



Published in final edited form as:

Cold Spring Harb Perspect Biol. ; 14(10): . doi:10.1101/cshperspect.a040873.

Lung Regeneration: Cells, Models, and Mechanisms

Arvind Konkimalla^{1,2}, Aleksandra Tata¹, Purushothama Rao Tata^{1,3,4,5}

¹Department of Cell Biology, Duke University School of Medicine, Durham, North Carolina 27710, USA

²Medical Scientist Training Program, Duke University School of Medicine, Durham, North Carolina 27710, USA

³Duke Cancer Institute, Duke University School of Medicine, Durham, North Carolina 27710, USA

⁴Center for Advanced Genomic Technologies, Duke University, Durham, North Carolina 27710, USA

⁵Duke Regeneration Center, Duke University School of Medicine, Durham, North Carolina 27710, USA

Abstract

Lung epithelium, the lining that covers the inner surface of the respiratory tract, is directly exposed to the environment and thus susceptible to airborne toxins, irritants, and pathogen-induced damages. In adult mammalian lungs, epithelial cells are generally quiescent but can respond rapidly to repair of damaged tissues. Evidence from experimental injury models in rodents and human clinical samples has led to the identification of these regenerative cells, as well as pathological metaplastic states specifically associated with different forms of damages. Here, we provide a compendium of cells and cell states that exist during homeostasis in normal lungs and the lineage relationships between them. Additionally, we discuss various experimental injury models currently being used to probe the cellular sources—both resident and recruited—that contribute to repair, regeneration, and remodeling following acute and chronic injuries. Finally, we discuss certain maladaptive regeneration-associated cell states and their role in disease pathogenesis.

Lung epithelium serves as the largest interface between the environment and our body tissues and facilitates efficient gas exchange. Lifelong exposure to environmentally derived inhaled particles, pathogens, and toxic chemicals makes it vulnerable to local tissue damage, which can potentially spread to other tissues and organs. Therefore, respiratory health is vital for the well-being and survival of an organism. Whereas the composition and function of cells significantly vary along the proximal to distal axis of the respiratory tract, recent studies have demonstrated that cells from one region can contribute to the repair and regeneration of damaged tissues in a neighboring region by virtue of extreme plasticity mechanisms. Studies have revealed that the mechanisms of tissue repair significantly vary depending on the extent of damage (acute vs. chronic), site of damage (airway vs.

alveoli) and the type of damage causing agent (pathogen, particles, or toxins). Therefore, understanding the anatomy, cellular composition, and regenerative mechanisms following different forms of injuries is vital to harness the endogenous regenerative capacity of lung tissues.

ANATOMY OF THE LUNG

The architecture and complexity of the respiratory system have evolved to facilitate efficient gas exchange and meet the metabolic needs of the organism. The respiratory system can be divided into two major functional subdivisions—gas-conducting tubular airways and gas-exchanging alveolar sacs (Fig. 1A,B). The lower respiratory tract, starting from the trachea that bifurcates into mainstem bronchi, which repeatedly branches into increasingly smaller bronchi and bronchioles, all together constitutes the entire gas conducting tubular network (Weibel 1963, 2015; Crapo et al. 1982). The terminal airways lead into saccular alveoli, which facilitate gas exchange. While the basic organization of the lungs among mammals is similar, there are significant interspecies differences in tissue complexity and cellular composition across species. One striking example is the variable number of bronchial generations between different species. For instance, the human and pig tracheobronchial tree on average consists of 23 bronchial generations, whereas the number of generations in mice is about 13–17 (Weibel 2015). Another significant difference is the transitional zone between conducting airways and gas exchange units in various species. In human, monkey, dog, cat, and ferret lungs, the terminal bronchioles are followed by one to several generations of respiratory bronchioles (Weibel 1963, 2015; Castleman et al. 1975; Hyde et al. 1979; Pinkerton and Joad 2000). In contrast, respiratory bronchioles are absent in mice and rat lungs in which terminal bronchioles open directly into alveolar ducts (Schwartz et al. 1976; Zitnik et al. 1978).

In addition, the branching pattern of the airway tree varies between species. In mammals, including the mouse, rat, dog, pig, donkey, and horse, airway branching, although stereotypic in early phases, follows a monopodial branching pattern (Plopper et al. 1983; Onuma et al. 2001). In contrast, the humans and monkey lungs exhibit a dichotomous branching pattern, where branches separating from the parent airway are equal in size and diameter (Nikiforov and Schlesinger 1985). Furthermore, the localization and the density of submucosal glands (SMGs) vary among species (Choi et al. 2000). SMGs are embedded within the mesenchyme, open to the airway lumen and directly connect with surface epithelium (SE) through specialized ducts. In humans, SMGs are found in the trachea and extend into intralobular bronchi up to 8–10 bronchial generations. In contrast, SMGs in mice are localized only in the proximal part of the trachea (Borthwick et al. 1999; Liu and Engelhardt 2008).

CELLULAR COMPOSITION OF THE AIRWAY AND ALVEOLI

Classical studies using histological and ultrastructural analysis identified different cell types based on their morphology and ultrastructural features such as secretory granules, protrusions, or multicilia (on ciliated cells). In recent years, the advent of technologies such as single-cell RNA sequencing (scRNA-seq), spatial transcriptomics, multiplex in situ

analysis, and advanced three-dimensional imaging has led to the identification of novel cell types, subpopulations, cell markers, and changes in gene expression patterns in healthy and disease tissues.

The epithelium of large airways is a simple pseudostratified structure, populated by three main cell types—basal, secretory club, and ciliated cells (Fig. 1). Collectively, these three cell types constitute >90% of the airway epithelium (Mercer et al. 1994; Boers et al. 1998; Nakajima et al. 1998; Evans et al. 2001). Basal cells (expressing Transformation Related Protein 63 [TP63], keratin 5 [KRT5], and nerve growth factor receptor [NGFR]) are relatively small and tightly attached to the basement membrane and serve as progenitor cells (Evans et al. 1989; Borthwick et al. 2001; Rock et al. 2010). Classical immunostaining analysis revealed two morphologically indistinguishable basal cell subsets that differ in the presence or absence of expression of KRT14 (Hong et al. 2004a,b; Schoch et al. 2004). However, recent single-cell transcriptome data have revealed the presence of *Krt14* transcripts in all basal cells. Additionally, KRT14 protein expression is seen in all regenerating basal cells after airway injury. This suggests a role for posttranscriptional mechanisms that may underlie basal cell identity at homeostasis and injury repair. Single-cell transcriptome studies further revealed proliferating basal cells as well as another cell population, termed “hillock cells,” that expressed *Krt4* and *Krt13* (Fig. 1A,C; Montoro et al. 2018). These cells are organized in “hillocks” interspersed among other basal and luminal cells. Interestingly, immunostaining analysis on airways from smokers have revealed a significant increase in the number of KRT13-expressing cells in squamous metaplastic regions, suggesting that they provide a protective “barrier-like” function (Bolton et al. 2009; Duclos et al. 2019; Goldfarbmuren et al. 2020). The functional role for this spatially disparate organization and the role of these cells remains unknown. Columnar secretory club cells express secretoglobins including secretoglobin family 1A member 1 (SCGB1A1), secretoglobin family 3A member 1 (SCGB3A1), and proteins involved in the detoxification process, such as Cytochrome P450 Family 2 Subfamily F (CYP2F). Ciliated cells (marked by forkhead box J1 [FOXJ1] and tubulin β class IVA [TUBB4]) are another columnar cell population attached to the basement membrane and contain multiple cilia on their apical surfaces enfacing the airway lumen. The numbers of basal, club and ciliated cells vary among the mammalian species as well as along the proximodistal axis (Okuda et al. 2021).

The remaining infrequent cell populations of the airways include goblet, neuroendocrine (NE), tuft cells, and most recently identified ionocytes (Fig. 1A,C). Ionocytes are characterized by the presence of significantly higher expression of cystic fibrosis transmembrane conductance regulator (CFTR) protein and other markers, including transcription factor forkhead box I1 (FOXI1) (Montoro et al. 2018; Plasschaert et al. 2018; Scudieri et al. 2020). Previous studies have noted the presence of rare cells expressing high levels of *Cftr* transcripts in airway cells and termed them “CFTR hot cells” (Engelhardt et al. 1994). However, until recently, their molecular profiles and functions were unknown. The density of ionocytes is significantly higher in large airways, whereas CFTR expression can be readily detected in secretory cells of the small airways. This suggests that large and small airways use different cell types for CFTR-mediated airway luminal ionic balance and fluid transport. Additionally, ionocytes may have additional functions other than CFTR-mediated fluid transport. Goblet cells can be recognized by their characteristic goblet shape and

expression of mucins 5AC (MUC5AC) and 5B (MUC5B) (Whitsett 2018; Okuda et al. 2019). Recent single-cell transcriptome studies have identified distinct subsets within goblet, NE, and tuft cells. However the physiological relevance of these subsets remains unknown (Montoro et al. 2018; Plasschaert et al. 2018). NEs exist as solitary cells or in clusters and are characterized by the expression of calcitonin gene-related peptide (CGRP) and PGP9.5 (Franks et al. 2008; Song et al. 2012; Kuo and Krasnow 2015; Noguchi et al. 2015; Branchfield et al. 2016; Ouadah et al. 2019; Shivaraju et al. 2021). Tuft cells are predominantly found in the trachea and contain microvilli on the apical surface (Tizzano et al. 2006, 2011; Saunders et al. 2013; Montoro et al. 2018). Recent studies identified two subpopulations of tuft cells (Montoro et al. 2018). Whereas tuft, NE, and goblet cells are very rare during homeostasis, their numbers can rapidly expand in varying human disease states, directly impacting disease severity. For example, tuft cells increase in number after influenza infection, and they originate from basal cells (Rane et al. 2019). The mechanisms controlling such expansion of tuft cells in these injury models remain unknown.

Underneath the airway epithelium is a bed of fibroblasts and various supporting niche cells. The proximal airways are surrounded by cartilage rings or plates, which serve to provide structural support. Interspersed among these and underneath the SE are SMGs composed of distal acinar structures that connected to a highly branched tubular network, which in turn leads into ciliated conducting ducts that finally open into the airway lumen (Fig. 1A,C; Tos 1966; Choi et al. 2000; Widdicombe and Wine 2015). The structure of the conducting ducts closely resembles that of the SE and contains similar cell types, including basal, ciliated, and secretory cells (Meyrick et al. 1969). However, recent studies have shown some differences at the molecular level (sex determining region Y-box 9 [SOX9] expression in ducts but not on the SE) (Tata et al. 2018). The distal most regions of SMGs consist of saccular acini that are composed of mucous (MUC5B and trefoil factor 2 [TFF2]), serous (lactoferrin [LTF] and lysozyme [LYZ]), and myoepithelial cells (MECs) (actin α 2 [ACTA2], transformation-related protein 63 [TP63], and keratin 5 [KRT5]).

The alveolar compartment is organized to maximize gas exchange by thin, expansive alveolar type 1 epithelial cells (AT1s) that occupy about 95% of the total surface area (Fig. 1B,C; Crapo et al. 1982). Alveolar type 2 epithelial cells (AT2s), on the other hand, are cuboidal and sparsely distributed and are estimated to occupy about 5% of the alveolar luminal surface (Crapo et al. 1982; Mason 2006; Weibel 2015). Recent studies have identified heterogeneity within the AT2 population based on either the expression of Wnt target gene *Axin2* (axis inhibition protein 2) and, in another subpopulation, cluster of differentiation 44 (CD44) (Chen et al. 2017; Nabhan et al. 2018; Zacharias et al. 2018). Studies from preterm birth and other pediatric respiratory conditions have revealed a significant role for AT2s in lung function as some of their products, such as surfactants, are necessary for maintaining alveolar tension and preventing alveolar collapse (Whitsett et al. 2004). In recent years, subsets of AT2 and AT1 have been identified, with unique functional roles in alveolar renewal and regeneration, and these will be described in further detail below.

Underneath the airway and alveolar epithelium is a complex network of mesenchymal supporting cells composed of fibroblasts, endothelium, and resident immune cells. The full

heterogeneity of these cells is being explored with the advent of single-cell sequencing technologies and spatial transcriptomics and we refer the readers to other excellent papers and reviews that described these populations (Hogan et al. 2014; Zepp et al. 2017; Zepp and Morrissey 2019; Adams et al. 2020; Basil et al. 2020; Deprez et al. 2020; Habermann et al. 2020; Kobayashi et al. 2020; Liu et al. 2020b; Strunz et al. 2020; Travaglini et al. 2020; Vila Ellis et al. 2020; Delorey et al. 2021; Okuda et al. 2021).

EXPERIMENTAL MODELS OF LUNG INJURY AND REGENERATION

The adult mammalian lung epithelium is quiescent at homeostasis but quickly responds to injury to repair and regenerate damaged tissues (Hogan et al. 2014; Tata and Rajagopal 2017; Basil et al. 2020). Different types of injury-causing agents, most of which are environmentally derived, include pathogens, chemicals, and particulate matter and target different cells or tissues. Over the years, several experimental injury models have been developed. Most of these models were adapted from clinical observations of humans who had been exposed to different injury-causing agents (Table 1). For example, bleomycin, a deoxyribonucleic acid (DNA) intercalating agent, was prescribed as a chemotherapeutic agent but its side effects, including lung injury and fibrosis, led to a shift in its use to that of an experimental fibrosis-inducing agent (Adamson and Bowden 1974). Injury models can be divided into three categories, based on the agent used, which are either (1) environmentally derived pollutants and chemical agents, (2) pathogens (viruses, bacteria), and (3) treatments causing mechanical/physical/selective cell ablation, including surgical lung lobe removal. These different injury models revealed a surprisingly wide variety of region-specific stem/progenitor cell populations as well as cell plasticity mechanisms that participate in the regeneration of the airway and alveoli (Table 1; Matute-Bello et al. 2008).

Table 1 summarizes different forms of injury-causing agents (chemical, biological, and other), the route of administration, target cells, and responding cells.

Pollutants and Chemical Exposures as Injury-Repair Models

Whereas gaseous agents are more common than particulate ones, both have been widely used as injury models to study lung regeneration. Historically, most of these agents have come into the spotlight due to their use in chemical warfare and accidental or intentional release of toxicants from industries (Morabia et al. 1988). The target cell/tissue damage may depend on the degree of solubility of the agent or its derivatives and their mechanism of action in cells. For example, high and intermediate soluble chemicals (such as chlorine, SO₂, and bromine) tend to show damage primarily in upper and segmental bronchi, whereas poorly soluble chemicals (such as phosgene) (Summerhill et al. 2017) cause damage to the alveoli. Accordingly, different chemicals are being used as injury-causing agents to study cellular responses in large and small airways and alveolar tissues in small animal models such as mice and ferrets. Moreover, some of these chemicals recapitulated human lung disease pathophysiology and therefore have further evolved as tools to study and model certain lung diseases. For example, sulfur dioxide (SO₂), nitrogen dioxide (NO₂), cigarette smoke, and ozone (O₃) cause severe lung injury and development of respiratory diseases

such as asthma and chronic obstructive pulmonary disease (COPD) in humans (Lamb and Reid 1969; Riedel et al. 1988; Borthwick et al. 2001; Song et al. 2012).

