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Review

Pulmonary endogenous progenitor stem cell subpopulation: Physiology, pathogenesis, and progress

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ABSTRACT

Lungs are structurally and functionally complex organs consisting of diverse cell types from the proximal to distal axis. They have direct contact with the external environment and are constantly at risk of various injuries. Capable to proliferate and differentiate, pulmonary endogenous progenitor stem cells contribute to the maintenance of lung structure and function both under homeostasis and following injuries. Discovering candidate pulmonary endogenous progenitor stem cell types and underlying regenerative mechanisms provide insights into therapeutic strategy development for lung diseases. In this review, we reveal their compositions, roles in lung disease pathogenesis and injury repair, and the underlying mechanisms. We further underline the advanced progress in research approach and potential therapy for lung regeneration. We also demonstrate the feasibility and prospects of pulmonary endogenous stem cell transplantation for lung disease treatment.

Introduction

Lungs are important organs for gas exchange,[1] liquid electrolyte balance, and harmful substance elimination, which are consisted of more than 40 types of cells[2–5] (Figure 1). They are sensitive to various injuries, predisposing humans to major health concerns, such as coronavirus disease 2019 (COVID-19),[6–9] despite the availability of several valid therapies.[6,10] Recently, stem cell-based therapy for organ regeneration has attracted much attention.[6,10,11] Excitingly, pulmonary endogenous progenitor stem cells are distributed from proximal airways to distal alveoli,[11–16] which are static at normal conditions, yet can be activated by various injuries to proliferate and differentiate for repair[11,17] (Figure 2).

In this review, we describe pulmonary endogenous stem cell types that have been verified according to their anatomical positions along the respiratory tracts, analyze recent research progress, and promulgate the prospects of *ex vivo* and *in vitro* lung 3D models. Additionally, we describe the 3D model-based research methods and potential treatments for lung regeneration. We further provide preclinical evidence of pulmonary endogenous stem cell-derived cell transplantation therapy for lung disease treatment.

Pulmonary Endogenous Stem Cells

Proximal airway

Basal cells

The proximal conducting airway, comprising pseudostratified epithelia, is composed of cells, such as basal cells, ciliated cells, secretory cells, goblet cells, and neuroendocrine cells.[12] These cells work to essentially defend against inhalation of harmful substances.[18] To date, several studies have confirmed basal cells as multi-directional stem cells in the proximal conducting airway during homeostasis maintenance[14] and injury repair.[13,19,20] TRP63, KRT5, NGFR, and PDPN are commonly considered as markers of basal cells, and the heterogeneity of gene expression can further divide them into sub-populations. For example, krt5-positive basal cells with expression of p63 or krt14 seem to show differentiating potential, whereas qui-

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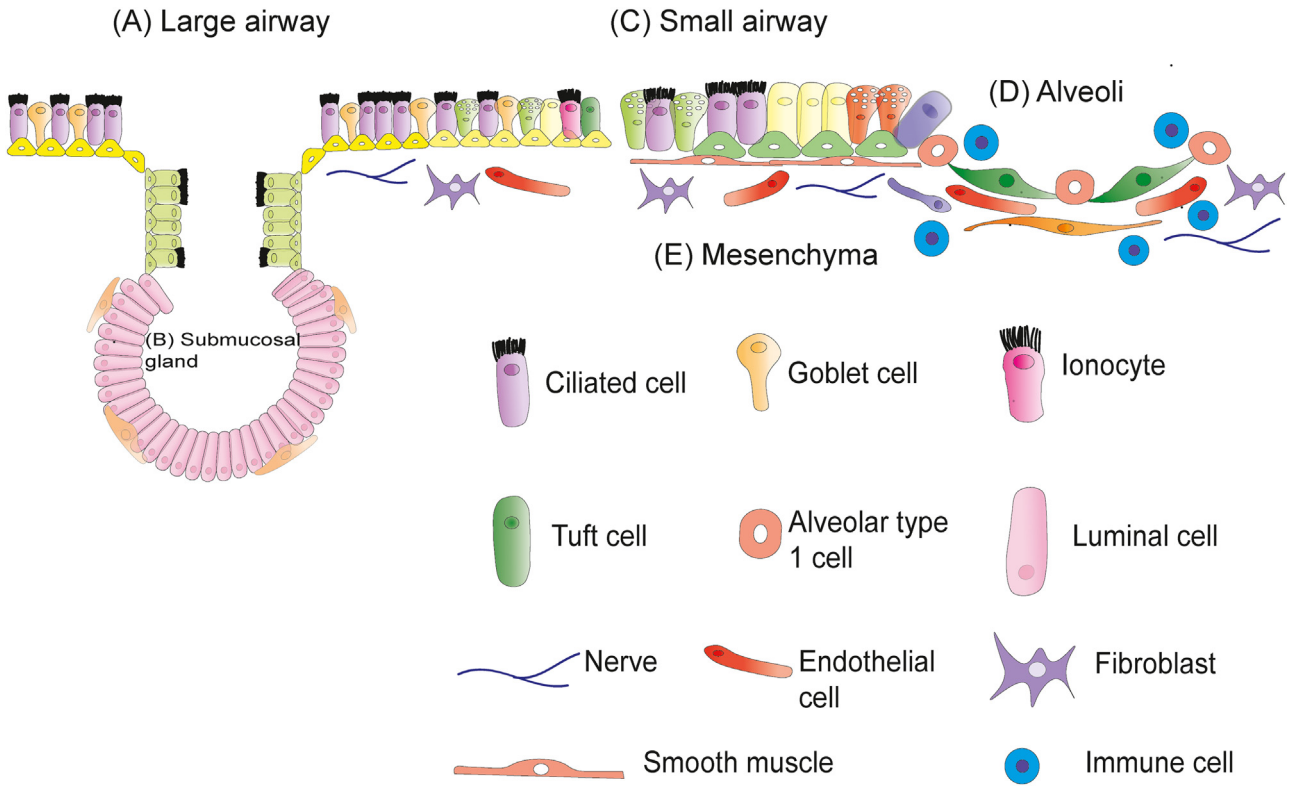

Figure 1. Multiple cell population components of the mouse lung airways. (A) Mouse large airways (trachea and main bronchus) mainly consist of basal cells, Clara cells, and ciliated cells. Few goblet cells, PNECs, tuft cells, and ionocytes line the mouse large airways. (B) A large number of SMGs exist beneath surface epithelia in the proximal trachea of mice but exist in whole cartilaginous airways of humans. SMGs can be divided into ciliated ducts, collecting ducts, terminal mucus, and serous tubules. Ciliated ducts are extensions of surface epithelia and change into collecting ducts and distal mucus and serous tubules, which consist of mucus and serous luminal cells. (C) Mouse bronchioles mainly contain Clara cells and ciliated cells. Mouse intrapulmonary airways lack basal cells, whereas basal cells exist in human conducting airways. (D) Alveoli are lined with two types of alveolar epithelial cells called AT1 and AT2 cells. (E) Lung airway epithelia are surrounded by mesenchymal tissue, including various mesenchymal cells (e.g., fibroblasts, pericytes), blood vessels, immune cells, smooth muscle, and nerves.
AT1: Alveolar type 1; AT2: Alveolar type 2; PNECs: Pulmonary neuroendocrine cells; SMGs: Submucosal glands.

