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Epithelial stem cells and niches in lung alveolar regeneration and diseases

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Abstract

Alveoli serve as the functional units of the lungs, responsible for the critical task of blood–gas exchange. Comprising type I (AT1) and type II (AT2) cells, the alveolar epithelium is continuously subject to external aggressors like pathogens and airborne particles. As such, preserving lung function requires both the homeostatic renewal and reparative regeneration of this epithelial layer. Dysfunctions in these processes contribute to various lung diseases. Recent research has pinpointed specific cell subgroups that act as potential stem or progenitor cells for the alveolar epithelium during both homeostasis and regeneration. Additionally, endothelial cells, fibroblasts, and immune cells synergistically establish a nurturing microenvironment—or "niche"—that modulates these epithelial stem cells. This review aims to consolidate the latest findings on the identities of these stem cells and the components of their niche, as well as the molecular mechanisms that govern them. Additionally, this article highlights diseases that arise due to perturbations in stem cell–niche interactions. We also discuss recent technical innovations that have catalyzed these discoveries. Specifically, this review underscores the heterogeneity, plasticity, and dynamic regulation of these stem cell–niche systems. It is our aspiration that a deeper understanding of the fundamental cellular and molecular mechanisms underlying alveolar homeostasis and regeneration will open avenues for identifying novel therapeutic targets for conditions such as chronic obstructive pulmonary disease (COPD), fibrosis, coronavirus disease 2019 (COVID-19), and lung cancer.

Keywords

Alveoli; Epithelium; Type II cell (AT2); Stem cell; Niche

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Introduction

The adult respiratory system comprises proximal airways—trachea, bronchi, and bronchioles, and distal alveoli. While the airways serve as conduits for air transport, the alveoli facilitate gas exchange between blood and air.[1,2] Lung alveoli contain alveolar type I cells (AT1) and alveolar type II cells (AT2), responsible for gas exchange and surfactant secretion, respectively.[3] These epithelial cells are adjacent to several types of mesenchymal cells such as fibroblasts, immune cells, and endothelial cells (ECs).

Lung epithelium faces ongoing exposure to environmental particles and toxins, necessitating robust maintenance mechanisms. Resident stem cells and epithelial–stromal cell crosstalk within an appropriate extracellular matrix (ECM) ensure proper epithelial integrity. AT2 cells, in particular, are central to alveolar repair. Dysregulation in alveolar homeostasis maintenance or repair can lead to pathologies like fibrosis[4] and chronic obstructive pulmonary disease (COPD).[5]

Recent technological innovations have enhanced our understanding of alveolar repair mechanisms. This review focuses on the reparative roles of alveolar progenitor cells, particularly AT2 cells. We explore the heterogeneity and plasticity of AT2 sub-populations and delve into the signaling pathways governing their maintenance and reparative functions. Additionally, we examine the interactions between AT2 cells and their microenvironmental niches and other cells involved in alveolar repair. Finally, we highlight recent advances in research methods and the implications for pathological conditions involving impaired lung repair.

Anatomical structure and cell types in airway and alveoli

The respiratory system can be categorized into two functional areas: proximal airways for air conduction and distal lungs for gas exchange. The proximal airways initiate from tracheal and bronchus, and branch into smaller airway called bronchioles.[1,2] The airway epithelium comprises basal cells, which function as stem cells, goblet cells for mucus secretion, and ciliated cells that move mucus and particulates.[3] Further distally, secretory club cells are present.[3] Species differences exist between human and mouse airways. In mice, bronchioalveolar duct junctions (BADJ) connect airways to alveoli and host a small population of multipotent progenitor cells named bronchioalveolar stem cells (BASCs).[6] In humans, a unique region called respiratory airways exists, which is absent in mice.[7] Recent single-cell RNA sequencing (scRNA-seq) studies have identified several cell types in this region, including “terminal and respiratory bronchioles secretory cells (TRB-SCs)” or “respiratory airway secretory (RAS) cells”, and alveolar type 0 cells (AT0 cells).[8,9] Additionally, $p63^+$ basal cells in humans are distributed more distally than in mice[7] (Fig. 1).

The distal lung consists of millions of alveoli, which form honeycomb-like structures facilitating extensive surface area for gas exchange.[1,2] The human alveoli offer a total surface area of about 70 m^2.[1,2] Alveoli are lined with squamous AT1 cells, responsible for gas exchange, and cuboidal AT2 cells.[10,11] This epithelial layer closely adjoins the lung

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microvascular endothelial layer, separated only by a 0.2–2.5 μm interstitial space, forming the gas–blood barrier[2] (Fig. 1).

AT1 cells are thin, flat, and squamous, each with an average volume of 1800 μm^3 and a surface area of 5000 μm^2. Remarkably, they constitute over 95% of the lung's gas exchange surface area.[10,12] Due to their expansive surface area, individual AT1 cells often extend across multiple alveoli. These pneumocytes are linked to adjacent epithelial cells by tight junctions.[10,12] Primary functions of AT1 cells include facilitating gas exchange and maintaining ionic and fluid balance in the alveolar region through the expression of various ion channels and pumps.[10]

In contrast, AT2 cells occupy only 2–5% of the alveolar surface, but are nearly twice as numerous as AT1 cells. These cuboidal cells have surface areas averaging around 180 μm^2 and volume of about 900 μm^3.[2,13] AT2 cells serve multiple functions: secreting surfactant, maintaining fluid balance, and modulating immune responses.[11] Crucially, they function as adult tissue stem cells, participating in lung homeostasis, repair, and regeneration.[14–16]

A pivotal function of AT2 cells is the production of pulmonary surfactant, essential for maintaining low surface tension in distal gas exchange units and required for effective respiration.[11] Pulmonary surfactant is a lipoprotein complex consisting of 90% lipids and 10% proteins, including Sp-A, B, C, and D, with phospholipids, especially phosphatidylcholine (PC), forming the majority of the lipid component.[11,17]

Human respiration involves the inhalation of 5–8 L of air per minute.[18] Alveolar epithelial cells, including AT2 cells, must thus respond to airborne particles and pathogens while modulating immune responses.[18,19] Surfactants from AT2 cells enhance macrophage chemo-taxis, bacterial uptake, and phagocytosis.[20,21] Additionally, AT2 cells produce cytokines and chemokines like tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1β, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1 α (MIP-1 α), and granulocyte-macrophage colony-stimulating factor (GM-CSF) in response to lung injuries.[22–26] These mediators allow AT2 cells to interact with various immune cells, such as macrophages, neutrophils, and T cells.[22–26]

Lastly, AT2 cells contribute to maintaining the alveolar fluid layer by expressing cation channels or pumps like the amiloride-sensitive epithelial sodium channel (ENaC).[27–29] Another focal point of this article is the stem/progenitor functions of AT2 cells, particularly their ability to self-renew and differentiate into AT1 cells.[14–16]

AT2s are the major source of stem cells in distal lung homeostasis and repair

Alveolar epithelial cells are under continuous assault from environmental factors including particles, toxins, and microorganisms. Their maintenance and repair are critical to the respiratory function of the lung. AT2 cells predominantly serve as the primary stem cells in alveoli, although AT1 cells and certain airway cell subsets can also participate in repair processes under specific conditions (Fig. 2A).

