Cellular plasticity and regenerative mechanisms in the lung

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Multiple cell types in the lung are capable of regenerating the lung tissue in response to injury. Understanding cellular and molecular mechanisms underlying this process will help promote tissue repair in the context of ageing or chronic lung disease. https://bit.ly/495ewkp

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Abstract

The adult lung is continuously exposed to environmental insults such as pathogens, pollutants and toxins, necessitating robust regenerative mechanisms to maintain tissue integrity and function. Epithelial regeneration relies on the activity and plasticity of resident stem and progenitor cell populations that are spatially distributed across airway and alveolar compartments. Basal cells in the conducting airways and alveolar type (AT) 2 cells in the alveoli act as regional stem cells, capable of self-renewal and multilineage differentiation. Additionally, variant club cells, bronchioalveolar stem cells (BASCs) and newly identified secretory and transitional cell types such as respiratory airway secretory and AT0 cells have emerged as critical players in lung repair. Cellular plasticity, the ability of differentiated cells to dedifferentiate or transdifferentiate, enables rapid adaptation to injury but may also contribute to chronic lung disease when dysregulated. Ageing and chronic injury reduce regenerative capacities, leading to failed repair, fibrotic remodelling or epithelial simplification, as seen in diseases such as idiopathic pulmonary fibrosis and COPD. Recent advances in single-cell and spatial transcriptomics have revealed cellular heterogeneity, novel progenitor states and transitional intermediates that underpin both normal repair and disease pathogenesis. In this review, we integrate findings from animal models and human lung studies to highlight conserved and divergent mechanisms governing cell fate decisions. We discuss how niche signals, transcriptional programmes and extrinsic cues shape epithelial regeneration and explore the therapeutic implications of targeting epithelial plasticity in chronic lung disease.

Introduction

The main function of the respiratory system is to exchange oxygen and carbon dioxide between the lungs and the blood. With this purpose, the adult mammalian lung is organised into two major compartments: the airways, which conduct oxygen from the atmosphere to the distal lung, and the alveoli where gas exchange occurs [1, 2]. Thus, the respiratory epithelium is continuously exposed to different environmental insults such as irritants, pollutants and pathogens, which can cause cellular damage [2]. However, despite their slow turnover under physiological conditions, the lung possesses a potent capacity to repair and regenerate the damaged tissue in response to frequent exposure to harmful agents and distinct types of injury, due to the presence of resident stem cell populations spatially and temporally restricted in the airways and the alveoli [1, 2]. While basal cells function as adult airway stem cells [3], alveolar type (AT) 2 cells behave as stem cells for the alveolar epithelium [4]. These cells are mature functional cells of the lung that serve as stem cells giving rise to the different pulmonary cell types present in their compartment [1–4]. Long-term

clonal analysis has delineated their behaviour in the maintenance of the homeostatic airway and alveolar epithelium, respectively [5, 6]. Upon injury, basal cells and AT2 cells act as the primary drivers of tissue regeneration, a biological process that restores tissue architecture and function [7]. Among multiple anatomical differences between the murine and the human lung, major differences are observed at the distal part of the small airways where bronchoalveolar duct junctions (BADJs) in the mouse hold a stem cell population (BASCs), while human respiratory bronchioles (RBs) are endowed with specific populations including terminal and RB secretory cells (TRB-SCs) and AT0 cells with the capacity to contribute to other cell types after injury [2].

It should be noted that, in the lung, adult stem cells are considered nonclassical stem cells, as they are not fully undifferentiated, but retain the ability to self-renew indefinitely and exhibit multipotent potential to generate differentiated cells within the same tissue. In addition, the lung harbours distinct progenitor cell populations, which are descendants of stem cells that arise during normal tissue development and turnover. These progenitor cells possess limited self-renewal capacity and are already committed to specific lineages. Notably, the adult lung comprises approximately 40 distinct cell types, each characterised by unique molecular, morphological and functional properties that enable them to perform specialised tasks. This remarkable cellular diversity encompasses multiple progenitor cell populations capable of proliferation and differentiation into distinct lineages, reflecting the high plasticity of the lung epithelium [8–11]. Cellular plasticity refers to the fundamental property that enables differentiated cells to adapt their identity in response to tissue damage. In the lung, epithelial cells display remarkable cell plasticity, undergoing lineage conversion in response to injury or other stimuli [8–11]. This plasticity can involve cells de-differentiating into stem-like cells, or transdifferentiating into other cell types and plays a crucial role in lung development, homeostasis and repair, but it can also contribute to disease development [8–11]. Thus, the regenerative potential of the lung critically depends on the plasticity of specific cell types.

During ageing, the regenerative capacity of stem cells is reduced. In fact, this exhaustion is one of the hallmarks of ageing [12]. The global population is steadily ageing [13]. Ageing is accompanied by cumulative functional decline across organ systems. In the lung, lifelong exposure to noxious stimuli such as household and ambient air pollution or tobacco smoke compounds exacerbates these intrinsic changes, leading to enlarged airspaces, diminished elastic recoil, impaired ventilatory control, reduced chest-wall compliance and respiratory-muscle weakness, all of which progressively compromise pulmonary function [14–17]. Ageing is therefore the predominant risk factor for chronic lung diseases such as COPD and idiopathic pulmonary fibrosis (IPF). In fact, both conditions present most of the hallmarks of ageing [12] and their rising incidence with age suggests a role for premature ageing in pathophysiology [18]. In this scenario, mechanisms of repair are inefficient and failed regeneration leads to epithelial simplification or scar tissue formation, respectively [2, 14, 19]. In addition, the emergence of aberrant epithelial cells contributes to the progression of pathology and impedes tissue repair [2, 19, 20].

Here, we review the most recent knowledge on the stem and progenitor cell populations of the airway and the alveolar epithelium, their intrinsic and extrinsic regulation by other cell types of their niche, and their contribution to disease.

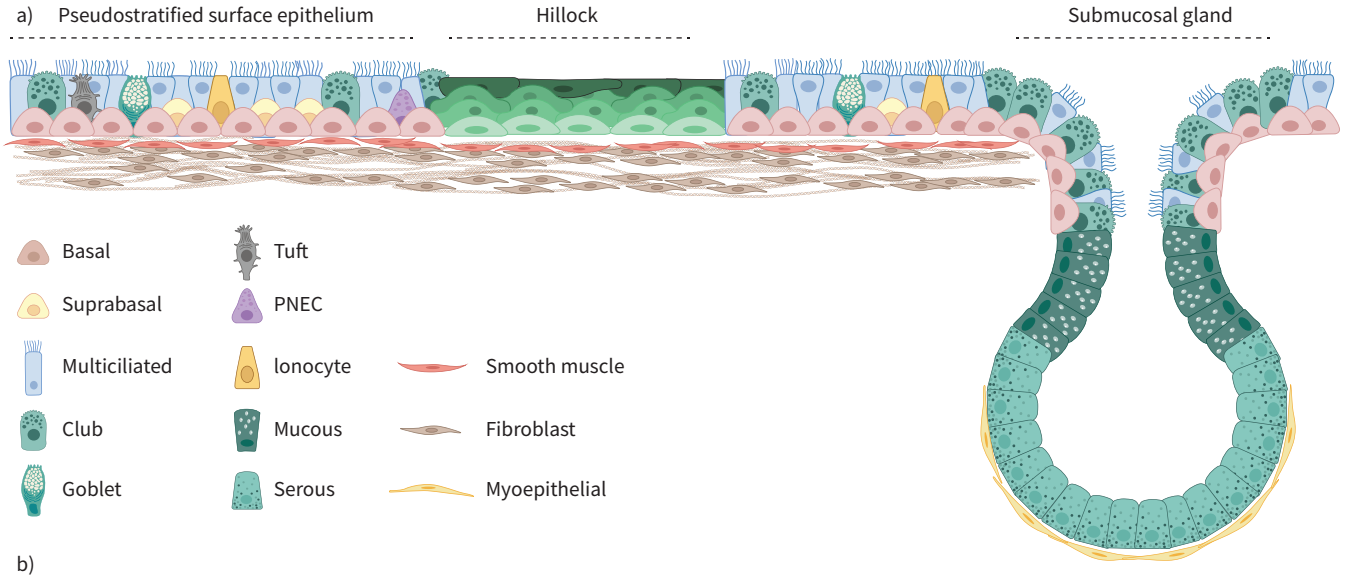
The airway epithelium

The airway epithelium forms a complex ecosystem, mainly composed of secretory cells including club cells, producing anti-microbial and anti-inflammatory peptides as well as goblet cells, secreting protective mucins on the luminal surface, and multiciliated cells (MCCs), evacuating mucus by the coordinated beating of their motile cilia. The airway epithelium also contains basal cells, playing a role in adhesion and stability of the epithelium [21–23] and rare cells such as ionocytes, neuroendocrine and tuft cells [24] (figure 1a). The balance between cell type proportions must be properly controlled in order to maintain an efficient mucociliary clearance, which is the main function of the airway epithelium together with serving as a powerful immunological barrier [2]. In the airways of patients suffering from chronic respiratory diseases, an over-representation of goblet cells and underrepresentation of MCCs are often observed, leading to worsening of symptoms. These diseases include COPD, primary ciliary dyskinesia, asthma and cystic fibrosis. These diseases, which collectively affect hundreds of millions of people, are characterised by frequent injuries, repair defects, tissue remodelling and altered mucociliary clearance [25–27].