escent basal cells tend to be krt5- and krt8-positive but p63-negative.[21]

Multiple molecular pathway components are actively involved in basal cell proliferation and differentiation. Conservative signal pathways, such as Wnt canonical signaling and Notch signaling, exert important regulatory functions in both human and murine basal cell-involved airway repair. Wnt signaling promoted their self-renewal and differentiation into ciliated cells via Wnt ligands dynamically, secreted by mesenchymal cells expressing PDGFRA and basal cells, respectively.[2] Additionally, β-catenin is indispensable for their proliferation and differentiation toward ciliated cells and Clara-like cells *in vitro*.[15] Inhibition of Notch signaling can hamper airway regeneration by increasing DNA damage checkpoint kinases, pATM and pCHK2, in human basal cells after irradiation, supporting the potential clinical usage of Notch inhibitors in anti-tumor therapy.[17] Bone morphogenetic protein (BMP) pathway components were reported to express dynamically during SO_2-induced airway epithelium injury repair, whose downregulation contributed to basal cell proliferation and differentiation through Smad1/5 transient decline but did not affect its lineage choice toward ciliated cells or secretory cells over the long term.[4] However, using SO_2-induced lung injury mouse models, Tadokoro et al.[3] revealed for the first time that interleukin (IL)−6 originating from mesenchymal cells promoted the differentiation of basal cells into ciliated cells but not secretory cells through its receptor IL-6RA and downstream signaling, JAK and STAT3. Moreover, long non-coding RNAs (lncRNAs) likely participate in basal cell differentiation and lung disease pathogenesis. For example, Meg3 in lung epithelial cells were significantly highly expressed in the lung tissues of patients with idiopathic pulmonary fibrosis (IPF) than normal lungs, acting on gene networks related to basal cell differentiation and epithelial mesenchymal transformation (EMT), including TP63, KRT14, STAT3, YAP1, and AXL.[22]

Although basal cell stem features work for epithelial regeneration, when misadjusted, they lead to an abnormal repair process. P63-expressing stem cells were demonstrated to generate a rare cell type called solitary chemosensory cell following influenza virus infection,[23] which could cause abnormal epithelial remodeling via ectopic appearance in the injured lung regions near Krt5-expressing cells and mediating crosstalk between inflammatory response and dysplasia repair.[23] Their dysfunction is involved in chronic obstructive pulmonary diseases (COPD) airway remodeling by hyperplasia and squamous metaplasia. Chronic exposure to nicotine can result in the downregulation of α7 nicotinic acetylcholine receptor, thus stimulating human airway basal cell proliferation but repressing differentiation.[24] Moreover, their over-proliferation can be promoted by trophoblast cell surface antigen 2 (TROP2) overexpression, followed by excessive inflammatory cytokines released

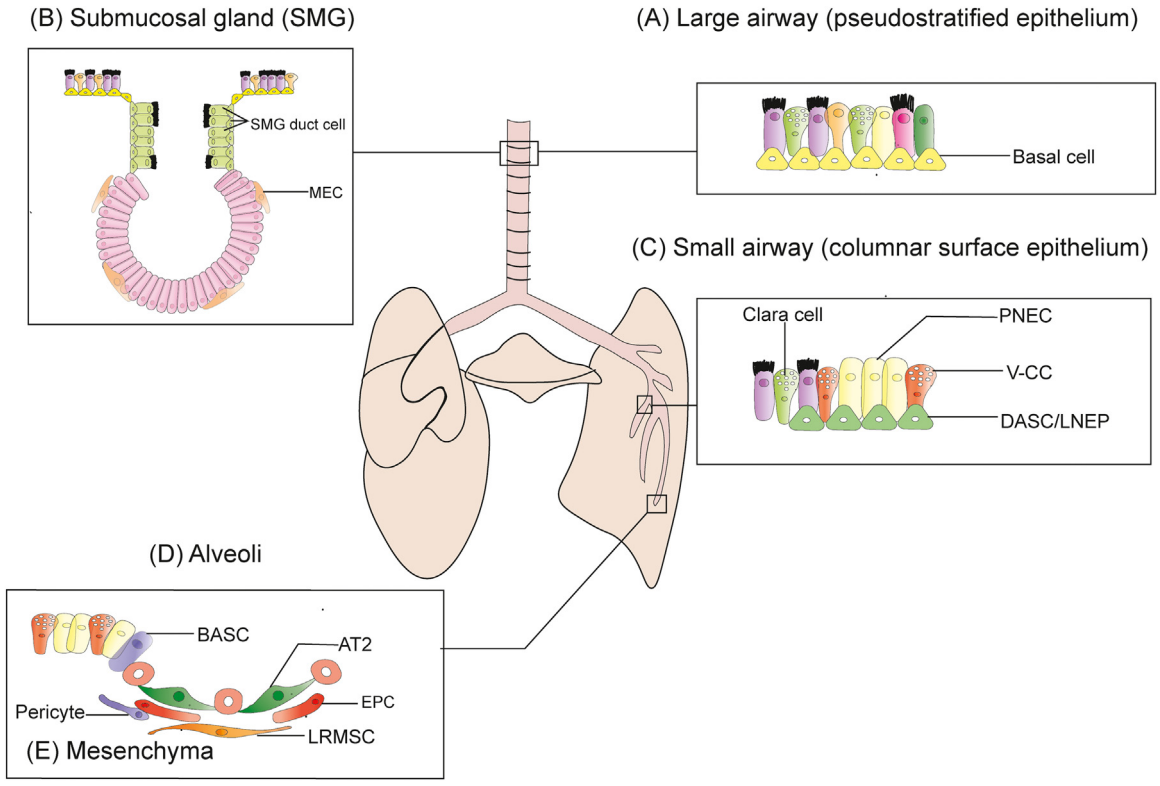

Figure 2. Main pulmonary endogenous stem cells within the mouse lung airways. (A) In large airways, basal cells are considered to be the dominant stem cells that can give rise to almost all epithelial cell types in large airways. (B) SMGs serve as a stem cell niche for SMG duct cells and MECs. SMG duct cells contain at least three subpopulations called basal cells of SMG duct cells, ciliated cells of SMG duct cells and non-basal cells, and non-ciliated cells of SMG duct cells. Contractile MECs are flat and thin and surround SMG tubules. (C) Clara cells are the main stem cells in the bronchioles. DASC/LNEPs, PNECs, and V-CCs are distributed in the distal airways. BASCs are considered to be potential stem cells located at the mouse BADJ, but no evidence supports their existence in human airways. (D) In alveoli, AT2 cells are widely known as progenitors of alveolar epithelium. (E) LRMSCs and pericytes are mesenchymal stem cells. Several EPCs are found within pulmonary circulation.