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The mechanisms underlying the maintenance and regeneration of lung epithelium are somewhat distinct from those governing other constantly exposed tissues such as the skin and the gastrointestinal (GI) tract. While these organs are characterized by rapid cellular turnover rates, often facilitated by specialized stem cells residing in dedicated niches,[30,31] the lung has a markedly slower turnover rate.[32] During homeostasis, only small subsets of AT2 cells are intermittently activated for either self-renewal or differentiation into AT1 cells, thus replacing damaged or aged epithelium. However, following lung injury, there is a considerable surge in the activation of AT2 cells. These cells transiently adapt to act as progenitor cells, playing a pivotal role in the repair and regeneration of alveolar structures.[33] Interestingly, AT2 cells are multi-functional. Alongside their conventional physiological roles such as the secretion of surfactant, they have the capacity for regenerative functions. These include both the self-renewal of their own population and differentiation into AT1 cells to restore the alveolar epithelium. Due to this duality in function, AT2 cells have been aptly described as “facultative stem cells”, highlighting their ability to serve both physiological and regenerative purposes when required.[34]

Heterogeneity and plasticity of AT2s

Recent studies revealed that AT2s constitute a heterogeneous population rather than a homogeneous one. In fact, distinct subsets of AT2s display varying regenerative potentials in different contexts, such as during basal homeostasis and post-injury repair.[33] Utilizing a mouse model with Cre recombinase expressed specifically in AT2 cells, researchers have discovered that over a 16-month period, AT2 cells were replenished by self-renewal, not derived from other cell types.[14,15] Interestingly, a minor fraction (~1%) of AT2 cells exhibited progenitor function. These cells produced small clusters of descendant cells, including both AT1s and AT2s, typically found in perivascular regions and at the lung periphery.[15] These specific locations appear to act as “hot spots” for alveolar cell renewal and differentiation.[15]

In line with this, we identified a small sub-population of AT2 cells, constituting approximately 3% of the total AT2 population, which expressed elevated levels of the hyaluronan receptor CD44, and are located primarily at these perivascular “hot spots”. These $CD44^{high}$ AT2s exhibited higher proliferation rates and increased potential for differentiation into AT1 cells, as well as for generating larger three-dimensional organoids, when compared to bulk AT2 cells. These suggest that the $CD44^{high}$ AT2s may play a critical role in steady-state maintenance of the lung epithelium in mice.[35] Intriguingly, the $CD44^{high}$ AT2s are also present in human lungs.[35] Furthermore, we found that the stem cell phenotypes of these $CD44^{high}$ AT2 cells seem to decline with age.[35]

Moreover, two other studies identified subset of AT2 cells with active Wnt signaling ($Axin2^{+}$) as progenitors during both homeostasis and post-injury repair.[36,37] However, these studies presented discrepancies concerning the proportion of $Axin2^{+}$ cells in AT2 populations during basal homeostasis. One study reported approximately 1% of AT2s being $Axin2^{+}$,[37] while another reported nearly 20%.[36] These disparities likely result from methodological variations, particularly different thresholds for Axin2 expression levels that were detected. Critically, both studies emphasized the role of Wnt signaling in regulating

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the stem cell functions of Axin2$^+$ AT2 cells, especially in *in vivo* lung repair scenarios.[36,37] Furthermore, human lung tissues have been found to contain a cluster of AT2 cells, referred to as AT2-s, which exhibit a distinct transcriptional profile and selective expression of Wnt signaling molecules, suggesting they are the human equivalent of Axin2$^+$ AT2 cells.[38]

While a small fraction of AT2s suffice for basal homeostatic maintenance, a more substantial subset is activated following lung injury to participate in repair processes, e.g., a significantly larger fraction of AT2s become Axin2$^+$ after lung injury.[37] Our research shows that in the aftermath of bacterial-induced lung injury, between 30% and 50% of AT2s begin to express the surface molecule stem cell antigen 1 (Sca1). These Sca1$^+$ AT2s exhibit both an elevated rate of proliferation and an increased propensity to differentiate into AT1s. Furthermore, these cells manifest activated Wnt signaling pathways.[39] Notably, the population of Sca1+ AT2s diminishes upon substantial completion of lung repair, suggesting that they represent a repair-engaged AT2 subset.[39]

Recent scRNA-seq studies have identified specific sub-populations of AT2s appearing post-injury,[40–45] which appear to be at various intermediate stages of the AT2-to-AT1 conversion process. These transitional cells exhibit the expression of markers like Krt8, Lgals3, and Krt19 and often show activation in transforming growth factor β (TGFβ) signaling, senescence pathways, and p53 pathway or inflammation-related pathways.[40–45] Different research groups have variously termed these cells as “cell cycle arrest” sub-populations,[42] “pre-alveolar type-1 transitional cell states (PATS)”,[41] “damage-associated transient progenitors (DATP),”[40] or “Krt8$^+$ alveolar differentiation intermediates (ADI)”.[43] Through analysis of these scRNA-seq data, we identified an overlap between the Sca1$^+$ AT2s and these intermediate AT2/AT1 cells.[39,43]

Therefore, depending on the environmental context—be it basal homeostasis or post-injury repair—AT2s display a significant degree of heterogeneity, representing various subgroups that reflect either distinct sub-populations or unique cellular states. Apart from the subsets mentioned, additional subgroups of AT2s have been reported as potential progenitor cells. These include the programmed cell death ligand 1 (PD-L1)-enriched SpC-low AT2s.[46]

As previously discussed, AT2s manifest remarkable plasticity, capable of differentiating into AT1 cells. Furthermore, a recent study reported that human AT2s can also transdifferentiate into basal-cell-like cells under the influence of pathological fibroblasts present in fibrotic lungs.[47]

Molecular mechanisms regulating AT2-mediated repair

Signaling molecules that regulate AT2 stem cell activities encompass growth factors, Notch, Wnt, bone morphogenetic protein (BMP), and Yes-associated protein (YAP). The repair processes mediated by AT2s involve a series of sequential steps: activation from a quiescent state, proliferation, transition into AT2/AT1 intermediate cells, and ultimately, differentiation into AT1s. Crucially, these signaling pathways are dynamically or temporally regulated during these phases (Fig. 2B). Various growth factors, including fibroblast growth factor (FGF) 7, FGF10, epidermal growth factor (EGF), and hepatocyte growth factor (HGF), have been well-documented to promote AT2 proliferation.[15,48–50]

