Defect of alveolar regeneration in pulmonary emphysema: Role of lung fibroblasts

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Abstract: Pulmonary emphysema is characterized by the irreversible loss of pulmonary alveoli. Despite recent advances in the understanding this disease, its treatment remains palliative. In this review, we will successively review the data suggesting (1) that alveolar regeneration systems are functional in the mammalian lung and have the potential to regrow lost alveoli, (2) that cigarette smoke, the main etiologic factor of emphysema, inhibits those systems under experimental conditions, and (3) that alveolar regeneration systems are dysfunctional in the human emphysematous lung and may be a target for therapeutic intervention in this disease. Special emphasis will be put on the role of alveolar fibroblasts in those processes.

Keywords: emphysema, repair, regeneration, HGF, fibroblasts

Introduction

Pulmonary emphysema is characterized by the progressive destruction of pulmonary alveoli. It is one of the most common diseases worldwide (Halbert et al 2006) and its incidence keeps growing along with the large diffusion of its main etiologic factor, tobacco smoking. The ever-increasing use of tobacco in the developing world allows for the prediction of a huge increase in the number of cases in the near and distant future. The prognosis of pulmonary emphysema is poor, with airflow limitation progressing to chronic respiratory insufficiency, disability and premature death.

To this day, therapeutic options for pulmonary emphysema are few and scarcely effective. Patients who stop smoking do benefit in terms of lung function and survival, but the decline in lung function is not reversed, merely slowed. Apart from long-term oxygen therapy, pharmacological intervention is unsatisfactory and is mainly limited to the control of symptoms related to frequently-associated chronic bronchitis (Sutherland and Cherniack 2004). Lung volume reduction surgery can be of interest in selected patients (Fishman et al 2003) as may be the implantation of endobronchial one-way valves (Wood et al 2007) but those techniques do not remedy the underlying condition. Lung transplantation, while beneficial in terms of symptoms-related quality of life, does not improve survival in patients with pulmonary emphysema (Hosenpud et al 1998). Overall, treatment of pulmonary emphysema is palliative.

Most studies of the pathogenesis of emphysema focused on the mechanisms involved in chronic injury induced by cigarette smoke to the pulmonary parenchyma. In particular, the role of protease-antiprotease imbalance, persistent inflammation, oxidative stress and excessive apoptosis of alveolar cells was demonstrated (Barnes et al 2003). However, while great advances were made towards the comprehension of those phenomenons, they have not translated into new therapeutic prospects until now.

Alveolar regeneration systems may be important actors in the course of emphysema. On the one hand, alterations or defects in those systems may account for the deficiency in tissue repair which is observed in the emphysematous lung. On the other hand, the induction of alveolar regeneration would be an interesting therapeutic target in

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this disease. In this review, we will successively assess the evidence (1) that alveolar regeneration systems are functional in the mammalian lung and have the potential to regrow lost alveoli, (2) that cigarette smoke, the main etiologic factor of emphysema, inhibits those systems under experimental conditions, and (3) that alveolar regeneration systems are dysfunctional in the human emphysematous lung and may be a target for therapeutic intervention in this disease. Special emphasis will be put on the role of alveolar fibroblasts in those processes.

Effective alveolar regeneration systems are functional in the adult mammalian lung

The potential for alveolar generation and regeneration in adult mammals was only recently demonstrated in rodents but also in larger animals. This phenomenon could be observed after alveolar depletion was induced either diffusely by starvation or locally by the surgical resection of lung parenchyma.

Alveolar regeneration has been observed in mice fed a normal diet after a starvation period. Starvation has long been known to induce the loss of pulmonary alveoli and the subsequent development of emphysema-like lesions in the lung as was recently shown in humans with anorexia nervosa (Coxson et al 2004). There are no data regarding the potential reversibility of these lesions in humans, however the return to a normal diet induces the complete resolution of starvation-associated emphysema in mice (Massaro et al 2004). This phenomenon represents a truly regenerative process as the restoration of normal numbers and surface of alveoli is accompanied by a sharp elevation of DNA synthesis in the lung, indicating cellular replication (Massaro et al 2007).

Alveolar regeneration was also demonstrated after a localized resection of lung parenchyma. Indeed, the knowledge that the remaining lung undergoes adaptative changes and compensatory alveolar generation after lung resection was acquired several decades ago (Cagle and Thurlbeck 1988). In rats, resection of the upper and middle lobes of the right lung (25% of the total lung volume) induces a sharp increase in the volume of the remaining lobes, while microscopically alveolar size and surface are close to those of unoperated animals, indicating the de novo generation of alveoli (Wandel et al 1983). In mice, post-resection alveolar generation involves all cellular components of the lung as epithelial, endothelial and mesenchymal cells proliferate (Voswinckel et al 2004). It must be emphasized that in this context the newly formed alveolar tissue seems to be functional and to participate in ventilation.