BADJ: Bronchoalveolar duct junction; BASCs: Bronchoalveolar stem cells; DASCs: Distal airway stem cells; EPCs: Endothelial progenitor cells; LNEPs: Lineage-negative epithelial progenitors; LRMSCs: Lung resident mesenchymal stem cells; MECs: Myoepithelial cells; PNECs: Pulmonary neuroendocrine cells; SMGs: Submucosal glands; V-CCs: Variant Clara cells.

from basal cells and EMT, as previously suggested in various types of stem cells of other tissue organs.[25] Similar to airway remodeling in COPD, airway remodeling in asthma[26] and cystic fibrosis[20,27] results from basal cell hyperplasia, goblet cell hyperplasia, and delayed ciliated cell differentiation. Interestingly, P63-positive basal cells can serve as progenitor cells of fibroblastic foci (FF) in IPF through EMT *in vitro*, demonstrating the connection between basal epithelia and mesenchymal cells.[28] Taken together, the above findings show that their potential roles in pulmonary diseases provide a prospective point of intervention for future therapies.

Single-cell RNA sequencing (scRNA-seq) technology promotes novel discoveries of several new basal cell subpopulations. A basal cell subpopulation called suprabasal cells was identified as a progenitor of Clara cells and goblet cells, which was characterized by low TP63 expression, decreased KRT5 expression, and increased KRT19 and NOTCH3 expression.[29] Moreover, basal cells in the normal lung included multipotent and secretory cell-primed subsets, which were regulated by Notch signaling, and the latter shared overlapping molecular signatures with basal cells in the distal lung of IPF.[30] In cystic fibrosis epithelia, a study found that the proliferating subset of basal cells marked by DNA topoisomerase II alpha (TOP2A) and Ki-67 decreased, which indicated stem cell exhaustion in airway repair.[31] Although such studies reveal significant heterogeneity and plasticity of basal cells under different injury types, the homeostatic mechanisms that determine their fate in the steady state and injured lung are largely unexplored.

Resident stem cells in the submucosal gland (SMG) niche

An SMG stem cell niche exists beneath the proximal airway epithelium, where two types of progenitor cells defined as myoepithelial cells (MECs)[32,33] and SMG duct cells[16,34,35] reside deeply. MEC can generate surface airway epithelial (SAE) basal stem cells and SMG-like cells following various severe injuries.[32,33] In some studies, severe naphthalene injury models were repeatedly established to verify certain transcription factors regulating MEC-derived repair processes. For example, Wnt-mediated Lef-1 and Sox9 induced inter-convention from MEC to basal cells[32] and migration into above SAE,[33] respectively.

MEC expressing K5, K14, and αSMA and SMG duct cells expressing TROP2 are both reported as multipotent progenitor cells within the SMG niche.[16] They can repopulate SAE, SMG, and SMG ducts in isolated human airway SMG duct cells[35] and tracheal transplantation-induced severe hypoxic-ischemic injury mice.[16] Based on those findings, Heagb et al.[34] further revealed that basal cells and SMG duct cells were likely to regenerate SAE initially post-transplantation, followed by MEC and clara cells. Collectively, given the abundance of SMG niche cells within the proximal airway, they may be a facultative stem cell

resource in correct settings.[34] The potential of epithelial cells in adult lung SAE in generating SMG remains unclear. Therefore, understanding the SMG niche-related factors that promote the progenitor ability of MEC and SMG duct cells is critical for regenerating complete SMG and SAE under appropriate conditions.

Clara cells in the bronchioles

Clara cells are defined as major progenitor cells located in bronchioles.[36–38] Secretoglobin family member 1a (Scgb1a1) and cytochrome P450 family 2, subfamily f, polypeptide 2 (Cyp2f2)[39] are their common marker genes. Recently, potential new markers, including claudin-10 (CLDN10), flavin monoxygenase 3 (FMO3), paraoxonase 1 (PON1), and aldehyde oxidase 3 (AOX3) have emerged.[40,41] Reduction and deletion of these cells contribute to the occurrence and exacerbation of many lung diseases, including post-lung transplantation bronchitis obliterans,[42] radiation-induced lung injury,[43] and naphthalene-stimulated bronchial fibrosis.[44]

In terms of differentiation, Clara cells can generate airway goblet cells and ciliated cells, which can secrete or clear mucus. The mechanisms underlying Clara cell differentiation lineage choice have been partly revealed. Hoxa5 deficiency can promote their transdifferentiation into goblet cells by upregulating Notch signaling,[45] while inhibiting JAG ligand 1-NOTCH 2 receptor axis signaling can strengthen their ciliated cells generation.[46] In another study, Foxm1 transcription factor deletion increased goblet cells but reduced ciliated cell generation, causing airway fibrosis and remodeling.[37] Intriguingly, tracheal Clara cells (Scgb1a1-positive secretory cells), which are differentiated from dominant basal stem cells, could dedifferentiate into basal stem cells, following basal cell ablation.[47]

Alveolar epithelial type 2 (AT2) cells were reported to repopulate from bronchial Clara cells through an intermediate phase when AT2 cell marker, pro-surfactant protein C (pro-SPC), was detected.[48,49] However, the reconstitutive capacity of Clara cells following severe alveolar epithelial damage remains controversial.

Using bleomycin-induced lung fibrosis mouse models, Zheng et al.[48] indicated that new AT2 cells were regenerated for alveolar repair, whereas Yokoyama et al.[50] revealed that pulmonary fibrosis (PF) could be alleviated following their denudation by naphthalene injury. Furthermore, accumulating studies have confirmed that they contribute to PF through a phenotypic conversion to achieve alveolar migration and proliferation capacity,[40] which was defined as alveolar bronchiolization.[51] In the lung tissues of patients with IPF, aberrantly proliferative Clara cells can not only repress epithelial restoration through tumor necrosis factor-related ligand (TRAIL)-dependent apoptosis[51] but also activate profibrotic αSMA signaling by interacting with fibroblasts.[40] Moreover, in hyperoxia-induced PF models, dysplastic alveolar epithelial cells originated from Clara cells in *ATP8B1* gene mutation mice.[41] Collectively, the above results indicate that the conversion of bronchial Clara cells into alveolar epithelial cells is like a double-edged sword. Cellular mechanisms that drive lineage commitment remain to be identified to further clarify its exact roles in alveolar injury repair.

