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Review Article

Cell–cell interactions and communication dynamics in lung fibrosis

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ABSTRACT

Cell–cell interactions are essential components of coordinated cell function in lung homeostasis. Lung diseases involve altered cell–cell interactions and communication between different cell types, as well as between subsets of cells of the same type. The identification and understanding of intercellular signaling in lung fibrosis offer insights into the molecular mechanisms underlying these interactions and their implications in the development and progression of lung fibrosis. A comprehensive cell atlas of the human lung, established with the facilitation of single-cell RNA transcriptomic analysis, has enabled the inference of intercellular communications using ligand–receptor databases. In this review, we provide a comprehensive overview of the modified cell–cell communications in lung fibrosis. We highlight the intricate interactions among the major cell types within the lung and their contributions to fibrogenesis. The insights presented in this review will contribute to a better understanding of the molecular mechanisms underlying lung fibrosis and may guide future research efforts in developing targeted therapies for this debilitating disease.

Introduction

Homeostasis in the lung relies on the collaborative effort of the functions of multiple cell types. This is governed by cell–cell interactions (CCIs) between different cell types, as well as within the same cell type.[1] Consequently, investigations into both normal lung function and disease-induced alterations depend on a comprehensive understanding of the intricate interplay between cell types in the lung. Major cell types are distributed across the respiratory tract's significant segments, ranging from the conducting airways to the alveolar units, which are crucial for oxygen–CO_2 exchange.[2] These cell types can be broadly categorized into five primary groups: epithelial cells, mesenchymal cells, immune cells, endothelial cells (ECs), and the pulmonary nervous system (PNS). Here, we present an expanded list of major subcellular types within each primary cell category, along with their respective functions (Table 1). CCIs often involve reciprocal interactions between cells across primary categories or within the same category. Protein molecules, encompassing ligands, receptors, extracellular vesicles (EVs), and components of the extracellular matrix (ECM) are released by sender cells to interact with receptors on recipient cells. This thereby can trigger downstream signaling events that profoundly influence cellular functions and the recipient cell's microenvironment, ultimately impacting lung function. Understanding the activities and interactions of lung cells within a functional unit is dependent upon identifying the messages, including paracrine proteins and EVs that transverse between cells. Assessing the altered messenger molecules and their associated signaling pathways is critical to appreciate the physiological and pathological significance of CCIs in the lung. In this review, we mainly focus on recent research findings on CCIs between epithelial cell and mesenchymal cell in pulmonary fibrosis (PF).

Disruptions of lung epithelial cells and ECs occur during lung injury and fibrosis pathogenesis. In the alveoli, the injury/stress of alveolar epithelial type 2 (ATII) cells serves as an initiating event in idiopathic pulmonary fibrosis (IPF).[3] ATII cells, which produce surfactants and function as tissue stem/progenitor cells, become exhausted.[4] ATII cells lose their self-renewal function,[5] with inhibited and blocked differentiation into alveolar epithelial type 1 (ATI) cells, resulting in persistent epithelial progenitors (PEPs).[6] PEPs encompass subtypes with distinct transcriptomic expression profiles, and yet share common functional attributes. These subtypes include ATII transition cells,[7] damage-associated transient progenitors (DATPs),[8] the pre-alveolar type-1 transitional cell state (PATs),[9] and keratin 8 ($Krt8)^+$ alveolar differentiation intermediate (ADI).[10] In the airway, the process for basal cell generation of club and ciliated cells was arrested.[11] Basal cells acquired either secretory signatures or mesenchymal features,[12–14] which expanded to contribute to the honeycombing structures observed in the brochiolarization of IPF lungs.[2,15] Immune cells can be recruited and infiltrate into the injury area, but are dysfunctional when the epithelial barrier is reshaped. Macrophages are the most common immune cell type in the lung, functioning through efferocytosis of the dead, dying, and senescent cells and maintenance of the integrity and responsiveness of the epithelium.[16] Furthermore, activation and accumulation of mesenchy-

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Table 1
Major cell types that help maintain lung homeostasis and contribute to PF.

