et al*., 2012
	BALB/cByJ	~ 45 min/day (gradually increased in the first 5 days from 4 to 14 Cig/day and 14 Cig/day until day 72 except day 42, 52, 62) and 50 µL of 2 or 10 µg/mL LPS on day 42, 52, 62	Smoke exposure (whole body) Intra-tracheal instillation (LPS)	+	+	N.A.	Pelgrim *et al*., 2022
	C57BL/6	2.5 h/day (100 Cig), 5 days/ week, for 2 weeks and 100 µg Eln/Eln peptide in 50 µL saline every other day for 30 days	Smoke exposure (whole body), Intra-tracheal administration (Eln)	+	+	+	Zhou *et al*., 2020
	BALB/c	1.2 U of PPE on day 1 and 7 µg of LPS on day 4/week, for 4 consecutive weeks	Intra-nasal administration (PPE, LPS)	+	+	N.A.	Sohn *et al*., 2013

TSP, total suspended particulates; Cig, cigarette; Wt, weight; PPE, porcine pancreatic elastase; PM, particulate matter; DEP, diesel exhaust particles; 1-NP, 1-nitropyrene; TWPM, total wet particulate matter; Eln, elastin; +, determined; N.A., not applied.

sematous phenotype have been generated by deleting five *Serpina1a-e* genes or performing endothelial-specific deletion of hypoxia-inducible factor-2α gene (Borel *et al*., 2018; Pasupneti *et al*., 2020). Due to complex COPD pathophysiology, genetically engineered mouse models are not available since these models could not feature both chronic bronchitis-related and emphysematous changes.

HUMAN LUNG ORGANOID MODELS

Mouse models have provided the majority of understating about the development of human lung and COPD pathogenesis. However, these models do not completely represent the full capacity of a human lung. Thus, *in vitro* systems that might supplement mouse COPD models are needed for a better understanding of human COPD. Since 2D models have limits of not having physiological context or cell-to-cell communications across lung tissues, more interactive *in vitro* human organoid models need to be developed to better understand human lung maturation and pathophysiology of lung diseases and develop new therapeutic approaches. Thus, 3D culture systems that can recapitulate the microenvironment and specialized architectures of tissues *in vivo* have been tried. More committed adult stem cells derived from human lungs and multipotent stem cells derived from human induced pluripotent stem cells (iPSCs) are being used for different kinds of human lung organoid (Fig. 2B). Conducting airway organoids, alveolar organoids, and lung organoids have been developed. Airway organoids include nasospheres, tracheospheres, and bronchospheres, which are composed of pulmonary cells such as neuroendocrine, goblet, ciliated, basal, tuft, and club cells. Alveolar organoids are composed of AT1 and AT2 cells. Whole lung organoids are composed of both cell types (airway and alveolar).

Lung organoid cultures for studying lung diseases have been introduced using human adult stem cells derived from patient-specific samples or primary human bronchial epithelial cells (Table 2) (Tan *et al*., 2017; Tindle *et al*., 2021; Chan *et al*., 2022; Iakobachvili *et al*., 2022). When primary bronchial epithelial cells are combined with stromal cells (lung fibroblast and lung microvascular endothelial cells) in 3D culture conditions, mixed cells undergo rapid condensation and self-organization into epithelial and endothelial structures to generate airway organoids (Tan *et al*., 2017). In that study, both proximal and distal epithelial makers were observed over time from the proximal source of primary epithelium. A method using human broncho-alveolar resections or lavage material to establish long-term-expanding human airway organoids that consist of basal cells, multi-ciliated cells, mucus-producing secretory cells, and CC10-secreting club cells has been reported by Sachs *et al*. (2019). They also showed that the established human airway organoids could be used for studying hereditary, malignant, and infectious pulmonary diseases. Patient-derived adult stem cell-derived human lung organoid has been generated by a modified method of Sachs *et al*. (2019), but the discrete airway structure was not observed in the organoid, although it contained both proximal and distal epithelia (Tindle *et al*., 2021). Another group also reported a human bronchiolar organoid established from patient lung biopsy for testing mycobacteria-host interactions (Iakobachvili *et al*., 2022). It displayed a cystic structure that is composed of basal cells lined with ciliated and goblet cells on the lumen facing side. COPD organoids have been also established as nasopharyngeal and bronchial organoids from patient samples (Chan *et al*., 2022). Goblet cell hyperplasia and reduced ciliary beat frequency were observed in COPD organoids, which recapitulated hallmark features of the disease. They also showed that viral and bacterial exposure could induce greater proinflammatory responses in COPD organoids compared to heathy organoids, although organoids were generated from different individual donors rather that paired specimens due to limitation in sampling. Other researchers have also established several kinds of lung organoids including alveolar organoids as well as upper airway organoids, which can be applied in the establishment of COPD organoid (Rock *et al*., 2009; Barkauskas *et al*., 2013; Danahay *et al*., 2015; Zacharias *et al*., 2018; Tran *et al*., 2022; Alysandratos *et al*., 2023). Since one of the symptoms of COPD is emphysema, alveolar organoids can be useful for better understanding the pathology of COPD.