Distal airway

Distal airway stem cells (DASCs)

DASCs have been confirmed as candidate stem cells involved in both bronchiolar and alveolar epithelial regeneration.[52–55] DASCs are also defined as lineage-negative epithelial progenitors (LNEPs).[53] Although DASCs express Krt5 and P63-like tracheal basal stem cells, they are distinct populations since DASC are located within the distal airway and can uniquely generate an alveolar epithelial lineage.[52–54,56] In humans, N-terminally truncated isoform of p63 (ΔNp63) and thyroid transcription factor-1 (TTF-1) dual positive lung epithelial cells were demonstrated as DASC counterparts.[57] The differentiating direction of DASC seems inconsistent under different circumstances. For example, DASCs can give rise to Clara cells, alveolar type 1 (AT1) cells, and AT2 cells following influenza virus infection[54] but only generate AT1 cells following bleomycin-induced injury.[52]

An increasing number of studies have revealed the relative contributions of DASCs and AT2 cells – another putative progenitor discussed later in this review – to alveolar epithelial regeneration. In influenza H1N1 virus-induced lung injury, no DASC transition to AT2 cells was detected before 25 dpi, whereas AT2 cells underwent self-renewal and conversion to AT1 cells from 19 dpi.[53] Moreover, another study reported that DASCs were converted into AT1 cells as early as 90 dpi in influenza-associated pneumonia, and abolition of DASC transition to AT1 cells resulted in fibrotic structure formation and oxygenation function deficiency.[54] Those findings suggest that AT2 cells exerts crucial regenerative effects in the early recovery phase, whereas DASCs favor long-term alveolar tissue repair after influenza-associated pneumonia.

Both Notch and Wnt signaling pathways are involved in adjusting DASC differentiation into alveolar epithelial cells. Notch signal activation enabled DASCs to generate basal-like cell pods initially post-injury, but persistently activated Notch pathway prevented their subsequent alveolar epithelial fate, causing micro-honeycombing structure formation and failed alveolar regeneration.[58] In another study, both hypoxia-inducible factor (HIF) 1 deletion and enhanced Wnt/β-catenin activity could promote direct conversion of DASCs into AT2 cells without undergoing an intermediate basal-like cell state by inhibiting Notch signaling, thus improving lung regeneration quality.[59]

Taken together, the above studies show that even when the differences in the regenerative potentials of DASC/LNEP reflect distinct injury severity, differences in experimental design or co-fractionation of heterogeneous subprogenitors with an overlapping signature profile remains unknown.

Stem cells in the neuroendocrine body (NEB) niche

The lung is classically known for its essential gas exchange ability. Recent findings demonstrate the lung as a sensory organ due to its responses to various stimuli with other systems.[1] Pulmonary neuroendocrine cells (PNECs) expressing CGRP, PGP9.5, and ASCL1[1,60] are a kind of rare innervated epithelial cell-type that occupy about 0.5% of all pulmonary epithelial cells.[1] PNECs perform various functions, such as immune modulation, lung development regulation, and airway oxygen sensing.[61] They have been confirmed to serve as reserved stem cells in the distal airway, because they can contribute to Clara cells and ciliated cells after lung injury.[5,61]

In addition, single PNECs located along the conducting airway gather at the bronchoalveolar junction to form the NEB, which maintains as the stem cell niche for PNECs and variant Clara cells (V-CCs).[62] Intriguingly, no other types of cells can regenerate PNECs once they are deleted.[5]

Naphthalene-induced injury murine models are widely used to explore the mechanisms underlying PNEC regenerative processes. For example, Notch signaling was shown to mediate PNEC differentiation into Clara cells after injury,[61] and Notch receptor 1 was critical to the transition of PNECs to Clara cells by targeting its downstream gene, *Hes5*.[60] Moreover, Rb and p53 modulated PNCE proliferation.[5,63] Activated Fgf10-Fgfr2 signaling was identified in NEB,[64] and IL6-STAT3 signaling was crucial to PNEC transdifferentiation following naphthalene injury.[61]

V-CCs are another cell type found in the NEB niche.[5] They express classic Scgb1a1 but not Cyp2f2, enabling them to exhibit resistance to naphthalene injury.[65,66] In naphthalene-induced lung injury mouse models, murine double minute 2 (MDM2) promoted V-CC proliferation and bronchiolar epithelial repair by amplifying DNA replication through Akt signal phosphorylation,[67] whereas insulin-like growth factor 1 receptor downregulation led to excessive proliferation but reduced differentiation, contributing to partial epithelial restoration.[68]

Given that current research evidence on the regenerative potential of PNEC and V-CC is derived from murine experiments, whether these cells exhibit similar functions in human lung injury needs to be elucidated.

Other distinct stem cell types in the distal airway

Found among Clara cells in the distal airway, a rare type of progenitor cells, H2-K1^{high} cells, highly express MHC class I marker, H2-K1.[69] These cells exhibit mobility and differentiation into AT1 and AT2 cells in bleomycin-induced alveolar injury.[69] Uroplakin 3a-positive cells are another type of Nap-resistant progenitors enriched in NEB.[70] They contribute to Clara cells and ciliated cells under both airway homeostasis and Nap-induced airway injury conditions but only repopulate AT1 and AT2 cells following bleomycin-induced alveolar injury.[70] In human respiratory bronchioles, a distinct secretory cell population called respiratory airway secretory (RAS) cells was identified, which could regenerate AT2 cells via Notch and Wnt signaling rapidly and unidirectionally. An abnormal trajectory of RAS cells to AT2 cells was found in COPD, indicating its essential role in maintaining alveolar homeostasis.[71] In addition, another human progenitor, AT0, lining the vicinity of terminal and respiratory bronchioles (TRBs), can regenerate both TRB secretory cells and AT2 cells.[72] Further clarification of other potential progenitor cells in the human distal airway is urgently needed.

Bronchoalveolar stem cells (BASCs) in the bronchoalveolar duct junction (BADJ)

BASCs are progenitor cells residing in the BADJ, the distal end of terminal bronchioles.[73] BASCs express both Clara cell marker, Scgb1a1, and AT2 cell marker, SPC.[73,74] They can regenerate both bronchiolar and alveolar epithelial cells following lung injury.[73,75,76] In recent years, mechanisms underlying BASC proliferation and differentiation have attracted much attention. Following naphthalene-induced airway epithelial injury, loss of GATA6 (a type of zinc finger transcription factor) could directly downregulate Fzd2 (a kind of non-canonical Wnt receptor) expression, leading to increased canonical Wnt signaling pathway and BASC proliferation but impaired BASC differentiation.[77] XB130, a kind of novel adaptor protein, could enhance BASC self-renewal by PI3K/Akt/GSK-3β pathway phosphorylation.[78] Furthermore, BASC-derived repair could be driven by their stromal microenvironments. For example, BASC proliferation may be enhanced by plasma and local HGF by Met-receptor tyrosine phosphorylation.[79] BASC-derived BMP4 could upregulate TSP1 release from pulmonary endothelial cells by BMPR1a/NFATc1 signaling. TSP1 subsequently directed BASCs into alveolar epithelial differentiation.[80]

The cell differentiating direction of BASCs accurately adjusts to the injured pulmonary region. BASCs differentiated into Clara cells and ciliated cells following naphthalene-induced bronchiolar epithelial deletion but converted into AT1 and AT2 cells in bleomycin-induced alveolar impairment.[73,76] In bronchiole–alveoli double injuries induced by naphthalene and bleomycin[81] or influenza virus-induced lung injury,[82] BASCs could regenerate both distal bronchioles and alveoli. In addition, deletion of BASCs delayed the distal airway regeneration progress after naphthalene injury, suggesting that BASCs primarily repair distal bronchioles.[82]

