Tissue regeneration as next-generation therapy for COPD – potential applications

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Abstract: COPD is a major cause of chronic morbidity and mortality worldwide, and there is a need to develop more effective therapeutic strategies to replace specialized treatment such as lung transplantation. Recent studies suggest that recognition of apoptotic lung epithelial or endothelial cells may result in growth factors to stimulate cell replacement, and defects in these processes may contribute to the pathogenesis of COPD. Furthermore, recent animal and human studies have revealed that tissue-specific stem cells and bone marrow-derived cells contribute to lung tissue regeneration and protection, and thus administration of exogenous stem/progenitor cells or humoral factors responsible for activation of endogenous stem/progenitor cells may be a potent next-generation therapy for COPD.

Keywords: COPD, regenerative medicine, cell therapy, stem cell, bone marrow, growth factor

Introduction

COPD is a major cause of chronic morbidity and mortality worldwide, and the World Health Report 2004 from WHO estimated 2.75 million deaths by COPD in 2002 (WHO 2004). Pulmonary emphysema, a major component of the morbidity and mortality in COPD, is characterized by permanent enlargement of airspaces distal to the terminal bronchiole, accompanied by destruction of their walls (Wright and Churg 2006). Only cigarette smoking cessation and long-term oxygen therapy improve survival in COPD (Celli and MacNee 2004; Anthonisen et al 2005), and patients with severe COPD require invasive treatment such as lung transplantation. Therefore, there is a need to develop less invasive, more effective therapeutic strategies for COPD.

It has been demonstrated that all-trans-retinoic acid (ATRA), a metabolite of retinol associated with the process of alveolar septation (Ong and Chytil 1976), can restore the normal lung structure in rats with emphysema (Massaro and Massaro 1997), suggesting the possibility of tissue regeneration in COPD. Since then, a number of studies demonstrating lung repair by means of stem/progenitor cells or humoral factors have been reported in animal models. This article reviews recent advances in regenerative medicine in COPD and its potential application.

Lung repair mechanisms in COPD

Apoptotic clearance in COPD

It has been reported that cell death in epithelial and endothelial alveolar cells are increased in patients with pulmonary emphysema (Kasahara et al 2001; Yokohori et al 2004). In general, apoptotic cells are removed from tissues, followed by cell replacement to maintain homeostasis. Recognition of apoptotic cells initiates production of growth/maintenance factors for both epithelial and endothelial cells, and stimulates endothelial angiogenic responses (Weihua et al 2005). Thus, signals from apoptotic cell recognition may contribute to stimulation, release, and/or attraction of progenitor cells for tissue regeneration (Henson et al 2006). Therefore, the presence

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of apoptotic cells in COPD may imply defects in these clearance mechanisms (Henson et al 2006). In fact, there is increasing evidence that apoptotic clearance mechanisms are less effective in COPD lungs and that macrophages from such lungs show a defect in recognizing and ingesting such targets (Hodge et al 2003).

Tissue-specific stem cells in the lung

In the lung, epithelial stem cells include type II pneumocyte in the alveolus and the Clara cell in the bronchiole (Mason et al 1997; Majka et al 2005). Reddy et al demonstrated that E-cadherin-negative subpopulation of type II cells were proliferative and exhibited high levels of telomerase activity (Reddy et al 2004). Kim et al isolated a pulmonary stem cell population at the bronchioalveolar duct junction, and it may maintain alveolar type II cells of the distal lung and Clara cells in the bronchiole (Kim et al 2005). Hong et al also demonstrated that tracheal basal cells represent a multipotent progenitor cell type for renewal of the injured tracheal epithelium induced by naphthalene (Hong et al 2004). On the other hand, side population (SP) cells, which are more primitive adult stem cells and found in various tissues, have been recently identified in the lung tissue (Summer et al 2003; Summer et al 2004). SP cells comprise 0.03%–0.07% of mouse lung cells and are sca-1-positive, lineage-negative, and heterogenous at CD45. In addition, Giangreco et al indicated that sca-1-positive, CD45-negative SP cells from mouse lung had a molecular phenotype similar to neuroepithelial body-associated variant Clara cells (Giangreco et al 2004), which are label-retaining cells with multipotency (Hong et al 2001).

