

Alveolar epithelial type 2 cells in acute lung injury and acute respiratory distress syndrome: spatiotemporal fate and regulation

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Abstract

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) present significant challenges in critical care, with high mortality rates and limited treatment options. Alveolar epithelial type 2 (AT2) cells are central to lung repair and regeneration and play a vital role in maintaining lung homeostasis. However, the regulatory mechanisms governing AT2 cell fate during different stages of lung injury and repair remain incompletely understood. This review delves into the temporal and spatial heterogeneity of AT2 cells, highlighting their dynamic behavior in response to lung injury. We explore the pivotal role of AT2 cells across various stages of inflammation, repair, and fibrosis and discuss how these processes are influenced by factors such as aging, mechanical stress, and interactions within the alveolar microenvironment. This review also emphasizes the importance of integrating spatiotemporal multiomics approaches to uncover the molecular mechanisms underlying AT2 cell function and their potential therapeutic applications. Furthermore, we discuss novel strategies for enhancing the regenerative capacity of AT2 cells through targeted delivery systems, including protein, gene, and mitochondrial therapies. By advancing our understanding of AT2 cell biology and improving therapeutic approaches, we aim to pave the way for more effective treatments for lung injury diseases, particularly ALI and ARDS.

Keywords: Acute lung injury; Acute respiratory distress syndrome; Alveolar epithelial type 2 cells; Lung regeneration; Spatiotemporal multiomics; Alveolar epithelial type 2 cell fate; Targeted therapy

Highlights

- AT2 cell fate is precisely regulated by both temporal dynamics and spatial heterogeneity during lung injury and repair.
- AT2 cells regenerate alveolar structure by proliferating and differentiating into AT1 cells, a process coordinated by balanced Wnt/ β -catenin, Notch, and BMP, and related signaling pathways.
- Persistent AT2-derived transitional cell states, influenced by spatiotemporal factors including aging, metabolic reprogramming, and mechanical tension, are a key driver of fibrosis.
- Spatiotemporal multi-omics are critical for deciphering these mechanisms and advancing novel targeted therapies to enhance AT2 cell regeneration.

Background

The lung is susceptible to various critical diseases. Patients with severe trauma and other critical diseases often suffer from acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) [1]. ALI is pathologically caused by diffuse lung tissue damage due to uncontrolled inflammation, which progresses to ARDS in severe cases [2, 3]. Surprisingly, ~10% of ICU patients in hospitals suffer from ARDS, accounting for 4% of all hospital patients. Severe ARDS has a mortality rate as high as 46% [4]. Therefore, it is essential to treat ALI effectively to improve the treatment of severe trauma and other critical diseases. While traditional methods for

treating ALI, such as anti-inflammatory and antibiotic therapy, tracheal intubation, ventilator assistance, and extracorporeal membrane pulmonary oxygenation, can help alleviate disease progression, they lack therapeutic effectiveness and have a limited role in prognosis [3, 5]. The prognosis of ALI largely depends on the ability of the lungs to regenerate and repair.

Although studies have shown that multiple types of lung stem progenitor cells can mediate the regenerative repair of lung tissue after the onset of ALI [6], AT2 cells are the main stem and progenitor cells involved in the regeneration and repair of damaged lung tissue [7]. Lung development and

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damage repair are complex processes that involve different cell lineages and lung stem progenitor cells. Their origins and heterogeneity in different periods and conditions have been the focus of attention in recent years. Kim et al. defined a novel lung stem cell located at the bronchioalveolar duct junctions of mice in 2005. This cell type expresses both secretoglobin1a1 (Scgb1a1 or CC10), which is found in the airway epithelium, and surface active protein C (SPC, also known as Sftpc), expressed by alveolar epithelial cells. Therefore, this type of stem cell is named bronchioalveolar stem cell (BASC) [8, 9]. These cells can promote the repair of the airway and alveolar epithelium after damage by naphthalene and bleomycin [8, 10]. However, unlike AT2 cells, which account for 10% of total lung cells, BASCs account for < 1 in 10 000 lung cells. Moreover, BASCs or similar cell subpopulations have not yet been identified in human lung tissue. Therefore, BASCs may play a limited role in lung regeneration and repair in humans.

In the distal trachea, in addition to BASCs, other lung stem progenitors have been reported. Zuo et al. [11] reported that p63⁺/Krt5⁺ distal airway stem cells (DASCs) are key stem cells involved in the regeneration of the lungs of mice infected with H1N1 influenza virus. They reported that endogenous p63⁺/Krt5⁺ DASCs proliferate in the lungs of mice with influenza-induced lung injury and aggregate at the site of lung inflammation to differentiate into new alveoli. However, compared to AT2 cells, this group of cells appears almost exclusively in severely damaged areas with limited repair ability.

Alveolar epithelial type 1 (AT1) cells also act as stem progenitors of the lung in some cases. In a mouse model after partial pneumonectomy, a small number of cells expressing Hopx⁺ (a marker of AT1 cells) were found to be able to both proliferate normally and, in rare cases, give rise to Sftpc⁺ AT2 cells [12]. In 2018, Wang et al. [12, 13] revealed heterogeneity within the AT1 cell population, where the Igfbp2⁺ AT1 subpopulation demonstrated a certain plasticity that could further differentiate into mature Hopx⁺ Igfbp2⁺ AT1 cells or switch to an AT2 cell fate. Despite these findings, the overall contribution of AT1 cells to alveolar epithelial regeneration remains minimal compared with the robust regenerative capabilities of AT2 cells. Moreover, recent lineage tracing studies have confirmed that AT1 cells lack the ability to transdifferentiate into AT2 cells [14]. Furthermore, the study highlighted that previously used AT1 markers, such as HOPX and AGER, are not entirely specific to AT1 cells, as up to 60% of club cells can also be labeled by HOPX. Since club cells have the potential to differentiate into AT2 cells, this overlap likely led to earlier misinterpretations regarding AT1 cell plasticity.

In addition, several other stem progenitor cells have been reported to be involved in the regeneration of functional alveolar cells. These include CC10⁺ Foxj1⁺ EpCAM⁺ Sox2⁺ ITGB4⁺ Krt5⁺ LNEP epithelial progenitor cells [15, 16], Sox2⁺ Scgb1a1⁺ Krt5⁺ progenitor cells [17], ΔNp63⁺ TTF-1⁺ peripheral airway epithelial cells [18], Scgb3a2⁺ respiratory bronchial secretory cells [19], and H2-K1^{high} club cell subtypes [20]. Although these cells are relatively low in number compared to AT2 cells, their role in lung tissue repair may vary depending on the context. These multipotent transitional cells are rare in homeostatic lungs but show a significant increase in number following damage, suggesting a potential role in lung regeneration [14]. While the majority of these progenitor cells eventually differentiate into AT2

cells to perform their functions, recent studies indicate the possibility of alternative differentiation pathways, such as direct differentiation into AT1 cells [21]. AT2 cells play a central role in maintaining alveolar homeostasis. Beyond their established role in surfactant production, they contribute to immune regulation by secreting cytokines and interacting with macrophages and other immune cells. During lung injury, AT2 cell dysfunction—such as impaired secretion, senescence, or misdirected differentiation—can lead to barrier disruption, prolonged inflammation, and fibrotic remodeling. These findings highlight the complexity of AT2 cell fate regulation and its central role in lung repair. Enhancing the endogenous regenerative capacity of AT2 cells, therefore, represents a promising therapeutic strategy for maintaining lung structure and function in ALI. Under normal conditions, only ~1% of AT2 cells, marked by the proliferation indicator ki-67, proliferate, suggesting limited self-renewal during lung homeostasis. However, in response to lung injury, the proliferation rate of AT2 cells can dramatically increase to 10%, with nearly all newly formed AT1 cells in the damaged area originating from AT2 cells [7, 22]. However, regulation of the proliferation and differentiation of AT2 cells is very complex and differs across different stages of the life cycle of lung development, maintenance of lung homeostasis and ALI. The regulation of AT2 cell dynamics and fate in lung tissue after ALI is unclear in both injured and uninjured regions [6, 23–25].

