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CURRENT TOPICS REVIEW ARTICLE

# Molecular basis of lung tissue regeneration

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**Abstract** Recent advances have expanded our understanding of lung endogenous stem cells, and this knowledge provides us with new ideas for future regenerative therapy for lung diseases. In studies using animal models for lung regeneration, compensatory lung growth, and lung repair, promising reagents for lung regeneration have been discovered. Stem or progenitor cells are needed for alveolar regeneration, lung growth, and lung repair after injury. Endogenous progenitor cells mainly participate in alveologenesis. However, human lung endogenous progenitor cells have not yet been clearly defined. Recently discovered human alveolar epithelial progenitor cells may give us a new perspective for understanding the pathogenesis of lung diseases. In parallel with such basic research, projects geared toward clinical application are proceeding. Cell therapy using mesenchymal stem cells to treat acute lung injury is one of the promising areas for this research. The creation of bioartificial lungs, which are based on decellularized lungs, is another interesting approach for future clinical applications. Although lungs are the most challenging organ for regenerative medicine, our cumulative knowledge of lung regeneration and of endogenous progenitor cells makes clear the possibilities and limitations of regenerative medicine for lung diseases.

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## Introduction

Regenerative medicine is now coming of age; however, the field of lung research is still far behind this promising new medicine. The complicated three-dimensional structure of the lungs and the involvement of many cell types in lung function make research on lung regeneration challenging. Recent discoveries of lung tissue stem cells are now opening doors in this difficult but worthy area of research.

In this review, we describe animal models for lung regeneration and lung repair and detail recent knowledge about lung stem cells. Then, recent clinical approaches toward lung regenerative medicine are discussed.

## Alveolar regeneration and repair models

Two animal models—alveolar regeneration in lung emphysema and compensatory lung growth after a pneumonectomy—are well established and well characterized models for lung regeneration research. Many lung injury models are currently being utilized to analyze the repair process of the lungs.

### Lung emphysema model

Chronic obstructive pulmonary disease (COPD) is becoming a major cause of death worldwide. Pulmonary emphysema, one of the main forms of COPD, is characterized by the progressive and irreversible loss of pulmonary alveoli. Despite recent advances in new drugs and

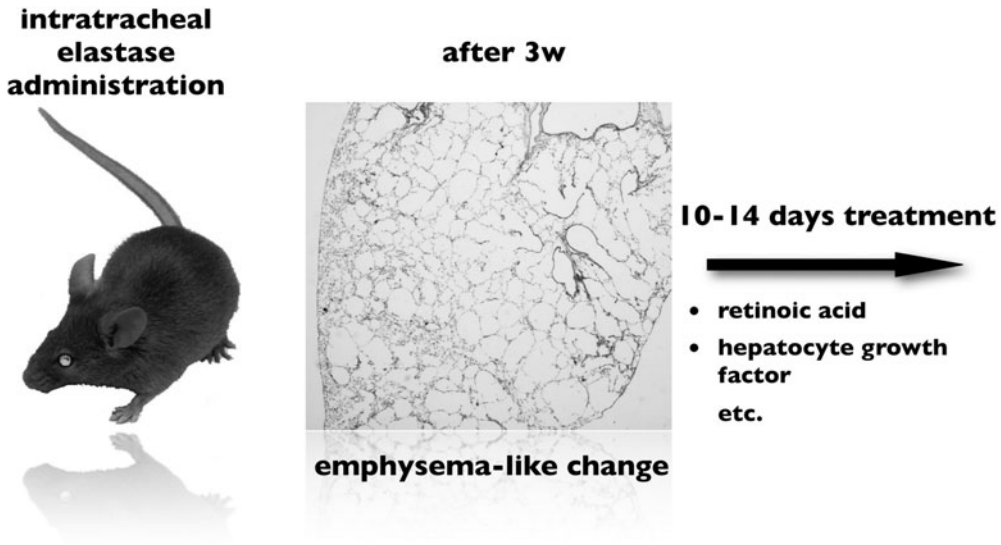


**Fig. 1** Elastase-induced lung emphysema model. *w*, weeks

in the understanding of this disease, its treatment remains palliative, and no therapy can restore the destroyed alveoli. Therefore, treatment of COPD is the most important target in lung regeneration research.

Several animal models have been developed to study COPD,[1] including the elastase model; the cigarette smoke exposure model[2]; the calorie restriction model[3]; the vascular apoptosis model[4]; and the genetic model.[5] The elastase-induced emphysema model is the most frequently used to study lung regeneration (Fig. 1). Intratracheal administration of elastase induces acute inflammation, neutrophil accumulation, and an increase in permeability. Three weeks after elastase treatment, there is no more acute inflammation, and the lung parenchyma is destroyed. Regenerative studies are performed after the development of emphysematous change.

*Reagents for lung regeneration*

Retinoic acid

Retinoic acid (RA) is involved in lung development, especially alveologenesis.[6] RA regulates embryonic branching morphogenesis[7] and genes involved in lung development and promotes alveolar septation. Deletion of RA receptors in mice causes the failure of alveologenesis, which is the formation of normal alveoli and alveolar elastic fibers.[8] Elastin synthesis in lung fibroblasts is increased by RA treatment.[9] These findings suggest the importance of RA in the morphology of the developing lung.

In 1997, Massaro et al. showed that all-trans retinoic acid (ATRA) reversed anatomical and functional disease indicators in a rat pulmonary emphysema model.[10] Since then, many studies have been performed in this area. Fourteen studies using RA in emphysema models have been reported; interestingly, eight of them observed successful lung regeneration after RA treatment, and the remaining six reported the failure of RA-induced lung regenerative capacity.[11] The reasons for this discrepancy might be (1) the difference in the species of the animal model and (2) the difference in the RA dose threshold. As described in the compensatory lung growth section below, small animals, such as rodents, have a better capacity for lung regeneration because their somatic growth continues throughout their lifespan. This may affect the results of RA treatment. Another factor is the required RA dose for lung regeneration. Stinchcombe and Maden evaluated the effect of RA on three strains of mice (TO, ICR, NIHS) and found that the RA dose threshold for inducing alveolar regeneration differed for each strain.[12]