Bone marrow-derived stem/progenitor cells

It has been demonstrated that circulating endothelial progenitor cells (EPC) are decreased in COPD patients and could be correlated with disease severity (Fadini et al 2006; Palange et al 2006). These results raise the possibility that bone marrow cells may be involved in lung regeneration or protection. EPC have been mobilized into the circulation in mice with lipopolysaccharide (LPS)-induced lung injury (Yamada et al 2004) and in patients with bacterial pneumonia (Yamada et al 2005), and large numbers of bone marrow-derived progenitor cells appeared in active fibrotic lesions in the lung of bleomycin-treated mice (Hashimoto et al 2004). However, whether EPC plays a role in the loss of capillaries, in the remodeling of the vascular bed or in the thickening of the small airway wall remains to be elucidated (Randell 2006). In human lung allografts, recipient-derived cells were integrated into pulmonary epithelium, and epithelial chimerism occurred in bronchi, type II pneumocytes and seromucous glands surrounding larger bronchi (Kleeberger et al 2003). Suratt et al examined human lung specimens from a retrospective cohort of female allogeneic hematopoietic stem cell transplantat recipients who received stem cells from male donors, and found significant rates of epithelial (3%–8%) and endothelial (38%–42%) chimerism (Suratt et al 2003).

Potential strategies for tissue regeneration in COPD

Administration of humoral factors responsible for lung regeneration

Because recognition of apoptotic cells by macrophages in COPD is related to the production of hepatocyte growth factor (HGF) by these macrophages, administration of HGF might compensate for the defects in apoptotic clearance in COPD macrophages (Morimoto et al 2001). It has been demonstrated that HGF induces angiogenesis in elastase-injured lung injury through mobilizing endothelial progenitor cells (Ishizawa et al 2004b). In addition, induction of HGF expression by a gene-transfection method resulted in improved pulmonary function via inhibition of alveolar cell apoptosis, enhancement of alveolar regeneration, and promotion of angiogenesis in rats with elastase-induced emphysema (Shigemura et al 2005).

Fibroblast growth factor-2 (FGF-2), an angiogenic factor indispensable for lung development and branching morphogenesis, has been shown to induce an increase in pulmonary blood flow in the damaged lung and a volume reduction in the emphysematous lung, and lead to recovery of pulmonary function in dogs with emphysema (Morino et al 2005); however, FGF-2 could not achieve parechymal regeneration and alveolar septation.

Vascular endothelial growth factor (VEGF) is a potent mediator of angiogenesis and vascular permeability (Ribatti 2005), and may be involved in many processes in COPD including pulmonary vascular remodeling and endothelial and epithelial cell apoptosis (Papaioannou et al 2006). The complexity of the roles of VEGF and its receptors suggests that strategies specifically targeting VEGF in COPD would have unpredictable and possibly deleterious effects on some of the different processes (Kanazawa 2007).

ATRA (Massaro and Massaro 1997; Belloni et al 2000) or granulocyte-colony-stimulating factor (G-CSF) promoted

lung regeneration and increased bone marrow-derived cell numbers in alveoli in mice with elastase-induced emphysema (Ishizawa et al 2004a). However, the effect of ATRA in the treatment of pulmonary emphysema in animals and humans remains controversial (Massaro and Massaro 2000; Mao et al 2002; Fujita et al 2004; March et al 2004; Roth et al 2006). The Feasibility of Retinoids for the Treatment of Emphysema (FORTE) study was established by the National Institutes of Health/National Heart, Lung, and Blood Institute as an initial step in evaluating the clinical feasibility of retinoid-based therapy (Roth et al 2006). No definitive clinical benefit related to the administration of retinoids was observed. However, time- and dose-dependent changes in D_{LCO}, CT density mask score, and health-related QOL were observed in subjects treated with ATRA, suggesting the possibility of exposure-related biological activity that warrants further investigation.