In this review, we focus on the temporally and regionally distinct AT2 cells that contribute to development and repair. The spatial and temporal heterogeneity of AT2 cells during lung development and the maintenance of lung homeostasis and after lung injury reflects the heterogeneity of their repair capacity. The repair ability of AT2 cells directly affects the prognosis of ALI/ARDS patients [6]. We explored the repair ability of AT2 cells from different dimensions, comprehensively analyzed their relationships with lung repair outcomes and key regulatory mechanisms, and explored new strategies for preventing and treating ALI/ARDS.

Review

Temporal heterogeneity of AT2 cells

The fate and function of AT2 cells are profoundly influenced by temporal factors, particularly in the context of lung injury and repair. These temporal variations are shaped by two primary factors: the different time stages following injury and the age of the individual at the time of injury. The specific timing after injury determines how AT2 cells respond and transition through phases of inflammation, repair, and fibrosis, while the age of the individual introduces additional complexity, as the regenerative capacity of AT2 cells diminishes with age. Together, these factors create a dynamic landscape in which the behavior and regulatory mechanisms of AT2 cells vary significantly over time, underscoring the importance of understanding these temporal dimensions to develop more effective treatments for lung injury and disease.

The fate of AT2 cells in different stages of lung injury, repair, and fibrosis

After lung injury, AT2 cells undergo distinct phases—characterized by inflammation, repair, and fibrosis—each influencing their function and destiny. Our group's study in a mouse ALI model revealed that lung homogenates

between 3 and 7 days postinjury had different effects on AT2 cell proliferation and differentiation, respectively [26]. These findings further confirm that AT2 cells have distinct fates at different stages of injury, with complex regulatory mechanisms governing their phenotypic changes (Figure 1).

Immunomodulation by AT2 cells during inflammation

AT2 cells play a critical role in maintaining lung immune homeostasis by balancing suppressive and proinflammatory signals. For example, CD200–CD200R signaling initiates anti-inflammatory responses [27]. Alveolar macrophages express CD200R, highlighting their role in regulating inflammation [28]. Increased CD200 on AT2 cells during heightened inflammation enhances resistance to infections such as influenza [29]. By binding to CD200R, CD200 effectively suppresses macrophage activation and reduces the production of pro-inflammatory cytokines, thereby minimizing damage to lung epithelial cells. In contrast, CD200 deficiency results in increased susceptibility to infection, delayed resolution, and higher mortality [29, 30]. Moreover, studies have shown that C3 from AT2 cells plays an important role in suppressing inflammation in the lungs, independent of the circulating blood C3 (mainly from the liver), and plays a key role in alleviating ALI [31].

A subset of AT2 cells with low SP-C and high MHC-II expression act as antigen-presenting cells [32], playing a crucial role in lung immune regulation by directing CD4⁺ tissue-resident memory T cells (CD4⁺ TRM cells) to specific lung regions. Ablation of MHC-II on lung epithelial cells disrupts CD4⁺ TRM cell localization and function, underscoring the lung-specific immune role of these AT2 cells [33]. MHC-II is also essential for PD-L1 presentation, which interacts with PD-1 to modulate CD4⁺ TRM cells, contributing to immunosuppression during peak inflammation in ALI and maintaining lung immune homeostasis [33].

Proliferation and differentiation in repair

AT2 cell fate during lung repair is governed by a network of signaling pathways. Initially, AT2 cells proliferate to maintain stemness; later, they differentiate into AT1 cells, which are essential for alveolar structure and air–blood barrier restoration. Wnt signaling is critical, with increased Wnt production postinjury driving AT2 cell proliferation through autocrine signaling. Lineage-tracing and AT2-specific gene-deficient mouse studies highlight the importance of Wnt/ β -catenin signaling in AT2 cell proliferation, while β -catenin knockdown triggers AT1 cell differentiation [22, 34].

The Notch signaling pathway also plays a key role in lung repair. In an ALI mouse model induced by *Pseudomonas aeruginosa*, the atypical Notch ligand-like homolog Dlk1 was shown to regulate AT2-to-AT1 cell differentiation. Early repair activates Notch signaling in AT2 cells, whereas Dlk1 inhibits it in later stages, with Dlk1 deletion leading to impaired AT1 differentiation due to sustained Notch activation [35]. Additionally, BMP signaling is integral to lung homeostasis and repair and is typically activated in AT2 and AT1 cells during homeostasis but downregulated during AT2 proliferation and upregulated during differentiation into AT1 cells after injury. BMP inhibition promotes AT2 proliferation, whereas BMP activation enhances AT1 differentiation [36]. The aforementioned signaling pathways primarily govern the proliferation or differentiation of AT2 cells by modulating gene expression at various stages. Typically, the signaling

pathways that stimulate the proliferation of AT2 cells are reduced, whereas the expression of pathways linked to differentiation is increased.

These pathways regulate AT2 cell proliferation and differentiation by modulating gene expression, typically reducing proliferation signals and increasing differentiation signals. However, transcription factors such as Tfc2l1 do not adhere to simple temporal patterns, complicating the regulatory process. Morrissey's team reported that the grainyhead transcription factor cellular promoter 2-like 1 (Tfc2l1) differentially regulates AT2 cell fate across injury stages—suppressing AT2 proliferation early (Day 10) but promoting AT2 cell proliferation and differentiation into AT1 cells later (Day 14) [23]. This complexity suggests that no single molecule can fully govern AT2 cell fate, as it requires coordinated interactions between intrinsic cellular needs and the microenvironment. Proper lung repair hinges on the interactions of various factors, including inflammation regulation [37], metabolic stabilization [38, 39], telomerase maintenance [40], and mechanistic environment control [41, 42]. Failure to meet these demands can lead to an unregulated AT2 cell fate, risking progression to fibrosis or mortality.

Transitional states and fibrosis

AT2 cells in various lung injury models exhibit transitional states during repair, a normal phenomenon that can predict poor outcomes if persistent [37, 42–46]. Pulmonary fibrosis may occur when AT2 cells are unable to meet the necessary demands during the repair phase, as previously mentioned. Conversely, successful transition to AT1 cells takes place if the conditions are optimal. In a lipopolysaccharide-induced alveolar regeneration model, AT2 cells progress through the proliferative, cell cycle arrest, and transitional phases [47]. Some AT2 cells in damaged lungs display low levels of AT2 markers without increased AT1 gene expression, indicating a 'transitional state' marked by the shared characteristics of both cell types, with high expression of Krt8, Krt18, and Cdkn1a [44, 46]. These cells, termed damage-associated transient progenitors (DATPs) or prealveolar type-1 transitional cells (PATs), represent a critical phase in cell fate determination.

Choi et al. [37] applied scRNA-seq and discovered a new population of AT2-lineage cells called DATPs, which form during alveolar regeneration. A fraction of AT2 cells expressing Il1r1 are primed for conversion into DATPs via HIF1 α -mediated glycolysis by interstitial macrophage-derived IL-1 β , which is necessary for mature AT1 cell development. However, chronic inflammation and persistent IL-1 β hinder AT1 differentiation by promoting DATP accumulation, impeding lung regeneration [37]. IL- β -induced ER stress has been suggested as a mechanism for DATP formation [38, 48, 49]. Auyeung et al. [49] found that IRE1 α , which is crucial in the unfolded protein response to ER stress, enhances DATP availability and function. Inhibiting IRE1 α , either through KIRA8 or gene knockout, reduces TGF- β signaling, the senescence-associated secretory phenotype (SASP), DATP numbers, and fibrosis in the bleomycin model. These findings suggest that IL-1 β -induced IRE1 α activation and ER stress drive DATP formation.