Almost all gaseous chemicals and their derivatives can directly cause tissue damage. For example, inhalation of high doses of SO₂, which rapidly solubilizes in airway per-ciliary fluid and converts into sulfuric acid that causes death and sloughing of luminal cells of large airway SE, leaves behind only a few basal and club cells (Lamb and Reid 1968; Riedel et al. 1988; Borthwick et al. 2001; Hong et al. 2004a; Kodavanti et al. 2006; Rawlins et al. 2009; Rock et al. 2009, 2011; Song et al. 2012; Tata et al. 2018). As described in detail in a later section, cell-tracing animal models have provided further insights into the relative contribution and potential of different cell populations in repairing damaged tissues (Borthwick et al. 2001; Rawlins et al. 2007, 2009; Rock et al. 2009; Barkauskas et al. 2013; Chung and Hogan 2018; Tata et al. 2018; Liu et al. 2019, 2020a; Salwig et al. 2019). In humans, exposure to high doses of NO₂ results in fatal pulmonary edema and rapid death, whereas lower doses of NO₂ lead to the development of bronchiolitis, pulmonary edema, bronchial pneumonia, and acute bronchitis (Grayson 1956; Lowry and Schuman 1956). Nevertheless, exposure to NO₂ leads to shedding of the airway epithelium, cilia damage, pulmonary edema, damage to AT1 cells, AT2 cell hyperplasia, and increased thickening of the alveolar wall (Evans et al. 1981; Ranga and Kleinerman 1981; Foster et al. 1985; Rombout et al. 1986; Barth and Müller 1999). Similarly, ozone (O₃) (Broeckaert et al. 1999; Sunil et al. 2013; Michaudel et al. 2018), chlorine (Tian et al. 2008; Tuck et al. 2008; Musah et al. 2012), bromine (Rupp and Henschler 1967; Morabia et al. 1988; Woolf and Shannon 1999); and povidocanol (a non-ionic detergent used as a sclerosing agent) (Borthwick et al. 2001; Farrow et al. 2018) exposures denude epithelial tissues and damage underlying endothelial cells and connective tissues. Some chemical agents themselves are not toxic but their metabolic derivatives are harmful and cause tissue damage. Low doses of naphthalene (NA), an aromatic hydro-carbon found in cigarette smoke, predominantly kills club cells in the airway SE, whereas high doses cause widespread damage of the SE and SMGs in the lung (Buckpitt et al. 1995; Stripp et al. 1995; Van Winkle et al. 1995; Hong et al. 2001; Giangreco et al. 2002; Kim et al. 2005; Rawlins et al. 2009; Lynch et al. 2018; Tata et al. 2018).

The most commonly used chemical agents for the alveolar injury are bleomycin, asbestos (Behrens 1951; Wagner 1963; Bozelka et al. 1983; Mossman et al. 1990; Manning et al. 2002; Cheresh et al. 2015; Kim et al. 2016), hypoxia (Pritchard et al. 2004; Bouvry et al. 2006; Yee et al. 2014, 2016; Vohwinkel et al. 2015), hyperoxia (Frank et al. 1978; Smith 1985; Rawlins et al. 2009; Kallet and Matthay 2013; Desai et al. 2014; Nabhan et al. 2018; Penkala et al. 2021), diacetyl (Harber et al. 2006), and acid aspiration (Mendelson 1946; Hudson et al. 1995). As alluded to above, bleomycin has been used to induce lung injury and mimic pulmonary fibrosis (Table 1; Aso et al. 1976; Bigby et al. 1985; Isakson et al. 2001; Kim et al. 2005; Moore and Hogaboam 2008; Barkauskas et al. 2013; Vaughan et al. 2015; Kathiriya et al. 2020; Kobayashi et al. 2020). Similarly, the use of hydrochloric acid (HCl) came from clinical observations as one of the important risk factors involved in the development of acute respiratory distress syndrome (ARDS) is aspiration of gastric content (Mendelson 1946; Hudson et al. 1995). Because gastric content has low power of hydrogen (pH), HCl has been used to study lung damage in animal models. HCl injury by

instilling acid to the trachea or bronchi induces damage to the alveolar cells and increases capillary permeability, which leads to the development of acute lung injury and pulmonary fibrosis (Kennedy et al. 1989; Modelska et al. 1999; Marinova et al. 2019; Tavares et al. 2019). Diacetyl, a toxic flavoring chemical used in the food industry was shown to cause airway damage and lead to bronchiolitis obliterans and small airway obstruction (Harber et al. 2006). Subsequently, it was used in animal models and caused severe epithelial damage including necrosis and sloughing SE, with development of bronchial and bronchiolar fibrotic lesions (Colley et al. 1969; Hubbs et al. 2008; Morgan et al. 2008; Palmer et al. 2011; McGraw et al. 2020).

Biological Agents Used to Study Lung Injury Repair

Numerous viruses, bacteria, fungi, allergens, and microbial products have been shown to cause damage to lung tissues, which can progress to life-threatening diseases. Over the years, some of these clinically relevant pathogens or their derivatives have been adapted to study injury repair processes in model organisms. Lipopolysaccharide (LPS) is a glycolipid present in the cell wall of Gram-negative bacteria, including *Escherichia coli*. LPS is one of the widely used biological agents to study sepsis in rodent models. LPS when administered through intranasal inhalation or tracheal installation, can alter alveolar membrane permeability and recruit of immune cells such as activated macrophages and neutrophils to the site of injury (Stolk et al. 1992; Brass et al. 2008; Rittirsch et al. 2008; Sagiv et al. 2018; Yang et al. 2019). The site of damage and cellular responses, including inflammation, can vary depending on the bacterial strains used. Among the viruses, influenza virus infection in rodent models has been widely used to study lung damage, as this model recapitulates human ARDS and respiratory failure. After the 1957 global influenza pandemic (Asian flu), an observation made on human lung samples revealed the dramatic cytopathogenic changes in the epithelium of the respiratory tract but also described the regeneration of the airway and alveolar epithelium into an extended monolayer of the epithelium (Hers et al. 1958). To study the pathogenesis of influenza infection, mouse models were developed, and several influenza strains were tested, including a mouse-adapted version of a pandemic Hemagglutinin Type 1 and Neuraminidase Type 1 (H1N1) Puerto Rico 8 strain (PD8) (Loosli et al. 1975; Kumar et al. 2011; Zheng et al. 2012, 2014; Zuo et al. 2015; Kanegai et al. 2016; Tata et al. 2018; Zacharias et al. 2018). This mouse-adapted strain has been widely used to study viral infection-induced injury repair and disease pathogenesis.

Certain allergens, including house dust mites (HDMs) can induce airway inflammation, obstruction, and remodeling, which can lead to chronic lung diseases such as asthma (Bousquet et al. 2000). Some of the widely used allergens such as HDM, cockroach extracts, and ovalbumin (OVA), can induce a robust allergic inflammation in animal models (Herbert et al. 1995; Woolf and Shannon 1999; Sarpong et al. 2003; McMillan and Lloyd 2004; Locke et al. 2007; Royce et al. 2014; Shi et al. 2017). OVA is derived from chicken eggs and when introduced to immunized model animals through inhalation causes chronic airway inflammation and structural remodeling (Sarpong et al. 2003; Johnson et al. 2004).

Mechanical and Radiation-Induced Lung Injury-Repair Models

In 1931, the first successful total pneumonectomy (PNX)—surgical removal of a lung lobe—was performed by Rudolph Nissen, and since then, PNX has become a valuable clinical intervention for lung cancer patients (Nissen 1980). Clinical follow-up studies of patients after PNX lead to the observation that the remaining lungs expand by hypertrophy and hyperplasia, both contributing to physiological compensation. The degree of compensatory growth has been well documented and established in clinical follow-up examination as documented based on serial computed tomography (Butler et al. 2012). Experimental PNX in animal models similarly induces regrowth of remaining lung and restoration of pulmonary function (Brody et al. 1978; Uhal and Etter 1993; Chamoto et al. 2013; Jain et al. 2015; Vaughan et al. 2015; Chung et al. 2018; Wang et al. 2018). Lineage tracing coupled with PNX have revealed epithelial, endothelial, and mesenchymal cell contribution to compensatory lung growth. Additionally, this “injury model” is very valuable for studying mechanosignal transduction pathways (Liu et al. 2016).

Radiation pneumonitis and radiation fibrosis are two clinical manifestations that were initially observed in patients who underwent radiation therapy to treat cancers (Tsoutsou and Koukourakis 2006; Hanania et al. 2019). Follow-up studies revealed that radiation causes damage to alveolar epithelial cells, breakdown of vascular capillaries, collagen accumulation, and fibroblast proliferation (Karvonen et al. 1987; Franko et al. 1991; Johnston et al. 1998; Theise et al. 2002; Citrin et al. 2013). Since then, different types of radiation (X-rays and gamma rays) at different doses have been used to study lung injury repair and to model chronic lung diseases such as lung fibrosis.

CELL LINEAGES AND PLASTICITY IN LARGE AIRWAY EPITHELIAL REGENERATION

In the proximal airways, basal cells can self-renew and generate all cell types constituting the pseudostratified epithelium, as demonstrated by *in vivo* lineage tracing as well as *ex vivo* reconstruction of airway cellular lineages in air–liquid interface cultures (Fig. 2A; Randell et al. 1991; Liu et al. 1994; Hong et al. 2004b; Rock et al. 2009, 2010, 2011). Previous models of airway regeneration suggested that basal cells first go through a transient intermediate population, which then bifurcates into secretory or ciliated cells via a Notch-dependent mechanism (Rock et al. 2011). However, recent time course analysis coupled with molecular mapping has revealed that cell fate segregation occurs in the basal cells without going through a transient intermediate state. Such bifurcation into ciliated and secretory primed basal cell subsets can be identified based on the expression of activated Notch (NICD1) and *c-Myb* expression, respectively. This model was further supported by Notch loss of function studies in which basal cell fate was skewed toward ciliated cells. Clonal lineage-tracing studies further revealed that the basal cell population is heterogeneous (Pardo-Saganta et al. 2015; Watson et al. 2015). More recently, *in vivo* lineage tracing in adult mouse trachea coupled with scRNA-seq (termed Pulse-seq) provided a comprehensive lineage dynamic and revealed that basal cells can give rise to all cell types on the SE including the rare populations such as the NE, tufts, and ionocytes (Fig. 2A; Rock et al. 2011; Mori et al. 2015; Montoro et al. 2018; Plasschaert et al. 2018; Rane et al. 2019; Ruiz García et al. 2019).

In addition, *in silico* lineage prediction studies identified a novel basal-to-club progenitor population, characterized by the expression of *Krt4* and *Krt13* (Montoro et al. 2018). Future studies using *Krt13* promoter-driven lineage tracing studies are needed to empirically test the potential progenitor function of these cells (Fig. 2A).

Although basal cells are the main source of cells contributing to large airway regeneration following injury, other cell types can contribute to tissue regeneration. Club cells normally function to detoxify inhaled chemicals but they can also proliferate to some extent and generate ciliated cells after injury, thereby serving as facultative progenitors (Fig. 2A; Rawlins et al. 2009). Furthermore, early observations from allergen-induced mouse injury models and human disease lungs showed that goblet cell metaplasia preferentially occurs distally, suggesting that club cells in proximal and distal regions within the large airway may have functional differences (Pardo-Saganta et al. 2013). This was partly explained by the observation that distal club cells are enriched for *Muc5b*, *Notch2*, and *Il13ra1* (interleukin 13 receptor $\alpha 1$), all prominent mediators and markers for goblet cell metaplasia (Montoro et al. 2018). How and why these distal club cell variants are established needs to be further studied, as well as the mechanisms guiding their potential expansion in disease states. Additionally, following ablation of basal cells in the airway epithelium, mature club cells can proliferate and dedifferentiate to basal cells (Fig. 2A; Rawlins et al. 2009; Tata et al. 2013). These newly formed basal cells similarly appear to maintain all the characteristics of wild-type basal cells in that they are activated after secondary injury and capable of normal differentiation pathways and regeneration of all airway epithelial cell types (Tata et al. 2013).

As described above, cartilaginous airways contain mucin-rich glandular tissues that are embedded deep within the submucosal tissues. The main function of SMGs is the secretion of aqueous fluids containing many distinct macromolecules including mucins, surfactants, and antimicrobials (Widdicombe and Wine 2015). Several lines of evidence reveal that SMGs are the source of multipotent stem cells that contribute to tissue repair and regeneration (Fig. 2A; Borthwick et al. 2001; Hegab et al. 2011, 2012; Xie et al. 2011; Lynch et al. 2016, 2018; Tata et al. 2018). Multiple experimental approaches, including *in vivo* injury/repair mouse models and *ex vivo* cell cultures and tissue explants, showed that MECs serve as a stem cell population that can proliferate in response to injury and generate other SMG cells including serous and mucous cells. Interestingly, following severe injury, MECs can also migrate to the luminal surface and regenerate basal, club, and secretory cells (Fig. 2A). Using scRNA-seq in combination with lineage-tracing mouse models and multiple injuries (NA, influenza infection, and SO₂) revealed that ACTA2-expressing myoepithelial cells have a regenerative potential to repair the damaged tissue, and thereby serve as multipotent reserve stem cell of the airways (Lynch et al. 2018; Tata et al. 2018). The recruitment of MECs to the surface is followed by dramatic changes in MEC cell shape and down-regulation of contractile proteins such as ACTA2 (Lynch et al. 2018; Tata et al. 2018). Although SMGs are confined to the very proximal regions of the trachea, SMG-derived MECs were able to contribute distally up to 10 cartilage rings. Contrary to mice, human airways contain SMGs of up to 13–15 generations of airways. This suggests that SMGs may serve as a large reservoir of stem cells in human lobular airways. Indeed, studies from experimental chlorine-induced injury in pig lungs have suggested that SMG-derived

myoepithelial cells contribute to lobular airways. Longer-term lineage-tracing studies in large animals are needed to experimentally test the potential contribution of SMG-derived cells to lobular airways and potentially to distal alveoli (Yu et al. 2019).

SMALL AIRWAY EPITHELIAL STEM CELL CONTRIBUTION TO LUNG REGENERATION

In small airways lacking cartilage (bronchioles), multiple cell populations including secretory (club), NE, rare TP63⁺ cells, and bronchioalveolar stem cells (BASCs) have been proposed to contribute to both airway and alveolar regeneration (Fig. 2B; Van Winkle et al. 1995; Giangreco et al. 2002; Reynolds et al. 2002; Kim et al. 2005; Rawlins et al. 2009; McQualter et al. 2010; Kumar et al. 2011; Lee et al. 2014; Vaughan et al. 2015; Zuo et al. 2015; McConnell et al. 2016; Guha et al. 2017; Xi et al. 2017; Yang et al. 2018; Liu et al. 2019; Kathiriya et al. 2020; Strunz et al. 2020).

First descriptions of club cell contribution to small airway regeneration came from environmental toxicology studies that used NA as an injury-causing agent (Van Winkle et al. 1995). Following NA-induced injury, histological and cell-specific marker analysis revealed that the residual SCGB1A1-expressing cells proliferate extensively followed by the emergence of ciliated cells. These observations led to subsequent lineage-tracing studies that provided experimental evidence that *Scgb1a1*-expressing club cells serve as the major source of cells for homeostatic turnover of the bronchiolar epithelium (Reynolds et al. 2002; Rawlins et al. 2009). However, over the years, multiple subsets of cells within SCGB1A1⁺ club cells including variant club cells and uroplakin 3A (UPK3a⁺) club cells, have been proposed to exhibit regeneration potential following lung injury (Fig. 2B; Van Winkle et al. 1995; Rawlins et al. 2009; McQualter et al. 2010; Guha et al. 2017).

Contemporary studies using scRNA-seq described a new subpopulation of club cells (~5% of total) characterized by high expression of major histocompatibility complex (MHC) class I (H2-K1 [histocompatibility 2, K1, K region]) (Kathiriya et al. 2020). Based on marker analysis, it was hypothesized that this cell population significantly expands in response to bleomycin-induced injury. Subsequent studies revealed that a purified H2-K1-enriched population of cells can expand and contribute toward alveolar lineages in engraftment assays (Fig. 2B; Kathiriya et al. 2020). Another study using scRNA-seq analysis proposed that an MHC-II⁺ subset of club cells, in response to lung injury give rise to AT2s and a Krt8^{hi} transitional alveolar state in response to bleomycin-induced lung injury (Fig. 2B; Strunz et al. 2020). Further analysis suggested that these two populations share common molecular signatures (Kathiriya et al. 2020; Strunz et al. 2020). Future studies will need to focus on further defining the molecular and functional differences and the potential of all of the above-described club cell subsets.