We have demonstrated that adrenomedullin (AM), a potent vasodilator peptide, improves elastase-induced emphysema (Murakami et al 2005). In this study, AM infusion significantly inhibited the increase in lung volume, static lung compliance, and mean linear intercept in mice given elastase. AM increased the numbers of mononuclear cells and sca-1-positive cells in circulating blood, and significantly increased the number of bone marrow-derived cells incorporated into the elastase-treated lung. In vitro, addition of AM attenuated elastase-induced cell death in alveolar epithelial cells and endothelial cells. These results suggest that AM improves emphysema at least in part through mobilization of bone marrow cells and direct protective effects on alveolar epithelial cells and endothelial cells. AM also ameliorated LPS-induced acute injury in rats (Itoh et al 2007), possibly through inhibition of inflammation, hyper-permeability, and alveolar wall cell apoptosis.

Administration of exogenous stem/progenitor cells

After intravenous administration of lacZ-labeled bone marrow-derived cells into wild-type recipient mice with bleomycin-induced lung injury, cells were engrafted in recipient lung parenchyma with the phenotype of type I pneumocytes of the alveolar epithelium (Kotton et al 2001). On the other hand, male whole bone marrow cells or $CD34^{+}lin^{-}$ bone marrow cells differentiated into bronchiolar epithelia and type II alveolar cells after transplantation into lethally irradiated female mice (Krause et al 2001; Theise et al 2002). Because differentiation into inflammatory cells was not observed, these findings suggest the potential application of bone marrow-derived cells for therapy of lung diseases including COPD. However, several groups did not find evidence of pulmonary repopulation via bone marrow-derived cells (Davies et al 2002; Wagers et al 2002; Kotton et al 2005; Zander et al 2006), raising the possibility of a dominant role of resident lung stem cells in alveolar repair and regeneration.

Mesenchymal stem cells (MSC) reside in bone marrow (Friedenstein et al 1976), adipose tissue (Gimble and Guilak 2003), and many other tissues (Pittenger and Martin 2004). A recent study suggested that MSC reside in virtually all post-natal organs and tissues, and may be localized to vessel walls (da Silva Meirelles et al 2006). MSC can differentiate not only into osteoblasts, chondrocytes, and adipocytes, but also other types of cells such as vascular endothelial cells (Pittenger et al 1999). It has been demonstrated that transplanted MSC home to the lung in response to bleomycin-induced lung injury and adopt phenotypes of alveolar epithelial cells, endothelial cells, fibroblasts and bronchial epithelial cells, and protect lung tissue through suppression of proinflammatory cytokines such as TNF-α and IL-1, and through triggering production of reparative growth factors (Ortiz et al 2003; Rojas et al 2005; Ortiz et al 2007). Shigemura et al demonstrated that autologous transplantation of adipose tissue-derived MSC ameliorates pulmonary emphysema in rats (Shigemura et al 2006b), and accelerates alveolar and vascular regeneration after lung volume reduction surgery in rats with emphysema (Shigemura et al 2006a).

However, whether exogenous administration of those cells improves COPD in humans remains to be elucidated. In addition, recent studies demonstrated that cellular senescence is observed in epithelium and endothelium as well as in fibroblasts (Muller et al 2006; Tsuji et al 2006), raising the possibility that stem/progenitor cells are also associated with senescence (Sharpless and DePinho 2007). Accordingly, it is not clear which type of cells (including adult cells or embryonic cells) could be adopted for the treatment of COPD, and if it will be possible to repair emphysema or to prevent it.