Kobayashi's work showed that AT2 cells enter a transitional state called PATs before maturing into AT1 cells, with these cells being more sensitive to DNA damage due to stretching during differentiation [50]. PATs cells exhibit increased

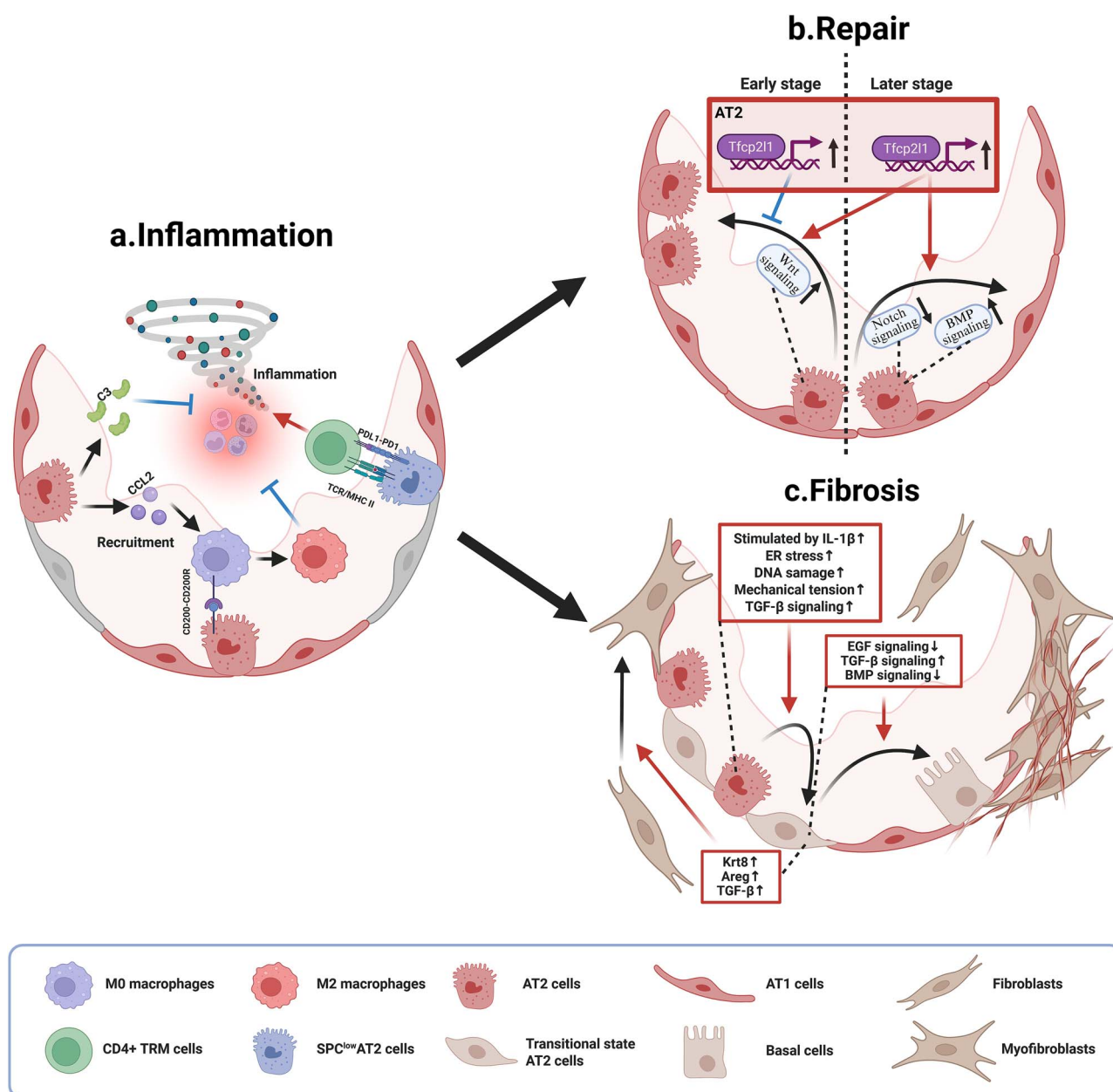


Figure 1. Temporal heterogeneity of AT2 cells during lung injury, repair, and fibrosis. **(a)** Inflammation Phase: AT2 cells play a central role in regulating inflammation by interacting with immune cells such as macrophages and CD4⁺ TRM cells. They release CCL2 to recruit macrophages and use CD200–CD200R signaling to control inflammation. **(b)** Repair Phase: Early-stage AT2 cells proliferate via Wnt signaling. As repair progresses, later-stage AT2 cells differentiate into AT1 cells through Notch and BMP pathways. The transcription factor Tfc211 is key during this stage, initially suppressing AT2 proliferation and later promoting both proliferation and differentiation at more advanced stages of repair. **(c)** Fibrosis Phase: In the fibrosis phase, persistent IL-1 β , TGF- β , and other injury-related signals drive AT2 cells into a damage-associated transitional state. In this state, AT2 cells secrete Areg and TGF- β , which stimulate fibrosis by activating myofibroblasts. Additionally, the downregulation of EGF and BMP signaling disrupts AT2 differentiation, leading to transdifferentiation into basal-like cells. This figure illustrates the heterogeneity of AT2 cell behavior throughout the stages of lung injury, repair, and fibrosis. AT2 alveolar type 2 cells, TRM tissue-resident memory, CCL2 C-C motif chemokine ligand 2, CD200R CD200 receptor, BMP bone morphogenetic protein, TGF- β transforming growth factor beta, IL-1 β interleukin 1 beta, EGF epidermal growth factor, Areg amphiregulin

levels of TP53, TGF- β , DNA damage-response signaling, and senescence, with TP53 directly regulating their transcription. Tang's team reported that mechanical tension transiently activates TGF- β signaling in these cells, with Cdc42 deficiency in AT2 cells leading to persistent mechanical tension, sustained TGF- β signaling loop activation, and progressive fibrosis [42, 47]. Although most studies suggest that TGF- β acts as a pro-fibrotic factor [51, 52], it also plays a critical role in regulating AT2 cell plasticity. During the differentiation of

AT2 cells into AT1 cells, the activation and deactivation of TGF- β signaling directly determine the progression of this process [47]. Furthermore, the role of TGF- β signaling in regulating AT2 cell fate is influenced by multiple factors, such as the counterbalance of BMP signaling [53] and the direct activation effects of the PCLAF-DREAM axis [54]. Therefore, the role of TGF- β signaling in lung repair is not straightforward, particularly in its spatiotemporal regulation of AT2 cell fate, which warrants further investigation.

Recent studies have shown that the transitional states of AT2 cells observed in mice also occur in human lung tissues [44, 55]. In human AT2 cells (hAT2s), transitional states occur during differentiation into both AT1 and Krt5⁺ basal cells. Neighboring CTHRC1hi/TGFB1hi pathogenic fibroblasts may enhance this process, where modulating TGF- β 1 and inhibiting BMP signaling facilitate hAT2 trans-differentiation into basal cells [45, 56]. Murthy and colleagues also confirmed alternative differentiation trajectories of AT2 cells. In regenerating lungs, AT2 cells transiently acquire an AT0 state (marked by SPC and SCGB3A2, which is similar to BASCs. However, SCGB3A2 and SCGB1A1 show distinct expression patterns in club cells, and their similarities and differences require further investigation) before differentiating into either AT1 cells or terminal and respiratory bronchiole-secretory cells, guided by a signaling hub of LGR5⁺ fibroblasts, particularly those involved in EGF signaling [46]. This highlights that the AT2 differentiation program in humans differs from that in mice, suggesting potential species-specific differences in AT2 cell fate that may complicate the translation of theoretical findings into clinical treatments.