Of major interest, compensatory alveolar generation after lung resection was also evidenced in growth–arrested species, albeit to a lesser degree than in rodents. In dogs as well as in humans, the intensity of post-pneumonectomy alveolar generation seems to depend on age. In young dogs, after left pneumonectomy removing less than 45% of total lung parenchyma, compensatory alveolar generation occurs vigorously and leads to the restoration of alveolar numbers similar to that of unoperated animals (Takeda et al 1999). By contrast, in fully adult dogs, a stronger stimulus such as right pneumonectomy (removing 55% or more of parenchyma) is needed to induce a weaker and incomplete response (Hsia et al 1994). In humans, post-pneumonectomy alveolar generation and lung growth do not seem to occur in adults but indirect clues point towards the existence of such phenomenon in children and to a lesser degree in adolescents. In particular, the ventilatory function at adulthood of patients pneumonectomized before the age of 4 is close to that of unoperated subjects, most likely indicating that the formation of new alveoli compensated for resected ones (Laros and Westermann 1987). Patients operated upon between 6 and 20 years of age still had better lung function parameters than those of patients who underwent pneumonectomy at adulthood (Laros and Westermann 1987). Altogether, those results indicate that alveolar regeneration systems are present in the lung of growth-arrested mammals such as humans, and that those systems are accessible to stimulation.

Mechanisms of alveolar regeneration, role of alveolar fibroblasts

Alveolar cells of every type (epithelial, endothelial, and mesenchymal) participate in alveolar multiplication and regeneration events since those require their equilibrated proliferation. Moreover, all three main cell types seem to possess the ability to drive lung growth since the administration of growth factors targeted towards either type leads to the acceleration of alveolar generation after lung resection. The hepatocyte growth factor (HGF), the first such agent that was identified as stimulating lung growth after lung resection (Yanagita et al 1993), targets both epithelial and endothelial cells. The epidermal growth factor (EGF) (Kaza et al 2000), activates mainly epithelial cells, whereas the platelet–derived growth factor-BB (PDGF-BB) (Yuan et al 2002) is a mitogen primarily for mesenchymal cells. While both the keratinocyte growth factor (KGF) (Kaza et al 2002), and the vascular endothelial growth factor (VEGF) (Sakurai et al 2006)

stimulate post-pneumonectomy alveolar multiplication, the former induces proliferation exclusively in epithelial cells while the latter essentially targets the endothelium. Altogether, those results suggest that the induction of proliferation in a particular cell population of alveoli may be accompanied by the simultaneous expansion of the other neighboring cell types, possibly through paracrine mechanisms. However, some elements indicate that mesenchymal cells may be first among equals and may play a leading part in those processes. Indeed, pulmonary fibroblasts are the main contributors to the synthesis and maintenance of the alveolar extracellular matrix which is the essential scaffold over which other cells proliferate and differentiate and which provides the lung with its essential mechanical characteristic, elasticity. Moreover, alveolar fibroblasts secrete growth factors targeting alveolar epithelial and endothelial cells.

Pulmonary fibroblasts are the main cell type responsible for the synthesis and secretion of the main components of the alveolar extracellular matrix, proteoglycan, collagen and elastin. Elastin in particular is an essential component of the alveolar extracellular matrix as it provides the lung with the elasticity needed for ventilation, and it is probable that elastin synthesis is an essential step in alveolar growth and regeneration. Supporting this hypothesis, Koh and colleagues (Koh et al 1996) showed that tropoelastin mRNA was sharply overexpressed in the lung of rats after pneumonectomy, and that its expression was localized to the ends, bends and junctions of alveolar walls, indicating that the *de novo* synthesis of elastic fibers was part of the process of alveolar growth and multiplication. In this study, the localization of lung elastin mRNA expression after pneumonectomy differed slightly from that observed in developing animals as in the latter case elastin mRNA expression was observed at the end and bend of alveolar walls but was not observed at the junctions of alveolar walls.

In addition to their role as extracellular matrix providers, alveolar fibroblasts seem to participate in the complex cellular interactions that lead to alveolar growth and multiplication both in the fetal lung (Warburton and Bellusci 2004) and in the adult lung. Fibroblasts interact both with endothelial cells and epithelial cells through the secretion of soluble factors that act in a paracrine fashion. In particular, fibroblasts are the main if not the only source of KGF in the distal lung and contribute greatly to the elevation of lung HGF levels in response to alveolar injury (Stern et al 2000; Marchand-Adam et al 2003, 2005; Cohen et al 2006). Moreover, although this point has not been demonstrated in adult animals so far, lipid-laden interstitial fibroblasts, a subpopulation of alveolar fibroblasts, are the main source of retinoic acid in the lungs of rodents undergoing alveolar septation (McGowan and Torday 1997). In adult animals, retinoic acid, the active metabolite of vitamin A, increases post-pneumonectomy lung growth in rodents (Kaza et al 2001) and enhances alveolar capillary formation after right pneumonectomy in dogs (Yan et al 2004).