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Our research establishes that Notch signaling is intrinsically required in AT2s for their stem cell function during alveolar repair. Importantly, Notch activity is temporally modulated in the two distinct phases during the AT2 to AT1 transition. In the initial phase, Notch activity is elevated in AT2s as they transition to an AT2/AT1 intermediate state, and this elevation of the Notch signal is required for these cells to survive at this stage.[51] Conversely, in the late phase, Notch activity must be suppressed by the non-canonical Notch ligand Dlk1 (delta-like 1) to enable the further conversion of AT2/AT1 intermediate cells into AT1s.[51]

Wnt signaling also plays a pivotal role, particularly in Axin2-positive AT2s.[36,37] Wnt signaling is requisite for the activation of AT2s, thereby allowing them to acquire stem cell properties. However, a subsequent attenuation of canonical Wnt/β-catenin signaling is necessary for the AT2 to AT1 transition.[37]

Moreover, the roles of TGFβ and BMP4 in AT2 self-renewal and transition to AT1 have been studied, albeit with some discrepancies.[52,53] One study employing a 2D AT2 culture suggested that TGFβ promotes AT2 to AT1 transition, while BMP4 exerts the opposite effect.[53] Another study using a 3D AT2-fibroblast culture and a mouse pneumonectomy model showed that BMP4 inhibits AT2 proliferation but facilitates their transition to AT1.[52] Therefore, the functions of TGFβ and BMPs may be both context and dose-dependent.

Numerous studies have demonstrated that the Hippo pathway molecule YAP plays a critical role in the transition from AT2 to AT1 cells.[54,55] This transition entails a significant change in cell shape, from cuboidal to flat and squamous, along with an expansion in surface area. Consistent with changes in YAP subcellular localization observed in other cell types—where YAP tends to translocate to the nucleus in elongated cells with larger surface areas,[56] YAP is usually found in the cytoplasm of cuboidal AT2 cells, however, it translocates to the nucleus when AT2s initiate the transition to AT1 cells and remain there in mature, flat AT1 cells.[54,55] Several factors may trigger YAP activation and subsequent nuclear translocation, leading to the AT2-to-AT1 conversion. The first is a change in mechanical tension, as evidenced in a mouse pneumonectomy model.[55] Additionally, alterations in cell–cell adhesion mediated by Claudin 18 could also activate YAP.[57] Moreover, our research indicates that following lung injury, microvascular ECs (miECs) release the lipid molecule sphingosine-1-phosphate (S1P), which activates YAP via the sphingosine-1-phosphate receptor 2 (S1PR2) receptor on AT2 cells.[58]

Immunity-related signaling molecules, such as Toll-like receptor 4 (TLR4) and IL1β, also regulate AT2 progenitor functions.[59,60] Furthermore, scRNA-seq studies have revealed the activation of p53 and cell senescence pathways in AT2-to-AT1 intermediate cells, suggesting their role in alveolar repair.[40–43] Recent research has also highlighted the role of mitochondrial metabolism, particularly pathways regulating Ca^{2+} uptake,[61] and glycolysis,[62] in the AT2-to-AT1 transition.

Lastly, various transcription factors, including FoxM1,[63] hypoxia inducible factor 1 α (HIF1 α),[64] Etv5,[65] and Tfcp2l1,[66] have been implicated in either AT2 self-renewal or cell fate conversion between AT2 and AT1. These transcription factors are likely downstream effectors of some of the signaling pathways previously discussed. Future studies should also

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explore how the regulation of these factors relates to epigenetic mechanisms affecting AT2 stem cell functions.[67,68]

Other epithelial progenitor cells that may participate in alveolar repair

While AT2 cells are primarily responsible for alveolar repair, other epithelial cells such as subsets of small airway cells, or AT1s, can also contribute in the repair process under certain injury conditions (Fig. 2A).

Upon exposure to H1N1 influenza viral infection, which induces severe lung injuries, specific $Sox2^+$ airway-derived cells[69] become activated and mobilized. These cells acquire markers such as Trp63 and Krt5 and migrate into the alveolar region where they form pod-like structures to temporarily seal denuded alveolar layers.[70,71] These specialized cells have been termed “distal airway stem cells” (DASCs)[70] or “lineage-negative epithelial progenitors” (LNEPs).[71] [ntriguingly, the activation of DASC/LNEP requires Notch signaling. However, after entering alveoli, these “pods” cells rarely differentiate into AT2s, unless the lungs were treated with a Notch inhibitor.[71,72] Thus, comparing with the roles of Notch during AT2/AT1 transition as discussed above,[51] a temporal upregulation of Notch activity may govern the activation of these quiescent progenitor cells to adopt an intermediate cellular state, and a subsequent down-regulation of Notch might be required for further differentiation into the destination cell types. In the case of AT2s, Notch suppression is needed when PATS/DATP/ADI intermediates become AT1s; in the case of DASC/LNEPs, this is needed for further differentiation into AT2s.

Besides DASC/LNEPs, subgroups of club-like secretory cells may also participate in alveolar repair.[73–75] Specifically, around 5% of $CGB1A1^+$ club cells, expressing higher levels of the MHC gene *H2-K1*, can differentiate into AT2s and AT1s through transplantation assays.[74]

Some cells at the transitional zone of bronchioles and alveoli display stem cell-like properties and contribute to alveolar repair. In mice, a group of putative stem cells called BASCs that co-express the AT2 marker Sftpc and Club cell marker Scgb1a1 are located in this region (termed BADJ in mice).[6,76] Lineage tracing studies using Sftpc and Scgb1a1 dual markers indicate that BASCs can give rise to alveolar epithelial cells, contributing to lung repair.[77,78] In humans, instead of BADJ, a structure termed the “respiratory airway” exists between conductive airways and alveoli.[7] Recent scRNA-seq studies have identified new progenitor cell subsets in this region, including TRB-SCs expressing secretoglobin family 3A member 2 (SCGB3A2), and another subset called AT0 cells co-expressing SCGB3A2 and surfactant protein C (SFTPC).[9] AT0 might represent a transient cell state with high plasticity, interestingly, AT2s could also convert to TRB-SCs through the AT0 state.[9] Another research group also identified similar progenitor cells in the respiratory airway that can give rise to AT2s and named them “respiratory airway secretory” (RAS) cells.[8]