Even more concerning is that transitional AT2 cells actively contribute to the progression of fibrosis. Wang et al. reported that the persistence of transitional AT2 cells through a Krt8-dependent mechanism leads to refractory fibrosis by regulating chemokine expression and macrophage recruitment, thereby reinforcing fibrogenesis [46]. Another study further suggested that transitional AT2 cells specifically express amphiregulin (Areg), leading to the abnormal activation of fibroblasts into myofibroblasts, which in turn causes severe fibrosis [57]. This team validated the correlation between Areg plasma levels and lung function in patients with IPF in their following human studies, and further validated it in IPF patient tissue sections. Furthermore, the team has developed a targeted drug for this target, PMG1015, which has now begun clinical trials. These findings indicate that transitional AT2 cells are not merely a transient phenomenon but may actively participate in the fibrotic process, contributing to the progression of fibrosis. In addition to AT2 cells, transitional states have also been identified in alveolar fibroblasts, particularly those characterized by Sfrp1 and Runx1. These fibroblasts interact with AT2 cells and coordinate the composition of the ECM, contributing to lung fibrosis [58, 59]. This highlights that different cell types undergo unique transitional states, underscoring the complexity of lung injury repair processes. In summary, AT2 cells are central to lung repair, and their fate is determined by complex signaling pathways and interactions within the lung environment. Transitional AT2 cells, while critical for regeneration, can also drive fibrosis if their transition is dysregulated.

In addition to their roles mentioned above, AT2 cells are crucial for alveolar liquid clearance (ALC) by regulating ENaC and Na-K-ATPase, which mediate sodium transport and fluid removal [60]. During conditions like influenza or ARDS, these channels are suppressed by cytokines (e.g. KGF, TNF- α), oxidative stress, and viral damage, leading to pulmonary edema. β 2-adrenergic agonists (e.g. salbutamol) have shown promise in preclinical models by enhancing ENaC/Na-K-ATPase function and restoring ALC [61]. Therefore, understanding the phenotypic and fate changes of AT2 cells after lung injury could offer valuable

insights for developing new therapeutic strategies for lung repair.

The fate of AT2 cells in aging lung

Lung tissue repair capacity declines markedly with age, leading to worse outcomes in elderly patients with lung injury, as seen during the COVID-19 pandemic and in animal models where aged mice exhibit higher mortality, greater fibrosis, and impaired alveolar regeneration [62–66]. While younger mice can regenerate alveoli after pneumonectomy, this capacity diminishes by 9 months of age [67, 68]. This regenerative decline is largely attributable to dysfunction in AT2 cells, which in aged lungs show reduced self-renewal, diminished differentiation capacity, and increased apoptosis [69, 70]. The regulation of AT2 cell fate by senescence is a complex process influenced by intrinsic factors such as metabolism and telomere dysfunction [69, 70], as well as intercellular interactions within the microenvironment [71] (Figure 2).

Telomere instability plays a critical role in AT2 cell aging. Specifically, FBW7-mediated degradation of telomere protection protein 1 (TPP1) leads to telomere uncapping, triggering DNA damage and cellular senescence, ultimately promoting fibrosis [40]. Therapeutic stabilization of TPP1 or inhibition of FBW7 improves AT2 cell numbers and lung function in aged or stressed lungs [72, 73]. In parallel, metabolic impairment—particularly AMPK–PFKFB2 pathway inactivation—suppresses glycolysis and AT2 cell differentiation, fostering the emergence of dysfunctional Krt8⁺ transitional cells. Additional contributors such as oxidative stress, lipid accumulation, and epigenetic alterations further impair AT2 cell regenerative capacity [74–77].

Beyond intrinsic aging, the alveolar niche—comprising mesenchymal and immune cells—also regulates AT2 cell fate. Mesenchymal–epithelial interactions are vital for stem cell maintenance and are modulated by growth factors [78, 79]. The role of senescent fibroblasts in AT2 cell repair is context-dependent. Retinoic acid-treated fibroblasts from aged mice can increase PDGFA secretion and promote epithelial differentiation through paracrine signaling [80]. Conversely, fibroblasts expressing the senescence marker p16^{INK4a} have shown both supportive and detrimental effects: while they may enhance regeneration under certain inflammatory conditions [81–83], their accumulation in chronic injury models, such as bronchopulmonary dysplasia, impairs alveolar repair—an effect reversible by targeted depletion of these cells [84]. Thus, p16^{INK4a}⁺ senescent fibroblasts are not always beneficial for lung repair. The senescence of immune cells also critically influences AT2 cell repair capacity. Regulatory T cells (Tregs) promote AT2 cell-mediated lung repair after injury by secreting factors such as KGF and Areg [85–87]. However, in aged mice, their ability to support this healing process is diminished [66]. Alveolar macrophages, which are essential for lung regeneration, also exhibit age-related changes, including reduced expression of cell cycle genes and a decreased population in aged mice [88, 89], although the precise mechanisms by which they influence AT2 fate remain unclear.

Mechanical stress, increasingly prominent in aged lungs due to ECM remodeling [76], tissue stiffening [90, 91], and structural alterations [92], significantly affects AT2 behavior [42, 93]. Post-resection mechanical tension activates JNK, p38, and YAP pathways through actin remodeling and requires Cdc42 activity for effective regeneration [93]. In aged mice,



Respiratory diseases impact various parts of the respiratory system, including the trachea, bronchi, bronchioles, and alveoli, with epithelial progenitor cells from different regions playing key roles in maintaining and repairing the epithelial layer under diverse conditions. Basal cells act as lung epithelial stem cells, rapidly proliferating and differentiating to restore the damaged epithelial layer of the trachea and bronchi [96–98]. In the bronchioles, club cells (Clara cells) can proliferate and differentiate into multiple epithelial cell types, including club and ciliated cells, and can revert to an undifferentiated state to transform into basal cells when needed, especially if basal cells in the tracheal area are compromised [99–101].

In the alveolar region, AT2 cells can rapidly proliferate and differentiate into AT1 cells to repair damage [7, 102].

Repair capacity of different regions of the alveoli

The alveolar region in ALI patients suffers significant epithelial damage, making the repair of alveolar epithelial cells crucial. The regenerative ability of AT2 cells, the primary seed cells in the alveoli [7], varies depending on the location of injury. Morrissey's team reported that AT2 cells in the injury junction region exhibit the highest proliferative activity, which is regulated by FGFR2 and promotes proliferation and inhibits differentiation. This mechanism is specific to the injury junction region, highlighting the varied repair capacities of AT2 cells in different lung regions [24].

Recent studies have shown that AT2 cell origins may differ by region postinjury. While traditionally believed to arise from AT2 cells themselves [7, 22], BASCs [10], and even AT1 cells [12, 13], Liu et al.'s research using dual recombinase-mediated genetic approaches revealed that in severe injuries, AT2 cells may be derived from existing AT2 cells, club cells, and BASCs but not AT1 cells. Notably, AT2 cells from club cells and BASCs are localized only to the bronchiolar region [10, 11].

The repair process in the alveolar region does not rely solely on AT2 cells; seed cells from other regions, such as bronchial basal cells, also contribute under certain conditions [10, 11]. The severity of injury across lung regions varies, leading to different inflammatory mediators, chemokines, and growth factors, affecting cell proliferation, differentiation, and participation in repair [78]. AT2 cells maintain their daily balance and repair modest damage to the distal lung parenchyma [7, 22]. However, severe lung injury can lead to AT2 cell death, hindering their regenerative ability. In such cases, bronchial basal cells, including DASCs, play a critical role in lung tissue restoration. These Krt5- and P63-marked cells can differentiate into secretory and ciliated cells in the bronchi, and they can migrate to the distal lung to repair damaged parenchyma [11, 15, 103]. Frank McKeon's team first identified these cells in the distal bronchi of mice in 2011 [103]. By 2015, they demonstrated that these Krt5-expressing bronchial basal cells undergo proliferation, migration, and differentiation post-influenza infection [94]. Zuo et al. further showed that removing bronchial basal cells in mice led to impaired lung function repair and fibrosis development, underscoring their essential role in lung repair [11].