Hepatocyte growth factor

Hepatocyte growth factor (HGF) was originally isolated as a potent mitogen for mature hepatocytes in primary cultures.[13] HGF is a pleiotropic growth factor that has been shown to have mitogenic, morphogenic, and protective effects via tyrosine kinase phosphorylation of its receptor, c-Met, after pulmonary injury[14] or during lung development.[15] In particular, it is a potent mitogen for alveolar type II epithelial cells in vivo[16] and in vitro.[17] In addition, HGF also activates migration and proliferation of endothelial cells and induces angiogenesis.[18]

Because of the potential described above, the effect of HGF on lung regeneration has been extensively studied. Intraperitoneal administration of HGF significantly increases the Sca-$1^+$/ Flk-$1^+$ fraction in peripheral mononuclear cells in mice. It also induces proliferation of both

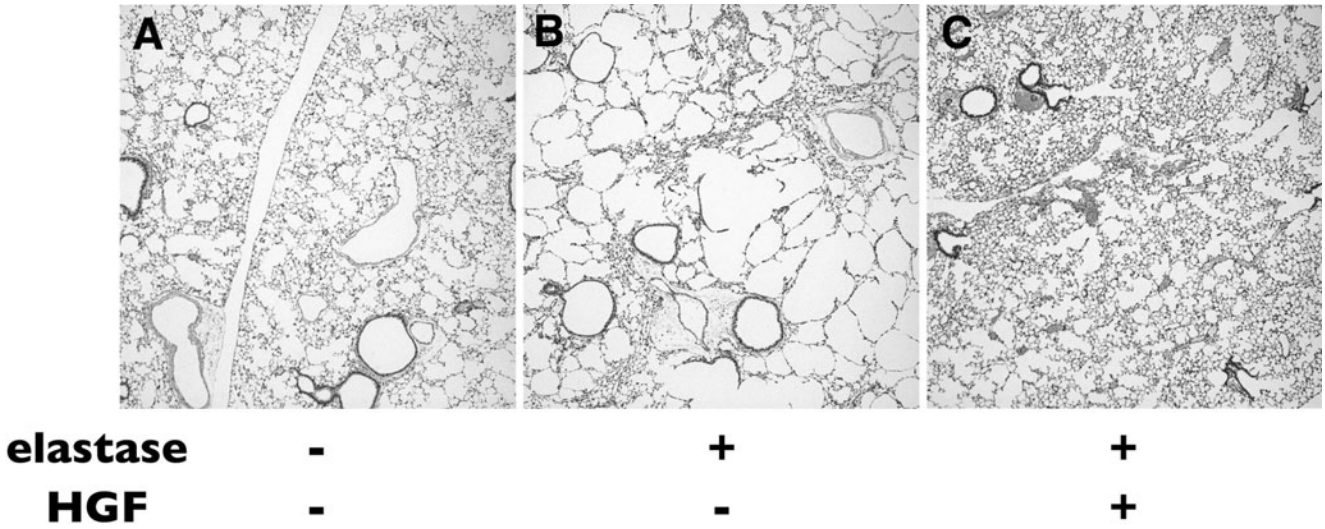


**Fig. 2** Hepatic growth factor (HGF) administration regenerates mouse emphysematous lungs. **A** Normal lung. **B** Elastase administration induces emphysematous changes in mouse lungs. **C** HGF inhalation markedly alleviates the emphysema. (×40) (Modified from Hegab et al.[21])

bone marrow-derived and resident endothelial cells in the alveolar wall, resulting in a reversal of elastase-induced pulmonary emphysema in mice.[18] Transfection of cDNA-encoding human HGF demonstrated efficient expression of HGF in alveolar endothelial and epithelial cells and resulted in a more extensive pulmonary vasculature and in inhibition of alveolar wall cell apoptosis in a rat emphysema model.[19] Intravenous injection of adipose tissue-derived stromal cells, which secrete HGF, improved emphysema in rats.[20] Hegab et al. reported that twice-weekly inhalation of HGF for 2 weeks significantly ameliorated elastase-induced enlargement of air spaces and alveolar wall destruction (Fig. 2), and that elevated static lung compliance returned to normal levels.[21]

## Granulocyte colony-stimulating factor

Granulocyte colony-stimulating factor (G-CSF) has been reported to enhance tissue regeneration and improve survival after myocardial infarction by mobilizing stem cells from the bone marrow into peripheral blood.[22] G-CSF also induces angiogenesis in postischemic tissues, such as the hindlimb.[23] In a mouse emphysema model, G-CSF treatment alone provides a significant reduction in emphysema. In G-CSF-treated mice, the alveolar mean linear intercept (Lm), used as a morphometric parameter of emphysema, showed a reduction when compared to vehicle-treated mice. This was the same degree of reduction observed in RA-treated mice. G-CSF increases circulating endothelial progenitor cells derived from the bone marrow. Combined treatment with G-CSF and RA exhibits an additive effect with an increased reduction in Lm.[24] Bone marrow-derived cells were shown to contribute partially to G-CSF-induced lung regeneration. These results suggest that the lack of circulating stem cells could be a limiting factor in elderly COPD patients.

## Keratinocyte growth factor

The keratinocyte growth factor (KGF) receptor is expressed in alveolar type II epithelial cells. KGF promotes survival, proliferation, and migration of type II cells.[25] Intratracheal administration of KGF induces alveolar type II hyperplasia.[26] Although pretreatment with KGF prevented elastase-induced emphysema, post-treatment with KGF (3 weeks after the elastase administration) did not reverse alveolar enlargement.[27] These results suggest that KGF has only an antiinflammatory effect and does not induce alveolar repair.

## Adrenomedullin

Adrenomedullin is a multifunctional regulatory peptide originally isolated from a human pheochromocytoma.[28] It is reported to induce cyclic AMP production, bronchodilation, cell growth regulation, survival of apoptosis, and angiogenesis and to have antimicrobial activity.[29] The adrenomedullin receptor is strongly expressed in basal cells of the airway epithelium and alveolar type II epithelial cells, both of which are involved in epithelial

regeneration of the lung.[30] Continuous infusion of adrenomedullin by a subcutaneous osmotic pump increases Sca-1$^+$ cells in peripheral blood and regenerates alveoli and vasculature in an elastase-induced emphysema model in mice.[31]

### Simvastatin

In addition to a cholesterol-lowering effect, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) have various pharmacological effects, such as an antiinflammatory effect and improving endothelial function. Statins also seem to have a beneficial effect on tissue regeneration.[32] Intraperitoneal injection of simvastatin was performed in an elastase-induced mouse emphysema model, and a reduction of the mean linear intercept and an increase in proliferating (PCNA$^+$) cells were observed.[33]

## Compensatory lung growth

Compensatory lung growth has been observed after lobectomy or pneumonectomy in children[34] and experimental animal models.[35–37] Pneumonectomy has been widely used to evaluate postnatal lung growth because the remaining lung can be kept intact and volume loss is easily controlled. In the pneumonectomized animal and even in aged humans, adequate gas exchange is maintained after lung resection. This physiological compensation is provided by an increase in diffusion capacity and blood flow in the remaining lung with or without compensatory lung growth.