While AT1 cells were once thought to be terminally differentiated and incapable of proliferation,[79] this paradigm has been questioned. New research shows that AT1 cells can, under specific conditions, proliferate and transdifferentiate into AT2-like cells.[80,81]

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Moreover, the plasticity of AT1 cells is likely to depend on age and different injury contexts. Lineage tracing studies using AT1 markers such as HopX (HOP home-obox) and Ager (advanced glycosylation end-product specific receptor) reveal that AT1 cells in newborn mice display plasticity, and can transdifferentiate into AT2 cells when the lungs are damaged by hyperoxia or hypoxia.[82] In contrast, mature AT1 cells marked by insulin-like growth factor-binding protein 2 (IGFBP2) appear unable to transdifferentiate into AT2 cells.[83] Therefore, it seems that adult lungs harbor two distinct AT1 cell subtypes: those that can transdifferentiate into AT2 cells ($HopX^{+}Igfbp2^{-}$) and those that cannot ($HopX^{+}Igfbp2^{+}$). Interestingly, this AT1-to-AT2 trans-differentiation process is regulated by mechanical stress induced by breathing movements, which lead to chromatin reorganization.[84]

Microenvironmental niches regulating AT2 stem cells

Much like other types of adult resident stem cells,[85,86] alveolar epithelial stem cells are influenced by their local microenvironment, or stem cell niche. In the context of AT2s, this niche is most likely comprised of fibroblasts, along with resident or recruited immune cells and ECs (Fig. 3). Notably, the elements constituting these niches seem to vary depending on the state of homeostasis or during the aftermath of lung injuries.

Subsets of fibroblasts in alveoli

Multiple studies have underscored the crucial role that fibroblasts play in supporting AT2 stem cell functions.[14,87] Recent technological advancements such as lineage tracing and scRNA-seq have revealed significant heterogeneity among fibroblasts. There is evidence to suggest that certain sub-populations of fibroblasts are uniquely responsible for supporting specific subsets of AT2s.

Based on function, transcriptomic profiles, and anatomical locations, fibroblasts in the alveolar regions can be categorized into multiple subpopulations. These include lipofibroblasts, myofibroblasts, adventitial fibroblasts, and pericytes.[88–90] Lipofibroblasts, often classified as “alveolar fibroblasts”, are situated adjacent to AT2 cells and contribute to lipid transport, which aids in surfactant production.[14,88,89] Myofibroblasts, identified by their expression of the contractile protein α-smooth muscle actin, provide mechanical strength to the alveoli septae and are a source of collagen deposited in the ECM.[89] Lastly, adventitial fibroblasts and pericytes are positioned near blood vessels and are believed to facilitate communication with ECs.[89]

Utilizing lineage or reporter-labeled mice as models, fibroblasts can be categorized into various subgroups based on their expression of specific signaling-related molecules. Some of these fibroblast sub-groups have demonstrated a capacity to function as alveolar niche cells, thereby supporting AT2 stem cell functions. For instance, platelet-derived growth factor receptor α (PDGFRα) positive fibroblasts, which also overlap with lipo-fibroblasts and are adjacent to AT2 cells, have been shown to facilitate AT2 proliferation and differentiation within an organoid culture system.[14] Further research has revealed that fibroblasts co-expressing PDGFRα and Axin2 act as niche cells for a subset of Wnt-responsive AT2 cells.[91] Moreover, certain fibroblasts labeled by Lgr5 and Lgr6 have also been implicated as potential niche cells.[92] However, it remains an open question how these

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signaling molecule-defined fibroblast subsets overlap with functionally and anatomically defined categories such as lipo-fibroblasts and myo-fibroblasts.

One mechanism by which fibroblasts contribute to alveolar epithelial regeneration involves the provision and modulation of the ECM and scaffolding for epithelial stem cells. Additionally, specific fibroblast subsets have been shown to offer various paracrine cues that influence the progenitor behaviors of adjacent AT2 cells. For example, PDGFRα$^+$ fibroblasts have been observed to modulate BMP/SMAD signaling, thereby supporting AT2 stem cell functions during *in vivo* mouse lung regeneration.[52] Furthermore, several fibroblast subsets provide Wnt ligands essential for the stem cell functions of Wnt-responsive AT2 cells, both in homeostasis and repair.[36,37,91] In line with the role of FGFs, particularly FGF10 and FGF7, as crucial signaling molecules during lung development,[93,94] adult lung fibroblasts have also been found to produce FGF ligands that regulate AT2 progenitor functions.[50]

Immune cells

Numerous tissue stem cells, such as those found in the intestine, liver, hair follicle, and muscle, can be activated by either resident or recruited immune cells to participate in tissue repair.[95,96] Similarly, AT2 cells in the lungs respond to immune-related signals like IL-1 β and TNF-α to initiate proliferation and transition into AT1 cells.[60] Following injury, subsets of AT2 cells in transition to AT1 cells display activation of the interleukin 1 receptor 1 (IL1R1).[40] Multiple immune cells, including macrophages and T cells, are either present in the quiescent lung or recruited after various lung injuries, serving to regulate the stem cell functions of AT2s.[97–99]

Lung macrophages, like AT2 cells and fibroblasts, also display significant heterogeneity and plasticity.[100] Among the critical subsets of residential macrophages that interact with AT2s are alveolar macrophages (AlvMs). These cells primarily function to suppress unnecessary inflammation in basal-state lungs[101] and aid in the catabolism of surfactants produced by AT2 cells.[18] On the other hand, AT2-derived cytokine GM-CSF is essential for AlvMs development and maintenance.[102] Following lung injury, AlvMs can be activated by microbial pathogen-associated molecular receptors (PAMPs) and subsequently interact with other immune cells by secreting various cytokines and growth factors.[100] Through factors such as TNF-α and IL-1 β, AlvMs may modulate AT2 progenitor activities.[103]

Another noteworthy subset of residential macrophages are interstitial macrophages (IntMs).[100] After lung injury, these macrophages can be activated and transition into either the pro-inflammatory M1 phenotype or the regenerative M2 phenotype.[104,105] Circulating monocytes can also be mobilized post-injury, recruited into the lungs, and become IntMs.[106] One study indicated that C-C motif chemokine receptor 2 (CCR2)$^+$ recruited monocytes are essential for AT2 proliferation and the AT2-to-AT1 transition following pneumonectomy.[97] In addition, some residential macrophages that adopted the M2 phenotype after lung injury likely also play important roles in regulating the AT2 progenitor functions.[97] Moreover, IntMs can produce IL-1 β, stimulating a subset of IL1R1-expressing AT2s and promoting their progenitor functions.[40]