Our team reported that connective tissue growth factor (CTGF) in the post-ALI microenvironment stimulated the growth of specific AT2 cell subpopulations [104]. CTGF facilitates the interaction between Krt5 and LRP6 in AT2 cells, activating the Wnt signaling pathway. These findings suggest that Krt5⁺ AT2 cells may originate from Krt5⁺ basal cells, with CTGF potentially facilitating their migration to the alveolar region and transformation into AT2 cells. Further investigation is needed to fully understand this process. Additionally, our analysis of bronchoalveolar lavage fluid from ARDS patients revealed a strong correlation between Krt5⁺ cells and disease severity, confirming that severe lung injury triggers Krt5⁺ cell activation, supporting the activation of regional lung stem progenitor cells in lung repair [104].

The regulatory role of spatial heterogeneity of mechanical tension on AT2 cell repair capacity

Mechanical tension in the lungs is unevenly distributed due to the structure of the lung and the influence of gravity, with alveoli at the periphery being more likely to be stretched.

Tang et al. [42] found that the spacing between AT2 cells is more uneven at higher lung pressures, particularly in the lung periphery, which may contribute to the progression of IPF from the periphery to the center. This differs from the previously mentioned ECM remodeling caused by aging factors and is more closely related to differences in lung anatomical positioning. However, this also highlights the critical role of spatiotemporal factors in regulating AT2 cell fate through mechanical tension. Stretched AT2 cells produce TGF- β ligands, activating TGF- β signaling in both AT2 and neighboring stromal cells. Persistent mechanical tension leads to a constitutively activated TGF- β signaling loop in AT2 cells, driving progressive lung fibrosis. Reducing TGF- β signaling in AT2 cells has been shown to slow fibrosis progression [105].

However, proper mechanical stress was found to be one of the key factors in initiating alveolar regeneration. Increased mechanical tension in the alveolar epithelium, triggered by respiration following lung resection, promotes actin polymerization and YAP-dependent cell proliferation in AT2 cells [93]. Excessive mechanical tension may increase intracellular ROS levels in AT2 cells and activate the FasL/Fas exogenous death signaling pathway [106]. Moreover, mechanical stress also enhances endoplasmic reticulum stress in AT2 cells and increases the expression of chemokines, such as CCL2 and CCL4, which in turn enhance monocyte recruitment [107]. Hence, different degrees of mechanical tension may determine the different fates of AT2 cells.

However, further exploration is needed to determine the boundaries for assessing the advantages and disadvantages of mechanical stress in regulating the repair function of AT2 cells. Identifying an endogenous indicator of the mechanical tension response within AT2 cells may be essential. Cdc42 is currently recognized as a crucial molecule in the response to mechanical tension, although the specific threshold for its role remains unclear. *In vitro* experiments have demonstrated that primary AT2 cells exhibit significant upregulation of Cdc42 when stretched. This upregulation mediates cytoskeletal remodeling and activates YAP, promoting cell proliferation and chromatin remodeling. Additionally, nuclear-layer-chromatin interactions play a role in maintaining the alveolar structure by supporting the normal mechanical tension within the alveoli. However, in the absence of Cdc42, AT2 differentiation is impaired, leading to sustained mechanical tension that prompts AT2 cells to secrete TGF- β 1 in an autocrine manner, stimulating fibroblasts and compensating for the increased mechanical tension [42, 93]. Despite these findings, abnormal expression of Cdc42 has not yet been observed in fibrosis patients, suggesting that its role warrants further investigation. Clarifying the limits of mechanical tension on AT2 cells could provide guidance for setting parameters during mechanical ventilation in ARDS patients, ultimately enhancing treatment efficacy and prognosis for individuals with ARDS.

Influence of the spatial distribution discrepancies of other lung tissue cells on AT2 cell repair capacity

AT2 cells do not act in isolation but are embedded in a complex microenvironment that includes immune and stromal cell types, which dynamically interact to regulate lung homeostasis and repair. Fibroblasts, macrophages, and Treg cells play crucial roles in regulating AT2 cell fate, with evidence suggesting that these effects are influenced by their spatial positioning within the lung [25, 108]. The interstitial microenvironment of the lung is key to maintaining alveolar

homeostasis and promoting regeneration. For example, Fgf10 expression in distal mesenchyme cells influences nearby distal buds [109], and Wnt signaling from the mesenchyme promotes alveolar development [110]. Specific mesenchymal cell subpopulations, such as Lgr5⁺ cells, support alveolar lineages, whereas Lgr6⁺ cells are associated with bronchiolar lineages, reflecting their anatomical locations [111]. Axin2⁺Pdgfra⁻ mesenchymal cells, located farther from AT2 cells, contribute to the formation of fibrogenic myofibroblasts. In contrast, nearby Axin2⁺Pdgfra⁺ mesenchymal alveolar niche cells play a crucial role in promoting AT2 cell proliferation [112]. Similarly, research has confirmed that Pdgfra⁺ lipofibroblasts, which are positioned adjacent to AT2 cells, create a supportive niche that facilitates AT2 self-renewal and differentiation [113, 114]. This highlights the significance of spatial proximity in these regulatory processes. Further investigation is needed to determine whether these clusters represent distinct cell types with unique functional characteristics and to reveal the underlying mechanisms dependent on spatial proximity.

Macrophages are also essential in lung injury responses, and their interaction with AT2 cells is crucial for determining their fate. Interstitial macrophages influence AT2 cell destiny by secreting IL-1 β , whereas AT2 cells, in turn, influence AT2 cells through the release of CCL2, promoting AT2 cell proliferation [37, 115]. However, the crosstalk between macrophages and AT2 cells may depend on spatial positioning. A study by Westphalen et al. revealed that macrophages regulate AT2 cell AKT signaling through calcium ion exchange mediated by connexin 43 (Cx43), influencing immune modulation and reducing lung injury [116]. Additionally, mesenchymal stem cells interact with AT2 cells via Cx43, transferring mitochondrial support to increase their repair capacity [117].

In the lung injury microenvironment, the spatial arrangement of immune and stromal cells—such as alveolar and interstitial macrophages, Tregs, and fibroblasts—plays a pivotal role in regulating AT2 cell function. Direct interactions and paracrine signaling are heavily influenced by spatial proximity, yet our understanding of these spatially governed regulatory networks remains incomplete (Figure 3). As previously discussed, temporal factors can drive functional changes in other cell types, subsequently impacting AT2 cells. Likewise, within the microenvironment of lung injury, cell–cell interactions must be analyzed through the lens of both spatial and temporal dynamics. Developing a more comprehensive spatiotemporal blueprint to understand how these interactions influence AT2 cell repair could provide valuable insights and pave the way for precision-targeted therapeutic strategies.

Spatiotemporal multiomics resolution of heterogeneity in the repair capacity of AT2 cells

Single-cell spatial multiomics technology provides a powerful approach for deciphering the molecular landscape of AT2 cells and their microenvironment during tissue repair and regeneration [118, 119]. By integrating single-cell RNA sequencing (scRNA-seq) with spatial transcriptomics, researchers can explore the cellular diversity and spatial organization within tissues at unprecedented depths. By capturing transcriptomic profiles from thousands of individual cells, scRNA-seq allows the identification of distinct cell types and their functional states, revealing rare populations and specific transcriptional programs involved in repair [118, 120]. However, these methods lack spatial context, which is crucial for understanding cell interactions within their native environment.