Basal cells as the main stem cells of the large airways

To accurately characterise the cellular and molecular identity of progenitor cells of the airway epithelium that contribute to tissue regeneration at homeostasis and upon repair, lung epithelial cell fates have been investigated mainly in rodents, after acute injury of the epithelium with noxious agents (table 1). Lineage tracing studies using cytokeratin (Krt) 5 or TP63 (tumour protein p63), a basal cell-specific transcription

a)



b)

	Cell type	Markers	Properties – trajectories	References
Stem cells	Basal	TP63, KRT5, NGFR	Self-renewal, differentiate into all cell types of the airway epithelium	[3, 24, 28, 29]
Progenitor cells	Club	SCGB1A1, NOTCH2	Differentiate into goblet and multiciliated cells. Upon basal cell ablation, can dedifferentiate into basal cells and give rise to all cell types of the surface epithelium	[63, 66]
	Hillock basal	KRT13, KRT6a, DSG3	Resistant to chemical assaults, differentiate into all luminal cell types of the surface epithelium	[78, 79]
	Myoepithelial	ACTA2, KRT5/14	Upon injury, acquire basal cell characteristics to support regeneration of all cell types of both the SMG and the surface airway epithelium	[67, 80–81]

FIGURE 1 Stem and progenitor cell populations in the airway epithelium. a) Schematic representation of the basal cell-containing airway epithelium indicating the different cell types in the surface epithelium, the submucosal glands and the mesenchyme. b) Table summarising the described populations acting as stem or progenitor cells of the airway epithelium. PNEC: pulmonary neuroendocrine cell; SMG: submucosal gland.

factor (TF), to label basal cells together with *in vitro* analysis using the air–liquid interface (ALI) model have identified basal cells as the main airway stem cells in mouse and human airways, as they display self-renewal capacities and ability to differentiate into secretory, multiciliated, as well as into rare cell types [3, 24, 28, 29] (figure 1b). Importantly, basal cells in mice are abundant in the trachea and in the main bronchi, but they are absent in smaller airways [1, 2, 21]. However, in humans, they populate the whole airways, though showing a two-fold decrease in numbers in smaller airways, *i.e.* with diameters below 0.5 mm [30], suggesting that mouse airways below the main bronchi are not an adequate model to study mechanisms taking place in the human airways.

Basal cells do not form a homogeneous population. Several markers and properties have defined basal cell subsets, such as Krt14 which is expressed in a subset of basal cells at homeostasis but increases both in repair after injury and in squamous metaplasia [31–33]. Long-term clonal dynamics analysis revealed that in the murine trachea at homeostasis, the basal cell population comprised stem cells and luminal progenitors that lived for approximately 10–12 days before differentiating into secretory cells and later into ciliated cells [5]. These progenitors occupy around 50% of the entire basal cell population and are morphologically identical to basal stem cells, but show some transcriptional differences and express low

TABLE 1 Comparative overview of experimental models used to identify stem/progenitor cell populations and to study regeneration and cellular plasticity

Model	Principle	Key findings	Representative studies	PMID
SO_2 injury	Depletion of secretory and ciliated cells in the trachea, widely used model to study basal stem cells	Basal cells proliferate and repair the tracheal epithelium They are specified early into ciliated and secretory lineages	Rock *et al.* [3] Pardo-Saganta *et al.* [34]	19625615 25658372
Naphthalene injury	Depletion of mature club cells in the intrapulmonary airways	Identification of naphthalene resistant "variant club cells" as progenitors of the intrapulmonary airways	Reynolds *et al.* [85]	10623675
Bleomycin-induced lung injury	DNA-damage inducing agent Injures the distal lung (AT1 and 2)	Triggers AT2 proliferation and differentiation into AT1 Reveals transitional $Krt8^+$ cell states Widely used model for fibrosis and regeneration	Strunz *et al.* [119] Kobayashi *et al.* [122]	32678092 32661339
Pneumonectomy (surgical removal of lung lobe)	Stimulates compensatory lung growth and induces AT2 proliferation	A noninflammatory model of lung regeneration, highlighting the role of mechanical cues and Wnt signalling in AT2 proliferation	Lechner *et al.* [155] Ahmadvand *et al.* [116] Wu *et al.* [169]	28506464 33863742 31866069
Genetic ablation of AT1 or AT2 cells	Expression of diphtheria toxin receptor in a cell-type-specific manner (SFTPC, Hopx, Ager)	Demonstrates AT2/AT1 plasticity and capacity for alveolar regeneration Identifies injury-induced intermediate states	Zacharias *et al.* [127] Konkimalla *et al.* [161]	29489752 37922879
Influenza or viral infection models	Model severe epithelial injury	Used to study epithelial repair and uncover immune-epithelial crosstalk and basal-like cell emergence	Zuo *et al.* [222] Vaughan *et al.* [125]	25383540 25533958
Organoid cultures	Prospective isolation of epithelial stem cells, followed by culturing in three-dimensional matrices, alone or in combination with other niche cells	Enable clonal analysis of basal cells and AT2 stemness Recapitulate differentiation and immune or mesenchymal interactions *in vitro*	Barkauskas *et al.* [4] Chanda *et al.* [223]	23921127 34528872
Precision-cut lung slices	*Ex vivo* model retaining lung architecture	Used to study epithelial behaviour, injury responses, and drug screening	Lehmann *et al.* [224] Lang *et al.* [203]	39499861 38055803

levels of Krt8 [5], a marker typically expressed by luminal cells. Interestingly, few basal cells at homeostasis expressed TFs of luminal cells: 5% of basal cells showed N2ICD (Notch2 intracellular domain) that is characteristic of secretory cells and 7% of basal cells expressed c-myb, which is a ciliated cell marker [34]. Whether these subsets belong to basal luminal progenitors remains unknown. Upon chemical injury, basal cells segregate to express one of these proteins in a mutually exclusive manner. C-myb-expressing basal cells directly differentiate into MCCs, whereas $N2ICD^+$ basal cells commit to the secretory pathway [34]. Basal cells in the mouse and human trachea also display regional heterogeneity, with ventral cells being enriched in Tgm2 and Isl1, while dorsal cells express higher levels of CD44, Cav1 and Krt17 [35]. These two populations differ in their self-renewal and differentiation capacities. Suprabasal cells have also been considered as a basal cell subcluster. Suprabasal or parabasal cells, situated between basal and luminal cells within the airway epithelium, exhibit a morphology comparable to that of basal cells. Under homeostatic conditions, most proliferating epithelial cells belong to this population [32, 36] which is characterised by the expression of Notch3, which is activated by ligands produced by basal cells [37]. The absence of Notch3 or of the signal provided by the Notch ligands resulted in aberrant expansion of basal cells and altered pseudostratification [37].

Regulation of basal cell behaviour at homeostasis and repair

The Notch signalling pathway plays a key role in directing epithelial cell fate decisions, particularly in balancing goblet cell and MCC differentiation. Inhibition of Notch signalling in basal cells is necessary to preserve their stem cell properties while constitutive activation of the Notch1 intracellular domain results in loss of basal cells and goblet cell hyperplasia in adult murine trachea [38]. While activation of Notch promotes goblet cell specification, its inhibition is essential for commitment to the multiciliated lineage [38–42]. Similarly, hyperactivation of the Wnt–β-catenin pathway has been shown to suppress multiciliogenesis in favour of goblet cell formation, mediated through interactions between β-catenin and

the transcriptional co-activators CREB-binding protein (CBP) and p300 [43]. Sonic hedgehog (SHH) signalling has been implicated in the regulation of mucociliary clearance by controlling the balance between multiciliated and goblet cells as well. Pharmacological inhibition of SHH signalling in fully differentiated, human ALI cultures resulted in a marked reduction in both ionocytes and MCCs, alongside goblet cell hyperplasia [44]. A potent driver of goblet hyperplasia in chronic respiratory diseases such as asthma and COPD is T-helper 2 (Th2) inflammation. Interleukin (IL)-4/13 secreted by type 2 lymphocytes and eosinophils activate transcriptional programs (*via* signal transducer and activator of transcription 6 (STAT6)) leading to goblet cell metaplasia and mucus overproduction [42, 45, 46]. In addition, epidermal growth factor receptor (EGFR) ligands also contribute to the differentiation into goblet cells, especially in response to various stimuli: tobacco smoke, viral infections or in chronic respiratory diseases such as COPD and asthma [47–50].

Transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP)/small mother against decapentaplegic (SMAD) signalling is suppressed in basal cells and active in suprabasal and luminal cells [51]. SMAD inhibition using *in vivo* and *in vitro* models has shown to promote basal cell expansion impeding luminal cell formation [51]. However, the specific role of BMP signalling in basal cell self-renewal and differentiation remains controversial. While BMP inhibition seems to impede MCC differentiation in a model of human airway organoids [52, 53], contrary observations have also been reported [54]. These opposite results may arise from the distinct roles of different BMP factors or from experimental conditions such as specific components of the culture media that modulate other pathways interacting with BMP.

The stromal niche also plays a critical role: following injury in mice, IL-6 [55] or Wnt ligands [56] secreted by Pdgfrα^+ cells in the intercartilaginous region are crucial for basal cell response and efficient airway regeneration. Notably, during ageing, glandular-like epithelial structures derived from basal cells form within this region, contributing to airway maintenance and regeneration [55]. In noncartilaginous mouse airways, peribronchial “repair-supportive mesenchymal cells” have been identified [57] and, more specifically, a Pdgfrα^+/Gli1$^+$ fraction that is crucial for airway epithelial repair after chemical depletion, through Fgf10 signalling [58]. The airway epithelium also controls the peribronchiolar mesenchyme through SHH which suppresses proliferation. During repair, this signalling is downregulated, permitting mesenchymal cell expansion and enabling reciprocal crosstalk that promotes basal cell proliferation and epithelial regeneration [59]. Immune cells also support basal cells. In particular, a unique immune cell population, intraepithelial airway macrophages (IAMs), has been identified in the trachea and proximal bronchi of mice and has been shown to be required for airway regeneration [60]. During repair, the C-C motif chemokine receptor 2-dependent recruitment of monocyte-derived macrophages is required for epithelial regeneration [60, 61]. Interestingly, a reciprocal interaction exists and basal cells are essential to maintain IAMs, serving as an immune niche [62], similar to their role maintaining their own progeny [39].