Importance of cigarette smoke cessation and exercise

Cigarette smoke causes apoptosis and an inflammatory response in the lower respiratory tract, impairs the repair functions of fibroblasts, epithelial cells and mesenchymal cells, and induces senescence of lung fibroblasts (Spurzem and Rennard 2005; Nyunoya et al 2006; Rennard et al 2006). Although a reduction in inflammation is observed

after smoking cessation in COPD patients, histopathological studies show persistent airway inflammation (Willemse et al 2004). Since cigarette smoke cessation improves respiratory symptoms and slows the rapid FEV_1 decline in COPD patients, it is apparent that cigarette smoke cessation is the primary therapeutic intervention for these patients, not only as therapeutic basis but also as ethical and economical viewpoints. However, the exact mechanism of the reduction in airway inflammation and the relationship with lung function and regeneration after cigarette smoke cessation remain to be established.

Exercise might be another approach, because mobilization of endothelial progenitor cells occurs after exercise (Rehman et al 2004). In addition, several studies have provided evidence that exercise improves several of the variables associated with poor outcomes such as exercise capacity and dyspnea as well as the multidimensional BODE index (Celli et al 2004; Cote and Celli 2005; Nici et al 2006). Therefore, it is interesting to speculate that the gain in exercise tolerance is achieved through mobilization of endothelial progenitor cells. Palange et al reported that circulating progenitor count was unchanged after endurance exercise in patients with COPD; however, this might have been due to insufficient intensity and/or duration of the test (Palange et al 2006).

Conclusions

Growing evidence obtained from basic and translational research on regenerative medicine in COPD suggests that, in addition to cigarette smoking cessation and exercise, administration of humoral factors responsible for activation of endogenous stem/progenitor cells or administration of exogenous stem/progenitor cells may be a potential therapeutic tool in the treatment of COPD (Table 1). However, in addition to clarifying beneficial effects and their mechanism of tissue repair and regeneration by administration of humoral factors or exogenous stem/progenitor cells, harmful aspects including tumor formation should be carefully assessed. Therefore, we are at a very initial stage, and tissue regeneration for COPD patients, although very attractive, is still a far away option.

Table 1 Potential strategies for tissue regeneration in COPD

1. Administration of growth/differentiation factors
ATRA
FGF-2
2. Activation of endogenous stem/progenitor cells
HGF
AM
G-CSF
3. Administration of exogenous stem/progenitor cells
MSC (bone marrow, fat tissue)
Bone marrow-derived cells (?)
EPC (?)

Abbreviations: G-CSF, granulocyte-colony stimulating factor; HGF, hepatocyte growth factor; AM, adrenomedullin; MSC, mesenchymal stem cells; EPC, endothelial progenitor cells; FGF-2, fibroblast growth factor; ATRA, all-trans retinoic acid.

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Disclosures

Neither author has any conflicts of interest to disclose.

References

Anthonisen NR, Skeans MA, Wise RA, et al. 2005. The effects of a smoking cessation intervention on 14.5-year mortality: a randomized clinical trial. *Ann Intern Med*, 142:233–9.

Belloni PN, Garvin L, Mao CP, et al. 2000. Effects of all-trans-retinoic acid in promoting alveolar repair. *Chest*, 117:235S–41S.

Celli BR, Cote CG, Marin JM, et al. 2004. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. *N Engl J Med*, 350:1005–12.

Celli BR, MacNee W. 2004. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J*, 23:932–46.

Cote CG, Celli BR. 2005. Pulmonary rehabilitation and the BODE index in COPD. *Eur Respir J*, 26:630–6.

da Silva Meirelles L, Chagastelles PC, Nardi NB. 2006. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci,* 119:2204–13.

Davies JC, Potter M, Bush A, et al. 2002. Bone marrow stem cells do not repopulate the healthy upper respiratory tract. *Pediatr Pulmonol*, 34:251–6.

Fadini GP, Schiavon M, Cantini M, et al. 2006. Circulating progenitor cells are reduced in patients with severe lung disease. *Stem Cells,* 24:1806–13.