The degree of compensatory lung growth differs among species and varies with age. Small animals, such as rodents, have a better capacity for compensatory growth than larger animals. Postpneumonectomy compensation usually occurs rapidly and completely in rodents. For example, the weight of the remaining lung doubles within 14 days after pneumonectomy in rats,[38] whereas a period of 28 days is needed in rabbits[39] and 5 months in dogs.[35] Age is another factor in the capacity for lung growth. In the adult dog lung, compensatory lung growth is slow and incomplete, but extensive lung resection in an immature dog stimulates rapid and vigorous compensatory growth, resulting in complete normalization of lung function at maturity.[36] This suggests that postpneumonectomy lung growth is maturity-dependent. Because somatic growth in rodents continues throughout their lifespan, lung tissue can grow throughout the experiments.

Mechanical stresses and a variety of growth factors and hormones are thought to be the key inducers that initiate postpneumonectomy lung growth.

### *Mechanical stress*

Mechanical stress changes cell function. Stretch stimulation on lung cells induces cell proliferation,[40] growth factor production,[41] and changes in gene expression. In contrast, decreased mechanical stress attenuates lung development, causing lung hypoplasia.[42] Positive airway pressure induces cell proliferation and extracellular matrix remodeling.[43] Taken together, these results indicate that mechanical stress plays an important role in lung development and growth.

Pneumonectomy induces many anatomical changes in the remaining lung and the thoracic cavity. Shifting of the mediastinum toward the vacated thoracic compartment increases mechanical stress to the remaining lung, and these stresses induce cell proliferation and expression of genes such as early growth response gene-1.[44] The remaining lung inflates more in the increased space of the thoracic cavity. This stretch signal enhances cAMP expression, which plays a role in the early phase of the lung growth response.[38]

In addition, loss of the vascular bed due to pneumonectomy increases pulmonary perfusion per unit of remaining lung tissue.[45] Chronic capillary distension and increased shear stress induce endothelial cell growth and septal remodeling. The increased blood flow and shear stress also enhance alveolar growth.[46]

It is believed that compensatory lung growth occurs only in alveoli and not in respiratory bronchioles. However, Hsia et al. reported that the number of respiratory bronchiole segments and branch points increase in immature lungs.[47]

### *Growth factors, hormones, and other factors in compensatory lung growth*

As in the lung emphysema model, the role of RA was extensively examined in compensatory lung growth. RA treatment enhances compensatory lung growth[48] and alveolar capillary formation after pneumonectomy.[49] However, RA does not improve lung function in mature pneumonectomized dogs.[50] Hsia et al. demonstrated that 55% resection of the lungs by right pneumonectomy induces compensatory growth and increased lung function.[35,49] RA enhances this compensatory lung growth in dogs. A 45% resection by left pneumonectomy, however, did not induce lung growth.[51] These results suggest that RA is a promoter of existing alveolar growth but not an initiator of alveolar growth.[51]

After pneumonectomy, HGF increases in the remaining lung as well as in the liver and kidney.[52] c-Met, the HGF receptor, is transiently up-regulated in alveolar

type II cells. Neutralization of endogenous HGF suppresses compensatory DNA synthesis in lung epithelial cells, suggesting that HGF has a role in postpneumonectomy compensatory lung growth.

Epidermal growth factor (EGF) plays an important role in prenatal and postnatal lung development. Alveolar type II epithelial cells express the EGF receptor, and EGF induces epithelial maturation and regeneration. In EGF receptor-deficient mice, lungs are immature and show impaired branching and deficient alveolarization.[53] Kaza et al. reported that EGF administration to pneumonectomized rats induces significant increases in lung volume and in the weight of the remaining lung.[54]

The effects of growth hormones on lung volume have also been reported.[55,56] Treatment with human growth hormone in children or with excessive hormone in acromegalic adults induces an increase in lung volume. This increase is correlated with standing height, suggesting that the effect of growth hormone is systemic growth,[56] including enlargement of the thoracic cavity, rather than a direct effect on lung growth.

Alveolar hypoxia induces the expression of many genes, such as vascular endothelial growth factor (VEGF) and hypoxia-induced mitogenic factor (HIMF). Along with the neoalveolization, proper vascular growth should occur to avoid a V/Q mismatch. During lung development, VEGF is deposited in the subepithelial matrix at the leading edges of the branching airways, suggesting its stimulus effect on angiogenesis.[57] After pneumonectomy, alveolar type II cells express VEGF protein[58] and its receptor, flk-1, which are increased in the lungs.[59] Sakurai et al. demonstrated that administration of VEGF accelerated angiogenesis and lung growth after pneumonectomy.[37] HIMF, another protein induced under hypoxic condition, is up-regulated in the lung during the early hyperplastic period of compensatory lung growth. In addition, intratracheal instillation of recombinant HIMF protein induced epithelial, endothelial, and muscular proliferation in the lungs.[60] These data suggest that alveolar hypoxia and its related proteins also play a role in compensatory lung growth.

Kenzaki et al. implanted fetal lung tissue fragments into adult rat lungs. The implanted lung tissue was connected to the pulmonary circulation, and its alveolar spaces were opened. These changes were enhanced when the recipient lungs were partially resected. However, lung fragments obtained from adult rats did not expand after implantation.[61] These observations suggest that mechanical forces and premature lung cells and/or growth factors produced from premature cells are key elements for lung regrowth.