Club cells as important players in the airway epithelial cell lineage

The consensus emerging from lineage tracing studies suggests that, at homeostasis, basal cells usually differentiate first into suprabasal cells and then into club cells before committing to the multiciliated lineage [5, 39, 40]. Club cells are widespread from mouse trachea to bronchioles and are also found in human airways, though at a much less abundance, and enriched in the terminal and RBs [63]. The most extensively used marker of club cells is the anti-inflammatory secretoglobin SCGB1A1. Lineage tracing studies have used Scgb1a1 to determine the fate of club cells and have shown that they are capable of giving rise to MCCs and goblet cells at homeostasis and in repair [64]. Club cells play a particularly important role in airway regeneration in the distal airways of mice, where basal cells are absent. *NOTCH2* is expressed and activated in every club cell [39], also in agreement with studies identifying this receptor as crucial for secretory lineage establishment [65]. Club cells, despite being mature and functional cells, demonstrate high plasticity as they are able to proliferate and dedifferentiate upon basal cell ablation, resulting in basal cells able to regenerate the whole surface epithelium [66].

The rare epithelial cell lineages

Rare cell types found in the airway epithelium include ionocytes, recently identified as the primary source of CFTR, a cystic fibrosis transmembrane conductance regulator and member of the ABC transporter family [67], pulmonary neuroendocrine cells (PNECs), which function as oxygen and mechanosensors and secrete calcitonin gene-related peptide, a neuropeptide involved in regulating metabolism, inflammation and blood pressure [68], and tuft cells, chemosensory epithelial cells that contribute to microbial defence and may support mucociliary clearance [69]. These rare cells were initially thought to differentiate directly from basal cells [4] upon hypoxic conditions stimulating this differentiation [70]. However, early single-cell RNA sequencing (scRNAseq) atlases of the human airway epithelium suggested that they may

also be present under homeostatic conditions in healthy airways [36]. More recent studies, using transgenic ferret models and human ALI cultures, have identified a transitional progenitor population co-expressing FOXI1 (forkhead box I1) and POU2F3 (POU class 2 homeobox 3), which are lineage-defining TFs for ionocytes and tuft cells, respectively [44, 71, 72]. Diversity has been described within PNECs, including classical PNECs expressing neuropeptides only, PNECs expressing neuropeptides together with βIII-tubulin (TUBB3), and PNECs expressing TUBB3 only. In human airways, all three types are found whereas in mouse, $TUBB3^+$ PNECs only reside in the trachea and classical PNECs only reside in intrapulmonary airways. $TUBB3^+$ PNECs arise from basal cells, probably through an $ASCL1^+$ (achaete-scute family bHLH transcription factor 1) progenitor [73]. Given that several diseases emerge from PNEC alterations, additional studies would be necessary to clarify the lineage from basal cells towards ionocytes, tuft cells and the different subtypes of PNECs.

Another type of rare cells found in the airway epithelium are microfold (M) cells, which are well recognised for their role in capturing materials from the lumen and delivering them to underlying immune cells within gut-associated lymphoid structures, such as Peyer's patches [74, 75]. In the airway epithelium, M cells have been predominantly observed following influenza infection or stimulation by RANKL (receptor activator of nuclear factor κB ligand) [76]. However, they are also present at low levels under baseline conditions, where they are thought to originate from club cells [77].

Epithelial repair mediated by airway hillocks

Hillock structures were first identified in 2018 due to their distinctive morphology [24]. They are composed of stratified, flat squamous cells that sit above basal cells. These squamous cells are commonly linked to barrier functions, similar to those found in the epidermis. Both *in vivo* in mice and *in vitro* in human, basal cells from hillocks were found to be highly resistant to several assaults and were able of clonal expansion and differentiation towards multiciliated, secretory, tuft, neuroendocrine and ionocyte cells to repair large surfaces of the epithelium [78]. Moreover, in the context of vitamin A deficiency, squamous metaplasia commonly observed in tobacco-induced airway injury emerged through the expansion of hillock structures rather than through the transition of typical airway epithelial cells revealing the relevance of hillock basal cells in repair processes [78, 79].

Contribution of the submucosal glands for repair of the airway epithelium

The airway epithelium not only covers the surface of the respiratory tract but also extends into the submucosal glands (SMGs) found in the cartilaginous airways [21]. These glands contain serous cells, which secrete antimicrobial peptides such as lactoferrin and lysozyme, as well as mucous cells that produce MUC5B mucin, contributing to the lung's defence mechanisms [67, 80, 81]. SMGs are surrounded by myoepithelial cells, identified by their expression of smooth muscle actin (ACTA2) and TP63. In mouse injury models, these myoepithelial cells exhibit notable plasticity, acquiring basal cell characteristics to support regeneration of both the SMG and the surface airway epithelium. SOX9 (SRY-box transcription factor) is required for myoepithelial cell plasticity. SMG-like cells were also found in the airway epithelium from the trachea and bronchioles in a porcine model following chemical injury [82].

The distal airway epithelium

Progenitor cells in the murine bronchioalveolar region

Despite recent advances in spatial and single cell technologies, most knowledge about pulmonary progenitor cells in transitional regions of distal airways comes from rodent injury models. Early evidence is based on the emergence of regenerative foci of Scgb1a1-expressing cells in distinct anatomical niches after severe naphthalene-induced bronchiolar injury [83] (table 1). Such niches harbour pollutant-resistant variant club (vClub) cells, which survive toxin exposure due to low expression of xenobiotic-metabolising enzymes and serve as starting points for subsequent regenerative processes [65, 84–86]. While vClub cells situated at airway bifurcations juxtaposed to neuroepithelial bodies express secretory markers and Uroplakin3A [87], surviving cells at BADJs frequently co-express Scgb1a1 and surfactant protein C (Sftpc), a classical marker for AT2 cells, highlighting diversity within the pollutant-resistant population.

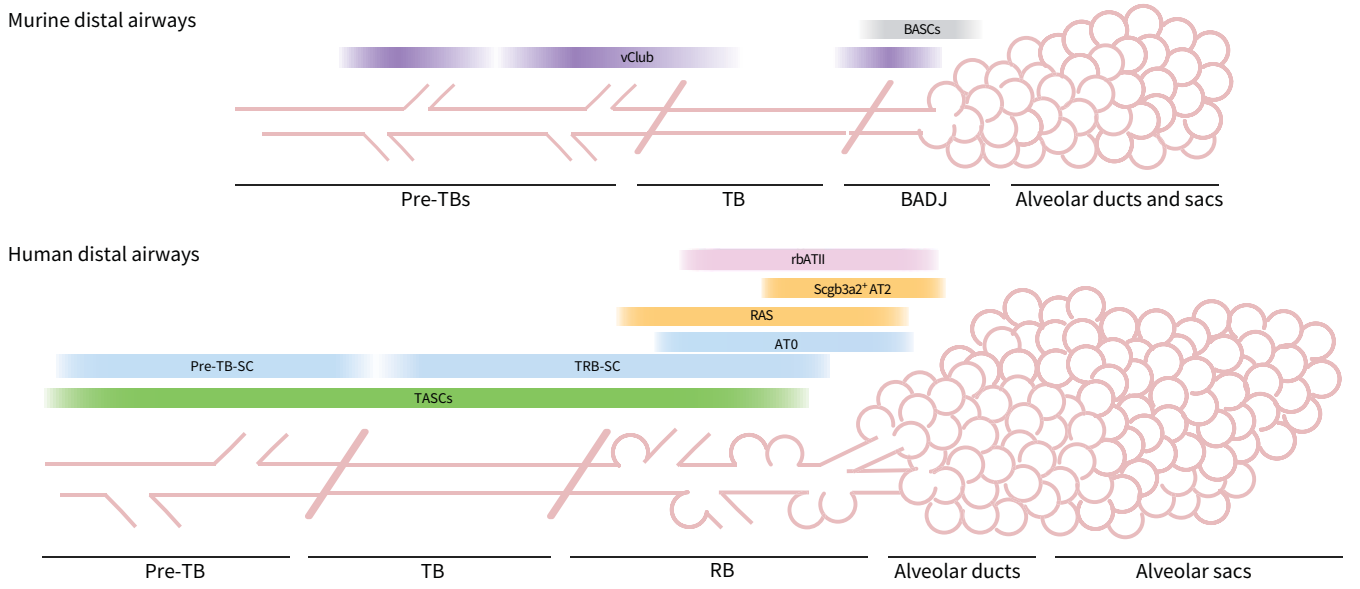
Distal pollutant-resistant cells include rare BADJ-associated bronchioalveolar stem cells (BASCs), which expand at early recovery stages and exhibit key stem cell properties such as self-renewal and multi-lineage differentiation potential *in vitro* [88–91]. Despite accumulating evidence for progenitor function, the biological role of BASCs remained elusive or even contradictory [64, 88, 92–95] until advanced intersectional genetics (ISG) approaches provided the specificity needed for rigorous lineage-tracing of $Scgb1a1^+$/$Sftpc^+$ cells *in vivo* [96, 97]. Selective BASC targeting was either achieved by the combinatorial use of orthogonal recombinases (Cre-loxP/Dre-rox) [96] or by utilising split-intein moieties to reconstitute effector molecules that become active upon co-expression exclusively in target cells (split-Cre/split-tTA) [97].