Name	Functions
Alveolar epithelial cells	
ATII	ATII cells produce surfactant to reduce surface tension, preventing alveolar collapse, protecting against pathogens, and functioning as alveolar stem/progenitor cells for self-renewal and differentiation into ATI cells to regenerate lost alveolar epithelial cells. Diminished ATII cells were found in IPF lungs.[22]
PEPs	Intermediate transitional cells between ATII cells and ATI cells, which persist in human and mouse lung fibrosis.[6–10]
ATI cells	These cells are flat and line the gas-blood interface within the alveoli, facilitating gas exchange.
Airway epithelial cells	
Club cell	These cells secrete proteins that provide protection against toxins and oxidative stress, and also function as stem cells for regenerating the airway epithelium. They contribute to bronchiolization in IPF.[23]
Ciliated cell	Contains cilia that aid in moving mucus and trapped particles out of the airways. They contribute to aberrant multiciliogenesis in IPF. [24,25]
Basal cell	These cells act as stem cells, promoting airway epithelium repair and regeneration, and are observed to increase in number during lung fibrosis.[15,26]
Goblet cell	Produces mucus to lubricate and protect the airways.
Neuroendocrine cell	Releases hormones and neuropeptides that regulate airway functions.
Ionocyte	Involved in ion transport, maintenance of electrolyte balance, and regulation of mucus viscosity in the airways.
Tuft cell	Functions as a chemosensory cell and can initiate immune responses.
SMG cells[26]	
Myoepithelial cell	Residing above the basement membrane beneath the luminal cells of the glandular epithelium, they help expel secretions from the lumina of the secretory units and ducts.
Mucous cell	Produces mucus-secreting goblet cells.
Serous cell	Secretes watery fluid to help moisten the airways.
Duct cell[27]	Modifies the serous/mucous secretions flowing into them,[27] capable of regenerating SMG tubules, ducts, and surface epithelium.[28]
Mesenchymal cells	
Fibroblast	Central mediator of ECM deposition and proliferates during lung fibrosis.
Myofibroblast	Contractile fibroblast involved in wound healing and fibrosis.
Smooth muscle cells	Smooth muscle cells encircle lung blood vessels and airways, regulating their diameter.
Lipofibroblast	Lipofibroblasts store fat droplets and aid alveolar cell function, with reduced expression in human IPF lungs.[29]
Pericyte	These cells control lung blood flow and capillary stability, exhibiting enhanced migration in IPF.[30]
Mesothelial cell	Forms the mesothelium lining the lung's pleural cavity.
Immune cells[31]	
Macrophage	The most common type of immune cells in the lungs, composed of lumen-dwelling alveolar macrophages and interstitial macrophages.
Monocyte	Replenishes macrophages.
Dendritic cells	Dendritic cells gather, process, and display antigens to immune cells, and they accumulate in IPF-afflicted lungs.[32]
Natural killer cell	NK cells, which can eliminate infected or cancerous cells, are significantly reduced in number and function in IPF lungs.[33]
Neutrophil	Neutrophils, which are infection-fighting immune cells, are suggested as an indicator for disease progression in IPF based on their ratio with lymphocytes.[34]
Eosinophil	Eosinophils, which are important in defending the body against parasites, allergens, foreign bacteria, and outside organisms, were found to be increased and active in BALF in patients with IPF, suggesting their involvement in allergic reactions in this context.[35]
Basophil	Involved in allergic reactions and play a role in the body's defense against parasites.
$CD8^+$ memory/effector T cell	Cytotoxic T cells involved in cell-mediated immune responses.
$CD8^+$ naive T cell	Resting cytotoxic T cells awaiting activation.
Natural killer T cell	Specialized T cells that bridge the innate and adaptive immune responses.
T_{helper} cell	$CD4^+$ T cells that play a central role in orchestrating and regulating immune responses against infections, allergens, and other challenges.
T_{reg} cell	Plays a critical role in maintaining immune balance, preventing excessive inflammation, and promoting immune tolerance.
B cell	Produces antibodies and contributes to humoral immune responses.
Plasma cell	Plasma cells are specialized B cells that produce a lot of antibodies, and in mice, depleting these cells with bortezomib was found to lower bleomycin-induced lung fibrosis.[36]
Endothelium	
Artery cell	ECs lining the arteries in the lung.
Vein cell	ECs lining the veins in the lung.
Capillary cell	Capillary cells, which are typically a type of EC forming the smallest blood vessels, can indirectly contribute to IPF by participating in the microenvironment of the lung tissue.
Bronchial vessel	ECs in the bronchial vessels supplying blood to the lung.
Lymphatic cell	ECs forming lymphatic vessels involved in immune function.
PNS	
Intrinsic neuron	Neurons present within the lung tissue involved in regulating airway function.
Glial cell	Supports and protects neurons in the PNS.

ATI: Alveolar epithelial type 1; ATII: Alveolar epithelial type 2; CD: Cluster of differentiation; BALF: Bronchoalveolar lavage fluid; ECM: Extracellular matrix; EC: Endothelial cell; IPF: Idiopathic pulmonary fibrosis; PEPs: Persistent epithelial progenitors; PF: Pulmonary fibrosis; PNS: Pulmonary nervous system; SMG: Submucosal gland.

mal cells leads to excessive release of ECM, promoting fibrosis pathogenesis.[17] The cellular contribution to PF largely depends on single-cell functions and interactions between cells in the lung.[18]

Major lung cell types that are important in PF

The complex interplay among the diverse lung cell types and intricate processes they engage in plays a pivotal role in gas exchange efficiency, immune response modulation, tissue repair mechanisms, and the precise regulation of airway function. Understanding this is especially significant in the context of lung fibrosis, as it sheds light on the underlying mechanisms driving this condition and offers insights into potential avenues for therapeutic intervention. We briefly summarized the cell types within the lung, noting that some have been previously implicated in PF, while others may possess altered functions in PF, warranting further investigation (Fig. 1). To facilitate comprehension, we have categorized them into different functional groups based on their specific roles and characteristics.[19–21] For these cell types, we empha-