Alveolar stem cells

AT2 cells

The pulmonary parenchyma is composed of two types of alveolar epithelial cells, known as squamous AT1 cells expressing markers, such as PDPN, RAGE, HopX, and AQP5,[83] and cuboidal AT2 cells specifically expressing SPC.[84,85] AT1 cells exerts a dominant role in gas exchange and barrier function, as they cover 98% of the alveolar epithelium,[84,85] whereas AT2 cells is capable of pulmonary surfactant secretion and surface tension reduction.[86] In the perinatal period, mature AT1 or AT2 cells are derived from a subset of bipotent alveolar cells, in which YAP enhances AT1 cells numbers and AT1 cell marker, AGER, via NFIB/KLF5/NKX2–1 but decreases mature AT2 cell markers.[87] AT2 cells is also widely acknowledged as a main progenitor cell in the alveoli.[83–86,88–96] AT2 cells are composed of heterogeneous subpopulations that express different markers and possess unequal regenerative capacity.[97,98] For example, the subpopulation highly expressing CD44 acts as progenitor cells during the steady state,[98] whereas the Sca1-positive subpopulation exhibits higher regenerative potential than the Sca1-negetive subpopulation, which is reported to mediate alveolar regeneration following both *Pseudomonas aeruginosa* (PA) and bleomycin-induced lung injury.[93,97] In addition, an AT2 cells subset called PD-L1^{+} Tom^{Low} cells remains quiescent and immature under homeostasis but acquires self-renewal and differentiation to mature AT2 cell ability to respond to pneumonectomy and bleomycin-induced lung injury.[99] Moreover, Axin2^{+} Wnt-responsive AT2 cells subpopulation, which expresses AT2 cell marker genes and enriched Wnt targets, represents a major progenitor lineage by generating AT1 and AT2 cells following influenza injury.[88]

A growing body of literature has emerged to reveal signaling pathways that regulate AT2 cells proliferation. For example, the

Hippo-YAP pathway regulates alveolar regeneration in multiple lung injury models induced by mechanical tension, LPS, or hemorrhagic shock.[84] Furthermore, IL-6/STAT3, BMP, and FGF signaling pathways play functional roles in mediating AT2 cells self-expansion.[100] Some critical regulatory mechanisms during embryogenesis were reported to be reactivated during adult alveolar epithelium repair. The HIF signaling pathway, which promotes cell expansion during angiogenesis and tumorigenesis, activated VEGF signal and SDF1/CXCR4 signal to promote AT2 cells proliferation and spreading, respectively, in LPS and HCl injury mouse models.[85] Genes related to AT2 cells proliferation in fetal lung development were shown to be the targets of microRNAs (miRNAs)−302, promoting alveolar regeneration after *Streptococcus pneumoniae* (Sp) injury.[89] In PF, DNA repair enzyme, OGG1, enhances AT2 cells proliferation by inhibiting the NF-κB pathway, and thus attenuated canonical inflammatory response signaling.[91] AT2 cells surface hyaluronan (HA), an extracellular matrix (ECM) glycosaminoglycan, boosts AT2 cells expansion and prevented bleomycin-induced lung fibrosis.[92] In addition, the contribution of neutrophils to AT2 cells mitosis has been demonstrated, underscoring a link between stem cell-derived regenerative process and immune response.[90]

Wnt, Notch, and Hippo signaling pathways were demonstrated to be involved in the process of intact alveolar structure repair in various lung injury models. Wnt signaling drove AT2 cells conversion to AT1 cells in both bleomycin[86] and PA[97] lung injuries. Recent *in vitro* human AT2 cells studies identified Wnt4, Wnt5a, and Wnt7a as three important ligands for the AT2-to-AT1 cells conversion process.[101] In addition, ENO1, PDIA3, and CBR2 were the first identified proteins involved in Wnt/β-catenin pathway-mediated AT2 cells differentiation process following bleomycin injury,[94] whereas FoxM1 was identified as a transcription factor for regulating alveolar barrier repair by binding to the promoter of β-catenin signaling following PA injury.[93] An *in vivo* PA lung injury model was established to reveal the dynamic change of Notch signal in AT2-to-AT1 cells transition, in which DLK1 could switch Notch signaling from initial activation to subsequent inhibition, enabling generation of completely mature AT1 from AT2 cells.[83] Along with Wnt and Notch signaling pathways, the Hippo pathway component, TAZ, is critical to AT2 cells convention to AT1 cells, whereas YAP is dispensable, following bleomycin-induced PF.[95] In lung fibrosis, Wu *et al.*[102] revealed that impaired AT2-AT1 cells differentiation caused sustained elevated tension on alveoli, which subsequently activated a TGFβ signaling loop in AT2 cells and periphery-to-center progression of lung fibrosis. Interestingly, a link exists between the AT2-to-AT1 cells transition and bronchopulmonary dysplasia (BPD). Molecular hydrogen exhibited protective roles against hyperoxia-induced lung injury by promoting AT2 cells differentiation, which may be associated with VEGFA, PDGFB, IGFBP3, EDN1, NADPH oxidase, and the coagulation cascade.[96] Notably, underlying mechanisms balancing AT2 cells proliferation and differentiation have been partly revealed. Fgfr2 was found to promote AT2 cells proliferation but inhibit differentiation in actively repairing areas after acute influenza injury, thus restoring normal alveolar structure and function.[103]

Recently, several studies have focused on the contribution of senescent AT2 cells to the pathogenesis of lung diseases. A large number of senescent AT2 cells were found in COVID-19-induced acute lung injury (ALI),[104] ventilation,[105] influenza,[106] LPS,[107] and chronic lung diseases, such as lung fibrosis and COPD.[108,109] Senescent AT2 cells exhibited lower regenerative capacity, as demonstrated by their impaired regeneration-related signaling pathways,[108] decreased AT2 and AT1 cell markers,[110] minimal stemness markers,[111] and lower proliferation markers.[104] The mechanism linking AT2 cells senescence to impaired lung repair has also been partly revealed. For example, p21 could suppress AT2 cells self-renewal and AT2-AT1 cells differentiation by disturbing p300-β-catenin interaction.[111]

In summary, new AT2 cells subsets exhibiting progenitor capacity require further discoveries via new technologies, such as scRNA-seq, thus facilitating their classification, lineage tracing, and identification of their specific functional roles in alveolar epithelial maintenance and repair. Underlying mechanisms linking stress-induced premature AT2 cells senescence and defected regeneration represent a promising research direction in the future.