Both complementary studies demonstrated that rare Scgb1a1$^+$/Sftpc$^+$ cells reside predominantly at BADJs, remain mostly quiescent in the steady-state and only modestly contribute to the maintenance of the bronchioalveolar epithelium [96, 97]. Transcriptional profiling of BASCs revealed a distinct molecular signature partially shared by club and AT2 cells while putative unique markers were sparse and inconsistent, indicating that marker co-expression remains the most reliable approach to identify BASCs [88, 96–99]. Importantly, ISG-based fate-mapping of Scgb1a1$^+$/Sftpc$^+$ cells unequivocally demonstrated the multi-lineage differentiation potential of BASCs, which serve as progenitor cells for both bronchiolar and alveolar derivatives *in vivo* [96, 97]. Although BASCs appear more or less dispensable for tissue homeostasis, different injuries lead to robust activation of BASCs, which rapidly proliferate and differentiate in a context-dependent manner into more mature progeny. Following acute naphthalene-induced toxicity, BASCs not only survive toxin exposure but quickly adopt a bronchiolar cell fate to repopulate denuded airways with nascent club cells and ciliated cells. In contrast, severe bleomycin-induced alveolar damage (table 1) favours BASC differentiation into AT2 and AT1 cells [96, 97]. While stem cell motility appears to be critical for alveolar repair [94], further research needs to investigate the migratory capacity of BASCs and whether differentiation occurs *in situ* or after reaching the sites of injury. Pulse-chase labelling of individual BASCs indicates that expansion occurs clonally and that single BASCs possess a bi-potent differentiation potential [96, 97, 100]. Of note, following viral infection the vast majority of BASCs was lost, limiting their contribution to post-viral repair [101]. Diphtheria toxin (DTA)-mediated ablation of Scgb1a1$^+$/Sftpc$^+$ cells strongly delayed bronchiolar recovery, suggesting compromised regenerative capacity of the lung in BASC-deficient animals, although the epithelium eventually recovered. These results underscore the crucial role of BASCs for distal lung regeneration and further illustrate that effective epithelial repair depends on the joint action of multiple progenitor cell types. Surprisingly, DTA-mediated cell ablation failed to completely eradicate Scgb1a1$^+$/Sftpc$^+$ cells [97], suggesting that BASCs are constantly replenished to ensure niche occupancy with bi-potent cells. This interpretation supports a model of substantial cellular plasticity in the bronchioalveolar epithelium, allowing quick adaptation to microenvironmental changes. Cell fate decisions in BASCs are likely shaped by a variety of niche-derived factors, including direct cell–cell interactions, soluble mediators and properties of the extracellular matrix. For instance, Wnt signalling is activated in the BADJ niche following injury, promoting BASC expansion and, if sustained, impaired differentiation [102]. Notch signalling is essential for maintaining the secretory cell identity and differentially regulates the conversion of club and BASCs into AT2 cells during alveolar repair [39, 65, 103, 104]. Similarly, vClub cells in distal airways are sensitive to Notch perturbations and acquire a lineage-ambiguous state, further highlighting that cell fate is critically influenced by short-range signals in the microenvironmental niche [39]. Further investigations are needed to comprehensively delineate the molecular circuits underlying BASC regulation and to understand the molecular basis of the high adaptability and plasticity of BASCs, enabling them to adequately respond to local needs.

Scgb3a2$^+$ cells in terminal and respiratory bronchioles of larger mammals (including humans)

Owing to the lack of unique markers, definition of BASCs typically combines Scgb1a1$^+$/Sftpc$^+$ co-expression and niche-association. Accepting such criteria makes identification of a human BASC equivalent difficult, since important anatomical differences exist between mouse and human lungs [105]. Murine TBs end rather bluntly into alveolar ducts, whereas human distal airways show a more complex, zonal arrangement: each TB supplies several orders of RBs, where alveoli out-pocked from the bronchiolar wall, before transitioning into alveolar ducts and sacs. Recent attempts to explore the spatial distribution and molecular identity of cell types enriched in pre-TBs/TBs/RBs have provided a more comprehensive map of human distal airways (figure 2). Several groups identified region-specific populations of secretory cells in TBs/RBs by *in situ* transcriptomics and single cell profiling, which differ from more proximal secretory cells by expression of Scgb3a2 [106–108].

Distal airway dissection approaches to delineate region-specific COPD-associated changes uncovered a unique population of Scgb3a2$^+$/Sftpb$^+$/Scgb1a1$^+$ cells enriched in pre-TB/TB/RB regions [108]. Terminal airway-enriched secretory cells (TASCs) integrate features of secretory and alveolar cells and represent lumen-facing nonciliated secretory cells, which gradually change their morphology from a dome-shaped appearance to a cuboidal shape at TB-RB junctions [108]. Interestingly, TASCs were dramatically reduced in pre-TBs/TBs of COPD patients, coinciding with an increased density of CD8$^+$ T-cells and higher interferon (IFN)-γ signalling in the airway surface epithelium. Distal basal cells, which serve as progenitors for TASCs, show impaired differentiation in response to IFN-γ, suggesting that T-cell-derived IFN-γ may suppress the regenerative potential of distal basal cells and contribute to the structural loss of pre-TBs/TBs in COPD [108]. Similar to TB-RB associated TASCs, Scgb1a1$^+$/Scgb3a2$^+$ respiratory airway secretory (RAS) cells display a cuboidal morphology and are situated in RBs of humans and ferrets, forming small patches of airway epithelium interspersed with alveoli [106]. RAS cells share transcriptional profiles of



	TASC	Pre-TB-SC	TRB-SC	AT0	RAS cell	Scgb3a2$^+$ AT2 cells	rbATII	vClub	BASC
Species	Human	Human, macaque	Human, macaque	Human, macaque	Human, ferret	Human, ferret	Human	Mouse	Mouse
Localisation/niche	Pre-terminal and terminal airways, RBs	Distal airways proximal to TBs	Specific to terminal and RBs	Alveolar septae contiguous with RBs, rarely in distal alveolar sacs	RBs, interspersed with alveolar out-pouchings	RBs	Individual alveoli of RBs	Airway bifurcation, bronchioalveolar duct junction	Bronchioalveolar duct junction
Abundance (homeostasis)	+++	+++	+++	++	+++	+	+	+	+
Marker genes (selection)	Scgb3a2$^+$, Sftpb$^+$, Scgb1a1$^+$	Sftpb$^+$, Scgb3a2$^+$, Scgb1a1$^+$	Sftpb$^+$, Scgb3a2$^+$	Sftpb$^+$, Scgb3a2$^+$, Sftpc$^+$	Scgb3a2$^+$, Scgb1a1$^+$, Ceacam6$^+$ (CD66c)	Scgb3a2$^+$, Sftpc$^+$ or Lamp3$^+$	Multi-SCGB^{pos} (3A2/1A1/3A1), Sftpc$^+$	Scgb1a1$^+$, Upk3a$^+$, Cyp2f2^{low}	Scgb1a1$^+$, Sftpc$^+$
Cellular relationships/ predicted trajectories	Distal, but not proximal airway BCs can generate TASCs *in vitro*	Distal, but not proximal airway BCs give rise to Scgb3a2$^+$ cells in ALI-based differentiation cultures; *in silico* predictions and human AT2-derived alveolosphere cultures suggest that AT2s can revert to bi-potent AT0s (unidirectional), which then differentiate into AT1s or TRB-SCs			Primary and hESC-derived induced RAS cells serve as progenitors for the AT2 cell lineage, transition occurs rapidly and unidirectional		Contribution of club and RAS cells to emphysema-specific asATII, with rbATII serving as potential transitional population	vClub mainly serve as progenitors for club and ciliated cells; contribution to alveolar repair after bleomycin	BASCs serve as progenitor cells for bronchiolar (club, ciliated) and alveolar epithelial cells (AT1, AT2)
Regulation/ involved signalling pathways	IFN-γ suppresses TASC regeneration by inhibiting the normal distal BC differentiation potential	Reduced Wnt or EGFR signalling enhances AT2–AT0 conversion; downstream of EGFR, blockade of RAF and MEK, but not AKT or JNK, induces a bi-potential AT0 state			Inhibition of Notch and/or activation of Wnt signalling enhances (i)RAS to (i)AT2 transition		Pronounced ageing characteristics, elevated levels of senescence marker genes and reduced mitochondrial function scores	Wnt activation in vClub cells prevents ciliary differentiation upon acute Notch inhibition	Wnt activation leads to BASC expansion; inhibition of Notch enhances BASC–AT2 conversion
Disease relevance	Loss of TASCs in COPD, potentially caused by enhanced T-cell-derived IFN-γ signalling	Emergence of AT0 cells in distal alveolar sacs in response to lung injury, accumulation in IPF and COPD patients; TRB-SCs present in IPF, but not in COPD/acute lung injury; ectopic TRB-SCs mostly found in severely fibrotic areas, resembling "bronchiolised regions"			Dysfunction of RAS to AT2 cell differentiation in COPD patients/smoke-induced chronic injury leads to an accumulation of Scgb3a2$^+$ AT2 cells, suggesting more active and/or stalled RAS–AT2 transition		Accumulation of asATII specific to COPD patients; reduced progenitor potential of asATII compared to hATII and rbATII	Increased toxin-resistance due to low expression of xenobiotic-metabolising enzymes	Pollutant-resistant, but prone to viral infections; loss of BASCs delays repair processes
Reference	[108]	[107]			[106]		[109]	[84, 85, 87, 105]	[88, 96, 97]