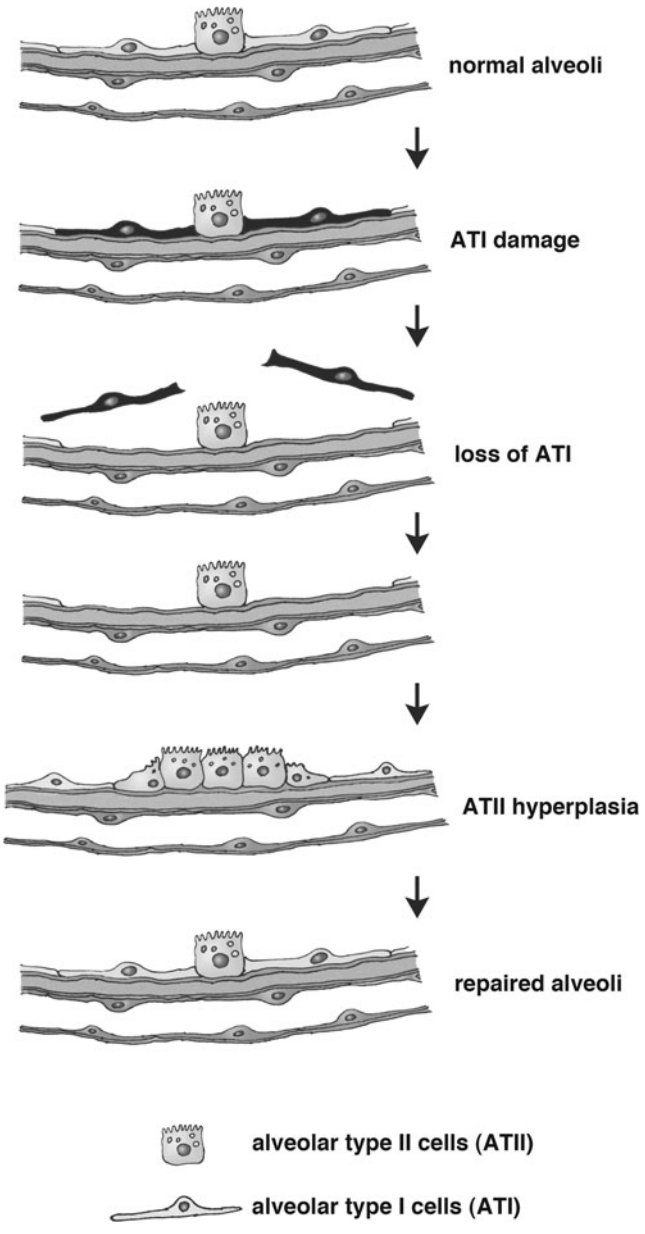


**Fig. 3** Alveolar repair. Severe alveolar damage induces apoptosis and necrosis of alveolar epithelial type I (*ATI*) cells. While in the recovery phase, hyperplasia of the alveolar type II (*ATII*) cells is observed. ATII cells are believed to then differentiate into ATI cells

## Repair process after acute lung injury and stem cells

Acute lung injury/acute respiratory distress syndrome is characterized by severe alveolar damage, including apoptosis and necrosis of alveolar epithelial and endothelial cells. In the recovery phase, hyperplasia of alveolar type II (ATII) cells is observed (Fig. 3). Newly differentiating and proliferating cells are required for lung repair and for replacement of damaged cells. ATII

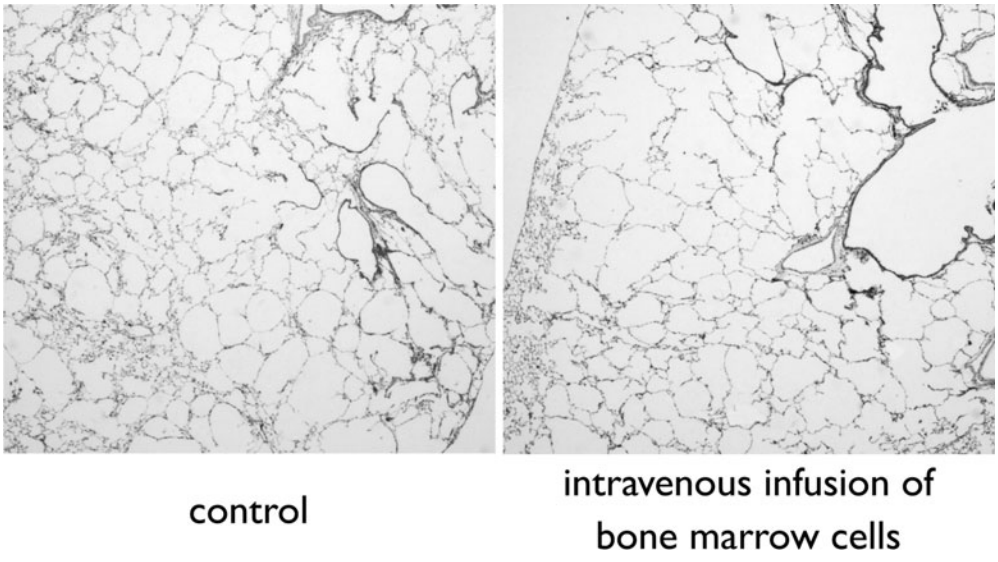


**Fig. 4** Administration of bone marrow-derived cells (BMDCs) does not ameliorate emphysematous changes in mice. BMDCs were intravenously administered in the elastase-induced emphysema model. Figures are representative lungs 3 weeks after BMDC treatment

cells can proliferate and are thought to be the source of type I cells. Even if the type II cells are damaged, two candidate sources of newly differentiating cells can undertake the repair process: circulating bone marrow-derived progenitor cells and lung endogenous stem cells.

Lipopolysaccharide (LPS) administration in animals is often utilized for studying acute lung injury. Initial LPS exposure induces severe acute inflammation in the lungs, but the lung structure is intact after injury, suggesting a proper repair process.[62] However, chronic exposure to LPS induces emphysema-like alveolar distraction in mice[63] and can be used as a lung repair model. Hyperoxia exposure[64] or repeated intratracheal bleomycin treatment[65] induces ATII cell hyperplasia; therefore, these can be good animal models to analyze the lung repair process.

## Cell sources for lung regeneration and repair

Stem or progenitor cells are needed for alveolar regeneration, lung growth, and lung repair after injury. Exogenous and endogenous progenitor cells are thought to participate in alveologenesis.