The establishment of protocols for generating human iPSC-derived lung organoids is a major advance in lung disease modeling, drug screening, and regenerative medicine (Table 3) (Vazquez-Armendariz and Tata, 2023). Efforts have been made to generate populations of immature lung epithelial and mesenchymal progenitors that can be highly expanded. In general, iPSCs are sequentially directed toward definitive endoderm, ventral anterior foregut endoderm cells (VAFECs), and NKX2-1+ lung progenitors (Green *et al*., 2011; Huang *et al*., 2014, 2015). Carboxypeptidase M-positive (CPM+) surface marker can be used to isolate lung progenitors from VAFECs (Gotoh *et al*., 2014). Organoids derived from VAFECs contain NKX2-1-and CPM double positive cells besides AT1 and AT2 cells. $CD47^{high}/CD26^{neg}$, a cell surface phenotype, has also been successfully used to isolate PSC-derived NKX2-1+ lung progenitors (Hawkins *et al*., 2017). Lung organoids derived from PSCs are generally generated from NKX2-1+ cells (Vazquez-Armendariz and Tata, 2023). Depending on specific factors added to the culture, NKX2-1+ cells can result in the formation of airway organoids, alveolar organoids, lung organoids, and bud tip organoids. PSCs-derived lung organoids have been used for modeling diseases including pulmonary fibrosis and hereditary diseases such as cystic fibrosis (Wong *et al*., 2012; Firth *et al*., 2015; Kim *et al*., 2021; Suezawa *et al*., 2021). Disease-specific lung progenitors have been generated from iPSCs derived from patients with cystic fibrosis to create a platform for understanding human lung diseases (Mou *et al*., 2012). Moreover, when single cell suspension dissociated from PSCs-derived airway organoids was replated in a 2D air-liquid interface culture, these cells displayed multilineage differentiation, showing a possibility of application for airway disease modeling (McCauley *et al*., 2017; Hawkins *et al*., 2021). In a recent study, a co-culture model of iPSC-alveolosphere with fibroblast derived from COPD patient lung was explored for studying epithelial-mesenchymal crosstalk in COPD (Dagher *et al*., 2024). Despite recent advances in organoid technology, a direct COPD model using iPSCs-derived lung organoids has not been reported yet.

FUTURE PERSPECTIVES

Despite pros and cons of mouse COPD models, these models are necessary to assess the development of human