FIGURE 2 Cell types identified in transitional regions of distal airways. The schematic depicts structural differences between murine and human distal airways and indicates the anatomical localisation of distinct cell subsets. In mice, terminal bronchioles (TBs) connect directly to alveolar ducts, with bronchioalveolar duct junctions (BADJs) representing the main transitional sites. In humans, TBs give rise to multiple generations of respiratory bronchioles (RBs), transitional regions where alveoli protrude from the bronchiolar wall, before continuing into alveolar ducts and sacs. The accompanying table summarises main characteristics of cell types with putative regenerative potential identified in distal airways of mice, humans and other RB-containing species. These cell subsets vary in distribution, abundance, marker expression, differentiation repertoire and disease-related changes, yet may represent partially overlapping cell populations along a shared continuum. asATII: alveolar sac alveolar type II cell; AT: alveolar type; AKT: protein kinase B; ALI: air–liquid interface; BASC: bronchioalveolar stem cell; BC: basal cell; EGFR: epidermal growth factor receptor; hATII: human alveolar type II cell; hESC: human embryonic stem cell; IFN-γ: interferon-γ; IPF: idiopathic pulmonary fibrosis; JNK: c-Jun N-terminal kinase; RAS: respiratory airway secretory; rbATII: respiratory bronchiole alveolar type II cell; RAF: rapidly accelerated fibrosarcoma; SC: secretory cell; TASC: terminal airway-enriched secretory cell; TRB: terminal and respiratory bronchiole; vClub: variant club cell.

canonical secretory cells and AT2 cells and function as progenitor for the AT2 cell lineage. *In vitro* differentiation of Ceacam6-enriched primary RAS and induced human embryonic stem cell-derived RAS (iRAS) cells demonstrates that RAS/iRAS rapidly downregulate Scgb3a2 and upregulate Sftpc as they mature into an AT2/iAT2 phenotype characterised by proper SFTPC processing and storage in lamellar bodies [106]. This RAS–AT2 cell differentiation involves a transient intermediate state co-expressing both markers, appears to be unidirectional and is enhanced by inhibition of Notch and/or activation of Wnt signalling [106]. Based on anatomical distribution and transcriptional signature, an independent study further subdivided Scgb3a2$^+$ cells in distal human airways into Sftpb$^+$/Scgb3a2$^+$/Scgb1a1$^+$ pre-terminal bronchiole secretory cells (pre-TB-SCs) localised in distal airways proximal to TBs, Sftpb$^+$/Scgb3a2$^+$ TRB-SCs restricted to TRBs and TRB-specific AT0 cells associated with alveolar septae contiguous to RBs, which share markers characteristic for airway and alveolar cells (Sftpb$^+$/Scgb3a2$^+$/Sftpc$^+$) [107]. Reciprocal signalling between distal basal cells and Lgr5$^+$ fibroblasts seems to establish a distinct mesenchymal niche essential to support the growth and maintenance of distal basal cells, which in contrast to proximal basal cells serve as progenitors for Scgb3a2$^+$ cells in *ex vivo* cultures, indicating that different basal cell subsets retain their regional memory [107, 108]. TRB-SC and AT0 cells are conserved in nonhuman primates and emerge dynamically during human lung development [107]. Unlike RAS cells, which act as unidirectional progenitor for AT2 cells, *in silico* predictions suggest distinct lineage trajectories indicating that AT0 cells originate from AT2 cells and subsequently differentiate into AT1 or TRB-SCs [106, 107]. Reduced Wnt or EGFR signalling promotes acquisition of an intermediate AT0 state, although bi-potent AT0 cells typically do not revert to AT2 cells [107]. Interestingly, the conversion of AT0 cells to TRB-SCs is characterised by a loss of Sftpc and a gain of RAS markers (*e.g.* Ceacam6) [107]. However, unlike TRB-SC, but consistent with TASCs, RAS cells express Scgb1a1 [106], suggesting that TASCs, TRB-SCs and RAS cells may represent partially overlapping cell populations.

In the context of disease, an accumulation of intermediate cell states is observed. While rarely detected in healthy donors, Scgb3a2$^+$ AT2 cells accumulate in lungs of COPD patients and smokers, correlating with increasing pack-year history. The presence of Scgb3a2$^+$ AT2 cells suggests an altered RAS–AT2 transition in diseased lungs, possibly reflecting either enhanced differentiation activity and/or stalled transition [107]. Trajectory analysis revealed that RAS cells are transcriptionally related to both mature, homeostatic AT2 cells, and Scgb3a2$^+$ AT2 subsets. However, it is also possible that the Scgb3a2$^+$ AT2 cells detected in COPD lungs correspond to AT0 cells, consistent with the findings of Kadur Lakshminarasimha Murthy *et al*. [107], who reported numerous AT0 (Scgb3a2$^+$ Sftpc$^+$) cells accumulating in the distal sacs of COPD and IPF patients, indicating the emergence of ectopic intermediate cell states in diseased lungs. This interpretation is further supported by deep profiling of alveolar epithelial cells from healthy and emphysematous human lungs, which identified uniquely enriched Scgb3a2$^+$ AT2 subsets [109]. Emphysema-specific alveolar sacs ATII cells (asATII) showed pronounced ageing characteristics and exhibited an impaired progenitor potential compared to healthy AT2 and respiratory bronchiole ATII (rbATII) [109]. Distinct from abundant asATII localised to enlarged distal sacs, rare rbATII were found in individual alveoli attached to RBs and co-expressed multiple secretoglobins (3A2/3A1/1A1) and Sftpc, closely resembling murine BASCs. Trajectory inference analysis supports a contribution of club and RAS cells *via* transitional rbATII to asATII, but neither rbATII nor asATII expressed the full scope of marker genes characteristic for AT0 or Scgb3a2$^+$ AT2 cells, suggesting partly divergent cell identities [106, 107, 109].

Shared features and strategic position – common (unifying) themes across species

Present data indicate a continuum of cell states along the differentiation trajectory from TB/RB-specific secretory cells towards alveolar epithelial cells, ranging from TASCs/pre-TB-SCs *via* RAS/TRB-SCs and AT0/rbATII to aberrant Scgb3a2$^+$ AT2/asATII that accumulate in diseased lungs (figure 2). Although molecular profiles and spatial distribution differ gradually, it seems plausible that these subsets represent partially overlapping populations within the same continuum. Distinct functional properties *in vitro* may reflect cell-specific intrinsic features and/or different degrees of maturation. Additional factors such as culture conditions, enrichment strategies, and stage of disease are likely to contribute to differences and merit further investigations. Although several identified cell states are conserved in RB-containing species, extrapolation to murine lungs is problematic due to existing anatomical differences, less restricted Scgb3a2 expression patterns [110] and the absence of unique markers or gene homologues (*e.g.* Ceacam6). However, from a broader perspective, (T/R/B)ASCs seem to fall into the same conceptual category: intermediate cells with shared features of secretory and alveolar epithelial cells, specific to transitional regions in distal airways, which serve important progenitor cell functions or whose loss and/or altered differentiation capacity is associated with impaired regeneration and disease. Future research should aim to harmonise and integrate available resources and increasingly incorporate functional parameters to accurately compare cell fate and biological relevance of candidate cell equivalents across species.

The alveolar epithelium

Stem cells of the alveolar epithelium

Gas exchange, the most essential function of the lung, takes place in the distal part of the lung, the alveolar compartment. The direct apposition of differentiated AT1 cells and the endothelial cells lining the alveolar capillary, create a very thin interface through which oxygen and CO_2 diffuse freely, ensuring adequate oxygenation of circulating blood. Although AT1 cells cover most of the alveolar space, number-wise they constitute a minority of the total epithelial cells in the distal lung. Indeed, AT2 cells are the most abundant epithelial cell type in the distal lung, playing a crucial function in lung homeostasis. They synthesise and secrete surfactant, a tension-active lipid–protein mixture that prevents alveolar collapse at the end of expiration [111, 112] as well as specific chemokines that attract immune cells following injury acting as a nonprofessional phagocyte [113–115]. Additionally, AT2 cells serve as *bona fide* stem cells, replacing AT1 cells during homeostasis and injury and repair [4]. Clonal analysis using lineage tracing Sftpc-CreER to recombine a fluorescent reporter demonstrated their stem cell properties in homeostasis and repair following either genetic ablation or distinct types of injury [4, 116, 117] (table 1). Moreover, their stemness ability has been widely validated in organoid forming assays [91, 107]. Together, the combination of *in vivo* injury/repair and *in vitro* assays confirmed AT2 cells as stem cells of the adult alveolar epithelium (figure 3).

The emergence of single-cell technologies allowed researchers to get a deeper, more granular view into the cellular composition of the alveolar epithelial compartments. Recent scRNAseq data in mouse and human demonstrated the existence of at least two separate states of AT2 cells [118]. AT2 cells exist in dynamic states, broadly categorised as nonactivated (homeostatic) and activated [119]. Nonactivated AT2s maintain alveolar homeostasis with high surfactant gene expression (Sftpc, Sftpb, NAPSA, Abca3, Lpcat1), while activated AT2s emerge in response to injury or stress, exhibiting proliferative and inflammatory profiles [119]. These activated states are critical for regeneration but can also contribute to pathological remodelling in diseases such as IPF [120–123].