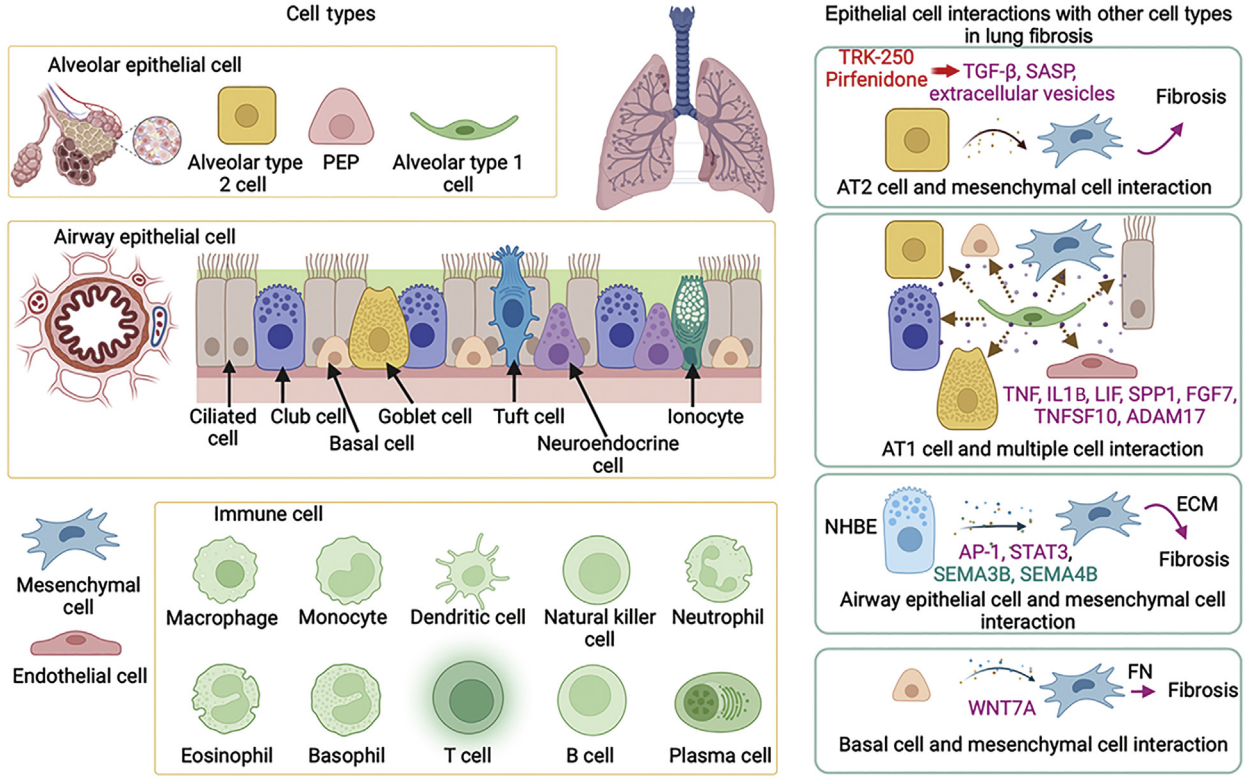

Fig. 1. Overview of the major cell types in lung homeostasis that potentially participate in lung fibrosis. Major cell types in the lung include alveolar epithelial cells (alveolar type 2 cells, PEPs, alveolar type 1 cells), airway epithelial cells (ciliated cells, club cells, basal cells, goblet cells, tuft cells, neuroendocrine cells, ionocytes), mesenchymal cells, ECs, and immune cells (macrophages, monocytes, dendritic cells, natural killer cells, neutrophils, eosinophils, basophils, T cells, B cells, plasma cells). CCIs between epithelial cells and other cell types in lung fibrosis: ATII cells release substances like TGF-β, SASP, and EVs that can stimulate mesenchymal cells and contribute to fibrosis. TRK-250 and pirfenidone, which target TGF-β, were tested in clinical trials. Single-cell RNA sequencing revealed that ATI cells can communicate with various other cell types by sending signals, such as TNF, IL1B, LIF, SPP1, FGF7, TNFSF10, and ADAM17. NHBE can trigger interactions with mesenchymal cells by using signals, like AP-1, STAT3, SEMA3B, and SEMA4B, which lead to increased ECM production and can promote fibrosis. Basal cells secrete WNT7A, which triggers mesenchymal cells to produce FN and promote fibrosis. ADAM17: A disintegrin and metalloproteinase 17; AP-1: Activator protein-1; ATI: Alveolar epithelial type 1 cells; ATII: Alveolar epithelial type 2 cells; CCIs: Cell-cell interactions; EC: Endothelial cell; ECM: Extracellular matrix; EVs: Extracellular vesicles; FGF7: Fibroblast growth factor 7; FN: Fibronectin; IL1B; Interleukin 1β; LIF: Leukemia inhibotry factor; NHBE: Normal human bronchiolar epithelial cells; PEPs: Persistent epithelial progenitors; SASP: Senescence-associated secretory phenotype: SEMA3B: Semaphorin 3B; SEMA4B: Semaphorin 4B; SPP1: Secreted phosphoprotein 1; STAT: Signal transducer and activator of transcription; TGF-β: Transforming growth factor-β; TNF: Tumor necrosis factor; TNFSF10: TNF superfamily member 10; WNT7A: Wnt family member 7A.

size their roles and contributions to PF, which are summarized in Table 1.

Epithelial cell interactions with other cell types in lung fibrosis

ATII cell-induced interactions with mesenchymal cells

The relationship between ATII cells and mesenchymal cells is crucial for maintaining lung homeostasis. ATII cells have a multifaceted role, acting as both surfactant protein secretors that are crucial for lubricating alveolar epithelial surfaces,[37] as well as stem/progenitor cells responsible for regenerating the alveolar epithelium (Fig. 1). However, ATII cells can falter when exposed to stressors that induce and support a fibrotic environment. This can disrupt their functionality, leading to their segregation, loss of self-renewal capability, hindered differentiation into ATI cells, and entrapment at the PEP stage.[7–10,12,38] This array of conditions effectively undermines their typical interactions with the supportive mesenchymal cells that encompass them.

Mesenchymal cells are able to sense ATII cell dysfunction through various factors secreted by ATII cells, including transforming growth factor-β (TGF-β),[39–41] senescence-associated secretory phenotype (SASP),[41,42] and EVs.[43,44] These signals can alert the mesenchymal cells, triggering their activation to assist in the situation. However, the outcome can be counterproductive in the context of disease. In their efforts to remedy ATII cell dysfunction, activated mesenchymal cells can generate additional ECM, inadvertently contributing to the complexity of fibrosis. This is particularly pronounced in aging conditions,[4] where the delicate balance becomes even more intricate. When both ATII and mesenchymal cells become senescent,[41] a delicate balance develops and can be disrupted by factors like viruses, smoking, or autoimmune conditions, potentially leading to more severe issues in IPF patients.

Understanding these interactions will therefore shed light on the delicate harmony that governs lung health. Exploring these intricate cellular relationships involves investigating the interdependence of ATII and mesenchymal cells, their responses to stressors, and the potential consequences.

ATI cell communication with other cell types

The alveoli of the lung are lined with thin ATI cells that cover over 90% of the surface area of the lung.[45] Utilizing publicly available single-cell RNA sequencing (RNA-seq) datasets, researchers combined the data and analyzed them to identify potential ligands involved in ATI cell interactions with other cell types during gas exchange in fibrotic lungs.[46] Two Gene Expression Omnibus (GEO) datasets, GSE135893[14] and GSE161685,[47] were examined, which revealed a distinctive pattern of ATI cell loss in IPF. This resulted in dysfunctional cell–cell communication signaling within the alveolar microenvironment. The R software

platform NicheNet algorithm was used as a bioinformatics approach to infer the cell–cell communication between ATI cells and other cell types within the alveolar microenvironment. ATI cells were examined as a receiver cell type, while sender cell types included ATI cells themselves, as well as ATII cells, basal cells, ciliated cells, club cells, goblet cells, ECs, fibroblasts, macrophages, lymphocytes, and mast cells. This analysis revealed that tumor necrosis factor (TNF), interleukin 1β (IL1B), leukemia inhibotry factor (LIF), secreted phosphoprotein 1 (SPP1), fibroblast growth factor 7 (FGF7), and TNF superfamily member 10 (TNFSF10) are important ligands on ATI cells for communication with other cell types in IPF. The a disintegrin and metalloproteinase 17 (ADAM17) ligand from macrophages, ATI cells, and ATII cells was enriched in the IPF single nuclear RNA-seq dataset, indicating that ADAM17 potentially functions in the communication between these cells.[46]