Other distinct cell types in pulmonary parenchyma

In addition to AT2 cells, other distinct candidate alveolar epithelial stem cell populations exist. An AT1 cells subtype expressing Hopx can self-renew and transdifferentiate toward AT2 cells both in pneumonectomy and single AT1 cells-cultured models.[112] Penkala et al.[113] further confirmed age-specific regeneration processes, in which AT1 cells could differentiate into AT2 cells in both neonatal and adult ALI, whereas AT2-AT1 cells transition only existed in the adult lung. YAP/TAZ deletion was sufficient to drive efficient AT1-AT2 cells reprograming without injury. Integrin $\alpha6\beta4$-positive cells residing within the bronchoalveolar junction and alveolar area represent progenitor potential owing to their transition capacity into AT2 cells following bleomycin insults.[114] In addition, a rare type of stem cells marked by EpCAM and CD73 that are distributed along epithelium basal membrane can generate pseudostratified airway epithelium and cystic-like alveolar structures in 3D organoid culture assay *in vitro*.[115]

Pulmonary mesenchyme

Lung resident mesenchymal stem cells (LRMSCs)

LRMSCs are different from bone marrow-originated MSCs for their distinct gene and cytokine profiles.[116–119] They express mesenchymal markers, including CD29, CD44, CD73, CD90, CD105, and CD13, but lack the expression of CD34, CD45, CD3, CD14/11b, and CD19/79.[116,120–122] LRMSCs can differentiate into multiple cell lineages,[119–124] including adipocytes, osteocytes, and chondrocytes, and even epithelial and endothelial cells. LRMSCs also secrete critical biologic factors for lung growth and development, such as FGF-7, FGF-10, interleukin-8, VEGF, and angiopoietin-1.[116,125] Considerable evidence suggest their immune regulatory and inflammation inhibitory properties.[126–128] In sum, their regenerative capacity along with their secretory and anti-inflammatory functions paves a promising way for LRMSC-targeted cell therapy to halt lung diseases.

Local factors from surrounding microenvironment can also convert LRMSC into myofibroblasts, leading to some pulmonary diseases, such as PF and BPD.

During the PF process, Wnt7b/10a ligands activate Wnt signal through Fzd9/10 receptors on LRMSCs, whose secretion is promoted by M2 macrophages through Frizzled-1 receptor on LRMSCs.[129] Furthermore, Hedgehog signaling component, Gli, in LRMSCs can exacerbate the lung fibrosis process by binding the promoter region of Wnt7b/10a to enhance their expression and secretion.[130] In contrast, small molecular ICG-001 disrupts Wnt signal through a Smad-independent manner to restrain LRMSC-myofibroblast transition.[124] Moreover, in EC-SOD deletion mice, LRMSCs were confirmed to convert into a contractile myofibroblast phenotype along with Wnt signal activation, suggesting a link between oxidative stress and LRMSC-derived microvascular remodeling.[123]

To date, a large number of miRNAs have been confirmed to play essential roles in LRMSC-myofibroblast transition. During the TGF-β-induced PF process, miR-152–3p, miR-140–3p, miR-148 b-3p, and miR-7a-5p were reported to suppress Kruppel-like factor 4 (*Klf4*) gene to inhibit LRMSC conversion into myofibroblasts by downregulating TGF-β1/Smad and Wnt pathways. miR-34a-5p, miR-27 b-3p, miR-323–3p, miR-27a-3p, miR34c-5p, miR-128–3p, and miR-224–5p target the inhibitor of growth family member 5 (*Ing5*) gene to enhance PI3K/AKT and Wnt/βcatenin pathways.[131] In bleomycin-induced PF mice, increased miR-497–5p in LRMSCs acts on reversion-inducing cysteine-rich protein with kazal motifs (*Reck*) gene, followed by LRMSC-myofibroblast conversion and fibrotic marker hyperexpression.[132] Endoplasmic reticulum stress can contributed to LRMSC-myofibroblast differentiation by facilitating the TGFβ/SMAD signaling pathway during PF.[133] *In vitro*, LRMSC from patients with BPD and hyperoxia-impaired LRMSCs facilitates myofibroblast phenotype conversion and leads to fibrotic reorganization.[116,125] Taken together, the above findings reveal LRMSC function heterogeneity under distinct settings of lung injuries. Further *in vitro* and *in vivo* studies recapitulating their spatial location and molecular and functional properties are needed to enrich our understanding of LRMSCs, thus improving autologous therapies for lung diseases.

Pericytes

Pericytes are supporting mesenchymal cells covering the blood vessels, which are embedded within surrounding considerable ECM.[134,135] Pericytes are also considered as an MSC subset, because they can generate chondrocytes, adipocytes, and osteocytes *in vitro*.[135] A wide range of proteins, including PDGFRβ, CD146, CD13, CD72, CD90, CD105, CD73, CD44, 3G5, Ang1, RGS5, contractile myofilaments (αSMA and SM22), and intermediate filaments (desmin and vimentin), have been reported as their markers.[135–138]

Pericytes are involved in the pathogenesis of chronic lung diseases, including PF and asthma, through detachment from microvascular endothelia and subsequent transdifferentiation into myofibroblasts. In the lung tissue of patients with IPF, pericytes exhibit stronger migration ability away from EC into interstitial space than in the healthy group,[136] and FF is attributed to pericyte-myofibroblast transition (PMT) via the TGF-β pathway.[137] Moreover, Notch1 promotes PMT by activating downstream PDGFR-ROCK1 signaling.[139] Based on these findings, inhibiting both TGF-β and PDGFβ pathways by depressing FUT8 catalyzed core fucosylation (CF)-exerts superior functions in retarding PF compared to blocking either the TGF-β or PDGFβ pathway.[140] Moreover, in house dust mite-induced chronic allergic asthma mice, deletion of PDGF-PFGFR signaling pathway led to pericyte migration from endothelial to airway subepithelial region, where they converted into myofibroblasts and mediated airway remodeling.[141] Pericyte migration can also be promoted by periostin responding to IL-13 and TGF-β in allergic asthma.[142] However, pericyte removal only retards acute lung inflammation without alleviating PF in bleomycin-induced lung injury, although they are important progenitors of myofibroblasts.[143] An efficient and specific lineage tracing system is urgently required to clarify the role of pericytes in lung development and disease responses.