Friedenstein AJ, Gorskaja JF, and Kulagina NN. 1976. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Exp Hematol*, 4:267–74.

Fujita M, Ye Q, Ouchi H, et al. 2004. Retinoic acid fails to reverse emphysema in adult mouse models. *Thorax*, 59:224–30.

Giangreco A, Shen H, Reynolds SD, et al. 2004. Molecular phenotype of airway side population cells. *Am J Physiol Lung Cell Mol Physiol*, 286:L624–30.

Gimble J, Guilak F. 2003. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. *Cytotherapy,* 5:362–9.

Hashimoto N, Jin H, Liu T, et al. 2004. Bone marrow-derived progenitor cells in pulmonary fibrosis. *J Clin Invest*, 113:243–52.

Henson PM, Vandivier RW, Douglas IS. 2006. Cell death, remodeling, and repair in chronic obstructive pulmonary disease? *Proc Am Thorac Soc*, 3:713–7.

Hodge S, Hodge G, Scicchitano R, et al. 2003. Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells. *Immunol Cell Biol*, 81:289–96.

Hong KU, Reynolds SD, Giangreco A, et al. 2001. Clara cell secretory protein-expressing cells of the airway neuroepithelial body microenvironment include a label-retaining subset and are critical for epithelial renewal after progenitor cell depletion. *Am J Respir Cell Mol Biol*, 24:671–81.

Hong KU, Reynolds SD, Watkins S, et al. 2004. In vivo differentiation potential of tracheal basal cells: evidence for multipotent and unipotent subpopulations. *Am J Physiol Lung Cell Mol Physiol*, 286:L643–9.

Ishizawa K, Kubo H, Yamada M, et al. 2004a. Bone marrow-derived cells contribute to lung regeneration after elastase-induced pulmonary emphysema. *FEBS Lett*, 556:249–52.

Ishizawa K, Kubo H, Yamada M, et al. 2004b. Hepatocyte growth factor induces angiogenesis in injured lungs through mobilizing endothelial progenitor cells. *Biochem Biophys Res Commun*, 324:276–80.

Itoh T, Obata H, Murakami S, et al. 2007. Adrenomedullin ameliorates lipopolysaccharide-induced acute lung injury in rats. *Am J Physiol Lung Cell Mol Physiol*.

Kanazawa H. 2007. Role of vascular endothelial growth factor in the pathogenesis of chronic obstructive pulmonary disease. *Med Sci Monit*, 13:RA189–95.

Kasahara Y, Tuder RM, Cool CD, et al. 2001. Endothelial cell death and decreased expression of vascular endothelial growth factor and vascular endothelial growth factor receptor 2 in emphysema. *Am J Respir Crit Care Med*, 163:737–44.

Kim CF, Jackson EL, Woolfenden AE, et al. 2005. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell*, 121:823–35.

Kleeberger W, Versmold A, Rothamel T, et al. 2003. Increased chimerism of bronchial and alveolar epithelium in human lung allografts undergoing chronic injury. *Am J Pathol*, 162:1487–94.

Kotton DN, Fabian AJ, Mulligan RC. 2005. Failure of bone marrow to reconstitute lung epithelium. *Am J Respir Cell Mol Biol*, 33:328–34.

Kotton DN, Ma BY, Cardoso WV, et al. 2001. Bone marrow-derived cells as progenitors of lung alveolar epithelium. *Development*, 128:5181–8.

Krause DS, Theise ND, Collector MI, et al. 2001. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*, 105:369–77.

Majka SM, Beutz MA, Hagen M, et al. 2005. Identification of novel resident pulmonary stem cells: form and function of the lung side population. *Stem Cells*, 23:1073–81.

Mao JT, Goldin JG, Dermand J, et al. 2002. A pilot study of all-trans-retinoic acid for the treatment of human emphysema. *Am J Respir Crit Care Med*, 165:718–23.