PEP cell-induced interactions with other cell types

ScRNA-seq analysis showed that Krt8$^+$ ADI cells have the largest number of receptor–ligand pairs with fibroblasts, macrophages, and general capillary (gCap) ECs.[10] Specifically, the capillary ECs received signals via the endothelin receptor (endothelin receptor type B, Ednrb) expressed by ECs upon binding to the endothelin 1 (Edn1) ligand secreted by Krt8$^+$ ADI cells. However, this mechanism has not yet been confirmed by further *in vitro* or *in vivo* experiments.[10] The interactions between Krt8$^+$ ADI cells with mesenchymal cells and macrophages are potentially through connective tissue growth factor (Ctgf), integrin subunit beta 6 (Itgb6), amphiregulin (Areg), heparin-binding EGF-like growth factor (Hbegf), endothelin 1 (Edn1), and galectin 3 (Lgals3), all of which are antifibrotic targets that have been tested in pre-clinical and clinical studies.[10] The expression patterns of Areg and Hbegf, as well as the integrin Itgb6, on Krt8$^+$ ADI cells were validated by flow cytometry and immunostaining. However, the interactions between Krt8$^+$ ADI cells and mesenchymal cells or macrophages warrant further confirmation.

Airway epithelial cell-induced interactions with mesenchymal cells

In the complex lung system, the airway epithelial cells form a vital layer atop the airway mesenchymal cells. Their interactions become especially intriguing in the context of fibrosis, where airway epithelial cells are believed to contribute to bronchiolized airspace formation in IPF.[48] In this scenario, the mesenchymal cells help synthesize components of the ECM, playing a role in remodeling of the lung architecture often seen in IPF patients.[49]

An *in vitro* model has been developed where airway epithelial cells and fibroblasts are cultured in isolation.[48] Notably, the presence of normal human bronchiolar epithelial (NHBE) cells was found to initiate an inflammatory response within primary normal human lung fibroblasts (NHLFs) merely 3 hours after co-culture.[50] This was followed by upregulated expression levels of key genes from the nuclear factor-κB (NF-κB) family, activator protein-1 (AP-1), and signal transducer and activator of transcription 3 (STAT3), supporting the subsequent activation of mesenchymal cells through TGF-β signaling and their synthesis of essential ECM components.

An interactome-based analysis of scRNA-seq data has highlighted a range of potential ligand–receptor (LR) interactions,[50] suggesting that the effects of NHBE cells on NHLFs might involve signaling mediated by semaphorins through plexin receptors. Intriguingly, increased expression levels of semaphorin 3A (SEMA3A), semaphorin 3B (SEMA3B), and semaphorin 4B (SEMA4B) in co-cultured NHBE cells could engage with their corresponding plexin receptors or neuropilin co-receptors, such as neuropilin 2 (NRP2), expressed by co-cultured NHLFs.[50]

This research offers insights into how early changes in airway epithelial cells can initiate inflammation in mesenchymal cells.[50] Recent scRNA-seq LR analysis suggested the involvement of epithelial cell-driven signaling, with multiple growth factors, cytokines, and chemokines transducing these signals through integrins, Wingless (Wnt) co-receptors, and other pathways.[14] The unfolding signaling interactions shown in the results demonstrate the complexity that is still only partially understood.

Basal cell-induced interactions with mesenchymal cells

Among the complexities of lung dynamics, the interplay between basal cells and mesenchymal cells draws our attention. Basal cells serve as pivotal stem/progenitor cells, nurturing the airway epithelium in the normal lung.[51] When injury occurs, they help replenish various airway epithelial cells, including the ciliated cells and secretory goblet cells. Because of this remarkable role, basal cells contribute to about 30% of the upper airway and tracheal epithelial cell population. However, when moving distally, their presence gradually tapers to a mere 6%, sometimes observed as clusters or even solitary entities.[52,53]

There is a transformative shift in fibrotic lungs, with basal cells undergoing a significant increase in number.[54] Yet, their exact location and functions in fibrosis remain shrouded in ambiguity. Notably, apart from their recognized features of hyperplasia and metaplasia across various disease contexts,[55] recent research has indicated that basal cells have secretory characteristics that contribute to mesenchymal cell activation in the context of lung fibrosis.[54] Intriguingly, canonical Wnt signaling is central to the orchestration of basal cell activities post-damage. This signaling pathway induces cell proliferation and guides their differentiation into either ciliated or secretory cells.[56,57] Further revelations have suggested a distinct role of basal cells in activating mesenchymal cells to release fibronectin (FN).[16] ScRNA-seq analysis identified *WNT7A* as a highly and specifically expressed gene in basal cells. Subsequent *in vitro* experiments involving neutralizing antibodies against *WNT7A* or a small molecule inhibitor targeting Frizzled signaling demonstrated effective inhibition of basal cell-induced fibroblast activation, as evidenced by reduced FN secretion.

As we explore the intricate mechanisms governing basal cells, pivotal questions emerge. What specifically triggers their expansion under different conditions and its potential link to inflammatory signals are points of interest. For example, the enrichment of IL-33 levels in basal cells post-virus infection raises intriguing possibilities.[58] Additionally, how basal cells can navigate to bronchiolization sites to interact with diverse cell types in fibrosis as well as how they are regulated to establish connections with mesenchymal cells has been questioned. Basal cell heterogeneity has become clearer in the context of both homeostasis in mouse lungs[59] and the complex realm of cystic fibrotic human lungs.[13] This indicates the potential richness of their interactions with other cell types, an area ripe for further exploration. Notably, a subset of basal cells, identified as cluster 2,[59] seems particularly intriguing. Found in normal Trp63 lineage-traced mouse lungs, this cluster boasts an enrichment in genes associated with the hedgehog/TGF-β/Wnt pathways crucial for squamous differentiation. This raises intriguing ideas about the transformative potential of basal cells, including their contribution to lung squamous cell carcinoma.