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Apart from macrophages, other immune cells also serve as niche components to support AT2 stem cell functions. Regulatory T cells (Tregs), for instance, infiltrate the lung after injuries induced by the influenza virus or lipopolysaccharide (LPS).[98,99] These cells produce keratinocyte growth factor to stimulate AT2 proliferation.[98] They also secrete the epidermal growth factor receptor (EGFR) ligand amphiregulin (Areg), activating a subset of $EGFR^+$ $Col14a1^+$ adventitial fibroblasts and further enhancing the reparative functions of AT2s.[99] Tissue-resident lymphocytes can suppress specific AT2 stem cell subsets via interferon signaling.[107] Lastly, neutrophil transmigration through epithelial cells appears to activate β-catenin signaling in AT2s, thereby facilitating their reparative functions.[108]

Pulmonary ECs

The lung is a highly vascularized organ with a large number of micro-blood vessels or capillaries situated close to alveolar epithelial cells, thereby facilitating blood–air gas exchange. ECs constitute approximately 40% of the total lung cell population.[13] These lung capillary ECs, also referred to as lung microvascular endothelial cells (LMVECs), and alveolar epithelial cells are separated by a thin ECM layer of less than 2 μm.[1] Besides serving as a barrier between the airspace and bloodstream, lung ECs are sensitive to micro-environmental changes and actively interact with various inflammatory cells.[109,110] Consequently, ECs are well-positioned to serve as niche components and regulate alveolar epithelial repair through the secretion of paracrine (angiocrine) factors.[111] LMVECs have been demonstrated to promote lung regeneration by producing the matrix metalloprotease MMP14, facilitating the presentation of EGF ligands to AT2 cells.[112] Additionally, LMVECs secrete other angiocrine factors like HGF[113] and thrombospondin[76] to further assist in lung regeneration. Our group has also shown that LMVECs produce and secrete a lipid-based angiocrine factor, S1P, into the interstitial space in alveoli after bacterial lung injury. This S1P acts on its receptor, S1PR2, expressed on AT2 cells, leading to the activation of downstream YAP signaling and promoting the AT2 to AT1 transition required for lung repair.[58]

Recent studies employing scRNA-seq have uncovered the heterogeneity of lung ECs. These studies identified multiple lung EC sub-populations, including macrovascular ECs, Plvap+ miECs (or called gCAP for “general capillary cells”), and $Car4^+$ ECs (or aerocytes) that are in close contact with AT1s and AT2s.[38,114,115] Another study revealed two major LMVEC sub-populations appearing after LPS-induced lung injury: one enriched in immune response gene products (immuneEC) and another with elevated expression of vascular development genes like *Sox17* (devEC).[116] The preferential roles of these EC subsets in regulating lung epithelial repair in various health and disease contexts remain to be elucidated.

Diseases associated with disruption of alveolar epithelial stem cells and their niches

Dysregulation in the functions of AT2 stem cells can contribute to a range of lung diseases. For instance, imbalances in lung homeostasis can impair the epithelial structure, leading to an emphysema-like phenotype. Ineffective post-injury repair can result in destructive remodeling, which may culminate in interstitial lung diseases like pulmonary

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fibrosis. Moreover, AT2s are vulnerable targets for respiratory viruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and can be the origin of lung adenocarcinomas.[12]

Emphysema is often observed in patients with COPD and is characterized by the gradual loss of alveolar surface area, often accompanied by chronic inflammation.[117,118] Disruptions in AT2 stem cell functions during steady-state maintenance and aging can lead to a gradual loss of epithelial cells, resulting in emphysema-like conditions.[119] Genome-wide association studies (GWAS) have linked increased expression of family with sequence similarity 13, member A (FAM13A) with COPD.[120] Further investigations revealed that FAM13A regulates AT2 stem cell functions via the Wnt/ β-catenin pathway.[120] A recent scRNA-seq analysis of lungs from COPD patients has identified an aberrant subpopulation of AT2 cells with altered cellular metabolism.[121] Additionally, abnormal expansion of resident T cells can suppress AT2 cell renewal, thereby contributing to the development of emphysema.[107]

Pulmonary fibrosis may stem from abnormal alveolar epithelial repair processes due to compromised AT2 progenitor functions post-lung injury.[4,122–126] scRNA-seq has identified aberrant subsets of alveolar epithelial cells in fibrotic foci.[123,124,127,128] These cells often express markers of AT2/AT1 intermediate cells and may activate surrounding fibroblasts via TGF β release.[41–43,45,129]

Over the past three years, the coronavirus disease 2019 (COVID-19) pandemic has posed a significant health crisis globally. This disease is caused by SARS-CoV-2,[130] a coronavirus primarily targets AT2 cells.[131–133] AT2s express key molecules like angiotensin converting enzyme 2 (ACE2), transmembrane protease, serine 2 (TMPRSS2), and Furin, which facilitate the virus entry.[134] In the lungs affected by COVID-19, cells expressing markers of AT2/AT1 intermediates have been observed.[135] Some AT2s in COVID-19 lungs also show signs of arrested progenitor state.[136,137] Thus, disrupted AT2 progenitor functions could contribute to the disease's progression.

AT2 cells are a major origin of lung adenocarcinoma.[138] Cells expressing AT2/AT1 intermediate markers have been detected in lung adenocarcinoma samples.[40] This suggests that repetitive injuries and aberrant repair mechanisms might be linked to the neoplastic transformation of AT2 cells.

Research methodologies in studying alveolar epithelial stem cells and niches

In this section, we explore the technological advancements for investigating the functionality of alveolar stem cells and their niches, focusing on recent innovations (Fig. 4).

Despite anatomical and cellular differences between rodents and human airways,[139] rodents are valuable in lung research. Diverse lung injury models exist: diphtheria toxin ablates AT2 cells or niche components in transgenic mice.[14,92] Chemicals like hyperoxia, acid, and cigarette smoke induce varying degrees of lung damage.[140] Bleomycin, a DNA damage agent, is used to model acute lung injury and transient fibrosis.[140] Pathological agents such

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as the H1N1 influenza virus,[140] LPS,[42] and bacteria (e.g., *Pseudomonas*, *Streptococcus pneumoniae*)[54,63] are also used to model lung injuries and repair. Furthermore, a unilateral pneumonectomy model can be used to study the compensatory regeneration.[140] In addition to injury models, mouse genetic techniques like lineage tracing enable the study of the fate of AT2 sub-populations or niche cells (e.g., specific subsets of fibroblasts) during aging or regeneration.[14,91] Conditional knockout models further allow investigation into the role of specific genes in targeted cell types during the injury repair process.[51]