March TH, Cossey PY, Esparza DC, et al. 2004. Inhalation administration of all-trans-retinoic acid for treatment of elastase-induced pulmonary emphysema in Fischer 344 rats. *Exp Lung Res*, 30:383–404.

Mason RJ, Williams MC, Moses HL, et al. 1997. Stem cells in lung development, disease, and therapy. *Am J Respir Cell Mol Biol*, 16:355–63.

Massaro GD, Massaro D. 1997. Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats. *Nat Med*, 3:675–7.

Massaro GD, Massaro D. 2000. Retinoic acid treatment partially rescues failed septation in rats and in mice. *Am J Physiol Lung Cell Mol Physiol*, 278:L955–60.

Morimoto K, Amano H, Sonoda F, et al. 2001. Alveolar macrophages that phagocytose apoptotic neutrophils produce hepatocyte growth factor during bacterial pneumonia in mice. *Am J Respir Cell Mol Biol*, 24:608–15.

Morino S, Nakamura T, Toba T, et al. 2005. Fibroblast growth factor-2 induces recovery of pulmonary blood flow in canine emphysema models. *Chest*, 128:920–6.

Muller KC, Welker L, Paasch K, et al. 2006. Lung fibroblasts from patients with emphysema show markers of senescence in vitro. *Respir Res*, 7:32.

Murakami S, Nagaya N, Itoh T, et al. 2005. Adrenomedullin regenerates alveoli and vasculature in elastase-induced pulmonary emphysema in mice. *Am J Respir Crit Care Med*, 172:581–9.

Nici L, Donner C, Wouters E, et al. 2006. American Thoracic Society/European Respiratory Society statement on pulmonary rehabilitation. *Am J Respir Crit Care Med*, 173:1390–413.

Nyunoya T, Monick MM, Klingelhutz A, et al. 2006. Cigarette smoke induces cellular senescence. *Am J Respir Cell Mol Biol*, 35:681–8.

Ong DE, Chytil F. 1976. Changes in levels of cellular retinol- and retinoic-acid-binding proteins of liver and lung during perinatal development of rat. *Proc Natl Acad Sci U S A*, 73:3976–8.

Ortiz LA, Dutreil M, Fattman C, et al. 2007. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proc Natl Acad Sci U S A*, 104:11002–7.

Ortiz LA, Gambelli F, McBride C, et al. 2003. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci U S A*, 100:8407–11.

Palange P, Testa U, Huertas A, et al. 2006. Circulating haemopoietic and endothelial progenitor cells are decreased in COPD. *Eur Respir J,* 27:529–41.

Papaioannou AI, Kostikas K, Kollia P, et al. 2006. Clinical implications for vascular endothelial growth factor in the lung: friend or foe? *Respir Res*, 7:128.

Pittenger MF, Mackay AM, Beck SC, et al. 1999. Multilineage potential of adult human mesenchymal stem cells. *Science*, 284:143–7.

Pittenger MF, Martin BJ. 2004. Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res*, 95:9–20.

Randell SH. 2006. Airway epithelial stem cells and the pathophysiology of chronic obstructive pulmonary disease. *Proc Am Thorac Soc*, 3:718–25.

Reddy R, Buckley S, Doerken M, et al. 2004. Isolation of a putative progenitor subpopulation of alveolar epithelial type 2 cells. *Am J Physiol Lung Cell Mol Physiol*, 286:L658–67.

Rehman J, Li J, Parvathaneni L, et al. 2004. Exercise acutely increases circulating endothelial progenitor cells and monocyte-/macrophage-derived angiogenic cells. *J Am Coll Cardiol*, 43:2314–8.

Rennard SI, Togo S, Holz O. 2006. Cigarette smoke inhibits alveolar repair: a mechanism for the development of emphysema. *Proc Am Thorac Soc*, 3:703–8.