Understanding the multifaceted role of basal cells and their intricate interactions with various cellular players, especially mesenchymal cells, is a complex journey filled with many unanswered questions. As we uncover additional details, we gain a deeper appreciation for the intricate balance that sustains lung health.

Mesenchymal cell interactions with other cell types in lung fibrosis

Mesenchymal cell-induced interactions with alveolar epithelial cells

In a state of homeostasis, mesenchymal cells support the epithelium by both forming an infrastructure and through soluble proteins and EVs (Fig. 2).[60] These EVs contain crucial messenger RNAs (mRNAs) that are translated into functional proteins after being taken up by the epithelial

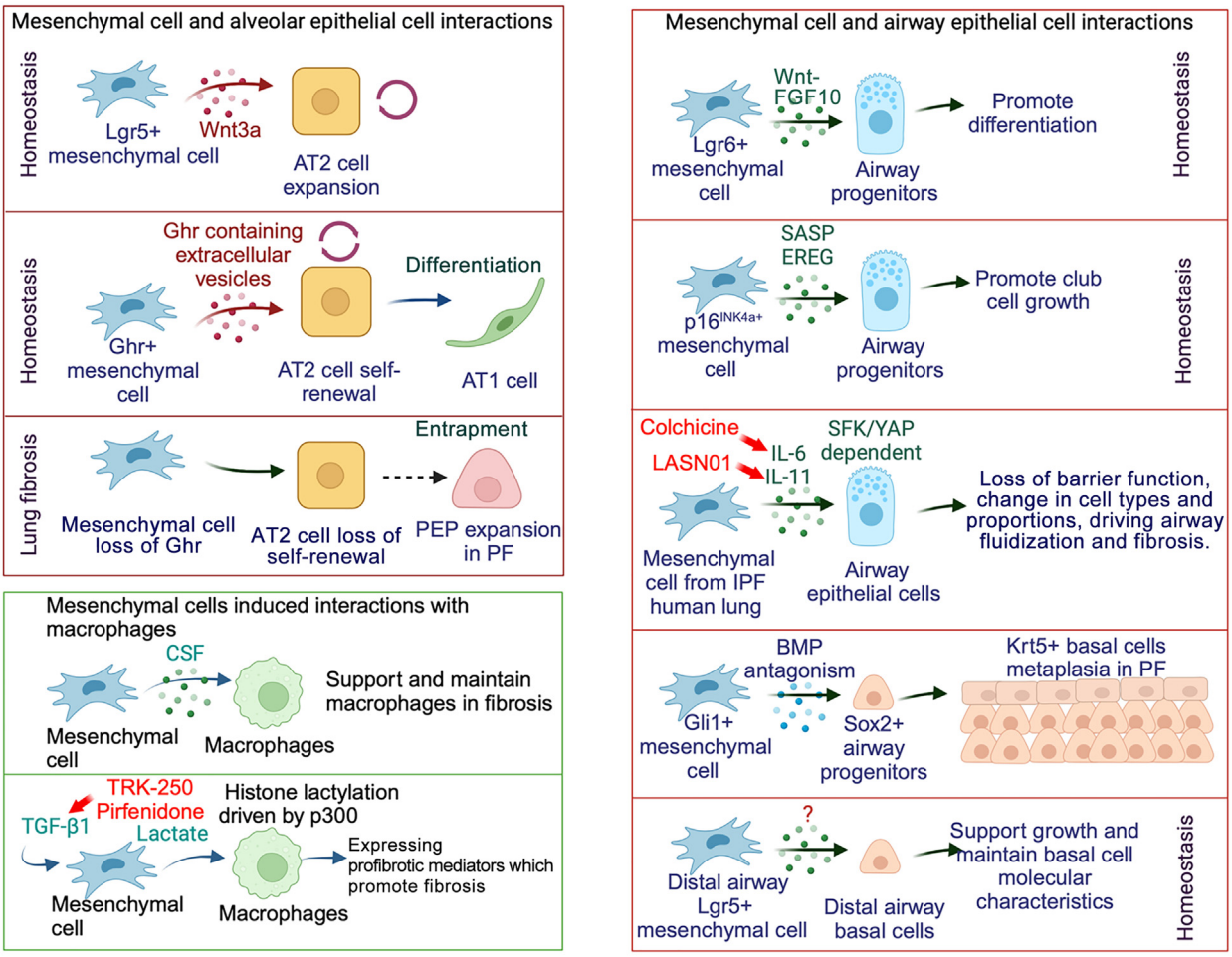

Fig. 2. CCIs between mesenchymal cells and other cell types in lung homeostasis and fibrosis. Lgr5$^+$ mesenchymal cells can promote ATII cell expansion through Wnt3a. In lung fibrosis, the loss of Ghr in mesenchymal cells hinders the self-renewal of ATII cells, potentially leading to their entrapment in the PEP stage rather than differentiation into mature ATI cells. Mesenchymal cells secrete CSF to support and maintain accumulated macrophages in PF. TGF-β1 triggers mesenchymal cells to produce lactate, leading to histone lactylation in macrophages through p300, resulting in increased expression of pro-fibrotic mediators and promoting fibrosis. Lgr6$^+$ mesenchymal cells can stimulate the differentiation of airway epithelial progenitor cells via the Wnt-FGF10 signaling pathway. P16^{INK4a+} mesenchymal cells induce airway progenitors to differentiate into club cells through SASP and EREG. IPF lung mesenchymal cells release IL-6 and IL-11 to disrupt airway epithelial cells, which can cause barrier function loss, cell type changes, altered proportions, and lead to airway fluidization in lung fibrosis. Colchicine, which targets IL-6, and LASN01, which targets IL-11, were evaluated in clinical trials. Gli1$^+$ mesenchymal cells promote Krt5$^+$ basal cell metaplasia from Sox2$^+$ airway progenitors through BMP antagonism in PF. Lgr5$^+$ mesenchymal cells in the distal airway support basal cell growth and help preserve their unique molecular traits. ATI: Alveolar epithelial type 1 cells; ATII: Alveolar epithelial type 2 cells; BMP: Bone morphogenetic protein; CCIs: Cell-cell interactions; CSF: Colony-stimulating factor; EREG: Epiregulin; FGF10: Fibroblast Growth Factor 10; Ghr: Growth hormone receptor; Gli1: GLI family zinc finger 1; IL: Interleukin; IPF: Idiopathic pulmonary fibrosis; Krt5: Keratin 5; Lgr5: Leucine-rich repeat-containing G-protein coupled receptor 5; Lgr6: Leucine-rich repeat-containing G-protein coupled receptor 6; PEP: Persistent epithelial progenitors; PF: Pulmonary fibrosis; SFK: Src family kinase; Sox2: SRY-box transcription factor 2; TGF-β: Transforming growth factor-β; SASP: Senescence-associated secretory phenotype; Wnt3a: Wnt family member 3a; YAP: Yes-associated protein.

cells. This exchange aids in the growth, self-renewal, and differentiation of alveolar ATII cells. In addition to other studies,[61] work by Lee et al[62] highlights the distinct presence of leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5)$^+$ mesenchymal cells in alveolar compartments, which are capable of activating Wnt signaling to promote alveolar differentiation of epithelial progenitors. Further insights from Xie et al[60] emphasize the supportive signals that ATII cells receive from their surrounding mesenchymal counterparts. A key player, the growth hormone receptor (GHR), was found to be predominantly expressed in mesenchymal cells, which were diminished in IPF lungs. Higher GHR expression levels correlated with improved lung function in IPF patients. Importantly, profibrotic mesenchymal cells exhibited an inhibitory effect on ATII cell growth and were associated with suppressed vesicular GHR expression. Interestingly, EVs enriched with Ghr facilitated ATII cell proliferation and mitigated PF in mesenchymal Ghr-deficient mice. This discovery underscores a previously unexpected mesenchymal paracrine signaling mechanism coordinated by GHR that is instrumental in supporting ATII progenitor cell renewal while curbing lung fibrosis severity.