Spatial transcriptomics addresses this gap by preserving the spatial coordinates of RNA molecules in tissue sections, enabling the discovery of spatial gene expression patterns and cell–cell interactions [121]. The combination of scRNA-seq and spatial transcriptomics offers a comprehensive view of gene expression in AT2 cells and neighboring cells, such as fibroblasts and immune cells, which are key players in the repair process.

This integrated approach uncovers spatially resolved gene expression dynamics and intercellular communication networks that regulate AT2 cell repair abilities [119]. Additionally, coupling these multiomics data with advanced computational tools enhances the identification of regulatory networks and signaling pathways critical for tissue repair, paving the way for novel therapeutic strategies targeting lung diseases with impaired regeneration, such as ARDS and fibrosis [112, 122–124].

Spatiotemporal multiomics studies of lung development

Pioneering work by Ljungberg et al. [125] introduced the use of spatial *in situ* hybridization to explore the spatial dynamics of lung development, with a focus on gene expression during murine alveolarization. With advances in high-throughput techniques, spatial transcriptomics has shifted the focus from animal models to human subjects, increasing clinical relevance.

Building on this foundation, He et al. [94] analyzed human lung samples from 5 to 22 weeks postconception via scRNA-seq, scATAC-seq, spatial transcriptomics, and mRNA *in situ* hybridization. They constructed a detailed single-cell atlas; identified 144 distinct cell types, including unknown progenitor and transitional cells; and revealed critical interactions within lung niches, such as the VEGFA–FLT1/FLT4/KDR interaction between AT2 cells and microvascular endothelial cells, which plays a key role in AT2 cell fate. Sountoulidis et al. [126] further advanced this field by combining scRNA-seq with spatial transcriptomics to create a spatiotemporal atlas of early human lung development. An analysis of 17 embryos from gestational weeks 5 to 14 revealed 83 distinct cellular states and highlighted key interactions among mesenchymal, epithelial, and neuronal cells. Similarly, Cao et al. [127] used single-cell and spatial transcriptomics, alongside single-molecule *in situ* hybridization, to map human lung development, revealing distinct transcriptional programs for proximal and distal lung cells. They also identified a subset of BDNF-expressing interstitial cells that secrete multiple signaling factors, such as BDNF, FGF9, and FGF10, thereby promoting AT2 cell proliferation and branching.

Many research teams utilizing scRNA-seq technology have consistently shown that the gene expression pattern of AT2 cells at E18.5 closely resembles that in adult mice, indicating that AT2 cells are largely developed at this stage [128]. Similar findings have been reported in human samples [94, 129], where a corresponding cluster of cells expressing both AT1 and AT2 marker genes has been identified, suggesting the presence of bipotential progenitor cells in human lung development as well. However, the persistence and potential of these cells as lung progenitors remain areas for further exploration [102, 130].

These studies provide a comprehensive spatiotemporal atlas of human lung development, highlighting key mechanisms of AT2 cell fate and epithelial differentiation. Understanding these developmental processes is essential for advancing regenerative medicine, as early-stage stem cells possess

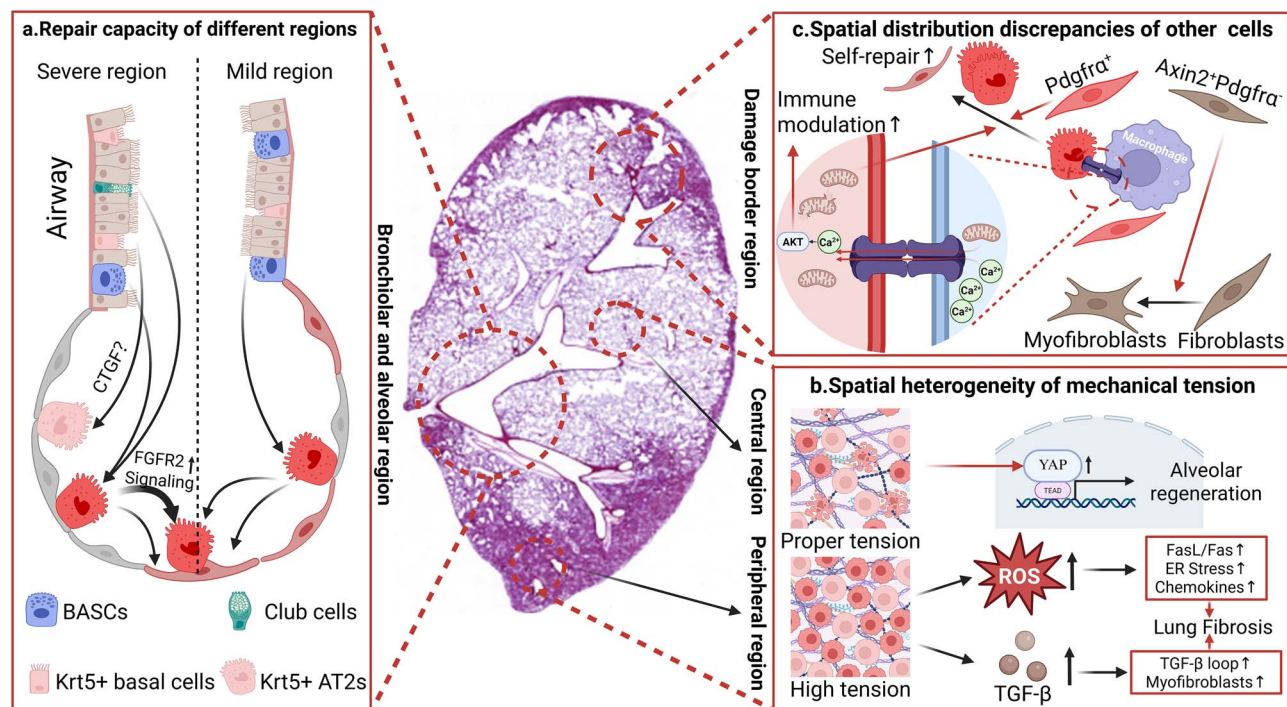


Figure 3. Spatial heterogeneity of AT2 cells in lung repair and regeneration. Spatial heterogeneity significantly impacts AT2 cell function across different regions of the lung. **(a)** Repair Capacity in Different Lung Regions: In the alveolar region, AT2 cells proliferate and differentiate into AT1 cells to repair damaged alveoli, with FGFR2 signaling enhancing proliferation in regions with severe damage, promoting efficient repair. In the bronchiolar region, Krt5+ basal cells, BASCs, and club cells play critical roles in regeneration, especially under severe injury conditions, differentiating into AT2 cells to aid local repair. **(b)** Spatial Heterogeneity of Mechanical Tension: In central lung regions, mechanical stress activates YAP signaling, promoting AT2 cell proliferation and alveolar regeneration. However, in peripheral lung regions, excessive mechanical tension leads to ROS production, triggering the FasL/Fas pathway, which induces AT2 cell apoptosis. Prolonged mechanical stress further enhances TGF- signaling, advancing fibrosis. **(c)** Spatial Distribution of Other Cells: The positioning of immune and stromal cells is crucial in regulating AT2 cell fate. $Pdgfr^+$ fibroblasts near AT2 cells create a supportive niche that enhances AT2 self-renewal and differentiation, while $Axin2+Pdgfr^-$ fibroblasts located farther away contribute to the formation of fibrogenic myofibroblasts, promoting fibrosis. Macrophages assist AT2 cell repair by transferring mitochondria via Cx43, supporting AT2 regeneration, while calcium signaling through Cx43 modulates AKT pathways, regulating immune responses and reducing lung injury. AT2 alveolar type 2 cells, FGFR2 fibroblast growth factor receptor 2, BASC bronchioalveolar stem cell, ROS reactive oxygen species, FasL Fas ligand, TGF- β transforming growth factor beta, CX43 connexin 43, $Pdgfr$ platelet-derived growth factor receptor alpha, EMT epithelial-mesenchymal transition

unique therapeutic potential owing to their increased stemness and self-renewal capabilities. This knowledge is critical for developing future therapies to repair lung tissue in pulmonary diseases.