Other distinct types of LRMSCs

In naphthalene and LPS injury models, a type of LRMSC expressing Dermo1 can generate intermediate airway progenitors, PNECs, followed by subsequent differentiation into ciliated cells, goblet cells, and Clara cells.[144] Another LRMSC subtype marked by Gli remained a potential source of myofibroblasts in the pathogenesis of kidney, heart, lung, and liver fibrosis.[145] Furthermore, in the latest literature, activated Hh signal in Gli-positive mesenchymal cells downregulated BMP signal of adjacent Sox-positive airway stem cells by secreting more BMP antagonists into the regeneration microenvironment, leading to alveolar bronchiolization and failed AT2 cells regeneration under fibrotic conditions.[146]

Endothelial progenitor cells (EPCs) in the endothelium

EPCs have been acknowledged to show characteristics of progenitor cells, owing to their proliferative and differentiative potential toward both blood and lymphatic vessels *in vivo* and *in vitro*.[147,148] EPCs are classically defined as CD34-positive mononuclear cells originating from bone marrow and displaying angiogenetic capacity.[149,150] In addition, a large number of lung-resident EPCs exist within pulmonary circulation, which are described as PVECs with progenitor identities, including high proliferative, colony-forming, and angiogenetic capacities.[148,149,151–153] Nucleosome assembly protein-1 is a key regulator of EPCs, because it enhances PVEC proliferative and angiogenetic ability without affecting their phenotypes.[153]

Commonly, EPCs in circulation from the bone marrow express various endothelial markers,[151,153,154] such as CD31 and CD144; von Willebrand factor[151,153,154]; and progenitor cell antigens, including CD34, CD133, C-kit, and Sca-1,[149,152,155,156] but lack the leukocyte marker, CD45.[151] However, specific lung-resident EPC surface markers still remain to be determined. Emerging studies have demonstrated that lung-resident EPCs and circulating EPCs share common surface markers. A cell population expressing C-kit (CD117) within lungs was shown to obtain the endothelial cell phenotype instead of epithelial cells in either cryo or pneumonectomy-induced mouse injury models.[154] In more recent literature, PVECs expressing CD133 (prominin-1) were likely to be potential lung-resident EPCs attributed to their stem characteristics, such as vascular repair functions responding to monocrotaline or hypoxia *in vitro*.[149]

Recently, studies exploring whether bone marrow-derived circulating EPCs or lung-resident EPCs play dominant roles in pulmonary vasculogenesis following endothelial injury have been reported. Proliferative PVECs in capillaries did not origi-

nate from bone marrow-derived EPCs post-hypoxia injury.[157] Pulmonary resident EPCs were suggested to contribute to vascular regeneration post LPS-induced endothelial cell injury *in vivo*.[155] To date, BM-derived EPCs cannot be distinguished from lung-resident EPCs. Future identification of lung EPC heterogeneity and their specific roles in vascular repair is urgently needed to pave the way for treating pulmonary vascular diseases.

Models and Methods for Research, and Potential Therapy Strategies for Lung Regeneration

Recently, several types of *ex vivo* and *in vitro* 3D models have been widely used for lung research. Furthermore, due to species difference between humans and animal models and ethical limitations in human experiments, they represent preferable model systems for pulmonary study.

Ex vivo lung explants are the simplest human tissue-based 3D models obtained from thoracic surgery, complete lobectomy, or pneumonectomy explants and whole lungs that cannot be used for transplantation.[158] They have been used to reveal that FGF7, 9, and 10 caused SOX9-positive cell expansion but SOX2-positive cells decrease, whereas SOX2 and SOX9 double positive progenitor cells were critical to lung branch development.[159] Furthermore, DNA protein kinase catalytic subunit (DNA-PKcs) downregulation caused SSEA4-positive mesenchymal progenitor cell proliferation, and full-length caveolin-1 scaffolding domain peptide (CSP) decreased AT2 cells apoptosis, providing a promising strategy for IPF treatment.[160,161] Precision cut lung slices (PCLS) from healthy or diseased human tissues represent another *ex vivo* 3D model with specific thickness of 100–500 μm.[158,162] Human PCLS have been used as model systems of IPF[163,164] and asthma[165] to reveal their pathogenesis and potential drugs for treatment. Mice-derived PCLS were generated to study AT2 cells regeneration roles and underlying mechanisms in IPF[166] and COPD.[167]

Lung organoids are defined as complex and self-organizing 3D models cultured *in vitro*, which can mimic critical aspects of lung structures and functions. Both airway and alveolar organoids were widely used for pulmonary studies to obtain important results.[168–174] Commonly, organoids can be generated from induced pluripotent stem cells (iPSCs) or adult stem cells (ASCs).[169,175,176] ASC-derived lung organoids have been used to investigate that pulmonary endogenous stem cell-mediated regeneration process impairment played a key role in lung disease pathogenesis. For example, a study focused on IL-1β-treated organoids revealed that sustained chronic inflammation inhibited AT2 cells transition to mature AT1 cells,[177] leading to IPF. In alveolospheres, AT2 cells regeneration ability was impaired by telomere dysfunction, causing IPF and emphysema.[171] In organoid assays, organoid-forming ability of distal lung epithelium progenitors was impaired in emphysema,[178] whereas AT2 cells-mediated alveolar epithelium repair was inhibited by cigarette smoke in COPD.[179] Furthermore, because retinoic acid (RA) inhibition and histone deacetylase (HDAC) inhibition promoted lung organoid proliferation and differentiation, respectively, their combination was a potential therapy strategy for COPD.[180]

Organoids can also serve as platforms for COVID-19 research. Using human airway organoids, HIF1α-glycolysis axis was found to contribute to SARS-CoV-2 infection in the airway epithelium.[181] Another study using human lung organoids confirmed that receptor-interacting serine/threonine-protein kinase 1 (RIPK1) induced host inflammatory response to promote SARS-CoV-2 replication.[182] Furthermore, ASC-derived complete lung organoid was recently established to confirm that airway cells were critical for sustained viral infection, whereas distal alveolar differentiation was crucial for mounting host immune responses.[183]

Recently, new research technologies, including scRNA-seq and gene editing, combined with potential 3D models have made tremendous progress. Comparing scRNA-seq results of lung *ex vivo* tissues from patients and normal persons cannot only help to identify diseased-associated gene changes and key signaling pathways in specific cell populations[184] but also reveal heterogeneous cell subpopulations roles in lung diseases.[185] ScRNA-seq and organoid combination were used to demonstrate the region-specific interactions between epithelial stem cells and neighboring mesenchymal cell subpopulations,[186] and the cellular pathway from AT2 to AT1 cells triggered by inflammation during bleomycin injury.[177] In a recent study, lung organoids and CRISPR/Cas9 were combined to reveal that SOX9 decreased organoid proliferative ability but was dispensable for human lung development.[187] Additionally, Schwank et al.[188] used the CRISPR/Cas9 system to correct *CFTR* gene of intestinal stem cell from patients with cystic fibrosis, providing opportunities for organoid transplantation therapy. Taken together, the above findings show that gene editing and organoid technology combination cannot only overcome the defects of mouse models with different genetic backgrounds but also create a promising strategy for gene therapy.

Pulmonary Endogenous Stem Cell-derived Therapy Strategies

With development of pulmonary endogenous stem cell research, preclinical evidence have emerged to support the feasibility of stem cell-based cell transplantation therapy for patients with lung disorders.

Basal cells

Basal cell transplantation is effective in some chronic lung injuries. Sun et al.[189] and Ma et al.[190] confirmed that human SOX9-positive basal cell transplantation improves lung structure and function in patients with bronchiectasis without mortality and adverse events in 1 year, indicating that it represents a safe and effective treatment therapy. Basal cell transplantation can also alleviate bleomycin-induced PF, and CoQ10 pretreatment on basal cells can help them adapt to high-oxidative stress microenvironment, thus strengthening their treatment effects.[191] Combination of gene editing and basal cell transplantation is a promising treatment therapy for hereditary lung diseases, such as cystic fibrosis, which is an autosomal recessive disease caused by cystic fibrosis transmembrane conduction regulator (CFTR) mutation.[192] Transplanted patient-specific *CFTR* gene-corrected basal cells can reconstruct airway by generating healthy secretory, ciliated cells, and ionocytes.[193,194] Denuding airway epithelia before basal cell transplantation with polydocanol (PDOC) improves transplantation levels.[195] More effec-

tive methods are needed to help transplanted basal cells reside at their normal basolateral location within airway and enhance the competition with endogenous CFTR-mutant basal cells.