The exact ways in which mesenchymal cells can influence the function of epithelial progenitors to promote fibrosis are not fully understood. In fibrotic lungs, the transition from ATII cells to ATI cells involves an intermediate stage called PEPs. However, the specific control mechanisms via mesenchymal cells and the conditions that enable ATI cell differentiation are unclear.[7–10,63]

The evolving interactions between mesenchymal and alveolar epithelial cells in the lung highlight the complex nature of lung biology. ScRNA-seq analysis has suggested that matrix-driven signaling through integrin receptors is the central mechanism through which mesenchymal lineages interact with epithelial cells in PF lungs.[14] These revelations, in their continuous refinement, underscore the intricate web that orchestrates human respiratory equilibrium.

Mesenchymal cell-induced interactions with airway epithelial stem/progenitor cells

Within the adult mouse lung dynamics, mesenchymal cells and airway epithelial stem/progenitor cells interact to support airway regeneration.[62] Specifically, the subset of Lgr6$^+$ mesenchymal cells, situated within the smooth muscle cell population encircling the airway epithelium, has a pivotal role. Through a cooperative Wnt–FGF10 mechanism, Lgr6$^+$ cells enhance the differentiation of airway epithelial progenitors, contributing to the airway's regenerative capacity. The importance of these cells is underscored by observations that the loss of Lgr6$^+$ cells can impede efficient airway injury repair.[62]

Another study, which involved the co-culturing of primary human lung fibroblasts (HLFs) from IPF lungs with an air-liquid interface culture of airway epithelial cells,[65] revealed that IPF HLFs produced increased levels of IL-6 and IL-11 compared with the controls. IPF HLF co-culture induced a diminished barrier function, change in cell types and proportions, and persistent fluidization in the airway epithelial cells. The study further found that IL-6 produced by mesenchymal cells is sufficient to induce epithelial dysfunction.

Similarly, research by Reyes et al[64] has shed light on the dynamic interplay between p16^{INK4a+} fibroblasts and airway stem cells. Following epithelial injury, p16^{INK4a+} fibroblasts exhibit an increased SASP. In 3D co-culture experiments, these fibroblasts enhanced the number and growth of airway stem cell organoids, particularly post-injury. Human experimentation further corroborated the pivotal role of p16^{INK4a+} fibroblasts in bolstering airway stem cell growth. Upregulated expression of epiregulin (EREG), a pivotal growth factor encoded by Ereg, in post-injury p16^{INK4a+} fibroblasts underscores its role in facilitating club cell growth. Experiments involving fibroblasts with Ereg being knocked out validated its significance, revealing challenges in secretoglobin family 1a member 1 (SCGB1A1)$^+$ cell regeneration post-injury. Furthermore, *in vivo* studies supplemented by senolytics accentuated the contribution of p16^{INK4a+} fibroblasts to epithelial cell regeneration.

Mesenchymal cell-induced interactions with Krt5$^+$ basal cells

Recognized for their role in contributing to myofibroblasts during scarring,[66] Gli1$^+$ mesenchymal stromal cells (MSCs) wield substantial influence over the fate of airway Sox2$^+$ progenitors, specifically guiding their differentiation into KRT5$^+$ basal cells and facilitating a metaplastic transformation.[67] Through hedgehog activation signaling during fibrotic repair, Gli1$^+$ MSCs can modulate the environment for airway progenitors by augmenting bone morphogenetic protein (BMP) antagonism (gremlin 2 [Grem2], follistatin [Fst], Wnt5a, and follistatin like 3 [Fstl3]). This modulation fosters metaplasia, shaping the regenerative milieu within the fibrotic context. By restoring the balance of BMP activation, the metaplastic differentiation of KRT5$^+$ basal cells is curtailed, paving the way for adaptive alveolar differentiation into an surfactant protein C (SFTPC)$^+$ epithelium. Notably, fibrotic human lungs exhibit perturbed BMP activation within the metaplastic epithelium, highlighting the pathological relevance of this regulatory mechanism.