Spatiotemporal multiomics studies of lung injury

Spatiotemporal multiomics approaches are now essential for studying lung injury, particularly for understanding the roles of AT2 cells in repair and regeneration. These methods allow for an integrated analysis of temporal and spatial factors, offering a comprehensive view of how lung tissue and AT2 cells evolve postinjury. While earlier studies relied on techniques such as *in situ* hybridization and laser capture microdissection, which laid important groundwork, these studies were limited in capturing the full complexity of cellular interactions [131]. The advent of spatial transcriptomics has overcome these limitations, enabling deeper insights into cellular responses to lung injury and informing therapeutic strategies. However, much of the research has focused on the inflammatory phase, with less emphasis on the subsequent repair processes.

For example, Boyd's group [124] identified three activation states in lung fibroblasts during respiratory viral infections—ECM-synthesizing, damage-responsive, and interferon-responsive. Notably, damage-responsive fibroblasts (DRFibs)

in inflammatory zones disrupt lung repair by promoting immune cell infiltration and fibrosis, with ADAMTS4 expression playing a key role in exacerbating fibrosis. Dinno et al. [132] used GeoMx DSP technology to study SARS-CoV-2-infected mice, revealing significant upregulation of inflammatory genes in alveolar and bronchial regions, particularly within the IFN, IL-1, and NF- κ B pathways. They observed that AT2 cells exhibited widespread infection with minimal apoptosis, leading to slower recovery in AT2-rich alveolar regions.

The COVID-19 pandemic further accelerated spatial omics research, with studies such as Butler et al. [133] using GeoMx to analyze spatial gene expression in COVID-19 lung samples. They found that spatially specific ACE2 expression was linked to the presence of SARS-CoV-2, along with increased macrophages and neutrophils, emphasizing the role of immune-mediated inflammation in COVID-19 pathology. Building on these findings, Delorey et al. [134] mapped the spatial transcriptomes of COVID-19 patient lungs, identifying alveolar type I and II cells as predominant in epithelial regions, whereas fibroblasts and myofibroblasts were more prevalent in nonepithelial regions. They also noted upregulated interferon- α/γ responses and oxidative phosphorylation genes in COVID-19 lungs, with downregulated TNF α , IL2-STAT5, TGF signaling, and tight

junction pathways, highlighting the complexity of COVID-19 lung pathology.

While these studies have advanced our understanding of the inflammatory response, repair studies, particularly after lung injury, have largely focused on fibrosis mechanisms. Murthy's group [56] used spatial and single-cell transcriptomics to reveal that postinjury differentiation of AT2 cells into distal cilium-secreting cells significantly impaired lung repair, underscoring the importance of maintaining AT2 cell functionality. Franzén et al. [135] provided further insights by creating detailed spatial maps of lung tissue from IPF patients and a bleomycin-induced model mice. They identified distinct fibrotic niches in IPF lungs where alveolar regeneration is hindered and discovered abnormal cell states, including a newly identified KRT5⁻/KRT17⁺ aberrant basaloid epithelial cell population, offering deeper insights into the cellular dynamics driving IPF. Notably, this specific subpopulation has not yet been identified in mice, indicating potential species-specific differences in the cellular mechanisms underlying lung fibrosis.

AT2 cells are essential for lung repair and regeneration and maintaining lung homeostasis. However, the mechanisms regulating their function and interactions during injury and repair are not fully understood. Spatiotemporal multiomics studies are crucial for uncovering these mechanisms, providing insights for developing new therapeutic strategies. By integrating spatial proteomics and metabolomics with transcriptomic data, researchers can achieve a deeper understanding of protein dynamics and metabolic processes in lung repair. For example, recent studies have shown that glycogen utilization is crucial in the transformation of fibroblasts into myofibroblasts within fibrotic lung regions [136]. This comprehensive approach could reveal novel therapeutic targets, paving the way for precision-targeted lung regeneration therapies.

New strategies and methods to increase the endogenous regenerative capacity of AT2 cells

Current therapeutic approaches for ALI and ARDS have shown limited effectiveness, prompting a focus on developing treatments that actively promote lung repair. Given the key role of AT2 cells in this process, they have become central to targeted therapeutic strategies. The primary challenge lies in developing delivery systems that can precisely target the lungs, specifically AT2 cells. Researchers have explored various platforms, including microvesicles [137, 138], liposomes [139–141], micelles [142], nanoparticles [143], and viral vectors [144, 145], engineered to enhance targeting to lung tissue and AT2 cells.

One effective approach is protein delivery systems. For example, Wnt3a has been shown to promote AT2 cell proliferation, which is crucial for lung repair. However, its hydrophobic nature limits its therapeutic application. Researchers have developed a novel approach using extracellular vesicles (EVs) to deliver Wnt3a, maintaining its biological activity and demonstrating significant therapeutic effects in lung injury models. Wnt3a-loaded EVs improved lung function, reduced abnormal alveolar structures, and promoted AT2 cell regeneration. However, the role of Wnt3a in fibroblast proliferation postinjury complicates its use, highlighting the need for selective targeting. This challenge is also relevant to other growth factors, such as EGF and FGF. Consequently, research has focused on protein delivery systems with enhanced specificity for AT2 cells. For

example, targeting pathological markers such as CD38 on AT2 cells with engineered mesenchymal stem cell-derived EVs has shown promise in reversing epithelial–mesenchymal transition and reducing age-related pulmonary fibrosis by restoring NAD⁺ levels in senescent AT2 cells.

Gene therapy approaches are also advancing, with mRNA and CRISPR technologies leading the way. The stability and safety of mRNAs, as recognized by the 2023 Nobel Prize, make them a preferred choice [146]. Lipid nanoparticles (LNPs) have been designed to deliver mRNAs encoding proteins such as BMP4 and CYB5R3, which are crucial for maintaining AT2 cell homeostasis and preventing fibrosis. In mouse models of lung fibrosis, these LNPs successfully reversed fibrotic changes and restored normal lung architecture, highlighting the potential of mRNA-based therapies [139].

CRISPR-Cas9 technology offers another powerful tool, especially for treating genetic lung diseases through precise gene editing. Viral delivery systems, such as AAV6.2FF, have shown high efficiency in targeting AT2 cells [144, 145]. For example, our laboratory has utilized the CRISPR-Cas13 RNA editing system with AAV6.2FF vectors to promote AT2 cell proliferation and enhance lung repair (unpublished data). However, safety concerns with viral vectors, particularly in lung diseases, have driven the exploration of safer alternatives such as lipid-based delivery systems. Recent studies have demonstrated that LNPs such as RCB-4-8 [140] and LUNG-SORT [141] can deliver CRISPR-Cas9 components via inhalation, achieving precise gene editing in lung epithelial cells. Although LNPs offer advantages such as a shorter half-life and the potential for repeat dosing, their targeting efficiency is currently lower than that of AAV-based systems, reaching ~70% [141] versus over 85% for AAVs [145]. Balancing the strengths of different delivery systems and optimizing their use will be crucial for future research.