DASCs

Transplanted DASC transdifferentiate into Clara, AT1, and AT2 cells in bleomycin-injured or influenza virus-injured murine lungs and bronchioles.[52,54,196] Moreover, human DASCs can be retained in the lungs of non-human primate macaque without causing adverse events.[196] DASC transplantation also provides a gene delivery platform for lung regeneration. For example, IL-37-DASCs, which produce IL-37, not only form the air sac-like structures expressing AT1 cell markers in bleomycin-injured lung but also expand bacterial clearance capacity of *Pseudomonas aeruginosa* (PA)-injured lung.[197] Collectively, endogenous DASC with multi-directional differentiation capacity is insufficient to repair injured lungs. Exogenous DASC supplementation alone or combined with genetic engineering may be more effective to lung regeneration.

BASCs

Intratracheal injection of iPSCs-derived BASCs engrafted within lung, maintain BASC phenotype and elevate Clara cells in naphthalene-injured lung.[198] Because BASCs only account for <1% of lung epithelia, generating BASCs from iPSC is a potential method for obtaining sufficient BASCs. Further studies on BASC transplantation application in several types of lung injury models are needed.

AT2 cells

The most attractive lung endogenous stem cell population for transplantation is AT2 cells. AT2 cells isolated from healthy donors,[199] iPSC-AT2 cells,[200] and umbilical cord blood MSC-AT2 cells[201] can improve lung structure and function in bleomycin-induced PF mice.[199–201] Transplantation is more effective in the fibrotic phase than in the inflammatory phase, even though PF developed.[199,200] In addition, Serrano-Mollar *et al.*[202] provided the first clinical evidence to reveal that patients with IPF receiving AT2 cells delivery had no deterioration in lung function, respiratory symptoms, or disease severity within 12 months, indicating that AT2 cells transplantation is safe and well-tolerated for IPF treatment.[203] In addition to IPF, a study found that intratracheal transplantation of mouse AT2 cells or human iPSC-AT2 cells could also inhibit hyperoxia-induced lung injury and avoid teratoma occurrence, which was induced by undifferentiated iPSC injection.[204] AT2 cells intratracheal transplantation also alleviated ALI induced by influenza virus, acid, and LPS-HCL.[205–207] In addition, it improved blood oxygen saturation following influenza injury.[173]

Notably, some studies confirmed that exogenous AT2 cells preferentially retained and expanded in injured lung actively[173] and maintained AT2 cells phenotype or differentiated into AT1 cells without dysplastic differentiation.[173,204] However, some studies emphasized that no AT2 cells engraftment was detected, and newborn AT2 cells was derived from endogenous lung cells self-renewal rather than transplanted AT2 cells, indicating that AT2 cells might retard lung injury through paracrine effects,[200,201] such as pulmonary surfactant and prostaglandin (PGE) secretion,[199,206] and some signaling pathways that inhibited lung epithelial cell apoptosis.[201] More preclinical studies are needed to elucidate the mechanisms underlying AT2 cells transplantation treatment. If the paracrine manner of AT2 cells plays dominant roles, AT2 cells-secretome or AT2 cells-exosome transplantation may be used to prevent adverse effects of stem cell transplantation.

LRMSCs

LRMSCs injected through the tail vein[126,208] and trachea[209] could engraft in murine lungs to alleviate LPS-ALI by upregulating Tregs/Th17 cell ratio and IL-10.[126,208] In addition, intraperitoneal injection of LRMSC protected against hyperoxia-induced lung injury, indicating that it was effective in BPD treatment.[210] Some studies have compared the therapeutic effects of MSC from different sources. In LPS-induced ALI, LRMSCs exhibited better therapeutic effects than bone marrow-derived MSCs (BM-MSC).[209] However, they were less effective than human chorionic-derived MSCs.[208] Compared with BM-MSCs, LRMSCs have lung-specific characteristics, including better lung engraftment ability, longer lung residence time, smaller volume, higher colony-forming ability, and weaker osteogenic capacity.[209,211]

Prospects for Future Research on Pulmonary Endogenous Stem Cell-derived Therapy Strategies

Pulmonary endogenous stem cell transplantation has attracted much attention, owing to their specific advantages. They cannot only continuously generate healthy lung cells via natural cell turnover processes[195,203] but also exhibit other functions, such as producing pulmonary surfactant, anti-inflammatory, and anti-bacterial substances.[203,205,212] Pulmonary endogenous stem cells are superior to differentiated lung cells[213] and stem cells from other sources[201] in therapeutic efficacy. Furthermore, they exhibit less risks of tumorigenesis and migration to other healthy tissues, as they are more differentiated and can only survive in the lung.[128,205] However, many disadvantages limit the clinical application of pulmonary endogenous stem cells. Cell therapy has high cost and safety concerns, such as tumorigenesis[196,200,214] and immunogenicity.[202,214] In addition, their abnormal proliferation and differentiation *in vivo* contribute to lung diseases.[200] Moreover, their colonization has not been fully confirmed,[200] suggesting that they may play protective roles through paracrine effects rather than progenitor characteristics.

Future research is urgently required to promote clinical application. The best time, optimal cell dose, and delivery route (via vein or trachea) for transplantation should be established. In addition, their colonization and underlying mechanisms remain to be revealed. After engraftment, ways to prevent abnormal regeneration are also critical. Cell transplantation with pharmacological operation may be a promising strategy to ensure normal repair. Moreover, protocols are needed to produce high-quality, xeno-free, and clinical-grade lung endogenous stem cells.

Conclusions

In this review, we presented the current candidate pulmonary endogenous stem cells, summarized recent findings on these cells, and identified the gaps in research. Pulmonary endogenous stem cell-derived therapy represents a promising direction, despite many unsolved problems. Due to differences between murine and human lungs, the translational significance of murine research must be carefully considered. Whether the heterogeneity of seemingly homogeneous stem cells is attributed to cofractionation of heterogeneous cells or homogeneous cells responding to distinct niche cues remain unexplored. Additionally, distinguishing reprogramed cells from genuine progenitors is challenging. In the future, more advanced tools, such as scRNA-seq, lineage tracing, and genetic manipulation, can not only reveal more distinct stem cell subpopulations and new progenitors but also deepen our understanding on their localization, cell fate, and non-epithelial lung compartment regulation of behavior. More basic studies and sound clinical approaches are needed to clarify mechanisms underlying lung regeneration and establish new therapies for the treatment of lung injury.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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