In another study,[68] researchers found that co-culture of distal basal cells with LGR5$^+$ fibroblasts alone was sufficient to support distal basal cell growth and maintain their molecular characteristics, suggesting that LGR5$^+$ fibroblasts serve as a niche for distal airway basal cells. However, how LGR5$^+$ fibroblasts specifically support distal basal cells, including through certain signaling pathways, warrants further investigation.

Mesenchymal cell-induced interactions with macrophages

Through secreted factors, activated fibroblasts can recruit monocytes or macrophages to these sites, contributing to the fibrotic response.[69] Myofibroblasts have been shown to produce colony-stimulating factor (CSF) to support and maintain the population of recruited macrophages in areas of fibrosis or injury, with healing, cold, and hot fibrosis potentially depending on the number of macrophages that are present.[70] Furthermore, activation of Yes-associated protein 1 (YAP1) in fibroblasts can elevate colony stimulating factor 1 (Csf1) expression levels and induce macrophage recruitment.[71]

In addition to secreted proteins, metabolites can participate in mesenchymal cell–macrophage intercellular communication during lung fibrosis.[72] With increased glycolysis, myofibroblasts can indirectly impact alveolar macrophages by releasing lactate. Lactate levels are elevated in the conditional media of lung myofibroblasts induced by TGF-β1 and in the bronchoalveolar lavage (BAL) fluids of mice with TGF-β1- or bleomycin-induced lung fibrosis. The lactate-rich environment promotes the expression of profibrotic mediators in macrophages. Mechanistically, lactate triggers histone lactylation in the promoters of profibrotic genes within macrophages, a modification seen in fibrotic lungs. This effect is driven by p300, evident from reduced levels in macrophages with p300 knockdown. These findings suggest that myofibroblast glycolysis can facilitate their interactions with macrophages and contribute to lung fibrosis pathogenesis.

Mesenchymal cell heterogeneity is involved in ECM component production and interactions with other cell types

Mesenchymal cell heterogeneity was first discovered when researchers performed clonal lineage analysis of mesenchymal progenitors expressing the early lung mesenchymal marker T-box transcription factor 4 (Tbx4).[73,74] The progeny of Tbx4$^+$ cells include multiple mesenchymal lineages, such as smooth muscle cells, myofibroblasts, fibroblasts, pericytes, lipofibroblasts, and mesothelial cells. Furthermore, using scRNA-seq, multiple studies have confirmed the heterogeneity of mesenchymal cells in both human and mouse lungs at the transcriptomic level.[75–78] Specific fibrosis-associated fibroblast subpopulations, including platelet-derived growth factor receptor beta (Pdgfrb) high,[75] hyaluronan synthase 1 (HAS1) high,[14] and collagen triple helix repeat containing 1 (Cthrc1)$^+$[78] fibroblasts, were observed. Myofibroblasts, lipofibroblasts, and fibrosis-associated fibroblast subpopulations are all important fibroblast subtypes that contribute to matrix deposition, as they highly express ECM genes and produce elevated levels of ECM components.[79] ScRNA-seq analysis has provided insights into mesenchymal cell subtype interactions with other cell types. For example, collagen type XIV alpha 1 chain (Col14a1)$^+$ fibroblasts were predicted to associate with gCap through collagen type I alpha 1 chain (Col1a1)–integrin subunit alpha 3 (Itga3) and Col1a2–Itga3 interactions.[79] More research is needed to investigate how different mesenchymal cell subtypes interact with other cell types at the molecular level.

Antifibrotic therapy drugs targeting CCIs in clinical trials

Several antifibrotic therapy drugs targeting various cytokines and growth factors involved in CCIs, as discussed in this review, are currently undergoing evaluation in clinical trials. We summarize these drugs in Table 2.

Computational algorithmic approaches unraveling CCIs in lung fibrosis

Pairing computational algorithms with experimental investigations has proven to be a potent strategy for elucidating the complex landscape of lung fibrosis. These computational methodologies offer researchers quantitative insights and predictive analyses that augment traditional experimental approaches. Particularly, with the advent of scRNA-seq, advanced algorithms have emerged to examine intricate CCIs, which has helped to predict their occurrence across spatial and temporal scales.

Several computational tools have stood out for deciphering these interactions and LR bindings, including the following:

Table 2
Relevant drugs that have been tested in clinical trials for antifibrotic therapy.

Target	Drug ID	Disease	Phase	NCT identifier	Status	Enrollment
IL-11	LASN01	IPF or PF-ILD or TED	Phase 1	NCT05331300	Recruiting	80
IL-6	Colchicine	COVID-19 and the development of post-COVID-19 PF	Phase 4	NCT04818489	Active, not recruiting	260
TGF-β1	TRK-250	IPF	Phase 1	NCT03727802	Completed	34
TGF-β1	Pirfenidone	Coal workers' pneumoconiosis (black lung) with PF	Phase 2	NCT04461587	Completed	50

COVID-19: Coronavirus disease 2019; IL: Interleukin; IPF: Idiopathic pulmonary fibrosis; PF: Pulmonary fibrosis; PF-ILD: Progressive fibrosing interstitial lung disease; TGF-β1: Transforming growth factor-β1; TED: Thyroid eye disease.