Efforts to establish AT1 and AT2 cell lines have fallen short of capturing the full range of characteristics observed in freshly isolated cells.[141] However, cultured primary AT1 and AT2 cells have yielded significant insights. A method for isolating AT2 cells using elastase digestion of lung tissue was pioneered by Dobbs *et al*[142] and later refined by other researchers.[143] To enhance cellular purity, fluorescence-activated or magnetic-activated cell sorting techniques have been employed.[144,145] Once isolated, AT2 cells can be cultivated in a 2D culture medium containing serum, and within three days, they begin to transit into AT1-like cells.[141,145–148]

Recent advances have propelled the development of organoids from stem or progenitor cells for modeling both organ homeostasis and disease states. An organoid is a 3D cellular structure, emerging from stem or progenitor cells, which undergoes cell sorting and lineage-specific development.[149] In the realm of pulmonary research, a plethora of culture systems have been established to create lung organoids.[150,151] Using a simplistic culture medium (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 [DMEM/F-12], 10% fetal bovine serum [FBS], insulin-transferrin-selenium [ITS]), AT2 cells co-cultured with fibroblasts in 3D Matrigel yield cyst-like structures that resemble alveoli.[14,87,145] Importantly, AT2 cells require mesenchymal factors from fibroblasts to successfully form these structures in this culture condition. More recently, a feeder-free, chemically defined alveolospheres culture system that includes more medium components, such as EGF and BMP4 antagonist Noggin, has been developed.[133,152–154] This system has been used to study the interactions between AT2s and SARS-CoV-2.[133,152–154] Beyond organoid cultures, alternative *in vitro* and *ex vivo* models for studying alveolar epithelium include precision-cut lung slices (PCLS)[155] and lung-on-a-chip technologies.[156,157]

The advent of scRNA-seq has marked a watershed moment in understanding the intricate cellular interactions during lung injury and repair. This technology enables the identification of unique subsets of epithelial and niche cells throughout dynamic injury–repair processes.[158,159] As discussed above, in addition to identifying AT2/AT1 intermediate cells, aberrant cell types linked to diseases like fibrosis, COPD, and COVID-19 have been discovered. This rich information base allows for an in-depth understanding of various lung pathologies and assists in the identification of novel drug targets.

Conclusion and future perspectives

Lung is an essential and complex organ, its homeostasis and repair is regulated through intricate mechanisms that involve both epithelial stem cells and their supporting niches. These mechanisms are modulated by diverse signaling pathways and intricate cellular

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interactions. Although substantial advances have been made in understanding lung maintenance and regeneration, several challenges persist.

Firstly, the remarkable cellular heterogeneity within both epithelial and mesenchymal compartments, as revealed by scRNA-seq, necessitates the identification of key stem cell sub-populations and niches involved in tissue repair. Additionally, these distinct sub-populations may have specialized roles depending on the type of injury or disease context.

Secondly, emerging data indicate a greater extent of cellular plasticity than previously appreciated; for example, subsets of airway cells and AT1s and AT2s can transdifferentiate.

Thirdly, the dynamic nature of repair processes necessitates temporally regulated signaling pathways, adding another layer of complexity. Different injuries, depending on the inducing agents, might also activate divergent signaling mechanisms for repair.

Fourthly, reciprocal interactions between epithelial cells and their niches further complicate the landscape of stem cell–niche communication. For instance, AT2 cells produce brain-derived neurotrophic factor (BDNF) to enhance the niche function of adjacent fibroblasts.[160]

Lastly, there are species-specific differences in lung repair mechanisms, which suggest a need for more physiologically relevant animal models, such as ferrets or rhesus monkeys, whose respiratory systems more closely resemble those of humans.[8,9]

In conclusion, refining current techniques and developing new models are essential for future investigations into lung stem cell functions. Such endeavors will likely pave the way for innovative therapeutic strategies aimed at enhancing alveolar repair and mitigating chronic diseases linked to impaired tissue regeneration.

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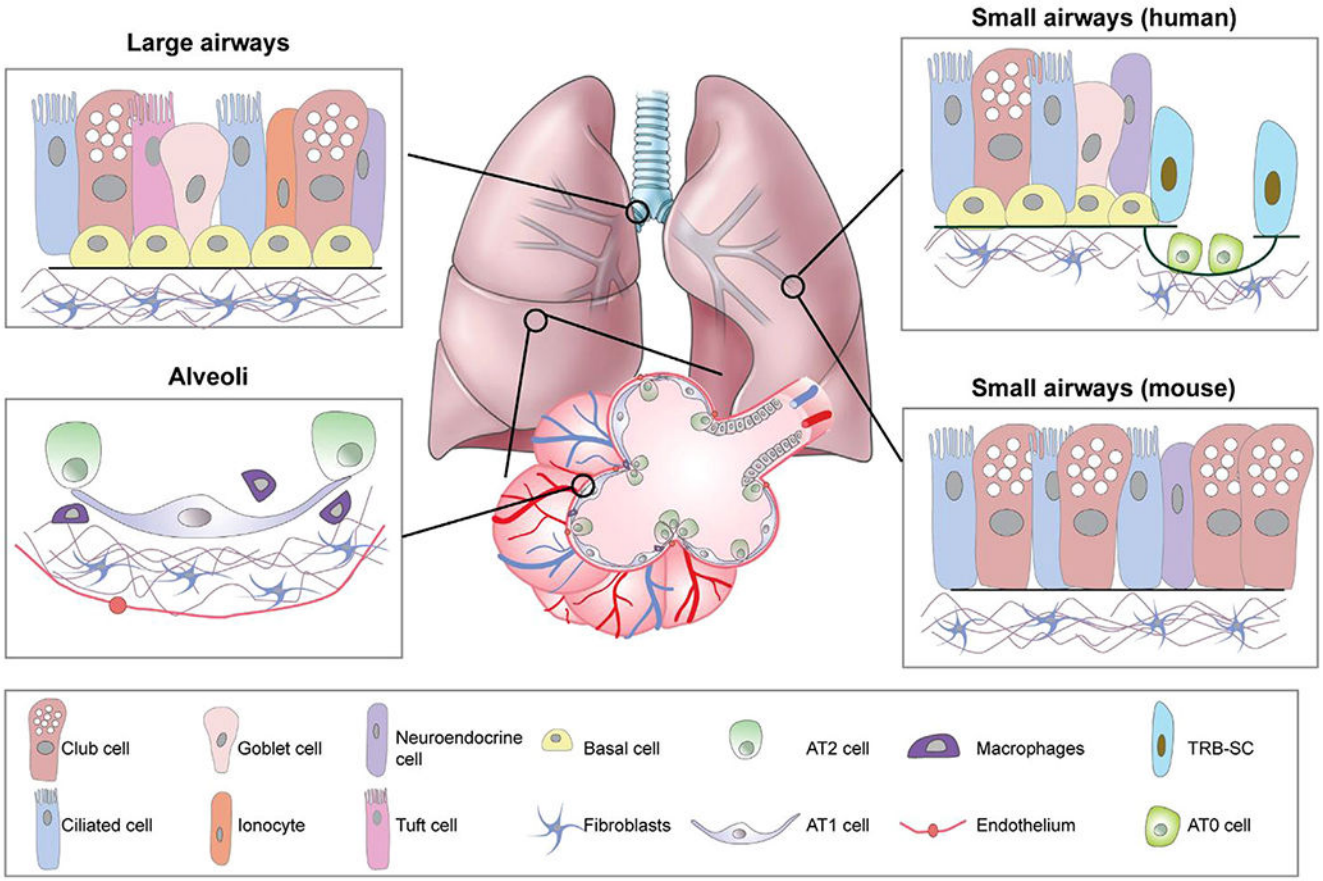