### Exogenous progenitor cells

The main source of exogenous progenitor cells is the bone marrow. In patients with bacterial pneumonia[66] or acute lung injury,[67] the number of circulating endothelial progenitor cells (EPCs) increases; the amount of the increase correlates with the disease outcome. This suggests that bone marrow-derived progenitor cells (BMDCs) are released into the circulation by inflammatory stimuli and that these cells facilitate resolution and repair of the inflammatory process. The contribution of BMDCs to alveolar regeneration after elastase-induced emphysema has been demonstrated in several reports described above. Treatment with G-CSF,[24] HGF,[18,21] or adrenomedullin[31] induces an increase in bone marrow-derived endothelial cells in the lung capillary walls during alveologenesis in a mouse emphysema model. However, the number of BMDCs seen in the alveolar walls gradually decreases after treatment. It is still an open question whether bone marrow cells differentiate into alveolar cells or simply fuse with the resident cells.

Because most of the reagents employed in lung regeneration studies increase circulating BMDCs, there is a potentially important role for these cells. In regeneration, however, exogenous infusion of bone marrow cells had no effect on alveolar regeneration (Fig. 4). This suggests that additional mediators, such as chemokines, adhesion molecules, growth stimulators, and extracellular matrix remodeling, among others, are needed for the bone marrow-derived cells to migrate, differentiate, and induce lung regeneration.

Administration of LPS induces severe damage to lung parenchymal cells, resulting in apoptosis and necrosis. Because these dead cells are not able to divide, other cells must replace them to repair the tissue and to maintain organ homeostasis. Seven days after LPS administration in green fluorescent protein (GFP) chimeric mice, thin, flat, GFP-positive BMDCs appear in the alveolar walls. These cells stain positive for cytokeratin as a marker of epithelial cells or for CD34 as a marker of endothelial cells.[62] This suggests that BMDCs differentiate or fuse with alveolar epithelial or pulmonary capillary endothelial cells, indicating a possible involvement of BMDCs

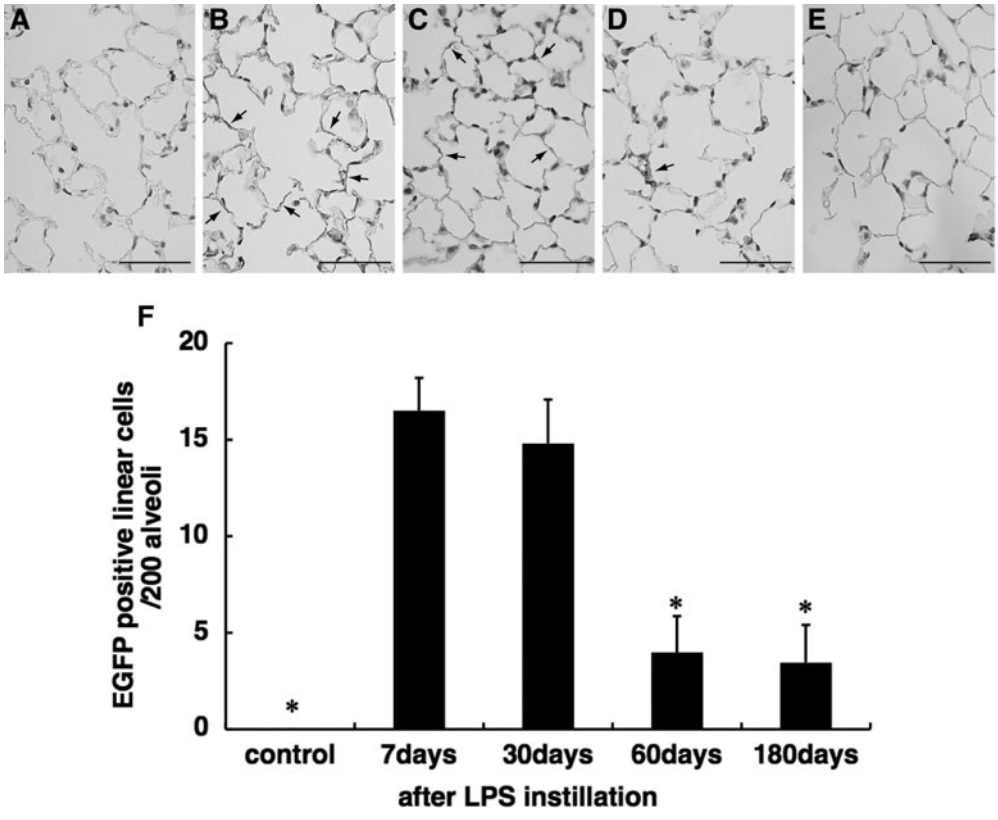


**Fig. 5** Presence of thin, flat BMDcs in the alveolar walls as a function of time after lipopolysaccharide (*LPS*) instillation. **A–E** Representative mouse lung sections immunostained for green fluorescent protein (GFP) 0 (**A**), 7 (**B**), 30 (**C**), 60 (**D**), and 180 (**E**) days after intranasal LPS administration. *Arrows* indicate thin, flat GFP-positive cells. *Bars* 50 μm. **F** The number of thin, flat GFP-positive cells in the alveolar walls of mouse lungs at the indicated time points after intranasal LPS administration. *Significantly fewer than in the day 7 group ($P < 0.05$)

in lung repair. However, the frequency of BMDCs gradually and significantly decreases over a prolonged period of time (Fig. 5).[68] These results suggest that BMDCs initially emigrate to the injured organ and differentiate into or fuse with the parenchymal cells of the organ. However, once BMDCs reach the damaged organ, they have little or no capacity to proliferate or to develop into new cells.

It has been reported that circulating progenitor cells do not contribute to compensatory lung growth.[69]

## Endogenous progenitor cells

### *Alveolar epithelial progenitor cells*

Administration of inflammatory stimuli, such as LPS and bleomycin, in the lungs results in an alveolar type I epithelium injury. Alveolar type II cells appear to proliferate and subsequently differentiate to replace the injured type I cells (Fig. 3). A portion of the type II cell population becomes hypertrophic. Both of these events are frequently observed in the diseased or damaged lung.

The experiments using GFP chimeric mice showed that the regenerated alveoli were composed of bone marrow-derived (GFP-positive) cells and cells of non-bone-marrow origin (GFP-negative)[18,21,24,31] This suggests that resident lung cells, including endogenous stem cells, contribute to alveologenesis. ATII cells can repair damaged alveolar epithelium.[74] However, the potential ability of lung endogenous stem cells to replace damaged ATII cells is not yet clear. Recently, mouse stem cell antigen (Sca)-1-positive cells have been proposed to be lung endogenous stem cells.[70–73] Hegab et al. reported that elastase-induced lung injury alone increased the number of cells with stem cell markers (e.g., Sca-1, CD34, c-kit) in the lungs before HGF administration (Fig. 6)[21] The numbers of Sca-1$^+$/SP-C$^+$ cells were markedly increased in response to either HGF or elastase, with a maximum increase in the groups treated with both. Most Sca-1$^+$ cells were lung endogenous stem cells, whereas most of the c-kit$^+$ cells were of bone marrow origin.