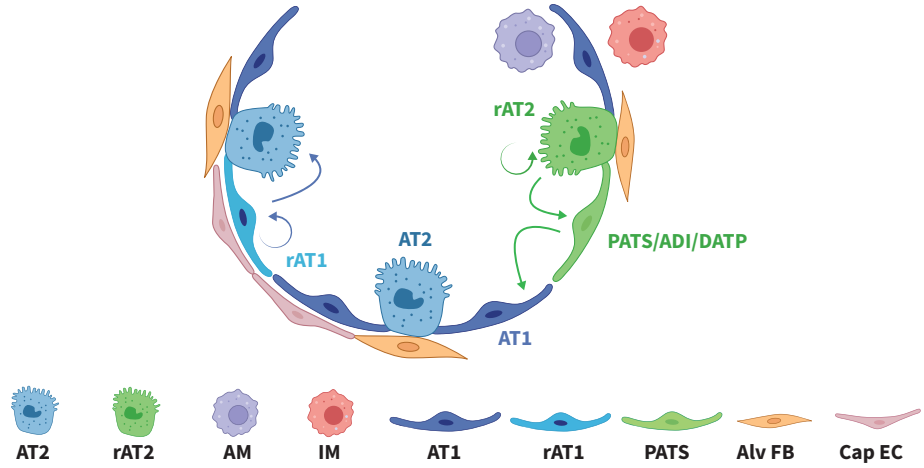
One of the lingering questions in alveolar cell biology was whether all AT2 cells have similar stem cell properties or whether stemness is reserved to only a few. Once believed to be a homogenous population, previous work identified distinct AT2 cell subpopulations with higher regenerative potential [118, 120, 124–129] (figure 3). Alveolar epithelial progenitors, characterised by Axin2 expression and detected by TM4SF1 (transmembrane 4 L six family member 1), represent 2–20% of the AT2 cell population and seem to have higher regenerative capacities in the mouse and human lung [127, 128]. Similarly, in the mouse, a population of epithelial stem cells expressing PD-L1 that expanded after pneumonectomy, was identified based on its low expression of the Rosa locus [116]. An equivalent population was found in human lung, and interestingly, it was found to be expanded in IPF [126]. In addition, AT2 cells expressing high levels of CD44 have been shown to be more proliferative [124, 129] and the subset of $IL1R1a^+$ AT2 cells seem to be the subpopulation able to regenerate since IL-1β is required to initiate alveolar regeneration [120]. Interestingly, an area of recent interest and controversy is represented by the apparent AT1 cell plasticity and their ability to replenish the AT1 and even AT2 pool after injury [130–133] (figure 3).

Regulation of AT2 cell fate – alveolar stem cell niche

The homeostatic maintenance of the AT2 progenitor cell fate is coordinated through several intrinsic and extrinsic mechanisms. Extrinsically, signal transduction pathways such as canonical Wnt and fibroblast growth factor (FGF) signalling play crucial roles [2, 128, 134, 135]. The homeostatic AT2 cell state is characterised by high Wnt, FGF, BMP and low Notch and TGF-β signalling [136]. Repair following injury is thought to occur through the re-activation of these developmental signalling pathways that are involved in AT2 cell lineage specification during embryonic development [137–140] and that ensure the exit from a differentiated state and activation of the progenitor programme [132, 136]. Little is currently known about the early events governing the differentiation/progenitor switch, but Notch activation has been shown to downregulate surfactant biogenesis, most likely through downregulation of the surfactant protein processing machinery in order to allow AT2 cells to initiate the progenitor programme [141, 142]. However, Notch activation has been shown to be required to initiate alveolar regeneration but to be silenced to allow terminal differentiation [143]. Interestingly, the two states, activated and nonactivated, represent very different metabolic states and we are just starting to understand the mechanisms of this metabolic regulation [144]. Most likely, epigenetic and chromatin remodelling factors play important roles in this rapid fate choice transition, representing a very important future direction of research.

AT2 cell fate in every instance is coordinated by the interacting cell types constituting their niche (figure 3a). AT2 cells interact with AT1 cells, fibroblasts, endothelial cells and macrophages in homeostasis, and these interactions are modulating their fate choice during regeneration and repair [136].

a)



b)

	Cell type	Markers	Properties – role	References
Mouse				
AT2	AEP	Sftpc, Sftpb, Lamp3, Abca3, Napsa, Axin2	Quiescent progenitors in homeostasis; lineage tracing shows regeneration of AT2 and AT1 following injury	[128, 129]
	IL1R1	Sftpc, Sftpb, Lamp3, Abca3, Napsa, Axin2, IL1R1a	Primed AT2s following bleomycin injury express IL-1R1, which, when activated by IL-1β secreted by interstitial macrophages, activates AT2 to proliferate and give rise to DATPs	[121]
	CD44	Sftpc, Sftpb, Lamp3, Abca3, Napsa, Axin2, CD44	Small (4%) population of AT2s in the perivascular niche with higher clonogenic potential in 3D organoid assays	[125, 130]
	IAAPs	Sftpclow, Rosa26low, Cd274 (PD-L1)	PD-L1^{+} alveolar progenitors; injury-activated subpopulation serving as an important reserve for lung regeneration	[116, 127]
Intermediates	PATS	Sfn, Cldn4, Krt8, Krt19, Tp53, Lgals3	Intermediates characterised by p53, TGF-β/DNA-damage response, and cellular senescence accumulating in lung fibrosis	[123]
	Krt8^{+} ADI	Krt18, Cldn4, Sfn, Lgals3, Fn1, S100a2, Sox4, Cald1, Prss2, Mmp7, Anxa2	Transitional cell state bridging AT2 to AT1 differentiation during alveolar regeneration; accumulation in IPF reflects defective cell fate progression	[120]
	DATP	Cldn4, Krt8, Ndrg1, Sprr1a, AW112010	Intermediate after bleomycin injury and in 3D organoid cultures; can give rise to both AT2 and AT1 *in vivo*	[121]
AT1	AT1	Pdpn, Hopx, Cav-1, Ager, Aqp5	Mature AT1s spontaneously differentiate into AT2 cells upon mechanical forces disruption or Hippo inhibition After pneumonectomy, Hopx^{+} AT1 cells give rise to AT2 cells AT1 cells contribute to regeneration after adult hyperoxia	[131, 132, 133]
	Plastic AT1	Pdpn, Hopx, Cav-1, Ager, Aqp5, Igfbp2^{neg}	Adult AT1 population includes Hopx^{+} IGFBP2^{+} (terminally differentiated) and Hopx^{+} IGFBP2^{-} (can transdifferentiate into AT2 during regeneration)	[134]
Human				
AT2	AEP	SFTPC, SFTPB, LAMP3, NAPSA, ABCA3, HTII-280, TM4SF1	3D organoid assays of hAEPs show clonogenic potential and differentiation into AT2 and AT1	[128]
Intermediates	Aberrant basaloid	TP63, KRT17, VIM, MMP7, KRT5^{neg}	Maladaptive alveolar intermediates contributing to persistent epithelial dysfunction and fibrosis progression	[122]

FIGURE 3 Alveolar stem and progenitor cells. a) Simplified schematic representation of the cellular composition of the alveolus. Specific subpopulations of alveolar type 2 (AT2) cells have been shown to have a higher regenerative capacity (rAT2) that differentiate into AT1 cells *via* intermediate cells (pre-alveolar type 1

transitional state (PATS)/alveolar differentiation intermediate (ADI)/damage-associated transient progenitor (DATP)). rAT2 cells include alveolar epithelial progenitors (AEPs), IL1R1^{+}, CD44^{hi} and injury-activated alveolar progenitors (IAAPs). Similarly, alveolar type 1 (AT1) cells show cell plasticity and can contribute to replenish alveolar epithelial cells under specific circumstances (rAT1). b) Table summarising the stem and progenitor populations of the murine and human alveolar epithelium. Alv FB: alveolar fibroblast; AM: alveolar macrophage; Cap EC: capillary endothelial cell; IM: interstitial macrophage; rAT1: regenerative AT1 cell; rAT2: regenerative AT2 cells.

The most studied interaction is that one established with adjacent fibroblasts. Lipofibroblasts provide AT2 cells with lipids important for surfactant production [145] and have been demonstrated to be essential for AT2 cell maintenance [146] keeping their differentiated function through Wnt, FGF, TGF-β and BMP signalling [128, 146–148]. Activation of AT2 progenitors is highly coordinated by Wnt ligands secreted by the mesenchyme [128]. AT2 cells are also in direct physical contact with alveolar macrophages, a connection mediated through connexin 43 [149] and play an important function in their maintenance [150]. As mentioned previously, activated AT2 cells secrete chemoattractants such as CXCL8 (IL-8) and CCL2 for circulating monocytes, natural killer cells and neutrophils, as well as chemokines activating alveolar macrophages [151–153]. On the other hand, interstitial macrophages are required to initiate alveolar regeneration through the release of IL-1β and other cytokines in distinct types of injury [120, 154, 155].