Fig. 1.

Anatomic domains and cell types in human and mouse lungs. AT0 cells: Alveolar type 0 cells; AT1: Alveolar type I cells; AT2: Alveolar type II cells; TRB-SCs: Terminal and respiratory bronchioles secretory cells.

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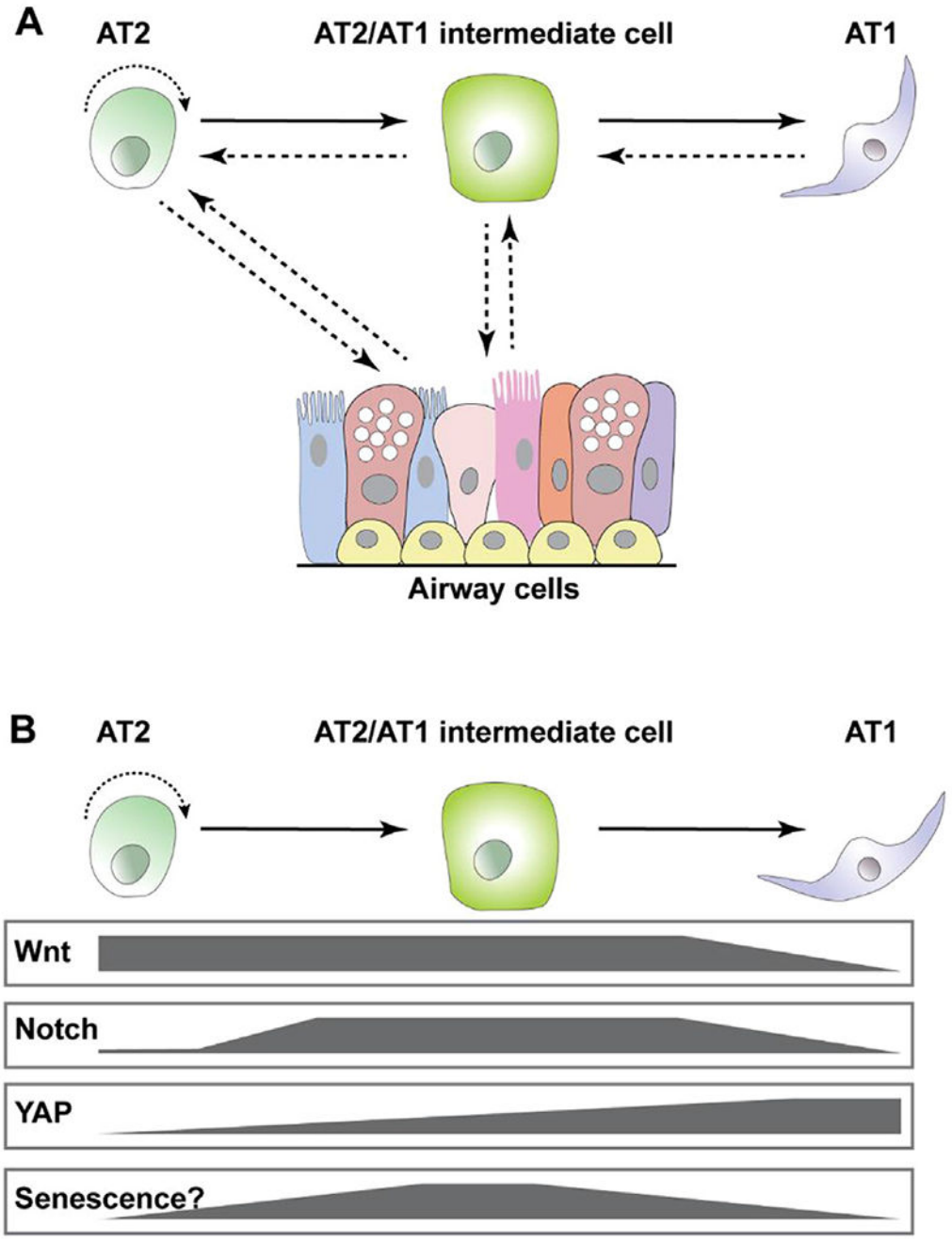

Fig. 2.

Cellular and molecular mechanisms regulating the epithelial progenitor cells during alveolar repair. (A) Plasticity of putative stem cells or progenitor cells that may contribute to alveolar repair. (B) Dynamic regulation of several signaling pathways during AT2 to AT1 transition. YAP: Yes-associated protein.