Ribatti D. 2005. The crucial role of vascular permeability factor/vascular endothelial growth factor in angiogenesis: a historical review. *Br J Haematol*, 128:303–9.

Rojas M, Xu J, Woods CR, et al. 2005. Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol*, 33:145–52.

Roth MD, Connett JE, D'Armiento JM, et al. 2006. Feasibility of retinoids for the treatment of emphysema study. *Chest*, 130:1334–45.

Sharpless NE, DePinho RA. 2007. How stem cells age and why this makes us grow old. *Nat Rev Mol Cell Biol*, 8:703–13.

Shigemura N, Okumura M, Mizuno S, et al. 2006a. Lung tissue engineering technique with adipose stromal cells improves surgical outcome for pulmonary emphysema. *Am J Respir Crit Care Med*, 174:1199–205.

Shigemura N, Okumura M, Mizuno S, et al. 2006b. Autologous transplantation of adipose tissue-derived stromal cells ameliorates pulmonary emphysema. *Am J Transplant*, 6:2592–600.

Shigemura N, Sawa Y, Mizuno S, et al. 2005. Amelioration of pulmonary emphysema by in vivo gene transfection with hepatocyte growth factor in rats. *Circulation*, 111:1407–14.

Spurzem JR, Rennard SI. 2005. Pathogenesis of COPD. *Semin Respir Crit Care Med*, 26:142–53.

Summer R, Kotton DN, Sun X, et al. 2004. Translational physiology: origin and phenotype of lung side population cells. *Am J Physiol Lung Cell Mol Physiol*, 287:L477–83.

Summer R, Kotton DN, Sun X, et al. 2003. Side population cells and Bcrp1 expression in lung. *Am J Physiol Lung Cell Mol Physiol*, 285:L97–104.

Suratt BT, Cool CD, Serls AE, et al. 2003. Human pulmonary chimerism after hematopoietic stem cell transplantation. *Am J Respir Crit Care Med*, 168:318–22.

Theise ND, Henegariu O, Grove J, et al. 2002. Radiation pneumonitis in mice: a severe injury model for pneumocyte engraftment from bone marrow. *Exp Hematol*, 30:1333–8.

Tsuji T, Aoshiba K, Nagai A. 2006. Alveolar cell senescence in patients with pulmonary emphysema. *Am J Respir Crit Care Med*, 174:886–93.

Wagers AJ, Sherwood RI, Christensen JL, et al. 2002. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science*, 297:2256–9.

Weihua Z, Tsan R, Schroit AJ, et al. 2005. Apoptotic cells initiate endothelial cell sprouting via electrostatic signaling. *Cancer Res*, 65:11529–35.

WHO. 2004. The world health report 2004. WHO.

Willemse BW, Postma DS, Timens W, et al. 2004. The impact of smoking cessation on respiratory symptoms, lung function, airway hyperresponsiveness and inflammation. *Eur Respir J*, 23:464–76.

Wright JL, Churg A. 2006. Advances in the pathology of COPD. *Histopathology*, 49:1–9.

Yamada M, Kubo H, Ishizawa K, et al. 2005. Increased circulating endothelial progenitor cells in patients with bacterial pneumonia: evidence that bone marrow derived cells contribute to lung repair. *Thorax*, 60:410–3.

Yamada M, Kubo H, Kobayashi S, et al. 2004. Bone marrow-derived progenitor cells are important for lung repair after lipopolysaccharide-induced lung injury. *J Immunol*, 172:1266–72.

Yokohori N, Aoshiba K, Nagai A. 2004. Increased levels of cell death and proliferation in alveolar wall cells in patients with pulmonary emphysema. *Chest*, 125:626–32.

Zander DS, Cogle CR, Theise ND, et al. 2006. Donor-derived type II pneumocytes are rare in the lungs of allogeneic hematopoietic cell transplant recipients. *Ann Clin Lab Sci*, 36:47–52.