Mechanisms of alveolar regeneration

Engagement of AT2 progenitors into the repair process in animal models of injury and repair (table 1) as well as in human disease was found to be a nonbinary (AT2–AT1) process, but rather a trajectory that spans intermediate states. One of the first AT2–AT1 transitional state was identified using scRNAseq after bleomycin injury as a state where typical AT2 cell markers are no longer expressed but the cells exhibit Krt8, a keratin otherwise not expressed in the alveolar epithelium [119, 120]. These cells were termed Krt8^{+} alveolar differentiation intermediates (ADIs) or damage-associated transient progenitors (DATPs) and their presence was studied in multiple models of lung injury [119, 120, 156]. Similarly, Kobayashi *et al.* [122] defined a similar state termed pre-alveolar type 1 transitional state (PATS). PATS are characterised by activation of p53, TGFβ/DNA-damage response and cellular senescence programmes, and their persistence has been observed in fibrotic lungs, indicative of stalled alveolar repair contributing to pathogenesis. Studies have shown that these intermediate cells accumulate specifically in IPF, surrounding fibroblastic foci and exhibiting markers like SFN, CLDN4, KRT17, and TP63, highlighting their key role in the initiation and progression of lung fibrosis [122]. Similar states have been identified in human where a distinct population of aberrant basaloid cells, which are absent in healthy controls, emerge in the lungs of IPF patients [121, 157]. These cells co-express markers of basal cells (*e.g.*, *TP63*, *KRT17*) and mesenchymal/inflammatory programs (*e.g.*, *VIM*, *MMP7*), along with features of senescence and failed differentiation. Positioned near fibroblastic foci, aberrant basaloid cells are thought to represent maladaptive alveolar intermediates that contribute to persistent epithelial dysfunction and fibrosis progression [121]. While the two states defined in mouse (KRT8 ADIs/PATS/DATPs) and human (aberrant basaloid cells) may share a common origin or initial trajectory as AT2 cells responding to injury, aberrant basaloid cells are thought to represent a dysfunctional end-point of transitional states like KRT8^{+} ADIs, driven by chronic injury, niche abnormalities, or impaired resolution mechanisms [123, 158–161]. Noteworthy, Kathiriya *et al.* [158] observed that human AT2 cells transdifferentiate into basal cells through intermediate states (ABI, alveolar basal intermediates) and demonstrated that these AT2-derived basal cells resemble IPF metaplastic basal cells [159]. More comparative work (*e.g.*, cross-species lineage tracing, multi-omic mapping) is needed to definitively link or distinguish their functional roles. Transitional states are not unique to epithelial cells but occur in almost every cell type that changes fate during the repair and regeneration process. Importantly, fibroblasts undergo similar fate transition from various identities to the myofibroblasts present in fibrotic lung [141, 161–163]. Konkimalla *et al.* [161] demonstrated that alveolar injury induces the co-emergence of transitional epithelial cells and specialised fibroblast states, both essential for proper tissue remodelling, with RUNX1 acting as a key regulator of the fibroblast response. Recently, a similar state expressing RUNX2 has been shown to have an important role in fibrogenesis [164]. Disruption of this epithelial–mesenchymal coordination leads to pathological outcomes, either fibrosis with persistent transitional states or emphysema-like tissue simplification when these states are lost.

Alveolar epithelial dysfunction in lung disease

Functioning at the interface with the environment, the lungs are continuously exposed to injurious agents. Epithelial integrity, which is necessary for gas exchange, is impaired in chronic lung diseases, severely

compromising lung function [18]. In IPF, repeated alveolar injury leads to impaired AT2-mediated regeneration, due to either intrinsic defects, such as mutations in *SFTPC*, *ABCA3* or *SFTPA2*, or extrinsic factors such as altered interactions with immune cells and fibroblasts [165–167]. This widespread AT2 cell dysfunction reduces surfactant production, increasing the risk of alveolar collapse promoting further injury [142, 167]. IPF is marked by AT2 stem cell loss, accumulation of transitional states [161, 168, 169], and depletion of AT1 cells [170]. In addition, injured AT2 cells secrete pro-fibrotic signals like TGF-β, which activate fibroblasts into myofibroblasts, driving extracellular matrix deposition, alveolar thickening, and scarring [161, 162].

Alveolar stem cells play also a crucial but impaired role in COPD [171]. Normally responsible for alveolar maintenance, AT2 cells in COPD exhibit a reduced regenerative potential and altered epithelial differentiation, as shown by epigenetic dysregulation of IFN and Wnt signalling in patient-derived cells [172–175]. scRNAseq has identified a distinct inflammatory AT2 subpopulation (termed AT2i) in COPD lungs, marked by elevated chemokines (*e.g.*, CXCLs, CCL2), whose frequency correlates with disease severity and may contribute to persistent alveolar inflammation [176]. Additionally, COPD lungs contain AT2-like cells derived from airways with impaired regenerative capacity, suggesting pathological recruitment of nonalveolar progenitors [108, 176]. Together, these findings highlight a dual challenge in COPD: reduced homeostatic repair due to AT2 dysfunction and emergence of pro-inflammatory or aberrant states that may exacerbate disease progression.

Impact of ageing on AT2 cells and implications in disease

López-Otín *et al.* [176] initially proposed nine "hallmarks of ageing", each meeting three core criteria, as follows: 1) they increase with chronological age, 2) their experimental intensification accelerates ageing, and 3) their attenuation delays it. This concept has since been refined and expanded; the most recent update defines 12 interconnected hallmarks of ageing, which include telomere attrition, mitochondrial dysfunction and cellular senescence [13]. Many of these hallmarks directly or indirectly impair stem cell function and have been identified in AT2 cells within diseased lungs [14].

Although studies of "healthy aged" lungs remain limited, available data suggest that an imbalance in developmental signalling pathways promotes cellular senescence in AT2 cells in aged mouse lungs, thereby contributing to diminished regenerative capacity [177, 178]. Epigenetic regulation controls progenitor cell balance in the lung and deteriorates with age, thereby reducing AT2 cell activity and impairing alveolar repair while increasing frequency of BASCs [179]. Similarly, in aged human lungs, a monotonic decline in alveolar expansion and surfactant secretion has been observed [180], along with features of inflammaging and reduced epithelial identity [181, 182]. A senescent phenotype has been reported in various structural lung cell populations, with AT2 cells appearing particularly vulnerable [183]. Moreover, lungs from patients with chronic lung diseases including IPF and COPD exhibit a greater abundance of ageing hallmarks in AT2 cells compared to age-matched controls, suggesting a phenotype of accelerated ageing.

Telomere attrition emerges as an early and pervasive insult. Heterozygous loss-of-function variants in TERT (telomerase reverse transcriptase), TERC (telomerase RNA component) or RTEL1 (regulator of telomere elongation helicase 1) lead to critically short telomeres, end-to-end chromosomal fusions and cGAS (cyclic GMP–AMP synthase)–STING (stimulator of interferon genes) activation, thereby predisposing to familial IPF [184–186]. In addition, sporadic IPF and COPD lungs, despite lacking these germ-line defects, display pronounced telomere shortening and shelterin loss in AT2 cells [187–189], underscoring the centrality of replicative senescence in seemingly "sporadic" disease.

Proteostatic balance is similarly compromised. Missense mutations in *Sftpc* misfold within the endoplasmic reticulum (ER), triggering a chronic unfolded-protein response that culminates in AT2 apoptosis and fibrotic remodelling [165, 190–193]. Even in the absence of canonical surfactant-gene variants, ageing epithelia exhibit persistent ER stress [194, 195]. Concurrently, autophagic flux diminishes, AMP-activated protein kinase (AMPK) signalling wanes and mechanistic target of rapamycin complex-1 (mTORC1) becomes hyperactive, a constellation that suppresses mitophagy and promotes fibroblast proliferation [196–198]. Mitochondrial dysfunction amplifies this pathological loop. Ageing and cigarette-smoke exposure induce mitochondrial fragmentation, excess reactive oxygen species and release of mitochondrial DNA; emphysematous AT2 cells are particularly rich in damaged, reactive oxygen species-producing mitochondria [199]. Deletion of the complex-I subunit NDUFS2 (NADH dehydrogenase (ubiquinone) iron–sulfur protein 2) arrests AT2 cells in a senescence-prone transitional state, thereby exacerbating fibrogenesis [200].

Functionally, these convergent insults manifest as stem-cell exhaustion. In COPD, AT2 proliferation declines and metabolically compromised "emphysema-specific" AT2-like cells predominate [106, 109, 201, 202]. In IPF, DNA-damaged AT2 cells stall in Krt8-positive basaloid intermediates that display signs of accelerated ageing including a senescence signature and emit Wnt, Notch and TGF-β ligands, recruiting myofibroblasts rather than reconstructing alveolar architecture [120, 157, 203]. The senescence-associated secretory phenotype (SASP), rich in IL-8, MMP-7 (matrix metalloproteinase 7) and GDF15 (growth differentiation factor 15), produced by these aberrant epithelial cells sustains chronic inflammaging [204]. Many SASP components are trafficked *via* extracellular vesicles, which are increased in diseased lungs [205–208]. Additionally, extracellular vesicles enriched in miR-34a propagate senescence to neighbouring cells [209].

Taken together, telomere erosion, sustained ER stress, and mitochondrial dysfunction drive AT2 pneumocytes toward a senescent or maladaptive transitional state, ultimately resulting in stem cell exhaustion and impaired alveolar regeneration, with an impact in the development of chronic lung disease.

Discussion

Tissue regeneration relies on the cellular plasticity of differentiated cells. In the lung, this plasticity is particularly remarkable providing a pool of progenitor cells capable of contributing to tissue repair. The different cell types along the respiratory tree that perform this function are described in this review and summarised in figure 4. The regenerative process includes the differentiation into another cell type. Accurate identification of cell types has long been debated with advances in next-generation sequencing and single-cell technologies further revealing additional layers of complexity [210]. Traditionally, cell types were defined by shared morphology and function, but it is now clear that cell identity is governed by the interplay between extrinsic signals and downstream gene networks during development and in adult life [210]. External stimuli influence gene expression, which in turn dictates cell phenotype and function [211, 212]. Ultimately, a cell's phenotype reflects a specific gene-expression program, highlighting TF networks as central determinants of identity.

The notion that differentiated cell identity is established and maintained by a defined set of TFs was proposed decades ago [213]. Co-expression of the complete set of TFs induces and preserves the full repertoire of differentiation genes [211]. These core TFs and interacting factors, known as core regulatory circuits (CRCs), provide a comprehensive framework for defining cell identity, as they regulate the gene-expression programmes necessary for maintaining cell-type-specific functions [214, 215]. Arendt *et al*. [216, 217] further extended these models and introduced the concept of core regulatory complex (CoRC). Interestingly, the origin of a new cell type in evolution coincides with the occurrence of a unique CoRC, suggesting that cell types can only be delineated and compared within and between species when considered under an evolutionary perspective.