Over a decade ago, BASCs were identified in murine lungs at the bronchioalveolar duct junction and were thought to contribute to lung repair and regeneration (Giangreco et al. 2002; Kim et al. 2005). First descriptions of BASCs functional properties were based on a combination of cell-type-specific marker expression in defined anatomical regions (i.e., at the bronchioalveolar duct junction) and ex vivo characterization of purified cell populations

(Giangreco et al. 2002; Kim et al. 2005). Lack of markers specific to BASCs has limited definitive lineage-tracing experiments to assess their contribution to normal turnover and regeneration following injury. Recently, two groups developed intersectional lineage tracing mouse models to lineage trace BASCs in vivo (Liu et al. 2019, 2020a; Salwig et al. 2019). One study used highly sensitive intein-mediated complementation of split-cre recombinase (Cre) or split-tet-transactivator (tTA) allowed for specific labeling of dual marker expressing BASC (club marker, *Scgb1a1*; and AT2 cell marker, *Sftpc*) and revealed that BASCs contribute to distal airway and alveolar regeneration depending on the type of injury (i.e., airway, alveoli, or both) (Salwig et al. 2019). These findings were further supported by another study that developed intersectional lineage tracing using two different recombinases (*Cre* and *Dre*) to label BASC (Liu et al. 2019). This study revealed that BASCs are able to contribute to club and ciliated cells of the airway as well as AT1 and AT2 cells in alveoli (Fig. 2B; Liu et al. 2019). This was further confirmed with the use of a multicolor dual-recombinase reporter system to simultaneously label BASCs, club, and AT2s (Liu et al. 2020a). Nevertheless, it remains unknown whether a BASC-equivalent population exists in human lungs. Apart from club cells, CGRP-expressing NE cells, another bronchiolar luminal cell population, has been shown to contribute to both club and ciliated cells following injury (Fig. 2B; Song et al. 2012). However, depletion of NE cells had no consequence on club or ciliated cell recovery, suggesting that they are dispensable for airway epithelial regeneration (Song et al. 2012).

In addition to luminal cells, a rare population of TP63-expressing cells in the bronchioles have received significant attention for their contribution to distal airway and alveolar repair. Kumar et al. first reported the presence of TP63- and KRT5-expressing cells in distal airways and alveolar following severe damage to the airways and alveoli caused by infection with mouse-adapted influenza virus (H1N1 Puerto Rico 8 [PR8] strain). The authors made attempts to lineage trace these cells using a transgenic KRT14-creER-driven, lineage-tracing model. However, due to inefficient labeling of this driver line prior to injury, the authors administered tamoxifen for 7 days following injury, at which time KRT14 expression was significantly higher in injured regions. Nevertheless, using this experimental model and other complementary ex vivo cultures, the authors concluded that a rare cell population in terminal bronchioles could contribute to airway and alveolar regeneration following severe injury (Kumar et al. 2011). This population was referred to as distal airway stem cells (DASCs). Subsequent studies revealed that these TRP63-expressing cells either lack or express low levels of KRT5 but activate its expression following injury. Using more stringent lineage-tracing studies and in vivo engraftment of purified cells (based on integrin subunit $\beta 4$ [ITGB4⁺] CD200⁺ CD14⁺), it was shown that these cells can migrate to damaged alveolar regions. However, the contribution of these migrated cells is very minimal or almost negligible toward AT2 and AT1s, respectively (Fig. 2C; Vaughan et al. 2015; Yang et al. 2018). Interestingly, these migrated cells persist for many months following injury and developed into metaplastic structures that resembled pathological tissues found in human fibrotic lungs. This suggests that acute lung injury caused by influenza virus and other agents may predispose lungs to fibrosis and other lung diseases (Vaughan et al. 2015; Kanegai et al. 2016). Pathways controlling the fate of such cell populations may offer therapeutic windows to reverse such pathological metaplastic tissues. Indeed,

genetic modulation in mice following influenza injury revealed that temporal modulation of hypoxia, bone morphogenetic protein (BMP), and Notch pathways can enhance the contribution of TP63-expressing cells toward alveolar epithelial lineages (Vaughan et al. 2015; Liu et al. 2019).

Alveolar Epithelial Regeneration

As discussed above, the alveolar compartment is composed of two epithelial cell types—AT2s and AT1s. During development, AT2 and AT1 cellular lineages are distinct, with minimal plasticity between the two cell types (Frank et al. 2019; Gonzalez et al. 2019). However, in the adult regenerating lung, early studies using isotope pulse-chase labeling suggested that surfactant-producing AT2s can proliferate and generate AT1s (Evans and Bils 1969; Evans et al. 1978, 1973). Subsequent studies using *Sftpc*-promoter-driven, creER-based lineage tracing coupled with injury-repair models further corroborated these findings (Chapman et al. 2011; Barkauskas et al. 2013; Desai et al. 2014).

Although AT2s were thought to be a homogeneous cell population, recent studies have identified molecularly and functionally distinct subsets of AT2s. Two independent studies have identified a Wnt-responsive AT2 subset (Nabhan et al. 2018; Zacharias et al. 2018), as demonstrated by the expression of *Axin2*. However, the proportion of *Axin2*-expressing cells significantly (2% vs. 20%) varied between these studies. Notably, these studies used two different mouse lines one based on *Axin2* promoter-driven reporter and another using *Axin2-CreER*-based, lineage-tracing models and used varying doses of tamoxifen, likely contributing to the discrepancy. Regardless, both studies have suggested that *Axin2*-expressing AT2s “preferentially” expand in ex vivo cultures, and they require sustained Wnt signaling for their maintenance. Another study revealed a subset of AT2s that can be identified based on differential expression of CD44. About 3% of AT2s expressed high levels of CD44 (CD44^{hi}) and exhibited high proliferation rates both in vivo and ex vivo cultures (Chen et al. 2017). It is currently unknown whether *Axin2*-expressing and CD44^{hi} AT2s represent the same cell population, or they entirely represent two distinct subsets.

Multiple signaling pathways, including activation of BMP, Notch, Yap/Taz, and transforming growth factor β (TGF- β) signaling, and decreased Wnt pathways and mechanosignal transduction have been implicated in AT2 differentiation into AT1s (D’Alessio et al. 2009; Flozak et al. 2010; Al Alam et al. 2011; Aumiller et al. 2013; Lee et al. 2014; Liu et al. 2016; Li et al. 2018; Finn et al. 2019; Wu et al. 2020). However, until recently, the precise mechanisms that control AT2s differentiation from a small cuboidal shape to large and extremely thin AT1 cells was unknown.

Using scRNA-seq of injured mouse lungs, multiple groups have identified a transition state between AT2 and AT1s, termed pre-alveolar type 1 transitional cell state (PATS) or damage-associated transient progenitors (DATPs) or KRT8^{hi} alveolar differentiation intermediates (ADIs) (Fig. 2C; Aspal and Zemans 2020; Choi et al. 2020; Jiang et al. 2020; Kobayashi et al. 2020; Strunz et al. 2020). This unique cell state can be characterized based on unique molecular signatures. In multiple mouse models of lung injury, including left-lobe PNX, bleomycin injury, and an AT1-ablation injury model (in which AT2 cells rapidly differentiate), PATS-associated molecular signatures arise in the regenerative phase

and gradually recede as the tissue injury resolves (Kobayashi et al. 2020). In mice, two subsets of PATS have been identified—PATS-1 (connective tissue growth factor/claudin 4/stratifin/keratin 19 [*Ctgf/Cldn4/Sfn/Krt19*]) and PATS-2 (Lectin, galactoside-binding, soluble 3/cysteine and glycine-rich protein 1/S100 calcium binding protein A14/claudin 18 [*Lgals3/Csrp1/S100a14/Cldn18*]), and lineage-tracing studies have further demonstrated that PATS in mouse injury models progress into fully differentiated into mature AT1 cells (Kobayashi et al. 2020). Perhaps most importantly, identification of the PATS state enabled further understanding of the processes AT2s undergo in differentiation, including activation of DNA damage repair pathways (e.g., TP53 signaling), cellular senescence, and TGF- β signaling (Kobayashi et al. 2020; Wu et al. 2020; Yao et al. 2021). Experimental evidence further demonstrated that loss of these pathways abrogates AT2 differentiation potential, and thereby provides a therapeutic avenue for mitigating degenerative diseases such as lung fibrosis.

Interestingly, studies have found that there is a significant overlap in marker expression between PATS and “hyperplastic epithelial cells” found in fibrotic lung tissues. Indeed, single-cell analysis of fibrotic human lungs has revealed a population that resembles PATS and that has therefore been referred to as “PATS-like” cells. This population is marked by the expression of KRT17⁺, TP63⁺, and SFN but lack KRT5 (Reyfman et al. 2019; Adams et al. 2020; Habermann et al. 2020; Kobayashi et al. 2020; Strunz et al. 2020). In human lungs, PATS-like cells are enriched around regions of active fibrosis. It remains to be seen whether PATS-like cells are the “chicken or the egg” in human fibrosis—are they a result of inefficient injury response and AT2 differentiation, caused by fibrosis, or do they drive myofibrogenesis via increased TGF- β signaling?

Alveolar type 1 cells are extremely large and classically seen as terminally differentiated cells. However, recent studies using homeodomain only protein homeobox (HOPX), a marker for AT1 cells, promoter-driven lineage tracing studies revealed plasticity of AT1 cells. During PNx-induced compensatory lung growth and hyperoxia-induced lung, a few injuries have revealed that lineage-labeled AT1 cells undergo dedifferentiation and acquire AT2 cell identity (Fig. 2C; Jain et al. 2015; Penkala et al. 2021). Further analysis revealed that these lineage-reverted cells are functionally equivalent to resident AT2s. Mechanistic analysis revealed that tonic activity of yes-associated protein (YAP) and tafazzin (TAZ), transcription factors of the hippo signaling, are required to maintain AT1 cell identity. Genetic loss of YAP and TAZ results in the reversion of AT1s to AT2s. Interestingly, the reversion of AT1 to AT1 goes through a transitional cell state that emerges during AT2 to AT1 differentiation (Little et al. 2021; Penkala et al. 2021). This suggests that alveolar epithelial tissue homeostasis is actively maintained, and that plasticity is an integral part of tissue regeneration.

CONCLUSION

Work from many groups in the past two decades have significantly improved our understanding of lung cell composition in different anatomical sites, and their activities in normal and regenerating tissues. Further, recent advancements in single-cell biology have

led to the identification of previously unknown cell populations, and subsets within known cell populations among epithelial, mesenchymal, endothelial, and immune cells.

Numerous challenges and unanswered questions remain. Current understanding of cell lineages and their contribution to regeneration have come almost exclusively from mouse models. However, there are significant differences in the anatomy, cell composition, and physiology of human lungs compared to mice. As discussed, lack of respiratory bronchioles in mouse lungs limits our understanding of the constituent cells of this region in human lungs. Therefore, we need to develop human lung tissue-based ex vivo models to study such populations. Currently, three-dimensional cell and tissue cultures are being used to study some aspects of human lung cell properties (Katsura et al. 2020). However, the culture conditions are either not optimal or do not recapitulate the full repertoire of tissue interactions. One possibility to overcome this limitation is to engraft cultured, genetically engineered human cells into either immunocompromised animal models or animals with human immune system. Alternatively, development of large animal-based genetic tools can potentially serve as a surrogate to better understand human lung tissue regeneration. Indeed, recent advancements in genome editing technologies have significantly improved methods to generate large animal-based genetic tools to label and trace cells and to modulate signaling pathways (Yu et al. 2019).

Much of our understanding of tissue repair and regeneration is reductionist in nature, focusing on a pair of cell types or pathways. However, most human lung diseases involve dysregulation of a complex network of cellular interactions and signaling pathways. Additionally, integration of mechanical cues and extracellular matrix-derived signals is vital to realize a functional and physiological regeneration. Efforts are being made to improve single-cell-based “omics” approaches coupled with advanced computational tools to establish an integrated view of tissue dynamics at homeostasis, injury repair, and diseases. Such comprehensive interactome maps will undoubtedly pave the way for enhancing the endogenous regenerative potential of the lung and developing new approaches to realize personalized regenerative therapies.

ACKNOWLEDGMENTS

We thank Tata laboratory members and Brigid Hogan for fruitful discussions. A.K. is supported by a medical scientist training program fellowship from NHLBI/NIH (F30HL143911). This work was supported by funding from NHLBI/NIH (R01HL146557 and R01HL153375) and pilot grant funds from Kaganov-MEDx Pulmonary Initiative to P.R.T. at Duke University. This work was partially supported by funds from Whitehead foundation and P.R.T. is a Whitehead Scholar at Duke University.

REFERENCES

- Adams TS, Schupp JC, Poli S, Ayaub EA, Neumark N, Ahangari F, Chu SG, Raby BA, DeIuliis G, Januszyk M, et al. 2020. Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. *Sci Adv* 6: eaba1983. doi:10.1126/sciadv.aba1983
- Adamson IY, Bowden DH. 1974. The pathogenesis of bleomycin-induced pulmonary fibrosis in mice. *Am J Pathol* 77: 185–197. [PubMed: 4141224]
- Al Alam D, Green M, Tabatabai Irani R, Parsa S, Danopoulos S, Sala FG, Branch J, El Agha E, Tiozzo C, Voswinckel R, et al. 2011. Contrasting expression of canonical Wnt signaling reporters

TOPGAL, *BATGAL* and *Axin2^{LacZ}* during murine lung development and repair. *PLoS ONE* 6: e23139. doi:10.1371/journal.pone.0023139 [PubMed: 21858009]