Nolen-Walston et al. evaluated the response of lung endogenous stem cells (Sca-1$^+$/SP-C$^+$/CCSP$^+$/CD45$^-$ cells) and ATII cells during compensatory lung growth after pneumonectomy in mice.[74] They found that the number of Sca-1$^+$ and ATII cells increased during compensatory growth and peaked at 220% and 124% of the baseline, respectively. The contribution of Sca-1$^+$ cells to compensatory lung growth was 0–25%, whereas the ATII cells were necessary for regrowth, based on a cell kinetic model.

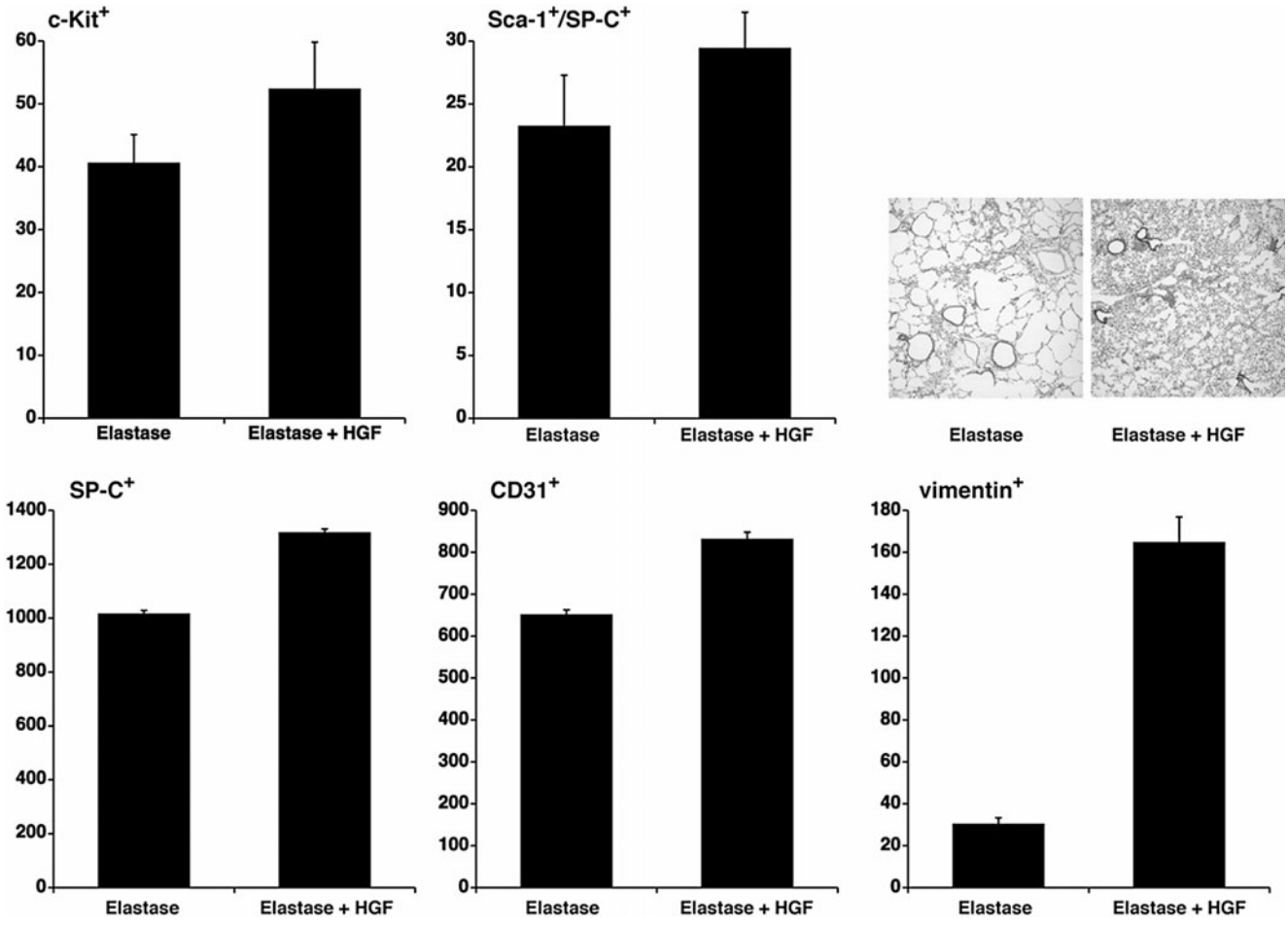


**Fig. 6** Cells with stem cell markers are increased during HGF-induced lung regeneration. (Modified from Hegab et al.[21])

In contrast to the increasing reports of lung endogenous stem cells in mice, the knowledge of stem cells in human lungs is limited. There are several reasons for this limitation: (1) there is no specific marker for human lung endogenous stem cells, and (2) the availability of human lung specimens is limited. Therefore, we developed a new preservation solution for tissue stem cells. Using this solution, called StemSurvive (Fig. 7), human lung tissues can be stored for up to 7 days without any adverse effect on tissue stem and niche cells (manuscript in preparation). Then, we isolated human alveolar progenitor cells (AEPCs) from the StemSurvive-preserved human lungs.[75] AEPCs represent an epithelial phenotype with mesenchymal stem cell character. According to microarray analyses, AEPCs share many genes with mesenchymal stem cells (MSCs) and ATII cells (Fig. 8), suggesting an overlapping phenotype of both alveolar epithelium and mesenchyme for these cells. Interestingly, AEPCs increased in fibrotic lungs and in some types of adenocarcinoma. The transitional phenotypes between the mesenchyme and epithelium observed in AEPCs suggest that these cells act as lung tissue stem cells in tissue repair and carcinogenesis. For involvement in alveolar repair, mesenchymal properties such as antiapoptotic activity and motility may be beneficial for a functional epithelial progenitor. Further examination is needed to clarify the pathophysiological role of AEPCs in lung diseases.