A key challenge in single-cell transcriptomics is distinguishing cell "types" from transient "states", as there is no consensus on whether cells undergoing state changes should be considered the same type. Defining cell identity through CRCs helps differentiating cell types from states, while combining TF profiles with differentially expressed genes can resolve distinct states within a given cell identity cluster [218]. Pseudotime analyses provide insights into differentiation trajectories in adult stem cells and disease, ordering cells by transcriptomic similarity to reflect relative differentiation or maturation [219, 220]. Although pseudotime approaches cannot replace lineage tracing techniques, the two approaches are complementary and should be used to validate each other [221]. In fact, most of our current understanding of respiratory epithelial lineage hierarchies has been gained through these strategies. As summarised in this review, distinct stem and progenitor cell populations residing in different regions of the respiratory tree are responsible for maintaining tissue homeostasis and orchestrating repair after injury (figure 4a, b).

In response to injury, functional tissue regeneration is normally achieved; however, in the context of disease, dysfunctional repair takes place (figure 4c). In the airway epithelium, failed regeneration results in an imbalance between secretory and MCCs, giving rise to an abnormal epithelium composition. This is evident in mucous metaplasia and squamous metaplasia, which are associated with airway diseases such as asthma, cystic fibrosis and COPD [33]. In the distal lung, diseases such as IPF and COPD are characterised by the accumulation of intermediate cell states that normally mediate physiological regeneration [1, 2, 67]. This includes Scgb3a2^{+} cells and AT0 cells, which, instead of differentiating into their final lineage (AT2 in the first case, and TRB-SC and AT1 in the second), remain arrested in an intermediate state [109]. The persistence of these intermediates may reflect either an increased attempt of the lung to repair the tissue, or stalled regeneration under disease conditions. Pathological regeneration also encompasses the emergence of aberrant cell circuits, leading to disease-associated cell states that actively contribute to the initiation and progression of pathology [109, 158] (figure 4c).

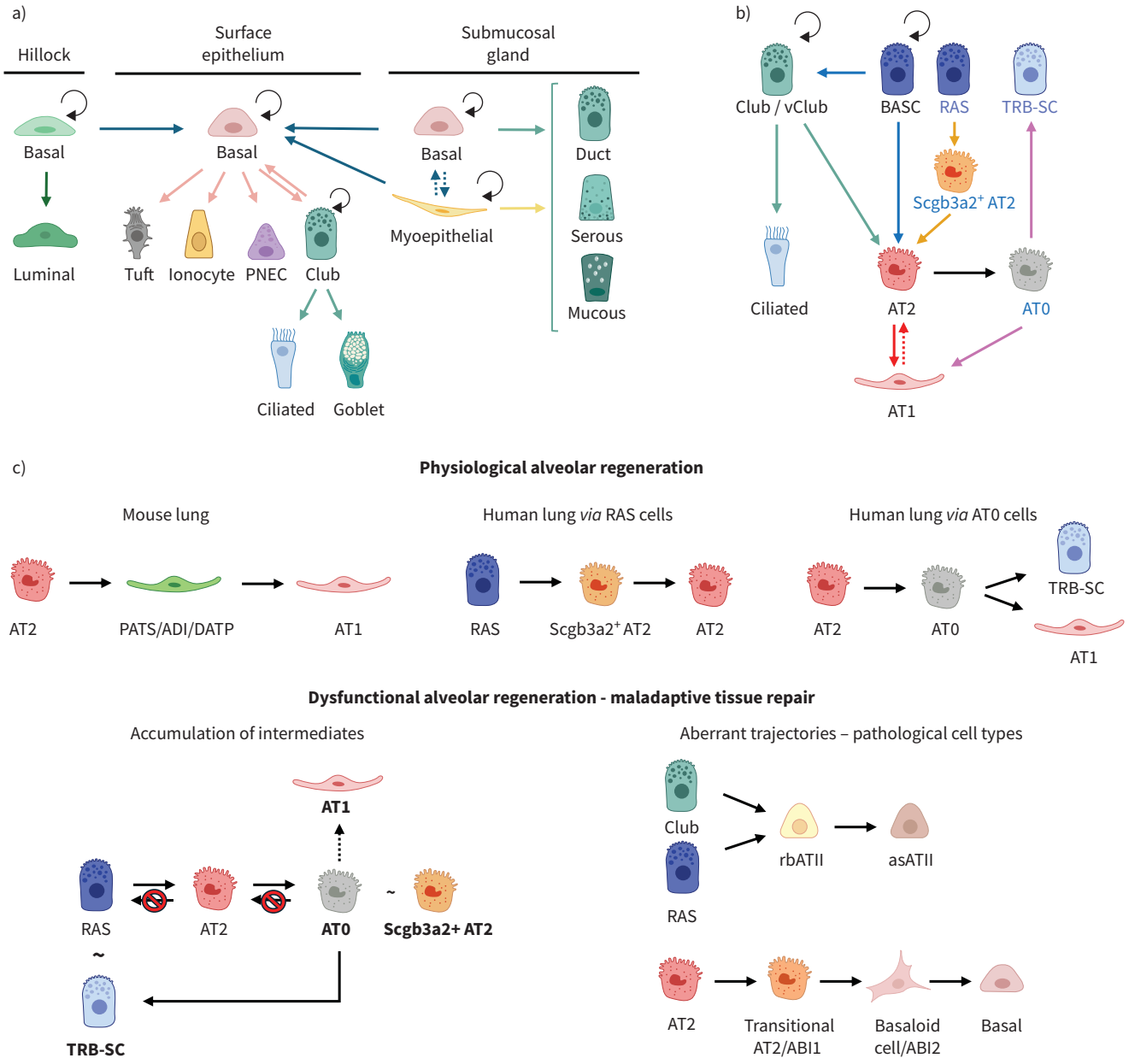


FIGURE 4 Lineage hierarchy of lung epithelial cells in homeostasis and lung regeneration. Epithelial lineage hierarchies in the a) proximal airway and in the b) distal lung including the distal airway epithelium and the alveolar epithelium. Solid arrows represent differentiation steps demonstrated by lineage tracing in animal models or inferred from single-cell technologies. Dashed arrows indicate controversial transitions that remain incompletely understood. Circular arrows denote self-renewal. c) Physiological alveolar regeneration follows a stepwise transitional process in which alveolar type (AT) 2 cells differentiate into AT1 cells through defined intermediate states (pre-alveolar type 1 transitional state (PATS) [122], Krt8$^+$ alveolar differentiation intermediate (ADI) cells [119], damage-associated transient progenitors (DATPs) [120] in the mouse lung and Scgb3a2$^+$ AT2 cells or AT0 cells in the human lung). Dysfunctional regeneration occurs when these intermediate cells (bold) accumulate and persist without completing their transition toward AT1 cells. Dashed arrows indicate impaired or absent differentiation steps. In this context, Scgb3a2$^+$ cells may in fact correspond to AT0 cells. Moreover, aberrant trajectories give rise to pathological epithelial populations such as alveolar sac alveolar type II (asATII) and basaloid cells, which ultimately contribute to disease progression. ABI: alveolar basal intermediate [158]. BASC: bronchioalveolar stem cell; PNEC: pulmonary neuroendocrine cell; RAS: respiratory airway secretory; SC: secretory cell; TRB: terminal and respiratory bronchiole; vClub: variant club cell.

Conclusion

Stem and progenitor cells are essential for tissue homeostasis and regeneration. Their plasticity enables repair but also contributes to disease when dysregulated. Clarifying their behaviour, niche interactions and

transitional states offers a promising avenue for therapeutic innovation in chronic lung diseases. Importantly, elucidating how ageing affects stem cell populations and how their plasticity can be restored in this context is critical for fostering tissue regeneration and repair in age-associated diseases such as COPD and IPF.

Questions for future research

Recent advances in single-cell and spatial transcriptomics, genetic lineage tracing and organoid systems have greatly expanded our understanding of epithelial cell plasticity and progenitor dynamics in the lung. However, several key questions remain unresolved and will shape the next decade of research in lung regeneration and repair:

- Functional heterogeneity among stem cells: not all basal or AT2 cells appear to contribute equally to repair. Understanding the molecular determinants of functional stemness and their dynamics within heterogeneous populations remains a major challenge.
- Proliferation *versus* differentiation: whether epithelial progenitors must divide prior to differentiation or if direct transdifferentiation occurs *in vivo* is unclear. How the cells sense damage and how they respond to it remains unknown. Do they first proliferate, or activate and then migrate? Or do they migrate first and then differentiate? Is every stem cell able to migrate? In addition, the molecular signals governing this decision require further elucidation.
- Plasticity of differentiated cells: the ability of AT1 cells, club cells or other differentiated cells to revert to a progenitor state is increasingly recognised in mice but remains poorly understood in humans and across disease contexts.
- Aberrant transitional states: transitional cell states, such as $Krt8^{+}$ ADIs/PATS/DATPs, are crucial for regeneration but can persist in chronic lung diseases, contributing to fibrosis, emphysema or carcinogenesis. Unravelling the molecular mechanisms underlying their persistence or maladaptation in disease will help finding efficient therapeutic approaches.
- Niche regulation and remodelling: how stromal, endothelial and immune cells dynamically influence progenitor fate and plasticity during injury is not fully defined. Additionally, understanding how these interactions fail in chronic lung disease is key to restore tissue structure and function.
- Ageing: age-related changes in proliferation, differentiation and niche signalling may underpin stem cell exhaustion in chronic lung disease. Finding strategies to reverse physiological or premature ageing will contribute to accomplish adequate tissue regeneration and repair.
- Therapeutic modulation of plasticity: a major future goal is to identify strategies that enhance beneficial regeneration while avoiding pathological remodelling.

Answering these questions will be essential for developing regenerative therapies for chronic lung diseases.

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