- Aso Y, Yoneda K, Kikkawa Y. 1976. Morphologic and biochemical study of pulmonary changes induced by bleomycin in mice. *Lab Invest* 35: 558–568. [PubMed: 62893]
- Aspal M, Zemans RL. 2020. Mechanisms of ATII-to-ATI cell differentiation during lung regeneration. *Int J Mol Sci* 21: 3188. doi:10.3390/ijms21093188
- Aumiller V, Balsara N, Wilhelm J, Günther A, Königshoff M. 2013. WNT/ β -catenin signaling induces IL-1 β expression by alveolar epithelial cells in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 49: 96–104. doi:10.1165/rcmb.2012-0524OC [PubMed: 23526221]
- Barkauskas CE, Cronce MJ, Rackley CR, Bowie EJ, Keene DR, Stripp BR, Randell SH, Noble PW, Hogan BLM. 2013. Type 2 alveolar cells are stem cells in adult lung. *J Clin Invest* 123: 3025–3036. doi:10.1172/JCI68782 [PubMed: 23921127]
- Barth PJ, Müller B. 1999. Effects of nitrogen dioxide exposure on Clara cell proliferation and morphology. *Pathol Res Pract* 195: 487–493. doi:10.1016/S0344-0338(99)80052-1 [PubMed: 10448665]
- Basil MC, Katzen J, Engler AE, Guo M, Herriges MJ, Kathiriya JJ, Windmueller R, Ysasi AB, Zacharias WJ, Chapman HA, et al. 2020. The cellular and physiological basis for lung repair and regeneration: past, present, and future. *Cell Stem Cell* 26: 482–502. doi:10.1016/j.stem.2020.03.009 [PubMed: 32243808]
- Behrens W 1951. Experimental asbestosis. *Schweiz Z Pathol Bakter* 14: 275–297.
- Bigby TD, Allen D, Leslie CG, Henson PM, Cherniack RM. 1985. Bleomycin-induced lung injury in the rabbit. Analysis and correlation of bronchoalveolar lavage, morpho-metrics, and fibroblast stimulating activity. *Am Rev Respir Dis* 132: 590–595. [PubMed: 2412475]
- Boers JE, Ambergen AW, Thunnissen FB. 1998. Number and proliferation of basal and parabasal cells in normal human airway epithelium. *Am J Respir Crit Care Med* 157: 2000–2006. doi:10.1164/ajrccm.157.6.9707011 [PubMed: 9620938]
- Bolton SJ, Pinnion K, Oreffo V, Foster M, Pinkerton KE. 2009. Characterisation of the proximal airway squamous metaplasia induced by chronic tobacco smoke exposure in spontaneously hypertensive rats. *Respir Res* 10: 118. doi:10.1186/1465-9921-10-118 [PubMed: 19930705]
- Borthwick DW, West JD, Keighren MA, Flockhart JH, Innes BA, Dorin JR. 1999. Murine submucosal glands are clonally derived and show a cystic fibrosis gene-dependent distribution pattern. *Am J Respir Cell Mol Biol* 20: 1181–1189. doi:10.1165/ajrcmb.20.6.3475 [PubMed: 10340937]
- Borthwick DW, Shahbazian M, Todd Krantz Q, Dorin JR, Randell SH. 2001. Evidence for stem-cell niches in the tracheal epithelium. *Am J Respir Cell Mol Biol* 24: 662–670. doi:10.1165/ajrcmb.24.6.4217 [PubMed: 11415930]
- Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. 2000. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 161: 1720–1745. doi:10.1164/ajrccm.161.5.9903102 [PubMed: 10806180]
- Bouvry D, Planès C, Malbert-Colas L, Escabasse V, Clerici C. 2006. Hypoxia-induced cytoskeleton disruption in alveolar epithelial cells. *Am J Respir Cell Mol Biol* 35: 519–527. doi:10.1165/rcmb.2005-0478OC [PubMed: 16741163]
- Bozelka BE, Sestini P, Gaumer HR, Hammad Y, Heather CJ, Salvaggio JE. 1983. A murine model of asbestosis. *Am J Pathol* 112: 326–337. [PubMed: 6311019]
- Branchfield K, Nantie L, Verheyden JM, Sui P, Wienhold MD, Sun X. 2016. Pulmonary neuroendocrine cells function as airway sensors to control lung immune response. *Science* 351: 707–710. doi:10.1126/science.aad7969 [PubMed: 26743624]
- Brass DM, Hollingsworth JW, Cinque M, Li Z, Potts E, Toloza E, Foster WM, Schwartz DA. 2008. Chronic LPS inhalation causes emphysema-like changes in mouse lung that are associated with apoptosis. *Am J Respir Cell Mol Biol* 39: 584–590. doi:10.1165/rcmb.2007-0448OC [PubMed: 18539952]
- Brody JS, Burki R, Kaplan N. 1978. Deoxyribonucleic acid synthesis in lung cells during compensatory lung growth after pneumonectomy. *Am Rev Respir Dis* 117: 307–316. [PubMed: 637412]

- Broeckeaert F, Arsalane K, Hermans C, Bergamaschi E, Brustolin A, Mutti A, Bernard A. 1999. Lung epithelial damage at low concentrations of ambient ozone. *Lancet* 353: 900–901. doi:10.1016/S0140-6736(99)00540-1
- Buckpitt A, Chang AM, Weir A, Van Winkle L, Duan X, Philpot R, Plopper C. 1995. Relationship of cytochrome P450 activity to Clara cell cytotoxicity. IV: Metabolism of naphthalene and naphthalene oxide in microdissected airways from mice, rats, and hamsters. *Mol Pharmacol* 47: 74–81. [PubMed: 7838135]
- Butler JP, Loring SH, Patz S, Tsuda A, Yablonskiy DA, Mentzer SJ. 2012. Evidence for adult lung growth in humans. *N Engl J Med* 367: 244–247. doi:10.1056/NEJMoa1203983 [PubMed: 22808959]
- Castleman WL, Dungworth DL, Tyler WS. 1975. Intrapulmonary airway morphology in three species of monkeys: a correlated scanning and transmission electron microscopic study. *Am J Anat* 142: 107–121. doi:10.1002/aja.1001420108 [PubMed: 804810]
- Chamoto K, Gibney BC, Ackermann M, Lee GS, Konerding MA, Tsuda A, Mentzer SJ. 2013. Alveolar epithelial dynamics in post-pneumonectomy lung growth. *Anat Rec (Hoboken)* 296: 495–503. doi:10.1002/ar.22659 [PubMed: 23408540]
- Chapman HA, Li X, Alexander JP, Brumwell A, Lorizio W, Tan K, Sonnenberg A, Wei Y, Vu TH. 2011. Integrin $\alpha 6\beta 4$ identifies an adult distal lung epithelial population with regenerative potential in mice. *J Clin Invest* 121: 2855–2862. doi:10.1172/JCI57673 [PubMed: 21701069]
- Chen Q, Suresh Kumar V, Finn J, Jiang D, Liang J, Zhao YY, Liu Y. 2017. CD44^{high} alveolar type II cells show stem cell properties during steady-state alveolar homeostasis. *Am J Physiol Lung Cell Mol Physiol* 313: L41–L51. doi:10.1152/ajplung.00564.2016 [PubMed: 28473330]
- Cheresh P, Morales-Nebreda L, Kim SJ, Yeldandi A, Williams DB, Cheng Y, Mutlu GM, Budinger GRS, Ridge K, Schumacker PT, et al. 2015. Asbestos-induced pulmonary fibrosis is augmented in 8-oxoguanine DNA glycosylase knockout mice. *Am J Respir Cell Mol Biol* 52: 25–36. doi:10.1165/rcmb.2014-0038OC [PubMed: 24918270]
- Choi HK, Finkbeiner WE, Widdicombe JH. 2000. A comparative study of mammalian tracheal mucous glands. *J Anat* 197: 361–372. doi:10.1046/j.1469-7580.2000.19730361.x [PubMed: 11117623]
- Choi J, Park JE, Tsagkogeorga G, Yanagita M, Koo BK, Han N, Lee JH. 2020. Inflammatory signals induce AT2 cell-derived damage-associated transient progenitors that mediate alveolar regeneration. *Cell Stem Cell* 27: 366–382.e7. doi:10.1016/j.stem.2020.06.020 [PubMed: 32750316]
- Chung MI, Hogan BLM. 2018. Ager-CreERT2: a new genetic tool for studying lung alveolar development, homeostasis, and repair. *Am J Respir Cell Mol Biol* 59: 706–712. doi:10.1165/rcmb.2018-0125OC [PubMed: 30011373]
- Chung MI, Bujnis M, Barkauskas CE, Kobayashi Y, Hogan BLM. 2018. Niche-mediated BMP/SMAD signaling regulates lung alveolar stem cell proliferation and differentiation. *Development* 145. doi:10.1242/dev.163014
- Citrin DE, Shankavaram U, Horton JA, Shield W, Zhao S, Asano H, White A, Sowers A, Thetford A, Chung EJ. 2013. Role of type II pneumocyte senescence in radiation-induced lung fibrosis. *J Natl Cancer Inst* 105: 1474–1484. doi:10.1093/jnci/djt212 [PubMed: 24052614]
- Colley J, Gaunt IF, Lansdown AB, Grasso P, Gangolli SD. 1969. Acute and short-term toxicity of diacetyl in rats. *Food Cosmet Toxicol* 7: 571–580. doi:10.1016/S0015-6264(69)80460-8 [PubMed: 5386601]
- Crapo JD, Barry BE, Gehr P, Bachofen M, Weibel ER. 1982. Cell number and cell characteristics of the normal human lung. *Am Rev Respir Dis* 126: 332–337. [PubMed: 7103258]
- D'Alessio FR, Tsushima K, Aggarwal NR, West EE, Willett MH, Britos MF, Pipeling MR, Brower RG, Tudor RM, McDyer JF, et al. 2009. CD4⁺CD25⁺Foxp3⁺ Tregs resolve experimental lung injury in mice and are present in humans with acute lung injury. *J Clin Invest* 119: 2898–2913. doi:10.1172/JCI36498 [PubMed: 19770521]
- Delorey TM, Ziegler CGK, Heimberg G, Normand R, Yang Y, Segerstolpe Å, Abbondanza D, Fleming SJ, Subramanian A, Montoro DT, et al. 2021. COVID-19 tissue atlases reveal SARS-CoV-2

pathology and cellular targets. *Nature* 595: 107–113. doi:10.1038/s41586-021-03570-8 [PubMed: 33915569]

- Deprez M, Zaragosi LE, Truchi M, Becavin C, Ruiz García S, Arguel MJ, Plaisant M, Magnone V, Lebrigand K, Abelanet S, et al. 2020. A single-cell atlas of the human healthy airways. *Am J Respir Crit Care Med* 202: 1636–1645. doi:10.1164/rccm.201911-2199OC [PubMed: 32726565]
- Desai TJ, Brownfield DG, Krasnow MA. 2014. Alveolar progenitor and stem cells in lung development, renewal and cancer. *Nature* 507: 190–194. doi:10.1038/nature12930 [PubMed: 24499815]
- Duclos GE, Teixeira VH, Autissier P, Gesthalter YB, Rein-ders-Luinge MA, Terrano R, Dumas YM, Liu G, Mazzilli SA, Brandsma CA, et al. 2019. Characterizing smoking-induced transcriptional heterogeneity in the human bronchial epithelium at single-cell resolution. *Sci Adv* 5: eaaw3413. doi:10.1126/sciadv.aaw3413
- Engelhardt JF, Zepeda M, Cohn JA, Yankaskas JR, Wilson JM. 1994. Expression of the cystic fibrosis gene in adult human lung. *J Clin Invest* 93: 737–749. doi:10.1172/JCI117028 [PubMed: 7509347]
- Evans MJ, Bills RF. 1969. Identification of cells labeled with tritiated thymidine in the pulmonary alveolar walls of the mouse. *Am Rev Respir Dis* 100: 372–378. doi:10.1164/arrd.1969.100.3.372 [PubMed: 5810808]
- Evans MJ, Cabral LJ, Stephens RJ, Freeman G. 1973. Renewal of alveolar epithelium in the rat following exposure to NO₂. *Am J Pathol* 70: 175–198. [PubMed: 4566990]
- Evans MJ, Dekker NP, Cabral-Anderson LJ, Freeman G. 1978. Quantitation of damage to the alveolar epithelium by means of type 2 cell proliferation. *Am Rev Respir Dis* 118: 787–790. doi:10.1164/arrd.1978.118.4.787 [PubMed: 707897]
- Evans MJ, Cabral-Anderson LJ, Dekker NP, Freeman G. 1981. The effects of dietary antioxidants on NO₂-induced injury to type 1 alveolar cells. *Chest* 80: 5S–8S. doi:10.1378/chest.80.1_Supplement.5S
- Evans MJ, Cox RA, Shami SG, Wilson B, Plopper CG. 1989. The role of basal cells in attachment of columnar cells to the basal lamina of the trachea. *Am J Respir Cell Mol Biol* 1: 463–470. doi:10.1165/ajrcmb/1.6.463 [PubMed: 2637758]
- Evans MJ, Van Winkle LS, Fanucchi MV, Plopper CG. 2001. Cellular and molecular characteristics of basal cells in airway epithelium. *Exp Lung Res* 27: 401–415. doi:10.1080/019021401300317125 [PubMed: 11480582]
- Farrow N, Cmielewski P, Donnelley M, Rout-Pitt N, Moodley Y, Bertocello I, Parsons D. 2018. Epithelial disruption: a new paradigm enabling human airway stem cell transplantation. *Stem Cell Res Ther* 9: 1–8. doi:10.1186/s13287-018-0911-4 [PubMed: 29291747]
- Finn J, Sottoriva K, Pajcini KV, Kitajewski JK, Chen C, Zhang W, Malik AB, Liu Y. 2019. Dlk1-mediated temporal regulation of Notch signaling is required for differentiation of alveolar type II to type I cells during repair. *Cell Rep* 26: 2942–2954.e5. doi:10.1016/j.celrep.2019.02.046 [PubMed: 30865885]
- Flozak AS, Lam AP, Russell S, Jain M, Peled ON, Sheppard KA, Beri R, Mutlu GM, Budinger GRS, Gottardi CJ. 2010. β -Catenin/T-cell factor signaling is activated during lung injury and promotes the survival and migration of alveolar epithelial cells. *J Biol Chem* 285: 3157–3167. doi:10.1074/jbc.M109.070326 [PubMed: 19933277]
- Foster JR, Cottrell RC, Herod IA, Atkinson HA, Miller K. 1985. A comparative study of the pulmonary effects of NO₂ in the rat and hamster. *Br J Exp Pathol* 66: 193–204. [PubMed: 3838681]
- Frank L, Bucher JR, Roberts RJ. 1978. Oxygen toxicity in neonatal and adult animals of various species. *J Appl Physiol Respir Environ Exerc Physiol* 45: 699–704. [PubMed: 730565]
- Frank DB, Penkala IJ, Zepp JA, Sivakumar A, Linares-Saldana R, Zacharias WJ, Stolz KG, Pankin J, Lu M, Wang Q, et al. 2019. Early lineage specification defines alveolar epithelial ontogeny in the murine lung. *Proc Natl Acad Sci* 116: 4362–4371. doi:10.1073/pnas.1813952116 [PubMed: 30782824]
- Franko AJ, Sharplin J, Ward WF, Hinz JM. 1991. The genetic basis of strain-dependent differences in the early phase of radiation injury in mouse lung. *Radiat Res* 126: 349–356. doi:10.2307/3577925 [PubMed: 1852022]