*Mesenchymal stem cells*

MSCs comprise a well-known population of endogenous stem cells. Many organs contain their own MSCs. MSCs have the capacity for self-renewal and an ability to differentiate into cells of mesenchymal lineage. Because the characteristics of the MSCs from different organs are not identical and there are no specific cell surface markers, the minimum criteria for MSCs have been defined (Table 1).[76]

Mesenchymal stem cells also exist in lungs and can be isolated from the neonatal lung[77] and bronchoalveolar

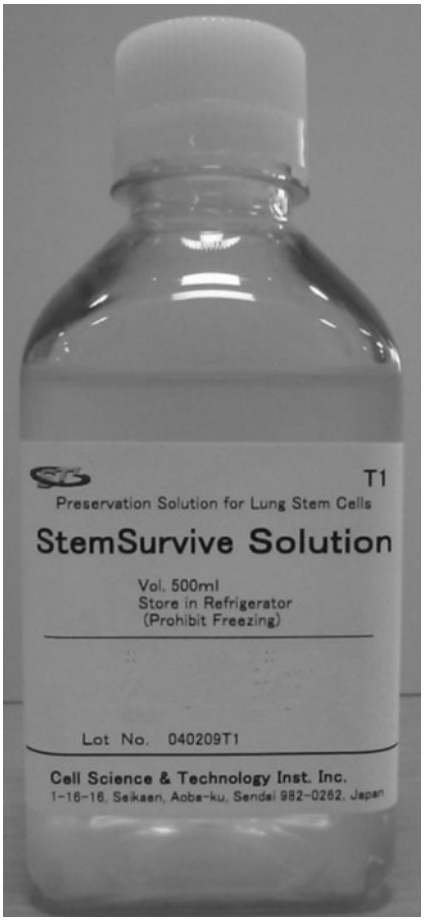


**Fig. 7** Preservation solution for tissue stem cells

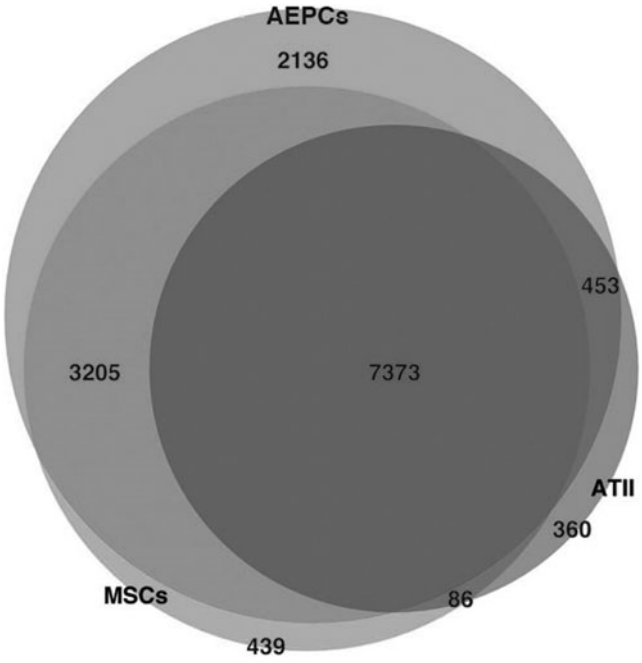


**Fig. 8** Venn diagram of expressed genes in human alveolar epithelial progenitor cells (*AEPCs*), human alveolar type II cells (*ATII*), and human bone marrow-derived mesenchymal stem cells (*MSCs*). AEPCs shared many genes with ATII and MSCs. (Modified from Fujino et al.[75])

**Table 1** Minimum criteria for identifying mesenchymal stem cells

1. Adherence to plastic in standard culture conditions
2. Cell surface markers
   - Positive
     - CD105
     - CD73
     - CD90
   - Negative
     - CD45
     - CD34
     - CD14 or CD11b
     - CD79α or CD19
     - HLA-DR
3. In vitro differentiation into osteoblasts, chondroblasts, and adipocytes

lavage (BAL) fluid.[78] Karoubi et al. also isolated MSCs from surgical human lung tissues and successfully differentiated them into aquaporin 5- and CCSP-expressing ATI cells.[79]

Although the role of MSCs in lung regeneration and growth is not yet clear, the beneficial effects of MSCs on lung injury have been extensively evaluated. MSCs are known to produce many cytokines and growth factors.[80] In addition, LPS-treated lung cells co-cultured with MSCs displayed reduced pro-inflammatory cytokine production,[81] suggesting that the inflammatory response is reduced by soluble factors produced by MSCs and/or by direct contact with MSCs. MSCs also have an immunomodulatory effect on immune cells, including T cells, B cells, and natural killer cells.[82] Interestingly, Spees et al. reported that mitochondrial DNA can be transferred from MSCs to other cells, which can restore mitochondrial function in the recipient cells.[83] Therefore, these inhibitory effects on lung injury are thought to be due to the antiinflammatory effects of MSCs rather than their capacity to differentiate into lung cells.[84]

*Lung cancer stem cells*

Cancer stem cells have recently attracted much attention. The endogenous progenitor cells described above may be candidates for lung cancer stem cells. Interestingly, several stem cell markers are expressed in lung cancer.[85,86] Some adenocarcinomas have shown a phenotype similar to that of alveolar epithelial progenitor cells.[75] These data suggest that some lung cancer cells have stem cell characteristics and thus may be cancer stem cells. Further research is needed in this area.

## Clinical approaches

### Retinoic acid

Based on the animal studies described previously, several clinical studies using RA to treat patients with pulmonary emphysema were performed.[87,88] Although oral administration of RA modulated the protease/antiprotease balance in COPD patients,[89] no statistical change was observed in lung function or CT density.[87,88] Currently, a clinical trial with Palovarotene (Roche, Basle, Switzerland), an orally active gamma selective retinoid agonist, in patients with $\alpha_1$-antitrypsin deficiency is ongoing.[90] However, because most COPD patients are elderly and their lungs are mature, they may have only a low number of stem cells, and the regenerative capacity of these cells might be low.

### Cell therapy

The MSCs are the most promising candidate for clinical cell-based therapy. MSCs are easy to isolate from the bone marrow and tissues. Allogenic MSCs are well tolerated from the recipient because of their low expression of major histocompatibility complex (MHC) I and II proteins and their lack of T-cell co-stimulatory molecules.[91] Therefore, autologous MSCs are not necessary, and MSCs can be stored until therapy is needed. In the United States, more than 100 clinical trials using MSCs are registered and ongoing. As described above, MSCs are expected to reduce tissue injury and to promote the repair process. These beneficial effects are thought to be based on the ability of MSCs to modulate the immune system and on their capacity to produce growth factors and cytokines,[92] such as keratocyte growth factor, HGF, and prostaglandin $E_2$.