Table 2. Human lung organoid models using adult stem cells for disease modeling

Organoid type	Stem cell	Cellular composition	Application	Reference
Tracheosphere	Basal cells	Basal cells (TP63+/KRT5+/KRT14+/NGFR+), ciliated cells (ACT+)	N.A.	Rock *et al*., 2009
Bronchosphere	Basal cells	Basal cells (TP63+), ciliated cells (ACT+), goblet cells (MUC5B+)	Goblet cell metaplasia modeling	Danahay *et al*., 2015
Bronchial/Bronchiolar/Airway organoid	Bronchial epithelial cells	Ciliated cells (ACT+), club cells (SCGB1A1+), goblet cells (MUC5AC+), AT1 cells (AQP5+/HOPX+/PDPN+), AT2 cells (SFTPC+)	Pulmonary fibrosis modeling	Tan *et al*., 2017
	Tracheobronchial epithelial cells	Ciliated cells (ACT+), club cells (SCGB1A1+), goblet cells (MUC5AC+/MUC5B+)	N.A.	Boecking *et al*., 2022
	Airway cells	Basal cells (TP63+), ciliated cells (ACT+), club cells (SCGB1A1+), goblet cells (MUC5AC+)	COPD modeling	Chan *et al*., 2022
		Basal cells (KRT5+), ciliated cells (ARL13B+), club cells (SCGB1A1+), goblet cells (MUC5AC+)	SARS-CoV-2 and influenza virus infection	Ekanger *et al*., 2022
		Basal cells (KRT5+), ciliated cells (ACT+), goblet cells (MUC5AC+), club cells (SCGB1A1+)	Mycobacteria infection	Iakobachvili *et al*., 2022
		Basal cells (KRT5+), ciliated cells (ACT+), goblet cells (MUC5AC+), club cells (SCGB1A1+)	Cystic fibrosis modeling and RSV infection	Sachs *et al*., 2019
		Basal cells (TP63+/KRT5+), ciliated cells (ACT+), club cells (SCGB1A1+)	Primary ciliary dyskinesia modeling	van der Vaart *et al*., 2021
		Basal cells (KRT5+), ciliated cells (ACT+), club cells (SCGB1A1+), goblet cells (MUC5AC+), AT1 cells (AQP5+), AT2 cells (SFTPC+/SFTPB+)	SARS-CoV-2 infection	Tindle *et al*., 2021
		Basal cells (TP63+/KRT5+), ciliated cells (ACT+), club cells (SCGB1A1+), goblet cells (MUC5AC+)	Influenza virus infection	Zhou *et al*., 2018
Alveolar organoid	EpCAM+/ HTII-280+ AT2 cells	AT2 cells (SPFTPC+/HTII-280+)	N.A.	Barkauskas *et al*., 2013
		AT2 cells (SPFTPC+/HTII-280+)	N.A.	Alysandratos *et al*., 2023
	EpCAM+ AT2 cells	AT1 cells (AQP5+), AT2 cells (HTII-280+)	N.A.	Tran *et al*., 2022
	HTII-280+/TM4SF1+ cells	AT1 cells (AQP5+), AT2 cells (SFTPC+)	N.A.	Zacharias *et al*., 2018

AT1, alveolar type 1 epithelial cell; AT2, alveolar type 2 epithelial cell; COPD, chronic obstructive pulmonary disease; RSV, respiratory syncytial virus; N.A., not applied.