- Franks TJ, Colby TV, Travis WD, Tuder RM, Reynolds HY, Brody AR, Cardoso WV, Crystal RG, Drake CJ, Engelhardt J, et al. 2008. Resident cellular components of the human lung: current knowledge and goals for research on cell phenotyping and function. *Proc Am Thorac Soc* 5: 763–766. doi:10.1513/pats.200803-025HR [PubMed: 18757314]
- Giangreco A, Reynolds SD, Stripp BR. 2002. Terminal bronchioles harbor a unique airway stem cell population that localizes to the bronchoalveolar duct junction. *Am J Pathol* 161: 173–182. doi:10.1016/S0002-9440(10)64169-7 [PubMed: 12107102]
- Goldfarbmuren KC, Jackson ND, Sajuthi SP, Dyjack N, Li KS, Rios CL, Plender EG, Montgomery MT, Everman JL, Bratcher PE, et al. 2020. Dissecting the cellular specificity of smoking effects and reconstructing lineages in the human airway epithelium. *Nat Commun* 11: 2485. doi:10.1038/s41467-020-16239-z [PubMed: 32427931]
- Gonzalez R, Leaffer D, Chapin C, Gillespie AM, Eckalbar W, Dobbs L. 2019. Cell fate analysis in fetal mouse lung reveals distinct pathways for TI and TII cell development. *Am J Physiol Lung Cell Mol Physiol* 317: L653–L666. doi:10.1152/ajplung.00503.2018 [PubMed: 31432712]
- Grayson RR. 1956. Silage gas poisoning: nitrogen dioxide pneumonia, a new disease in agricultural workers. *Ann Intern Med* 45: 393–408. doi:10.7326/0003-4819-45-3-393 [PubMed: 13363168]
- Guha A, Deshpande A, Jain A, Sebastiani P, Cardoso WV. 2017. Uroplakin 3a⁺ cells are a distinctive population of epithelial progenitors that contribute to airway maintenance and post-injury repair. *Cell Rep* 19: 246–254. doi:10.1016/j.celrep.2017.03.051 [PubMed: 28402849]
- Habermann AC, Gutierrez AJ, Bui LT, Yahn SL, Winters NI, Calvi CL, Peter L, Chung MI, Taylor CJ, Jetter C, et al. 2020. Single-cell RNA sequencing reveals profibrotic roles of distinct epithelial and mesenchymal lineages in pulmonary fibrosis. *Sci Adv* 6: eaba1972. doi:10.1126/sciadv.aba1972
- Hanania AN, Mainwaring W, Ghebre YT, Hanania NA, Ludwig M. 2019. Radiation-induced lung injury: assessment and management. *Chest* 156: 150–162. doi:10.1016/j.chest.2019.03.033 [PubMed: 30998908]
- Harber P, Saechao K, Boomus C. 2006. Diacetyl-induced lung disease. *Toxicol Rev* 25: 261–272. doi:10.2165/00139709-200625040-00006 [PubMed: 17288497]
- Hegab AE, Ha VL, Gilbert JL, Zhang KX, Malkoski SP, Chon AT, Darmawan DO, Bisht B, Ooi AT, Pellegrini M, et al. 2011. Novel stem/progenitor cell population from murine tracheal submucosal gland ducts with multipotent regenerative potential. *Stem Cells* 29: 1283–1293. doi:10.1002/stem.680 [PubMed: 21710468]
- Hegab AE, Nickerson DW, Ha VL, Darmawan DO, Gomperts BN. 2012. Repair and regeneration of tracheal surface epithelium and submucosal glands in a mouse model of hypoxic-ischemic injury. *Respirology* 17: 1101–1113. doi:10.1111/j.1440-1843.2012.02204.x [PubMed: 22617027]
- Herbert CA, King CM, Ring PC, Holgate ST, Stewart GA, Thompson PJ, Robinson C. 1995. Augmentation of permeability in the bronchial epithelium by the house dust mite allergen Der p1. *Am J Respir Cell Mol Biol* 12: 369–378. doi:10.1165/ajrcmb.12.4.7695916 [PubMed: 7695916]
- Hers JF, Masurel N, Mulder J. 1958. Bacteriology and histopathology of the respiratory tract and lungs in fatal Asian influenza. *Lancet* 272: 1141–1143. doi:10.1016/S0140-6736(58)92404-8
- Hogan BLM, Barkauskas CE, Chapman HA, Epstein JA, Jain R, Hsia CCW, Niklason L, Calle E, Le A, Randell SH, et al. 2014. Repair and regeneration of the respiratory system: complexity, plasticity, and mechanisms of lung stem cell function. *Cell Stem Cell* 15: 123–138. doi:10.1016/j.stem.2014.07.012 [PubMed: 25105578]
- Hong KU, Reynolds SD, Giangreco A, Hurley CM, Stripp BR. 2001. Clara cell secretory protein-expressing cells of the airway neuroepithelial body microenvironment include a label-retaining subset and are critical for epithelial renewal after progenitor cell depletion. *Am J Respir Cell Mol Biol* 24: 671–681. doi:10.1165/ajrcmb.24.6.4498 [PubMed: 11415931]
- Hong KU, Reynolds SD, Watkins S, Fuchs E, Stripp BR. 2004a. Basal cells are a multipotent progenitor capable of renewing the bronchial epithelium. *Am J Pathol* 164: 577–588. doi:10.1016/S0002-9440(10)63147-1 [PubMed: 14742263]
- Hong KU, Reynolds SD, Watkins S, Fuchs E, Stripp BR. 2004b. In vivo differentiation potential of tracheal basal cells: evidence for multipotent and unipotent subpopulations. *Am J Physiol Lung Cell Mol Physiol* 286: L643–L649. doi:10.1152/ajplung.00155.2003 [PubMed: 12871857]

- Hubbs AF, Goldsmith WT, Kashon ML, Frazer D, Mercer RR, Battelli LA, Kullman GJ, Schwegler-Berry D, Friend S, Castranova V. 2008. Respiratory toxicologic pathology of inhaled diacetyl in Sprague-Dawley rats. *Toxicol Pathol* 36: 330–344. doi:10.1177/0192623307312694 [PubMed: 18474946]
- Hudson LD, Milberg JA, Anardi D, Maunder RJ. 1995. Clinical risks for development of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 151: 293–301. doi:10.1164/ajrcem.151.2.7842182 [PubMed: 7842182]
- Hyde DM, Samuelson DA, Blakeney WH, Kosch PC. 1979. A correlative light microscopy, transmission and scanning electron microscopy study of the ferret lung. *Scan Electron Microsc* 3: 891–898.
- Isakson BE, Lubman RL, Seedorf GJ, Boitano S. 2001. Modulation of pulmonary alveolar type II cell phenotype and communication by extracellular matrix and KGF. *Am J Physiol Cell Physiol* 281: C1291–C1299. doi:10.1152/ajpcell.2001.281.4.C1291 [PubMed: 11546667]
- Jain R, Barkauskas CE, Takeda N, Bowie EJ, Aghajanian H, Wang Q, Padmanabhan A, Manderfield LJ, Gupta M, Li D, et al. 2015. Plasticity of Hopx⁺ type I alveolar cells to regenerate type II cells in the lung. *Nat Commun* 6: 6727. doi:10.1038/ncomms7727 [PubMed: 25865356]
- Jiang P, Gil de Rubio R, Hrycaj SM, Gurczynski SJ, Riemondy KA, Moore BB, Omary MB, Ridge KM, Zemans RL. 2020. Ineffectual type 2-to-type 1 alveolar epithelial cell differentiation in idiopathic pulmonary fibrosis: persistence of the KRT8^{hi} transitional state. *Am J Respir Crit Care Med* 201: 1443–1447. doi:10.1164/rccm.201909-1726LE [PubMed: 32073903]
- Johnson JR, Wiley RE, Fattouh R, Swirski FK, Gajewska BU, Coyle AJ, Gutierrez-Ramos JC, Ellis R, Inman MD, Jordana M. 2004. Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am J Respir Crit Care Med* 169: 378–385. doi:10.1164/rccm.200308-1094OC [PubMed: 14597485]
- Johnston CJ, Wright TW, Rubin P, Finkelstein JN. 1998. Alterations in the expression of chemokine mRNA levels in fibrosis-resistant and -sensitive mice after thoracic irradiation. *Exp Lung Res* 24: 321–337. doi:10.3109/01902149809041538 [PubMed: 9635254]
- Kallet RH, Matthay MA. 2013. Hyperoxic acute lung injury. *Respir Care* 58: 123–141. doi:10.4187/respcare.01963 [PubMed: 23271823]
- Kanegai CM, Xi Y, Donne ML, Gotts JE, Driver IH, Amidzic G, Lechner AJ, Jones KD, Vaughan AE, Chapman HA, et al. 2016. Persistent pathology in influenza-infected mouse lungs. *Am J Respir Cell Mol Biol* 55: 613–615. doi:10.1165/rcmb.2015-0387LE [PubMed: 27689795]
- Karvonen RL, Fernandez-Madrid F, Maughan RL, Palmer KC, Fernandez-Madrid I. 1987. An animal model of pulmonary radiation fibrosis with biochemical, physiologic, immunologic, and morphologic observations. *Radiat Res* 111: 68–80. doi:10.2307/3577022 [PubMed: 3602356]
- Kathiriyi JJ, Brumwell AN, Jackson JR, Tang X, Chapman HA. 2020. Distinct airway epithelial stem cells hide among club cells but mobilize to promote alveolar regeneration. *Cell Stem Cell* 26: 346–358.e4. doi:10.1016/j.stem.2019.12.014 [PubMed: 31978363]
- Katsura H, Sontake V, Tata A, Kobayashi Y, Edwards CE, Heaton BE, Konkimalla A, Asakura T, Mikami Y, Fritch EJ, et al. 2020. Human lung stem cell-based alveolospheres provide insights into SARS-CoV-2-mediated interferon responses and pneumocyte dysfunction. *Cell Stem Cell* 27: 890–904.e8. doi:10.1016/j.stem.2020.10.005 [PubMed: 33128895]
- Kennedy TP, Johnson KJ, Kunkel RG, Ward PA, Knight PR, Finch JS. 1989. Acute acid aspiration lung injury in the rat: biphasic pathogenesis. *Anesth Analg* 69: 87–92. [PubMed: 2742173]
- Kim CFB, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T. 2005. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 121: 823–835. doi:10.1016/j.cell.2005.03.032 [PubMed: 15960971]
- Kim SJ, Cheresh P, Jablonski RP, Morales-Nebreda L, Cheng Y, Hogan E, Yeldandi A, Chi M, Piseaux R, Ridge K, et al. 2016. Mitochondrial catalase overexpressed transgenic mice are protected against lung fibrosis in part via preventing alveolar epithelial cell mitochondrial DNA damage. *Free Radic Biol Med* 101: 482–490. doi:10.1016/j.freeradbiomed.2016.11.007 [PubMed: 27840320]
- Kobayashi Y, Tata A, Konkimalla A, Katsura H, Lee RF, Ou J, Banovich NE, Kropski JA, Tata PR. 2020. Persistence of a regeneration-associated, transitional alveolar epithelial cell state

in pulmonary fibrosis. *Nat Cell Biol* 22: 934–946. doi:10.1038/s41556-020-0542-8 [PubMed: 32661339]

Kodavanti UP, Schladweiler MC, Ledbetter AD, Ortuno RV, Suffia M, Evansky P, Richards JH, Jaskot RH, Thomas R, Karoly E, et al. 2006. The spontaneously hypertensive rat: an experimental model of sulfur dioxide-induced airways disease. *Toxicol Sci* 94: 193–205. doi:10.1093/toxsci/kf1087 [PubMed: 16929007]

Kumar PA, Hu Y, Yamamoto Y, Hoe NB, Wei TS, Mu D, Sun Y, Joo LS, Dagher R, Zielonka EM, et al. 2011. Distal airway stem cells yield alveoli in vitro and during lung regeneration following H1N1 influenza infection. *Cell* 147: 525–538. doi:10.1016/j.cell.2011.10.001 [PubMed: 22036562]

Kuo CS, Krasnow MA. 2015. Formation of a neurosensory organ by epithelial cell slithering. *Cell* 163: 394–405. doi:10.1016/j.cell.2015.09.021 [PubMed: 26435104]

Lamb D, Reid L. 1968. Mitotic rates, goblet cell increase and histochemical changes in mucus in rat bronchial epithelium during exposure to sulphur dioxide. *J Pathol Bacteriol* 96: 97–111. doi:10.1002/path.1700960111 [PubMed: 5667859]

Lamb D, Reid L. 1969. Goblet cell increase in rat bronchial epithelium after exposure to cigarette and cigar tobacco smoke. *Br Med J* 1: 33–35. doi:10.1136/bmj.1.5635.33 [PubMed: 5761894]

Lee J-H, Bhang DH, Beede A, Huang TL, Stripp BR, Bloch KD, Wagers AJ, Tseng Y-H, Ryeom S, Kim CF. 2014. Lung stem cell differentiation in mice directed by endothelial cells via a BMP4-NFATc1-thrombospondin-1 axis. *Cell* 156: 440–455. doi:10.1016/j.cell.2013.12.039 [PubMed: 24485453]

Li J, Wang Z, Chu Q, Jiang K, Li J, Tang N. 2018. The strength of mechanical forces determines the differentiation of alveolar epithelial cells. *Dev Cell* 44: 297–312.e5. doi:10.1016/j.devcel.2018.01.008 [PubMed: 29408236]

Little DR, Lynch AM, Yan Y, Akiyama H, Kimura S, Chen J. 2021. Differential chromatin binding of the lung lineage transcription factor NKX2–1 resolves opposing murine alveolar cell fates in vivo. *Nat Commun* 12: 2509. doi:10.1038/s41467-021-22817-6 [PubMed: 33947861]

Liu X, Engelhardt JF. 2008. The glandular stem/progenitor cell niche in airway development and repair. *Proc Am Thorac Soc* 5: 682–688. doi:10.1513/pats.200801-003AW [PubMed: 18684717]

Liu JY, Nettesheim P, Randell SH. 1994. Growth and differentiation of tracheal epithelial progenitor cells. *Am J Physiol* 266: L296–L307. [PubMed: 8166299]

Liu Z, Wu H, Jiang K, Wang Y, Zhang W, Chu Q, Li J, Huang H, Cai T, Ji H, et al. 2016. MAPK-mediated YAP activation controls mechanical-tension-induced pulmonary alveolar regeneration. *Cell Rep* 16: 1810–1819. doi:10.1016/j.celrep.2016.07.020 [PubMed: 27498861]

Liu Q, Liu K, Cui G, Huang X, Yao S, Guo W, Qin Z, Li Y, Yang R, Pu W, et al. 2019. Lung regeneration by multipotent stem cells residing at the bronchioalveolar-duct junction. *Nat Genet* 51: 728–738. doi:10.1038/s41588-019-0346-6 [PubMed: 30778223]

Liu K, Tang M, Liu Q, Han X, Jin H, Zhu H, Li Y, He L, Ji H, Zhou B. 2020a. Bi-directional differentiation of single bronchioalveolar stem cells during lung repair. *Cell Discov* 6: 1–4. doi:10.1038/s41421-019-0132-8 [PubMed: 31934347]

Liu X, Rowan SC, Liang J, Yao C, Huang G, Deng N, Xie T, Wu D, Wang Y, Burman A, et al. 2020b. Definition and signatures of lung fibroblast populations in development and fibrosis in mice and men. *bioRxiv* doi:10.1101/2020.07.15.203141

Locke NR, Royce SG, Wainwright JS, Samuel CS, Tang ML. 2007. Comparison of airway remodeling in acute, subacute, and chronic models of allergic airways disease. *Am J Respir Cell Mol Biol* 36: 625–632. doi:10.1165/rcmb.2006-0083OC [PubMed: 17237192]

Loosli CG, Stinson SF, Ryan DP, Hertweck MS, Hardy JD, Serebrin R. 1975. The destruction of type 2 pneumocytes by airborne influenza PR8-Avirus; its effect on surfactant and lecithin content of the pneumonic lesions of mice. *Chest* 67: 7S–14S. doi:10.1378/chest.67.2_Supplement.7S [PubMed: 1172777]

Lowry T, Schuman LM. 1956. Silo-filler's disease—a syndrome caused by nitrogen dioxide. *J Am Med Assoc* 162: 153–160. doi:10.1001/jama.1956.02970200001001 [PubMed: 13357306]