NicheNetR[80]: This method uses an inferential model supported by prior knowledge of how ligands can influence receiver cell gene expression patterns. NicheNetR selects from potential ligands based on the receptor expression levels in receiver cells. This approach has been used to analyze single-cell and single-nuclear datasets, revealing cell–cell communication in IPF lungs. For example, LR pairs were determined by analyzing ATI cell receptor expression and curated interaction evidence. Macrophages, fibroblasts, myofibroblasts, ECs, and alveolar epithelial cells are the likely sources of ligand production for signaling to ATI cells, which are altered in IPF tissues.[46] Using the NicheNet R package to construct an interaction map, the IPF distinct macrophage cluster, IPFeM$\surd$, displayed the unique interactions with cells in the fibrotic niche. IPFeM$\surd$ acted as sender cells, while myofibroblasts, vascular ECs, and aberrant basaloid epithelial cells were identified as receiver cells. This analysis uncovered specific ligand–receptor interactions and intracellular targets, including transforming growth factor beta 1 (TGFB1), TNF superfamily member 13b (TNFSF13B), SPP1, glycoprotein Nmb (GPNMB), and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta (PIK3CB), with implications for fibrotic signaling.[81] NicheNet was also used with spatial genomics data generated by utilizing the Nanostring GeoMx platform.[82] This study showcases the dynamic interplay between adjacent alveolar septae and immune cell infiltrates via factors such as bone morphogenetic protein 4 (BMP4), C-C motif chemokine ligand 2 (CCL2), cluster of differentiation 24 (CD24), hepatocyte growth factor (HGF), SPP1, plasminogen activator, urokinase (PLAU), TGF-β1, high mobility group box 1 (HMGB1), and others.

CellChat: CellChat[83] offers a comprehensive labeling function that includes approximately 2000 LR interactions, about 48% of which represent interactions of heterodimers. Its interactome analysis, combined with scRNA-seq, has been used to compare the control, IPF lung explants, and lung fibrosis associated with post-acute SARS-CoV-2 infection (PASC) datasets. This study created an inferred communication network of aggregated TGF-β signals that revealed how macrophage-driven TGF-β expression can target transitional ATII cells, promote fibrosis by disrupting ATII-to-ATI cell differentiation, and foster the accumulation of profibrotic transitional cells.[84] Using CellChat, researchers employed scRNA-seq data and LR expression to identify interacting cell types, revealing an increase in cell–cell communication after ionizing radiation (IR), particularly at higher doses (17 Gy *vs.* 10 Gy). This analysis highlighted the enhanced interactions between mesenchymal cells and ECs over time, particularly at later time points, supporting the role of collagen pathways in connecting mesenchymal cells to gCap during radiation induced pulmonary fibrosis (RIPF) development.[79]

CellPhoneDB[85]: This database houses around 900 receptor-ligand pairs, offering a systematic examination of interactions between various cell types. Using this database, researchers have revealed a complex CCI network predicting the roles of EC subpopulations in recruiting monocytes, promoting fibroblast proliferation, and shaping the ECM in bleomycin-induced lung injury. This study identified specific LR pairs, including those involving transforming growth factor beta-3 (TGFB3)_transforming growth factor beta receptor III (TGFBR3), neuropilin-1 (NRP1)_vascular endothelial growth factor A (VEGFA), AXL receptor tyrosine kinase (AXL)_growth arrest-specific gene 6 (GAS6), and collagen type IV alpha 5 chain (COL4A5)_integrin subunit alpha 1 (a1b1) complexes, with increased interactions in bleomycin-treated lungs.[86]

TALK[14]: Using the iTALK interactome-based analysis, researchers identified potential LR binding pairs in epithelial and mesenchymal cells, constructing interaction networks for mesenchymal-driven and epithelial-driven signaling in PF. This analysis found matrix-driven signaling via integrin receptors to be a central mechanism for fibroblast–epithelial cell interactions in PF, while epithelial-driven signaling involved a more intricate network that incorporated growth factors, cytokines, and chemokines through integrins, Wnt co-receptors, and other pathways.[14]

As these computational approaches continue to advance, they will play a pivotal role in uncovering the intricate web of cellular interactions that underlie lung fibrosis, offering valuable insights for potential therapeutic intervention.

Challenges and future directions

Lung fibrosis is a significant global healthcare burden, presenting formidable challenges for both patients and healthcare providers alike. To address these pressing issues, it is imperative to identify key therapeutic targets that hold high relevance to human fibrotic disease. Subsequently, the development of effective antifibrotic therapies targeting these specific targets must become a focal point in the future directions of research and medical intervention.

The intricate cellular interactions and communication dynamics that underlie lung fibrosis pose a compelling and complex challenge. Understanding this web of interactions is crucial for devising effective treatment methods. However, one of the major obstacles facing this goal involves the experimental validation of bioinformatics analysis results. Translating *in silico* findings into tangible molecular insights requires meticulous and rigorous experimental scrutiny.

To surmount this challenge, an innovative approach should be used that integrates scRNA-seq data from not only humans, but also from other mammalian species.[87] This would allow for cross-species comparisons that will help us identify conserved mechanisms, and also pinpoint species-specific nuances in the fibrotic processes.[75,88] Such cross-species insights would provide invaluable guidance in the quest for effective treatments.

Furthermore, the integration of spatial genomics data holds great promise for elucidating the spatial organization of different cell types within fibrotic lung tissue.[27] Understanding the spatial dimension of cellular interactions is paramount, as it can reveal critical insights into how resident lung cells interact with circulating cells, the tissue matrix, and the microbiome, which together can contribute to the development and progression of lung fibrosis.

It is important to recognize that the interrelationships between various cell types and the influence of matrix components on cellular behaviors are dynamic and constantly evolve throughout the course of fibrosis progression. The cell-specific LR pairs and their downstream molecular effectors and targets should also be defined in both *in vitro* and *in vivo* experimental systems. It therefore becomes imperative to develop innovative methodologies capable of capturing these real-time interactions. These methodologies must also allow for a thorough examination of the intricate influence of matrix components on cellular behaviors. Additionally, the use of novel tools, such as organoid and precision-cut lung slice cultures, humanized injury models, and pluripotent stem cell-

derived cell lines, can help reconcile these concepts and paradigms in preclinical studies.

As the fibrosis research field continues to advance, a comprehensive understanding of these intricate molecular dialogs will undoubtedly pave the way for the development of novel therapeutic intervention methods. These approaches will be designed to precisely target specific cell interactions and communication pathways within the context of lung fibrosis. Overall, the future is promising for precision medicine approaches that are tailored to address the unique challenges posed by this debilitating condition, offering hope and improved outcomes for patients worldwide.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT to check the grammar and make the manuscript read better. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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Declaration of competing 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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