In addition to protein and gene delivery systems, mitochondrial replacement therapy is emerging as a promising strategy, particularly for conditions such as pulmonary fibrosis. By engineering mesenchymal stem cells, researchers have developed methods for efficient mitochondrial transfer, restoring the function of damaged lung cells. This approach offers a novel therapeutic avenue for reversing pulmonary fibrosis and other related lung diseases [147]. Recent advancements have further enhanced this approach, such as the development of mitophagy-enhanced nanoparticles. These nanoparticles, engineered by encapsulating Parkin mRNA, facilitate the interaction between negatively charged isolated healthy mitochondria and positively charged nanoparticles, promoting mitochondrial autophagy and repair in lung epithelial cells [148].

In conclusion, advancements in protein, gene, and mitochondrial delivery systems present promising avenues for lung repair and regeneration, particularly through the targeting of AT2 cells (Figure 4). However, while developing these therapeutic strategies, it is crucial to consider the potential impact of drug delivery systems on pulmonary autoimmunity and the mucosal barrier. Viral vectors, such as AAV capsid antigens or transgene products, can be immunogenic and may trigger adverse immune responses, including the activation of T and B cells, potentially leading to lung inflammation and damage [149]. Similarly, non-viral carriers, such as lipid nanoparticles, which function similarly to adjuvants, may also interact with the immune system [150]. Furthermore, the mucosal barrier in the lungs poses a significant challenge, hindering

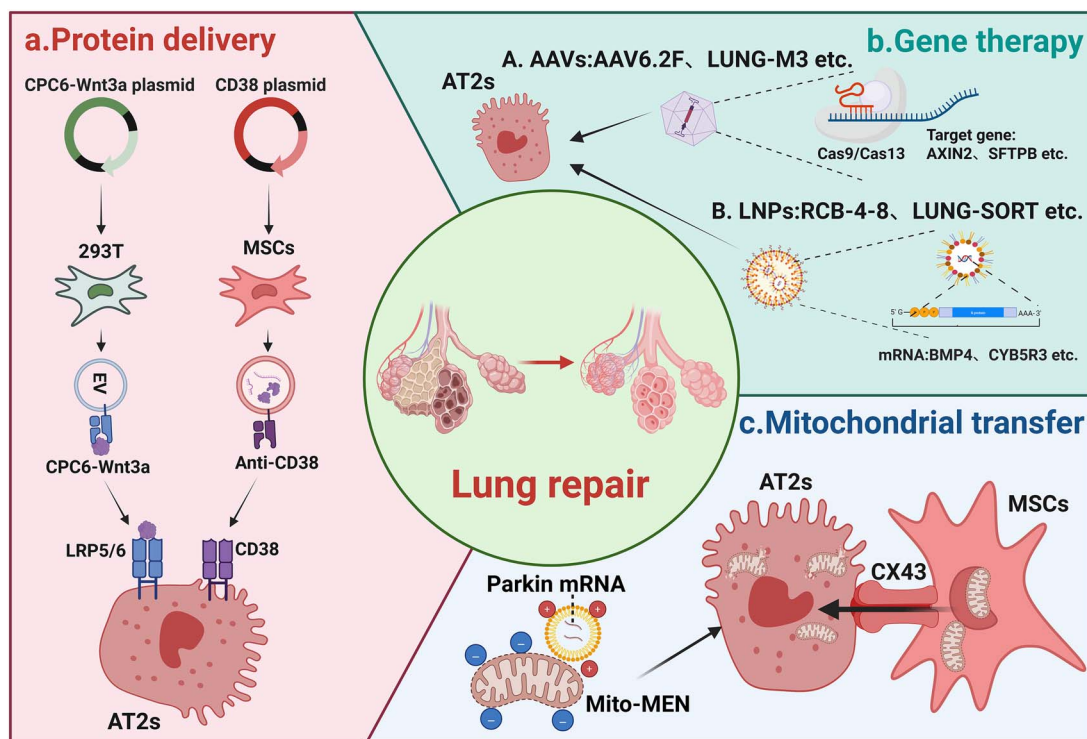


Figure 4. Therapeutic strategies for lung repair targeting AT2 cells. This figure illustrates various therapeutic strategies aimed at promoting lung repair by targeting AT2 cells. **(a) Protein Delivery:** The CPC6-Wnt3a plasmid is transfected into tool cells, which subsequently produce EVs encapsulating Wnt3a, enhancing protein activity and promoting AT2 cell regeneration in lung injury models. Similarly, anti-CD38 plasmid is transfected into MSCs to enhance their ability to target senescent AT2 cells via EVs, reversing EMT and reducing age-related pulmonary fibrosis. **(b) Gene Therapy:** AAV-based systems, such as AAV6.2F and LUNG-M3, deliver CRISPR/Cas9 and CRISPR/Cas13 components to edit genes like AXIN2 and SFTPB, which are crucial for AT2 cell function and fibrosis prevention. LNPs, such as RCB-4-8 and LUNG-SORT, deliver mRNA (e.g., BMP4 and CYB5R3) to support AT2 cell regeneration and homeostasis. **(c) Mitochondrial Transfer:** MSCs transfer mitochondria to AT2 cells via the CX43 channel, improving mitochondrial function, alleviating cellular stress, and supporting lung regeneration. Additionally, Mito-MEN, mitophagy-enhanced nanoparticles carrying Parkin mRNA, utilize electrostatic interactions between positively charged nanoparticles and negatively charged healthy mitochondria to facilitate mitochondrial autophagy and repair in AT2 cells. AT2 alveolar type 2 cells, MSC mesenchymal stem cells, CRISPR clustered regularly interspaced short palindromic repeats, AAV adeno-associated virus, LNPs lipid nanoparticles, BMP4 bone morphogenetic protein 4, CYB5R3 cytochrome b5 reductase 3, CX43 connexin 43, Mito-MEN mitophagy-enhanced nanoparticles, Parkin mRNA mRNA encoding Parkin, a protein involved in mitophagy

nanoparticles from effectively reaching target cells. Currently, strategies involving adjustments to lipid ratios, pH levels, or the incorporation of excipients are used to improve biocompatibility and stability [140, 151, 152]. Future research also should focus on the spatiotemporal regulation of AT2 cell fate during lung injury and repair. This includes developing targeted strategies for specific AT2 subpopulations and creating delivery systems capable of time-sequenced expression to align with the dynamic nature of lung regeneration. Addressing these challenges is essential for the progression toward more personalized and precise therapeutic interventions, ultimately improving outcomes for patients with lung injury and related conditions.

Conclusions

In conclusion, AT2 cells are central to lung repair and regeneration and play a pivotal role in addressing ALI and ARDS. Their regenerative capabilities are influenced by spatial heterogeneity and temporal dynamics, yet the mechanisms governing their fate and behavior remain poorly understood. Recent advances in spatiotemporal multiomics and other cutting-edge technologies have significantly expanded our comprehension of AT2 cell fate determination, findings that have been validated across both human and murine model systems. Nevertheless, multiple studies continue

to highlight the existence of species-specific differences. Therefore, identifying conserved regulatory mechanisms that drive AT2 cell function and pursuing clinical translation represent the paramount priorities. To improve therapeutic strategies, developing targeted delivery systems is crucial for effectively modulating AT2 cell functions. Approaches such as EVs for protein delivery and gene editing technologies show promise, but challenges in specificity and sustained delivery need to be addressed. Future research should focus on the spatiotemporal regulation of AT2 cells and their interactions with other cell types. By integrating insights from advanced technologies, we can deepen our understanding of lung tissue repair mechanisms. This knowledge is vital for creating personalized therapies for ALI, ARDS, and related lung diseases, ultimately improving patient outcomes and quality of life.

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Author contributions

J.J., L.Z., Z.W. and J.D. conceived the study, participated in coordination, and helped draft the manuscript. W.B., R.W., S.H., W.L. and H.Z. drafted the manuscript and substantively revised it. W.B. designed the figures. All the authors have read and approved the final manuscript.

Ethical approval statement

No ethical approval is required as the study does not contain animal or human studies.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Data availability

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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