- Lynch TJ, Anderson PJ, Xie W, Crooke AK, Liu X, Tyler SR, Luo M, Kusner DM, Zhang Y, Neff T, et al. 2016. Wnt signaling regulates airway epithelial stem cells in adult murine submucosal glands. *Stem Cells* 34: 2758–2771. doi:10.1002/stem.2443 [PubMed: 27341073]
- Lynch TJ, Anderson PJ, Rotti PG, Tyler SR, Crooke AK, Choi SH, Montoro DT, Silverman CL, Shahin W, Zhao R, et al. 2018. Submucosal gland myoepithelial cells are reserve stem cells that can regenerate mouse tracheal epithelium. *Cell Stem Cell* 22: 779. doi:10.1016/j.stem.2018.04.007
- Manning CB, Vallyathan V, Mossman BT. 2002. Diseases caused by asbestos: mechanisms of injury and disease development. *Int Immunopharmacol* 2: 191–200. doi:10.1016/S1567-5769(01)00172-2 [PubMed: 11811924]
- Marinova M, Solopov P, Dimitropoulou C, Colunga Biancatelli RML, Catravas JD. 2019. Acute exposure of mice to hydrochloric acid leads to the development of chronic lung injury and pulmonary fibrosis. *Inhal Toxicol* 31: 147–160. doi:10.1080/08958378.2019.1624895 [PubMed: 31232121]
- Mason RJ. 2006. Biology of alveolar type II cells. *Respirology* 11 (Suppl): S12–S15. doi:10.1111/j.1440-1843.2006.00800.x [PubMed: 16423262]
- Matute-Bello G, Frevert CW, Martin TR. 2008. Animal models of acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 295: L379–L399. doi:10.1152/ajplung.00010.2008 [PubMed: 18621912]
- McConnell AM, Yao C, Yeckes AR, Wang Y, Selvaggio AS, Tang J, Kirsch DG, Stripp BR. 2016. p53 regulates progenitor cell quiescence and differentiation in the airway. *Cell Rep* 17: 2173–2182. doi:10.1016/j.celrep.2016.11.007 [PubMed: 27880895]
- McGraw MD, Kim SY, Reed C, Hernady E, Rahman I, Mariani TJ, Finkelstein JN. 2020. Airway basal cell injury after acute diacetyl (2,3-butanedione) vapor exposure. *Toxicol Lett* 325: 25–33. doi:10.1016/j.toxlet.2020.02.012 [PubMed: 32112875]
- McMillan SJ, Lloyd CM. 2004. Prolonged allergen challenge in mice leads to persistent airway remodelling. *Clin Exp Allergy* 34: 497–507. doi:10.1111/j.1365-2222.2004.01895.x [PubMed: 15005746]
- McQualter JL, Yuen K, Williams B, Bertoncello I. 2010. Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung. *Proc Natl Acad Sci* 107: 1414–1419. doi:10.1073/pnas.0909207107 [PubMed: 20080639]
- Mendelson CL. 1946. The aspiration of stomach contents into the lungs during obstetric anesthesia. *Am J Obstet Gynecol* 52: 191–205. doi:10.1016/S0002-9378(16)39829-5 [PubMed: 20993766]
- Mercer RR, Russell ML, Roggli VL, Crapo JD. 1994. Cell number and distribution in human and rat airways. *Am J Respir Cell Mol Biol* 10: 613–624. doi:10.1165/ajrcmb.10.6.8003339 [PubMed: 8003339]
- Meyrick B, Sturgess JM, Reid L. 1969. A reconstruction of the duct system and secretory tubules of the human bronchial submucosal gland. *Thorax* 24: 729–736. doi:10.1136/thx.24.6.729 [PubMed: 5350723]
- Michaudel C, Fauconnier L, Julé Y, Ryffel B. 2018. Functional and morphological differences of the lung upon acute and chronic ozone exposure in mice. *Sci Rep* 8: 10611. doi:10.1038/s41598-018-28261-9
- Modelska K, Pittet JF, Folkesson HG, Courtney Broaddus V, Matthay MA. 1999. Acid-induced lung injury. Protective effect of anti-interleukin-8 pretreatment on alveolar epithelial barrier function in rabbits. *Am J Respir Crit Care Med* 160: 1450–1456. doi:10.1164/ajrccm.160.5.9901096 [PubMed: 10556104]
- Montoro DT, Haber AL, Biton M, Vinarsky V, Lin B, Birket SE, Yuan F, Chen S, Leung HM, Villoria J, et al. 2018. A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature* 560: 319–324. doi:10.1038/s41586-018-0393-7 [PubMed: 30069044]
- Moore BB, Hogaboam CM. 2008. Murine models of pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 294: L152–160. doi:10.1152/ajplung.00313.2007 [PubMed: 17993587]
- Morabia A, Sellegger C, Landry JC, Conne P, Urban P, Fabre J. 1988. Accidental bromine exposure in an urban population: an acute epidemiological assessment. *Int J Epidemiol* 17: 148–152. doi:10.1093/ije/17.1.148 [PubMed: 3384533]

- Morgan DL, Flake GP, Kirby PJ, Palmer SM. 2008. Respiratory toxicity of diacetyl in C57BL/6 mice. *Toxicol Sci* 103: 169–180. doi:10.1093/toxsci/kfn016 [PubMed: 18227102]
- Mori M, Mahoney JE, Stupnikov MR, Paez-Cortez JR, Szymaniak AD, Varelas X, Herrick DB, Schwob J, Zhang H, Cardoso WV. 2015. Notch3-Jagged signaling controls the pool of undifferentiated airway progenitors. *Development* 142: 258–267. doi:10.1242/dev.116855 [PubMed: 25564622]
- Mossman BT, Bignon J, Corn M, Seaton A, Gee JB. 1990. Asbestos: scientific developments and implications for public policy. *Science* 247: 294–301. doi:10.1126/science.2153315 [PubMed: 2153315]
- Musah S, Chen J, Hoyle GW. 2012. Repair of tracheal epithelium by basal cells after chlorine-induced injury. *Respir Res* 13: 107. doi:10.1186/1465-9921-13-107 [PubMed: 23170909]
- Nabhan AN, Brownfield DG, Harbury PB, Krasnow MA, Desai TJ. 2018. Single-cell Wnt signaling niches maintain stemness of alveolar type 2 cells. *Science* 359: 1118–1123. doi:10.1126/science.aam6603 [PubMed: 29420258]
- Nakajima M, Kawanami O, Jin E, Ghazizadeh M, Honda M, Asano G, Horiba K, Ferrans VJ. 1998. Immunohistochemical and ultrastructural studies of basal cells, Clara cells and bronchiolar cuboidal cells in normal human airways. *Pathol Int* 48: 944–953. doi:10.1111/j.1440-1827.1998.tb03865.x [PubMed: 9952338]
- Nikiforov AI, Schlesinger RB. 1985. Morphometric variability of the human upper bronchial tree. *Respir Physiol* 59: 289–299. doi:10.1016/0034-5687(85)90134-3 [PubMed: 3992063]
- Nissen R. 1980. Total pneumonectomy. *Ann Thorac Surg* 29: 390–394. doi:10.1016/S0003-4975(10)61496-8 [PubMed: 6987966]
- Noguchi M, Sumiyama K, Morimoto M. 2015. Directed migration of pulmonary neuroendocrine cells toward airway branches organizes the stereotypic location of neuroepithelial bodies. *Cell Rep* 13: 2679–2686. doi:10.1016/j.celrep.2015.11.058 [PubMed: 26711336]
- Okuda K, Chen G, Subramani DB, Wolf M, Gilmore RC, Kato T, Radicioni G, Kesimer M, Chua M, Dang H, et al. 2019. Localization of secretory mucins MUC5AC and MUC5B in normal/healthy human airways. *Am J Respir Crit Care Med* 199: 715–727. doi:10.1164/rccm.201804-0734OC [PubMed: 30352166]
- Okuda K, Dang H, Kobayashi Y, Carraro G, Nakano S, Chen G, Kato T, Asakura T, Gilmore RC, Morton LC, et al. 2021. Secretory cells dominate airway CFTR expression and function in human airway superficial epithelia. *Am J Respir Crit Care Med* 203: 1275–1289. doi:10.1164/rccm.202008-3198OC [PubMed: 33321047]
- Onuma K, Ebina M, Takahashi T, Nukiwa T. 2001. Irregularity of airway branching in a mouse bronchial tree: a 3-D morphometric study. *Tohoku J Exp Med* 194: 157–164. doi:10.1620/tjem.194.157 [PubMed: 11693664]
- Ouahad Y, Rojas ER, Riordan DP, Capostagno S, Kuo CS, Krasnow MA. 2019. Rare pulmonary neuroendocrine cells are stem cells regulated by Rb, p53, and Notch. *Cell* 179: 403–416.e23. doi:10.1016/j.cell.2019.09.010 [PubMed: 31585080]
- Palmer SM, Flake GP, Kelly FL, Zhang HL, Nugent JL, Kirby PJ, Foley JF, Gwinn WM, Morgan DL. 2011. Severe airway epithelial injury, aberrant repair and bronchiolitis obliterans develops after diacetyl instillation in rats. *PLoS ONE* 6: e17644. doi:10.1371/journal.pone.0017644 [PubMed: 21464978]
- Pardo-Saganta A, Law BM, Gonzalez-Celeiro M, Vinarsky V, Rajagopal J. 2013. Ciliated cells of pseudostratified airway epithelium do not become mucous cells after ovalbumin challenge. *Am J Respir Cell Mol Biol* 48: 364–373. doi:10.1165/rcmb.2012-0146OC [PubMed: 23239495]
- Pardo-Saganta A, Law BM, Tata PR, Villoria J, Saez B, Mou H, Zhao R, Rajagopal J. 2015. Injury induces direct lineage segregation of functionally distinct airway basal stem/progenitor cell subpopulations. *Cell Stem Cell* 16: 184–197. doi:10.1016/j.stem.2015.01.002 [PubMed: 25658372]
- Penkala IJ, Liberti DC, Pankin J, Sivakumar A, Kremp MM, Jayachandran S, Katzen J, Leach JP, Windmueller R, Stolz K, et al. 2021. Age-dependent alveolar epithelial plasticity orchestrates lung homeostasis and regeneration. *Cell Stem Cell* doi:10.1016/j.stem.2021.04.026

- Pinkerton KE, Joad JP. 2000. The mammalian respiratory system and critical windows of exposure for children's health. *Environ Health Perspect* 108 (Suppl. 3): 457–462.
- Plasschaert LW, Žilionis R, Choo-Wing R, Savova V, Knehr J, Roma G, Klein AM, Jaffe AB. 2018. A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature* 560: 377–381. doi:10.1038/s41586-018-0394-6 [PubMed: 30069046]
- Plopper CG, Mariassy AT, Lollini LO. 1983. Structure as revealed by airway dissection. A comparison of mammalian lungs. *Am Rev Respir Dis* 128: S4–S7. [PubMed: 6881706]
- Pritchard KA, Ou J, Ou Z, Shi Y, Franciosi JP, Signorino P, Kaul S, Ackland-Berglund C, Witte K, Holzhauser S, et al. 2004. Hypoxia-induced acute lung injury in murine models of sickle cell disease. *Am J Physiol Lung Cell Mol Physiol* 286: L705–L714. doi:10.1152/ajplung.00288.2002 [PubMed: 12972407]
- Randell SH, Comment CE, Ramaekers FC, Nettekheim P. 1991. Properties of rat tracheal epithelial cells separated based on expression of cell surface α -galactosyl end groups. *Am J Respir Cell Mol Biol* 4: 544–554. doi:10.1165/ajrcmb/4.6.544 [PubMed: 1711352]
- Rane CK, Jackson SR, Pastore CF, Zhao G, Weiner AI, Patel NN, Herbert DR, Cohen NA, Vaughan AE. 2019. Development of solitary chemosensory cells in the distal lung after severe influenza injury. *Am J Physiol Lung Cell Mol Physiol* 316: L1141–L1149. doi:10.1152/ajplung.00032.2019 [PubMed: 30908939]
- Ranga V, Kleinerman J. 1981. A quantitative study of ciliary injury in the small airways of mice: the effects of nitrogen dioxide. *Exp Lung Res* 2: 49–55. doi:10.3109/01902148109052302 [PubMed: 7346269]
- Rawlins EL, Ostrowski LE, Randell SH, Hogan BLM. 2007. Lung development and repair: contribution of the ciliated lineage. *Proc Natl Acad Sci* 104: 410–417. doi:10.1073/pnas.0610770104 [PubMed: 17194755]
- Rawlins EL, Okubo T, Xue Y, Brass DM, Auten RL, Hasegawa H, Wang F, Hogan BLM. 2009. The role of Scgb1a1⁺ Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. *Cell Stem Cell* 4: 525–534. doi:10.1016/j.stem.2009.04.002 [PubMed: 19497281]
- Reyfman PA, Walter JM, Joshi N, Anekalla KR, McQuattie-Pimentel AC, Chiu S, Fernandez R, Akbarpour M, Chen CI, Ren Z, et al. 2019. Single-cell transcriptomic analysis of human lung provides insights into the pathobiology of pulmonary fibrosis. *Am J Respir Crit Care Med* 199: 1517–1536. doi:10.1164/rccm.201712-2410OC [PubMed: 30554520]
- Reynolds SD, Reynolds PR, Pryhuber GS, Finder JD, Stripp BR. 2002. Secretoglobins SCGB3A1 and SCGB3A2 define secretory cell subsets in mouse and human airways. *Am J Respir Crit Care Med* 166: 1498–1509. doi:10.1164/rccm.200204-285OC [PubMed: 12406855]
- Riedel F, Krämer M, Scheibenbogen C, Rieger CHL. 1988. Effects of SO₂ exposure on allergic sensitization in the guinea pig. *J Allergy Clin Immunol* 82: 527–534. doi:10.1016/0091-6749(88)90961-X [PubMed: 3170998]
- Rittirsch D, Flierl MA, Day DE, Nadeau BA, McGuire SR, Hoesel LM, Ipaktchi K, Zetoune FS, Sarma JV, Leng L, et al. 2008. Acute lung injury induced by lipopolysaccharide is independent of complement activation. *J Immunol* 180: 7664–7672. doi:10.4049/jimmunol.180.11.7664 [PubMed: 18490769]
- Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, Randell SH, Hogan BLM. 2009. Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc Natl Acad Sci* 106: 12771–12775. doi:10.1073/pnas.0906850106 [PubMed: 19625615]
- Rock JR, Randell SH, Hogan BLM. 2010. Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis Model Mech* 3: 545–556. doi:10.1242/dmm.006031 [PubMed: 20699479]
- Rock JR, Barkauskas CE, Cronic MJ, Xue Y, Harris JR, Liang J, Noble PW, Hogan BLM. 2011. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proc Natl Acad Sci* 108: E1475–E1483. doi:10.1073/pnas.1117988108 [PubMed: 22123957]

- Rombout PJ, Dormans JA, Marra M, van Esch GJ. 1986. Influence of exposure regimen on nitrogen dioxide-induced morphological changes in the rat lung. *Environ Res* 41: 466–480. doi:10.1016/S0013-9351(86)80141-4 [PubMed: 3780645]
- Royce SG, Patel KP, Samuel CS. 2014. Characterization of a novel model incorporating airway epithelial damage and related fibrosis to the pathogenesis of asthma. *Lab Invest* 94: 1326–1339. doi:10.1038/labinvest.2014.119 [PubMed: 25264707]
- Ruiz García S, Deprez M, Lebrigand K, Cavard A, Paquet A, Arguel M-J, Magnone V, Truchi M, Caballero I, Leroy S, et al. 2019. Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures. *Development* 146: dev177428. doi:10.1242/dev.177428
- Rupp H, Henschler D. 1967. Effect of low chlorine and bromine concentrations on man. *Int Arch Arbeitsmed* 23: 79–90. [PubMed: 6075927]
- Sagiv A, Bar-Shai A, Levi N, Hatzav M, Zada L, Ovadya Y, Roitman L, Manella G, Regev O, Majewska J, et al. 2018. p53 in bronchial club cells facilitates chronic lung inflammation by promoting senescence. *Cell Rep* 22: 3468–3479. doi:10.1016/j.celrep.2018.03.009 [PubMed: 29590616]
- Salwig I, Spitznagel B, Vazquez-Armendariz AI, Khalooghi K, Guenther S, Herold S, Szibor M, Braun T. 2019. Bronchioalveolar stem cells are a main source for regeneration of distal lung epithelia in vivo. *EMBO J* 38: e102099. doi:10.15252/embj.2019102099 [PubMed: 31028085]
- Sarpong SB, Zhang LY, Kleeberger SR. 2003. A novel mouse model of experimental asthma. *Int Arch Allergy Immunol* 132: 346–354. [PubMed: 14707466]
- Saunders CJ, Reynolds SD, Finger TE. 2013. Chemosensory brush cells of the trachea. A stable population in a dynamic epithelium. *Am J Respir Cell Mol Biol* 49: 190–196. doi:10.1165/rmb.2012-0485OC [PubMed: 23526223]
- Schoch KG, Lori A, Burns KA, Eldred T, Olsen JC, Randell SH. 2004. A subset of mouse tracheal epithelial basal cells generates large colonies in vitro. *Am J Physiol Lung Cell Mol Physiol* 286: L631–L642. doi:10.1152/ajplung.00112.2003 [PubMed: 12959927]
- Schwartz LW, Dungworth DL, Mustafa MG, Tarkington BK, Tyler WS. 1976. Pulmonary responses of rats to ambient levels of ozone: effects of 7-day intermittent or continuous exposure. *Lab Invest* 34: 565–578. [PubMed: 933466]
- Scudieri P, Musante I, Venturini A, Guidone D, Genovese M, Cresta F, Caci E, Palleschi A, Poeta M, Santamaria F, et al. 2020. Ionocytes and CFTR chloride channel expression in normal and cystic fibrosis nasal and bronchial epithelial cells. *Cells* 9: 2090. doi:10.3390/cells9092090
- Shi N, Zhang J, Chen S-Y. 2017. Runx2, a novel regulator for goblet cell differentiation and asthma development. *FASEB J* 31: 412–420. doi:10.1096/fj.201600954r [PubMed: 27825108]
- Shivaraju M, Chitta UK, Grange RMH, Jain IH, Capen D, Liao L, Xu J, Ichinose F, Zapol WM, Mootha VK, et al. 2021. Airway stem cells sense hypoxia and differentiate into protective solitary neuroendocrine cells. *Science* 371: 52–57. doi:10.1126/science.aba0629 [PubMed: 33384370]
- Smith LJ. 1985. Hyperoxic lung injury: biochemical, cellular, and morphologic characterization in the mouse. *J Lab Clin Med* 106: 269–278. [PubMed: 2993457]
- Song H, Yao E, Lin C, Gacayan R, Chen MH, Chuang PT. 2012a. Functional characterization of pulmonary neuroendocrine cells in lung development, injury, and tumorigenesis. *Proc Natl Acad Sci* 109: 17531–17536. doi:10.1073/pnas.1207238109 [PubMed: 23047698]
- Stolk J, Rudolphus A, Davies P, Osinga D, Dijkman JH, Agarwal L, Keenan KP, Fletcher D, Kramps JA. 1992. Induction of emphysema and bronchial mucus cell hyperplasia by intratracheal instillation of lipopolysaccharide in the hamster. *J Pathol* 167: 349–356. doi:10.1002/path.1711670314 [PubMed: 1517904]
- Stripp BR, Maxson K, Mera R, Singh G. 1995. Plasticity of airway cell proliferation and gene expression after acute naphthalene injury. *Am J Physiol* 269: L791–L799. doi:10.1152/ajplung.1995.269.6.L791 [PubMed: 8572241]
- Strunz M, Simon LM, Ansari M, Kathiriya JJ, Angelidis I, Mayr CH, Tsidiridis G, Lange M, Mattner LF, Yee M, et al. 2020. Alveolar regeneration through a Krt8⁺ transitional stem cell state

that persists in human lung fibrosis. *Nat Commun* 11: 3559. doi:10.1038/s41467-020-17358-3 [PubMed: 32678092]