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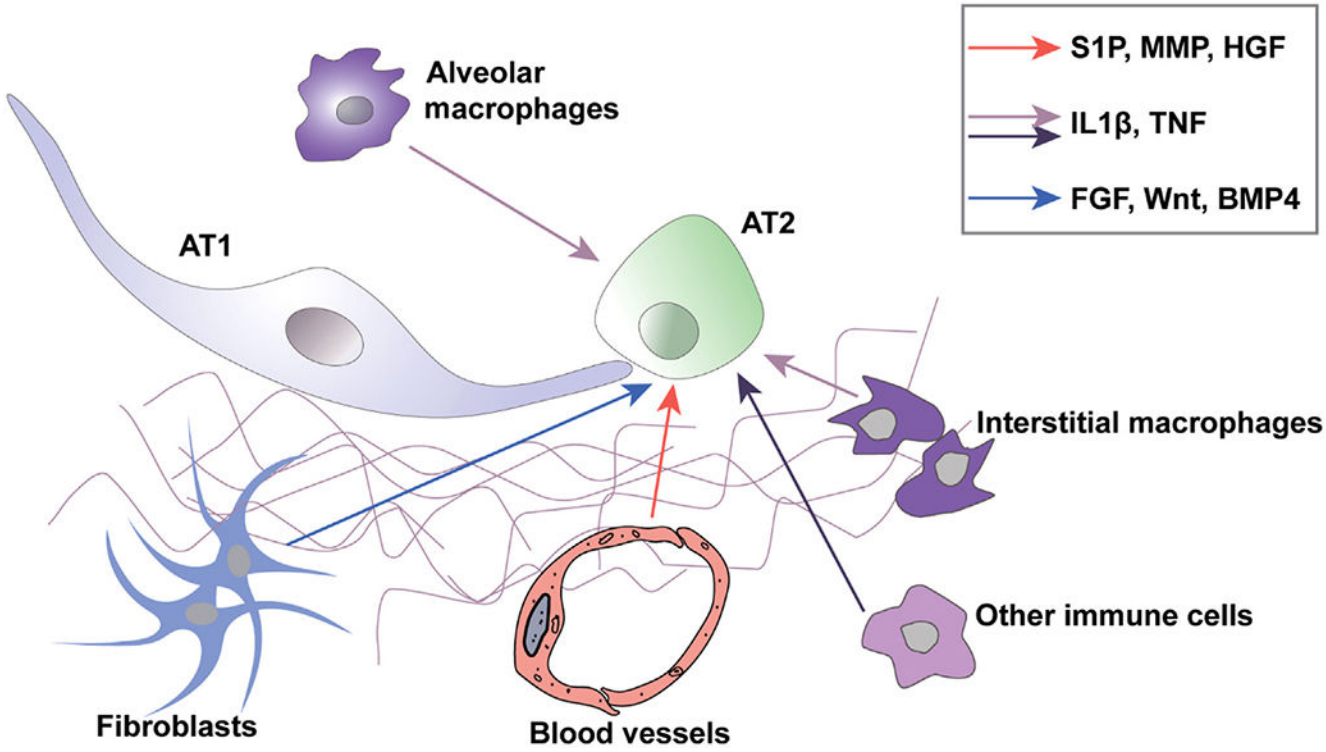

Fig. 3.

Components of micro-environmental niche that support AT2 stem cells. Arrows: examples of supportive paracrine molecules released by these niche cells. AT1: Alveolar type I cells; AT2: Alveolar type II cells; BMP4: Bone morphogenetic protein 4; FGF: Fibroblast growth factor; HGF: Hepatocyte growth factor; IL1*β*: Interleukin 1*β*; MMP: Matrix metalloprotease; S1P: Sphingosine-1-phosphate; TNF: Tumor necrosis factor.

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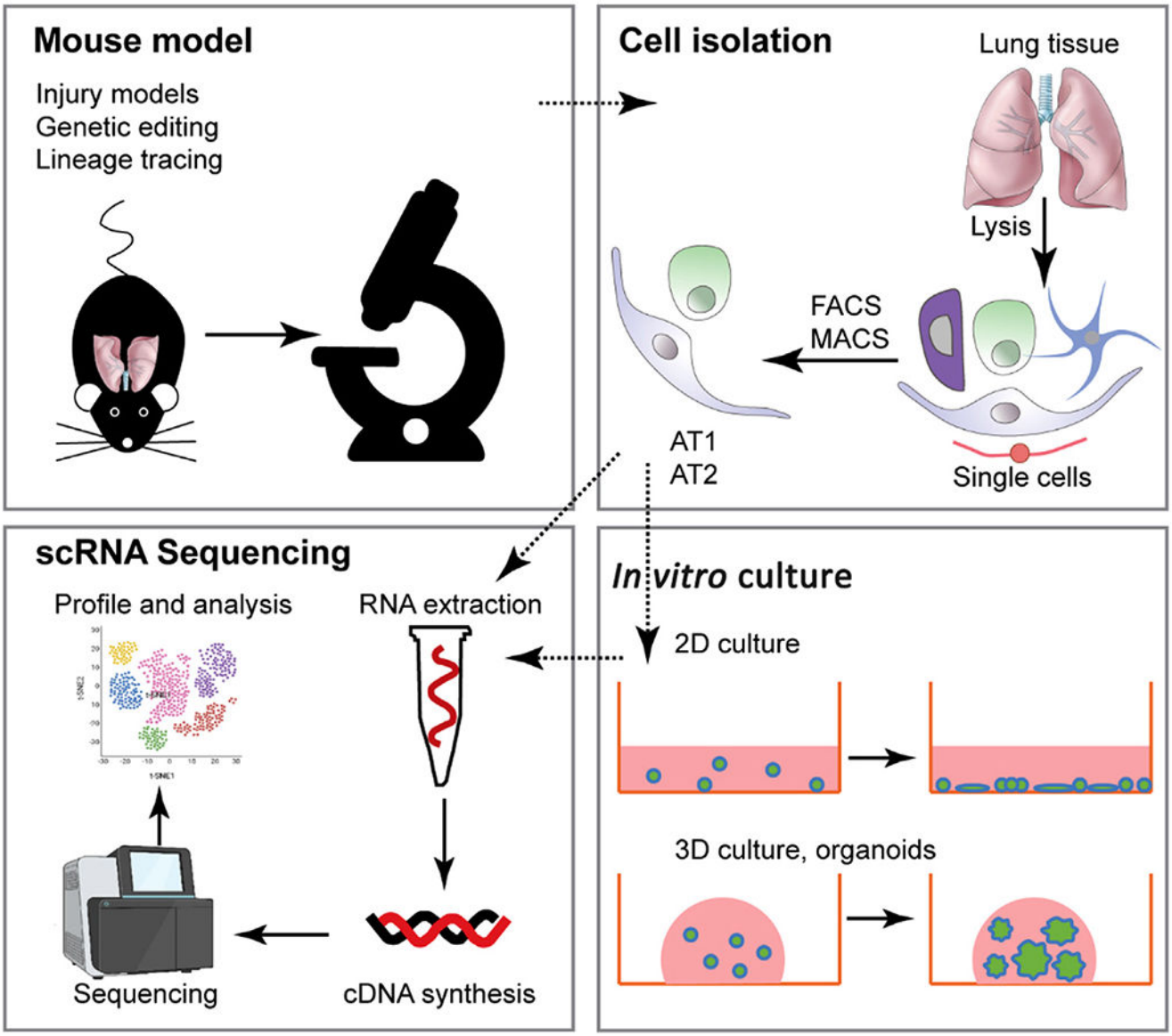

Fig. 4.

Major approaches used to study alveolar epithelial stem cells and niches. AT1: Alveolar type I cells; AT2: Alveolar type II cells; cDNA: Complementary DNA; FACS: Fluorescence-activated cell sorting; MACS: Magnetic-activated cell sorting; scRNA: Single-cell RNA.