Because of these antiinflammatory effects, the potential of MSCs for treating severe lung diseases, such as acute lung injury,[93] COPD,[73] pulmonary hypertension,[94] asthma,[95] and lung fibrosis,[96] has been extensively evaluated. In the experimental models, MSCs are administered into the injured lungs either intravenously or intratracheally. Intravenous[62] or intratracheal[97] administration of bone marrow cells or bone marrow-derived MSCs[81] ameliorated LPS-induced lung injury in mice; and bleomycin-induced inflammation, collagen deposition, and fibrosis were reduced by intratracheal or intravenous infusion of MSCs.[96]

In more recent work, we administered lung endogenous stem cells, which have the MSC phenotype, into elastase-injured mouse lungs. Intratracheal stem cell administration ameliorated elastase-induced lung injury and improved mortality (Fig. 9). Implanted stem cells

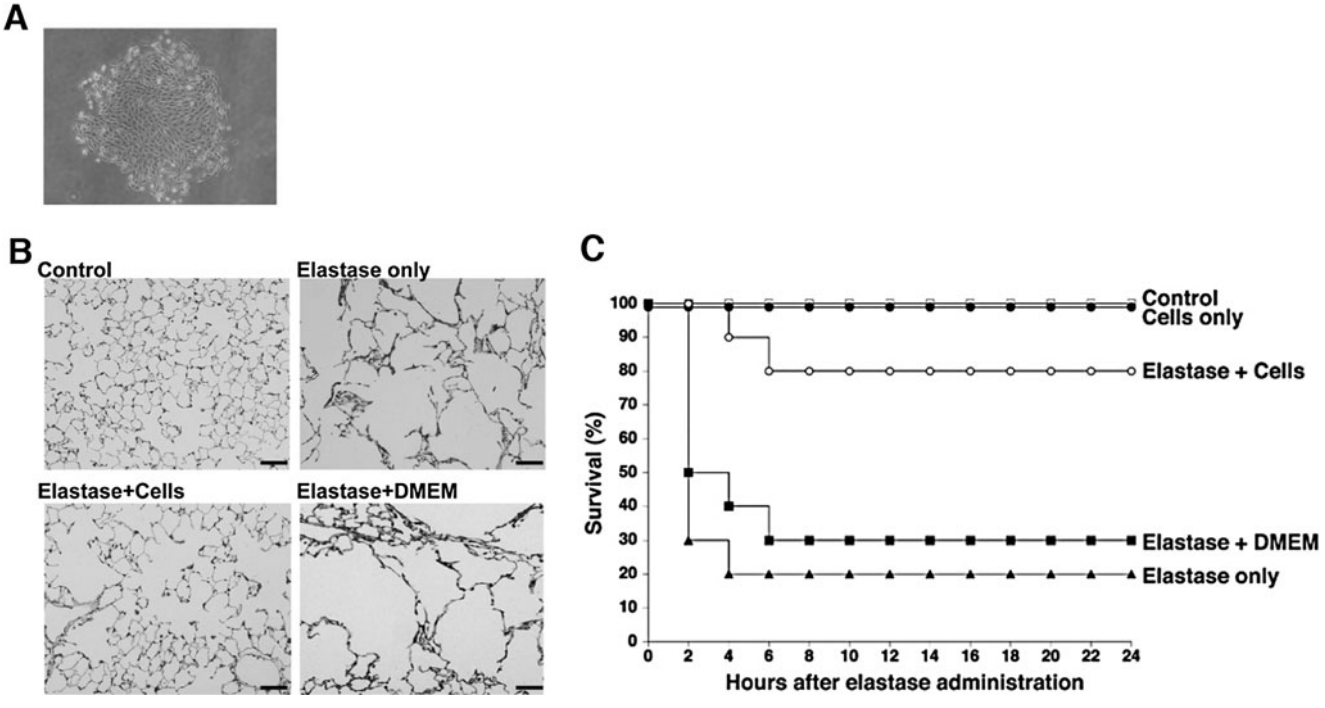


**Fig. 9** Cell therapy with multipotent lung stem cells (MLSCs) to treat elastase-induced lung injury in mice. **A** Colony formation of MLSCs derived from mouse lungs. **B** Intratracheal administration of MLSCs protects the lung against development of emphysema following administration of a sublethal dose of elastase. **C** Intratracheal treatment with MLSCs improves mouse survival following administration of a lethal dose of elastase. (Modified from Hegab et al.[21])

could reach the alveolar space; however, only a few cells remained in the alveolar walls.[73] These observations did not support differentiation of the cells but suggested an immunomodulatory effect of stem cells on lung injury.

### Bioartificial lungs

Artificially reconstructing the lungs is difficult because the lung is a complex organ containing more than 30 cell types within a three-dimensional structure. Recently, several bioartificial lung models were reported. All studies had used lungs that had been decellularized using detergent perfusion, into which new endothelial and epithelial cells were transplanted on the scaffolds.[98–100] Fetal lung cells, human umbilical cord endothelial cells, and a human lung adenocarcinoma epithelial cell line (A549) were utilized for reconstructing the artificial lungs. Although the cells employed in this study are not suitable for clinical use and these tissue-engineered lungs last only 6 h in vivo, the concept of the bioartificial lung could be a good candidate for future therapy.

## Conclusions

Our knowledge of lung regeneration and repair has been expanded by the development of suitable animal models. Regenerative medicine has become one of the most promising strategies to cure or treat intractable lung diseases. However, many challenges remain. Differences in the animal species used and changes in age are closely associated with responses to the regenerative reagents or stimuli. Most of the successful experiments were performed in rodents, in which the lungs and thorax continue to grow throughout the entire lifespan. Therefore, extrapolation to mature human lungs is difficult. In addition, in contrast to the mouse lung, our knowledge of endogenous stem cells in the human lung is more limited.

Despite these limitations, studies in animal models and the discovery of lung endogenous stem cells are essential to understanding the pathophysiology of lung regeneration and to the development of novel therapeutic strategies for intractable lung diseases. These studies provide valuable knowledge for the regenerative therapy of human lungs.

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