- Summerhill EM, Hoyle GW, Jordt S-E, Jugg BJ, Martin JG, Matalon S, Patterson SE, Prezant DJ, Sciuto AM, Svendsen ER, et al. 2017. An official American thoracic society workshop report: chemical inhalational disasters. *Biology of lung injury, development of novel therapeutics, and medical preparedness. Ann Am Thorac Soc* 14: 1060–1072. doi:10.1513/AnnalsATS.201704-297WS [PubMed: 28418689]
- Sunil VR, Vayas KN, Massa CB, Gow AJ, Laskin JD, Laskin DL. 2013. Ozone-induced injury and oxidative stress in bronchiolar epithelium are associated with altered pulmonary mechanics. *Toxicol Sci* 133: 309–319. doi:10.1093/toxsci/kft071 [PubMed: 23492811]
- Tata PR, Rajagopal J. 2017. Plasticity in the lung: making and breaking cell identity. *Development* 144: 755–766. doi:10.1242/dev.143784 [PubMed: 28246210]
- Tata PR, Mou H, Pardo-Saganta A, Zhao R, Prabhu M, Law BM, Vinarsky V, Cho JL, Breton S, Sahay A, et al. 2013. Dedifferentiation of committed epithelial cells into stem cells in vivo. *Nature* 503: 218–223. doi:10.1038/nature12777 [PubMed: 24196716]
- Tata A, Kobayashi Y, Chow RD, Tran J, Desai A, Massri AJ, McCord TJ, Gunn MD, Tata PR. 2018. Myoepithelial cells of submucosal glands can function as reserve stem cells to regenerate airways after injury. *Cell Stem Cell* 22: 668–683.e6. doi:10.1016/j.stem.2018.03.018 [PubMed: 29656943]
- Tavares AH, Colby JK, Levy BD, Abdunour REE. 2019. A model of self-limited acute lung injury by unilateral intra-bronchial acid instillation. *J Vis Exp* doi:10.3791/60024
- Theise ND, Henegariu O, Grove J, Jagirdar J, Kao PN, Crawford JM, Badve S, Saxena R, Krause DS. 2002. Radiation pneumonitis in mice: a severe injury model for pneumocyte engraftment from bone marrow. *Exp Hematol* 30: 1333–1338. doi:10.1016/S0301-472X(02)00931-1 [PubMed: 12423687]
- Tian X, Tao H, Brisolaro J, Chen J, Rando RJ, Hoyle GW. 2008. Acute lung injury induced by chlorine inhalation in C57BL/6 and FVB/N mice. *Inhal Toxicol* 20: 783–793. doi:10.1080/08958370802007841 [PubMed: 18645717]
- Tizzano M, Merigo F, Sbarbati A. 2006. Evidence of solitary chemosensory cells in a large mammal: the diffuse chemosensory system in *Bos taurus* airways. *J Anat* 209: 333–337. doi:10.1111/j.1469-7580.2006.00617.x [PubMed: 16928202]
- Tizzano M, Cristofolletti M, Sbarbati A, Finger TE. 2011. Expression of taste receptors in solitary chemosensory cells of rodent airways. *BMC Pulm Med* 11: 3. doi:10.1186/1471-2466-11-3 [PubMed: 21232137]
- Tos M 1966. Development of the tracheal glands in man; number, density, structure, shape, and distribution of mucous glands elucidated by quantitative studies of whole mounts. *Acta Pathol Microbiol Scand* 68 (Suppl. 185): 3.
- Travaglini KJ, Nabhan AN, Penland L, Sinha R, Gillich A, Sit RV, Chang S, Conley SD, Mori Y, Seita J, et al. 2020. A molecular cell atlas of the human lung from single-cell RNA sequencing. *Nature* 587: 619–625. doi:10.1038/s41586-020-2922-4 [PubMed: 33208946]
- Tsoutsou PG, Koukourakis MI. 2006. Radiation pneumonitis and fibrosis: mechanisms underlying its pathogenesis and implications for future research. *Int J Radiat Oncol Biol Phys* 66: 1281–1293. doi:10.1016/j.ijrobp.2006.08.058 [PubMed: 17126203]
- Tuck SA, Ramos-Barbón D, Campbell H, McGovern T, Karmouty-Quintana H, Martin JG. 2008. Time course of airway remodelling after an acute chlorine gas exposure in mice. *Respir Res* 9: 61. doi:10.1186/1465-9921-9-61 [PubMed: 18702818]
- Uhal BD, Etter MD. 1993. Type II pneumocyte hypertrophy without activation of surfactant biosynthesis after partial pneumonectomy. *Am J Physiol* 264: L153–L159. [PubMed: 8447427]
- Van Winkle LS, Buckpitt AR, Nishio SJ, Isaac JM, Plopper CG. 1995. Cellular response in naphthalene-induced Clara cell injury and bronchiolar epithelial repair in mice. *Am J Physiol* 269: L800–818. [PubMed: 8572242]
- Vaughan AE, Brumwell AN, Xi Y, Gotts JE, Brownfield DG, Treutlein B, Tan K, Tan V, Liu FC, Looney MR, et al. 2015. Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. *Nature* 517: 621–625. doi:10.1038/nature14112 [PubMed: 25533958]

- Vila Ellis L, Cain MP, Hutchison V, Flodby P, Crandall ED, Borok Z, Zhou B, Ostrin EJ, Wythe JD, Chen J. 2020. Epithelial Vegfa specifies a distinct endothelial population in the mouse lung. *Dev Cell* 52: 617–630.e6. doi:10.1016/j.devcel.2020.01.009 [PubMed: 32059772]
- Vohwinkel CU, Hoegl S, Eltzhig HK. 2015. Hypoxia signaling during acute lung injury. *J Appl Physiol* (1985) 119: 1157–1163. doi:10.1152/jappphysiol.00226.2015 [PubMed: 25977449]
- Wagner JC. 1963. Asbestosis in experimental animals. *Br J Ind Med* 20: 1–12. [PubMed: 13998251]
- Wang Y, Tang Z, Huang H, Li J, Wang Z, Yu Y, Zhang C, Li J, Dai H, Wang F, et al. 2018. Pulmonary alveolar type I cell population consists of two distinct subtypes that differ in cell fate. *Proc Natl Acad Sci* 115: 2407–2412. doi:10.1073/pnas.1719474115 [PubMed: 29463737]
- Watson JK, Rulands S, Wilkinson AC, Wuidart A, Ousset M, Van Keymeulen A, Göttgens B, Blanpain C, Simons BD, Rawlins EL. 2015. Clonal dynamics reveal two distinct populations of basal cells in slow-turnover airway epithelium. *Cell Rep* 12: 90–101. doi:10.1016/j.celrep.2015.06.011 [PubMed: 26119728]
- Weibel ER. 1963. *Morphometry of the human lung*. Springer, Berlin.
- Weibel ER. 2015. On the tricks alveolar epithelial cells play to make a good lung. *Am J Respir Crit Care Med* 191: 504–513. doi:10.1164/rccm.201409-1663OE [PubMed: 25723823]
- Whitsett JA. 2018. Airway epithelial differentiation and mucociliary clearance. *Ann Am Thorac Soc* 15: S143–S148. doi:10.1513/AnnalsATS.201802-128AW [PubMed: 30431340]
- Whitsett JA, Wert SE, Trapnell BC. 2004. Genetic disorders influencing lung formation and function at birth. *Hum Mol Genet* 13 (Spec. No. 2): R207–R215. doi:10.1093/hmg/ddh252 [PubMed: 15358727]
- Widdicombe JH, Wine JJ. 2015. Airway gland structure and function. *Physiol Rev* 95: 1241–1319. doi:10.1152/physrev.00039.2014 [PubMed: 26336032]
- Woolf A, Shannon M. 1999. Reactive airways dysfunction and systemic complaints after mass exposure to bromine. *Environ Health Perspect* 107: 507–509. doi:10.1289/ehp.99107507 [PubMed: 10339453]
- Wu H, Yu Y, Huang H, Hu Y, Fu S, Wang Z, Shi M, Zhao X, Yuan J, Li J, et al. 2020. Progressive pulmonary fibrosis is caused by elevated mechanical tension on alveolar stem cells. *Cell* 180: 107–121.e17. doi:10.1016/j.cell.2019.11.027 [PubMed: 31866069]
- Xi Y, Kim T, Brumwell AN, Driver IH, Wei Y, Tan V, Jackson JR, Xu J, Lee DK, Gotts JE, et al. 2017. Local lung hypoxia determines epithelial fate decisions during alveolar regeneration. *Nat Cell Biol* 19: 904–914. doi:10.1038/ncb3580 [PubMed: 28737769]
- Xie W, Fisher JT, Lynch TJ, Luo M, Evans TIA, Neff TL, Zhou W, Zhang Y, Ou Y, Bunnett NW, et al. 2011. CGRP induction in cystic fibrosis airways alters the submucosal gland progenitor cell niche in mice. *J Clin Invest* 121: 3144–3158. doi:10.1172/JCI41857 [PubMed: 21765217]
- Yang Y, Riccio P, Schotsaert M, Mori M, Lu J, Lee DK, García-Sastre A, Xu J, Cardoso WV. 2018. Spatial-temporal lineage restrictions of embryonic p63⁺ progenitors establish distinct stem cell pools in adult airways. *Dev Cell* 44: 752–761.e4. doi:10.1016/j.devcel.2018.03.001 [PubMed: 29587145]
- Yang J, Li M, Chen X, Lian Q, Wang Q, Gao F, Jin S, Zheng S. 2019. Lipoxin A4 ameliorates lipopolysaccharide-induced lung injury through stimulating epithelial proliferation, reducing epithelial cell apoptosis and inhibits epithelial–mesenchymal transition. *Respir Res* 20: 192. doi:10.1186/s12931-019-1158-z [PubMed: 31438948]
- Yao C, Guan X, Carraro G, Parimon T, Liu X, Huang G, Mulay A, Soukiasian HJ, David G, Weigt SS, et al. 2021. Senescence of alveolar type 2 cells drives progressive pulmonary fibrosis. *Am J Respir Crit Care Med* 203: 707–717. doi:10.1164/rccm.202004-1274OC [PubMed: 32991815]
- Yee M, Buczynski BW, O'Reilly MA. 2014. Neonatal hyperoxia stimulates the expansion of alveolar epithelial type II cells. *Am J Respir Cell Mol Biol* 50: 757–766. doi:10.1165/rcmb.2013-0207OC [PubMed: 24188066]
- Yee M, Gelein R, Mariani TJ, Lawrence BP, O'Reilly MA. 2016. The oxygen environment at birth specifies the population of alveolar epithelial stem cells in the adult lung. *Stem Cells* 34: 1396–1406. doi:10.1002/stem.2330 [PubMed: 26891117]
- Yu M, Sun X, Tyler SR, Liang B, Swatek AM, Lynch TJ, He N, Yuan F, Feng Z, Rotti PG, et al. 2019. Highly efficient transgenesis in ferrets using CRISPR/Cas9-mediated homology-independent

insertion at the ROSA26 locus. *Sci Rep* 9: 1971. doi:10.1038/s41598-018-37192-4 [PubMed: 30760763]

- Zacharias WJ, Frank DB, Zepp JA, Morley MP, Alkhaleel FA, Kong J, Zhou S, Cantu E, Morrisey EE. 2018. Regeneration of the lung alveolus by an evolutionarily conserved epithelial progenitor. *Nature* 555: 251–255. doi:10.1038/nature25786 [PubMed: 29489752]
- Zepp JA, Morrisey EE. 2019. Cellular crosstalk in the development and regeneration of the respiratory system. *Nat Rev Mol Cell Biol* 20: 551–566. doi:10.1038/s41580-019-0141-3 [PubMed: 31217577]
- Zepp JA, Zacharias WJ, Frank DB, Cavanaugh CA, Zhou S, Morley MP, Morrisey EE. 2017. Distinct mesenchymal lineages and niches promote epithelial self-renewal and myofibrogenesis in the lung. *Cell* 170: 1134–1148.e10. doi:10.1016/j.cell.2017.07.034 [PubMed: 28886382]
- Zheng D, Limmon GV, Yin L, Leung NHN, Yu H, Chow VTK, Chen J. 2012. Regeneration of alveolar type I and II cells from Scgb1a1-expressing cells following severe pulmonary damage induced by bleomycin and influenza. *PLoS ONE* 7: e48451. doi:10.1371/journal.pone.0048451 [PubMed: 23119022]
- Zheng D, Yin L, Chen J. 2014. Evidence for Scgb1a1⁺ cells in the generation of p63⁺ cells in the damaged lung parenchyma. *Am J Respir Cell Mol Biol* 50: 595–604. doi:10.1165/rcmb.2013-0327OC [PubMed: 24134540]
- Zitnik LA, Schwartz LW, McQuillen NK, Zee YC, Osebold JW. 1978. Pulmonary changes induced by low-level ozone: morphological observations. *J Environ Pathol Toxicol* 1: 365–376. [PubMed: 722197]
- Zuo W, Zhang T, Wu DZ, Guan SP, Liew A-A, Yamamoto Y, Wang X, Lim SJ, Vincent M, Lessard M, et al. 2015. p63⁺Krt5⁺ distal airway stem cells are essential for lung regeneration. *Nature* 517: 616–620. doi:10.1038/nature13903 [PubMed: 25383540]