Table 3. Human lung organoid models using iPSCs for disease modeling

Organoid type	Stem cell	Cellular composition	Application	Reference
Airway organoid	NKX2.1+TP63+NGFR+ basal cells	Basal cells (TP63+), ciliated-like cells (ACT+), club cells (SCGB1A1+)	Airway disease modeling	Hawkins *et al*., 2021
	CD47^{hi}CD26^{neg} lung progenitors	Basal cells (TP63+/KRT5+), ciliated cells (ACT+), club cells (SCGB1A1+)	Cystic fibrosis modeling	McCauley *et al*., 2017
	CPM+ VAFECs	Basal cells (KRT5+), ciliated cells (ACT+/SNTN+), neuroendocrine cells (CHGA+/SYN+), mucus-producing cells (MUC5AC+), club cells (SCGB1A1+)	N.A.	Konishi *et al*., 2016
	NKX2.1+CPM+ BTP-like cells	Basal cells (TP63+), goblet cells (MUC5A+), club cells (SCGB1A1+/SCGB3A2+), ciliated cells (ACT+), neuroendocrine cells (CHGA+/SYN+/ASCL1+)	N.A.	Hein *et al*., 2022
Alveolar organoid	CPM+ VAFECs	lung progenitor cells (NKX2.1+), AT2 cells (SFTPC+), AT1 cells (AQP5+)	N.A.	Gotoh *et al*., 2014
	CPM^{hi} NKX2.1+ cells	AT2-like cells (SFTPC+/SFTPB+) AT1-like cells (PDPN+/AQP5+)	Drug toxicology testing	Yamamoto *et al*., 2017
	CD47^{hi}CD26^{lo} lung progenitors	Lung progenitor cells (NKX2.1+), AT2 cells (SFTPC+/SFTPB+/MUC1+)	N.A.	Hawkins *et al*., 2017
	NKX2.1+CPM+ BTP-like cells	Lung progenitor cells (NKX2.1+), AT2 cells (SFTPC+/SFTPB+)	N.A.	Hein *et al*., 2022
	NKX2.1+ lung progenitors	Lung progenitor cells (NKX2.1+), AT2 cells (SFTPC+/SFTPB+)	Alveolar disease modeling	Jacob *et al*., 2017
		AT2 cells (SFTPC+/SFTPB+/ABCA3)	SARS-CoV-2 infection	Gandikota *et al*., 2024
		AT2 cells (SFTPC+)	SARS-CoV-2 infection	Dagher *et al*., 2024
Lung bud organoid	AFECs	Goblet cells (MUC5AC+), club cells (SCGB3A2+), AT2 cells (SFTPC+/HTII-280+/MUC1+)	RSV infection	Chen *et al*., 2017
Patterned lung organoid		Airway-like cells (SOX2+/MUC5AC+/SCGB1A1+), bud tip–like cells (SOX2+/SOX9+/SFTPC+/ID2+)	N.A.	Miller *et al*., 2018, 2019
Lung organoid		Basal-like cells (TP63+), ciliated cells (FOXJ1+), AT1 cells (HOPX+), AT2 cells (SFTPC+)	N.A.	

iPSC, induced pluripotent stem cell; BTP, bud tip progenitor; AT1, alveolar type 1 epithelial cell; AT2, alveolar type 1 epithelial cell; VAFEC, ventral anterior foregut endoderm cell; AFEC, anterior foregut endoderm cell; RSV, respiratory syncytial virus; N.A., not applied.

lung and COPD pathogenesis. Various experimental methods have been tried for studying COPD in mice. There is no standard protocol for parameters to be determined or experimental procedures to be performed. Methods for COPD induction and measurable and characteristic parameters should be further developed to better represent human COPD.

To complement mouse COPD models, human lung organoids can serve as a promising model system for advancing translational research. Organoids based on ASCs provide fundamental insights into generation and maintenance of limited lung tissues. In case of COPD modeling, pharyngeal and bronchial COPD organoids generated from ASCs derived from a patient can recapitulate the pathology of COPD disease. However, there are limitations in sampling and expanding ASCs. In this regard, lung organoids based on human healthy or patient PSCs can be a promising tool for studying COPD and other lung diseases and for screening drugs for drug discovery. PSCs-derived organoids are also useful for understanding development stages of a human lung organ.

Many useful organoid models of the lung have been reported using ASCs and PSCs for studying various aspects of lung development. Lung is a complex organ that functions as a respiratory system. Its structure is highly correlated with respiratory function. At present, specialized epithelial cell differentiation and cell-cell adhesion are mainly assessed and its morphological, structural differentiation for lung function has been poorly achieved in lung organoids. Especially, the intact structure of alveoli, an important gas-exchange unit, should be developed and assessed in generation of alveolar organoids to better understand COPD. Additional culture optimization is necessary to establish human organoids that can more accurately recapitulate the lung architecture. Until now, most organoids have been developed with limited lung tissues. Since rapid developments are being made in recent years, we can anticipate the generation of a whole lung model that can be translated into human use.

CONFLICT OF INTEREST

The authors have no competing financial interests to disclose.

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