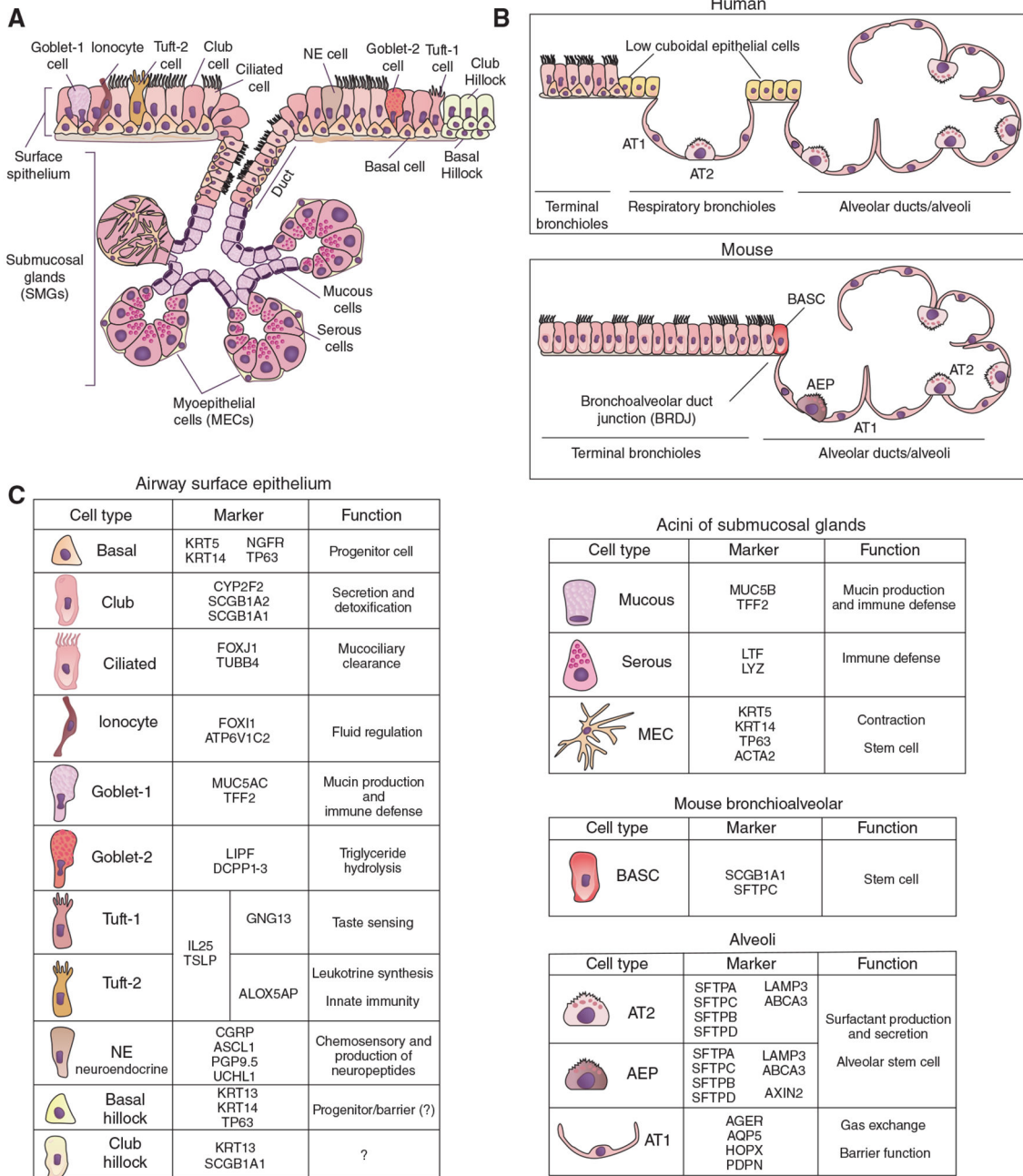


Figure 1. Structure and cellular composition of the lower respiratory system. (A) Schematic representation of the surface epithelium (SE) and submucosal glands (SMGs). SE of the trachea and bronchi consists of many distinct epithelial cell types including basal, club, and ciliated and rare cell types (ionocytes, neuroendocrine [NE] tufts [1 and 2], and goblet [1 and 2]). “Hillocks” are primarily composed of KRT13⁺ basal cells and club cells. Highly branched tubuloacinar SMGs are embedded within the mesenchyme and open to the SE through specialized ducts. The serous acini and mucous tubules are surrounded by stellate-shaped myoepithelial cells (MECs). (B) Comparison of human and mouse distal

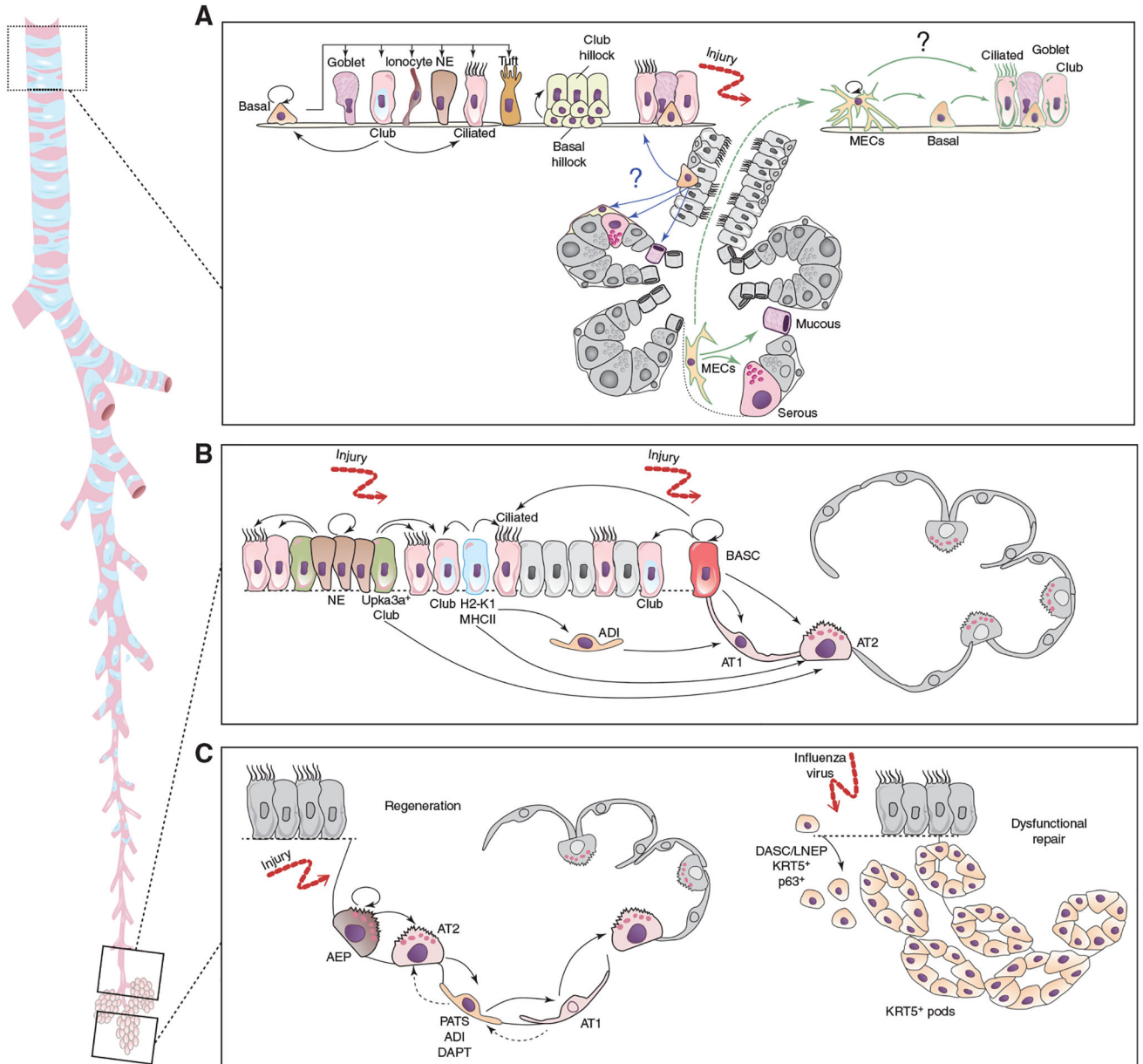
lungs at the airway and alveolar junctional region. Murine lungs lack respiratory bronchioles and terminal airways directly lead to alveolar ducts via bronchoalveolar duct junction (BADJ). Rare, bronchioalveolar stem cells (BASCs) are located in the bronchioalveolar duct junction in mouse distal airway. Schematic shows different epithelial cells and subsets (type 2 alveolar epithelial cells [AT2s], alveolar epithelial progenitors [AEPs], type 1 alveolar epithelial cells [AT1s]) of the alveoli. In human lungs, terminal bronchioles transition to alveolar ducts via a unique respiratory bronchiolar region. Basal cells and poorly characterized cuboidal epithelial cells are present in human distal airways. (C) Table depicting all described epithelial cells with their specific markers and function.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

**Figure 2.**

Epithelial cell lineage relationships in the large airways, small airways, and alveoli. (A) Regeneration of the trachea and large airways. Basal cells are the major cell types that can self-renew and give rise to all luminal cells present on the surface epithelium (SE) including club, ciliated, ionocytes, tufts, and goblet. Lineage-tracing studies have shown that basal “hillock” cells can generate club “hillock” cells. In response to damage, club cells give rise to ciliated cells. Club cells can also dedifferentiate into basal cells. Myoepithelial cells (MECs) of the submucosal glands (SMGs) can migrate to the SE, acquire basal cell characteristics, and give rise to club, ciliated, and goblet cells. MECs also have the potential to differentiate into mucous and serous in SMGs. (B) Multiple epithelial cell types respond to different injuries in small airways and the bronchioalveolar duct junction. Neuroendocrine

cells can self-renew and give rise to club and ciliated cells. In response to injury, H2-K1 and Upka3a-expressing club cells can regenerate luminal cells of SE and contribute to alveolar cell regeneration. Bronchioalveolar stem cells (BASCs) can self-renew and replenish injured club, ciliated, and alveolar type 2 epithelial cells (AT2s) as well as the neighboring club cells. (C) Regeneration and dysfunctional repair of the alveolar region. In response to loss of AT1 and AT2, alveolar epithelial progenitors (AEPs) proliferate and replenish injured AT2s and AT1s. AT2s differentiation into AT1 cells involves a novel transitional state termed pre-alveolar type 1 transitional cell state (PATS) (also called alveolar differentiation intermediate [ADI], damage-associated transient progenitors [DAPTs]). Hyperoxia and PNX-induced injury can induce AT1 cell dedifferentiation into AT2s. Following severe injury, such as influenza virus-induced injury, lineage-negative epithelial progenitors (LNEPs)/distal airway stem cells (DASCs) from distal airways migrate to damaged alveoli and form KRT5⁺ epithelial pods, which persist for the long term and generate dysplastic tissues.

Table 1.

Commonly used injury models to study lung regeneration

Injury model	Route of administration	Target cell/tissue and nature of damage	Responding epithelial cells	References
Chemical agents				
Sulfur dioxide (SO ₂)	Inhalation	Sloughing of airway epithelium	Basal, myoepithelial, and club cells	Lamb and Reid 1968; Riedel et al. 1988; Borthwick et al. 2001; Hong et al. 2004; Kodavanti et al. 2006; Rawlins et al. 2009; Rock et al. 2009, 2011; Song et al. 2012; Tata et al. 2018
Chlorine (Cl ₂)	Inhalation	Sloughing of airway epithelium, edema	Basal cells	Tian et al. 2008; Tuck et al. 2008; Musah et al. 2012
Nitrogen dioxide (NO ₂)	Inhalation	Sloughing of airway epithelium, damage to alveolar type I epithelial cells (AT1)	AT1, AT2, and club cells	Evans et al. 1981; Ranga and Kleinerman 1981; Foster et al. 1985; Rombout et al. 1986; Barth and Müller 1999
Ozone (O ₃)	Inhalation	Rapid disruption of the epithelial barrier, necrosis of ciliated and AT1 cells, degeneration of secretory cells	Club cells	Brockeaert et al. 1999; Sunil et al. 2013; Michaudel et al. 2018
Hypoxia	Inhalation	Epithelial damage	AT2 and club cells	Bouvy et al. 2006; Yee et al. 2014
Hyperoxia	Inhalation	Destruction of alveolar walls	AT2 and AT1 cells	Frank et al. 1978; Smith 1985; Rawlins et al. 2009; Kallet and Matthay 2013; Dessai et al. 2014; Nabhan et al. 2018; Penkala et al. 2021
Naphthalene	Intraperitoneal	Club, submucosal glands (SMGs), sloughing of epithelium	Basal, myoepithelial, club, and neuroendocrine (NE) cells	Buckpitt et al. 1995; Stripp et al. 1995; Van Winkle et al. 1995; Hong et al. 2001; Giangreco et al. 2002; Kim et al. 2005; Rawlins et al. 2009; Lynch et al. 2018; Tata et al. 2018
Polidocanol	Intranasal/ intratracheal	Sloughing of epithelium	Basal and SMG cells	Borthwick et al. 2001; Farrow et al. 2018
Hydrochloric acid (HCl)	Intratracheal/ intrabronchial	Damage of the alveolar cells	AT2 cells	Kennedy et al. 1989; Modelska et al. 1999; Marinova et al. 2019; Tavares et al. 2019
Diacetyl (DA)	Oral/intraperitoneal/ intratracheal/ intranasal	Sloughing of airway epithelium; loss of mucous, club, and ciliated cells	Basal cells	Colley et al. 1969; Hubbs et al. 2008; Morgan et al. 2008; Palmer et al. 2011; McGraw et al. 2020
Bleomycin	Intranasal/ intratracheal	Loss of airway and alveolar epithelium	AT2, bronchioalveolar stem cell (BASCs), H2-K1 ⁺ club cells	Aso et al. 1976; Bigby et al. 1985; Isaksson et al. 2001; Kim et al. 2005; Moore and Hogaboam 2008; Barakauskas et al. 2013; Vaughan et al. 2015; Kathirya et al. 2020
Asbestos	Intranasal/ intratracheal	Damage of alveolar cells	AT2 cells	Behrens 1951; Wagner 1963; Bozelka et al. 1983; Liu et al. 2013; Kim et al. 2016
Cigarette smoke	Intranasal	Damage of alveolar cells, goblet cells metaplasia	AT2 cells	Lamb and Reid 1969; Wright et al. 1992; Chung et al. 2008
Biological agents				
Influenza	Intranasal	Damage of airway and alveolar cells	Myoepithelial cells, basal cells, AT2, distal airway stem cells (DASCs)/lineage-	Loosli et al. 1975; Kumar et al. 2011; Zheng et al. 2012, 2014; Zuo et al. 2015; Kanegai et al. 2016; Tata et al. 2018; Zacharias et al. 2018

Injury model	Route of administration	Target cell/tissue and nature of damage	Responding epithelial cells	References
Lipopolysaccharide (LPS)	Intranasal/ intratracheal	Disruption of the alveolar/epithelial barrier	negative epithelial progenitors (LNEPs) AT2 and club cells	Stolk et al. 1992; Brass et al. 2008; Rittirsch et al. 2008; Sagiv et al. 2018; Yang et al. 2019
Ovalbumin (OVA)	Intranasal	Goblet cells metaplasia, airway hyperreactivity	Club cells	Sarpong et al. 2003; McMillan and Lloyd 2004; Locke et al. 2007; Royce et al. 2014
House dust mite (HDM)	Intranasal	Epithelial damage and loss of tight junctions	Club cells	Herbert et al. 1995; Shi et al. 2017; Woo et al. 2018
Other injury models				
Pneumectomy (PNX)	Surgical lung lobe removal	Lobe removal	AT1, AT2, BASCs	Brody et al. 1978; Uhal and Etter 1993; Chamoto et al. 2013; Jain et al. 2015; Vaughan et al. 2015; Chung et al. 2018; Wang et al. 2018
Irradiation	Whole body or lung exposure	Disruption to epithelial integrity, AT2 apoptosis	AT2 cells	Karvonen et al. 1987; Franko et al. 1991; Johnston et al. 1998; Theise et al. 2002; Citrin et al. 2013