Interestingly, the role of fibroblasts in the regulation of cell-cell interactions in alveoli may not be limited to the secretion of paracrine signal molecules as cytoplasmic expansions originating in those cells reach alveolar endothelial and epithelial cells through their respective underlying basement membranes (Sirianni et al 2003).

Overall, fibroblasts seem to play a central role in alveolar multiplication and regeneration and it may be assumed that the realization of such phenomenon requires proliferating and metabolically active fibroblasts.

Cigarette smoke represses fibroblast functions implicated in alveolar regeneration and repair

Cigarette smoke is responsible for an overwhelming majority of cases of pulmonary emphysema. The mechanisms by which chronic smoke exposure induces chronic lung injury leading to emphysema have been exhaustively described and include chronic inflammation (Hoidal and Niewoehner 1982), protease/antiprotease imbalance (Carp and Janoff 1978), oxidative stress (Church and Pryor 1985) and excessive death of bronchiolar and alveolar epithelial and endothelial cells (Jung et al 2000; Tuder et al 2000; Wickenden et al 2003). However, the role of cigarette smoke in the pathogenesis of emphysema does not seem to be limited to its participation in chronic injury to the lung. Indeed, cigarette smoke has been shown to reduce viability in human lung fibroblasts and to inhibit a number of fibroblast functions closely linked to alveolar regeneration, at least in vitro.

Cigarette smoke possesses cytotoxic properties towards lung fibroblasts as cigarette smoke extract reduces the viability of those cells in vitro (Ishii et al 2001). This effect has been linked to the induction of apoptosis (Carnevali et al 2003; Baglole et al 2006). Additionally, cigarette smoke inhibits lung fibroblast proliferation and their capacity to migrate (Nakamura et al 1995; Nobukuni et al 2002). Moreover, lung fibroblasts chronically exposed to cigarette smoke display a senescent phenotype characterized by an enlarged morphology and cell cycle arrest (Nyunoya et al 2006). Altogether, those results indicate that cigarette smoke probably induces a reduction in the number of metabolically active

fibroblasts available for alveolar growth and multiplication in the emphysematous lung.

In addition to its cytotoxic and antiproliferative effects, cigarette smoke inhibits key fibroblastic functions associated with alveolar growth and multiplication. In particular, elastin synthesis and cross-linking seem to be especially sensitive to exposure to cigarette smoke. While no data are available regarding adult lung fibroblasts, cigarette smoke extract down regulates tropoelastin mRNA in rat fetal lung fibroblasts (Gao et al 2005). In an acellular model, cigarette smoke inhibits elastin cross-linking (Laurent et al 1983), while the transcription of lysyl oxidase, a key effector of elastin cross-linking is reduced in fetal rat lung fibroblasts exposed to cigarette smoke extract (Gao et al 2005).

Whether cigarette smoke exposure antagonizes alveolar growth and multiplication in vivo has not been demonstrated directly. To our knowledge, no study has been dedicated to the description of the effect of cigarette smoke on post-pneumonectomy lung growth. However, data obtained in a different model of alveolar depletion, elastase-induced emphysema, indicate that cigarette smoke exposure may indeed induce a defect in elastin synthesis and cross-linking, alveolar growth, multiplication and/or repair. Indeed, while elastase instillation in the trachea induces a sharp elevation in elastin synthesis in the lung, this response is reduced by 40% in the lung of hamsters exposed to cigarette smoke for one week (Osman et al 1985). This reduction in elastin synthesis is accompanied by a seven-fold reduction in lysyl oxidase activity in the lung of those animals. This reduction in elastin synthesis and cross-linking may be of great pathophysiological significance as emphysema lesions are exaggerated in hamsters exposed to cigarette smoke after elastase instillation (Hoidal and Niewoehner 1983).

Are alveolar regeneration and repair systems defective in human emphysema? Abnormal phenotype of fibroblasts

Whether a defect in alveolar regeneration systems plays a role in cigarette smoke induced emphysema in humans remains unclear but some elements indicate that such systems do get activated in the course of this disease. For instance, contrary to what is observed in the normal lung (Shapiro et al 1991), a high elastin turnover is observed in the emphysematous lung (Stone et al 1995; Gottlieb et al 1996; Vlahovic et al 1999), indicating that in parallel with the excessive degradation of elastic fibers which is observed in this disease, elastin synthesis occurs in the lung. Interestingly, in line with a high rate of extracellular matrix synthesis associated with a high rate of extracellular matrix degradation, a high rate of cellular proliferation is associated with the elevated apoptosis of alveolar cells in the emphysematous lung (Imai et al 2005), adding more substance to the hypothesis that alveolar regeneration systems do get activated in the emphysematous lung even though they are probably not functional as the alveolar architecture is not restored. While an alteration of alveolar regeneration systems has not been directly evidenced in the emphysematous lung (Bonay et al 2005), it must be put forward that alveolar fibroblasts exhibit a defective phenotype in the emphysematous lung.

Available data regarding the in situ phenotype of pulmonary fibroblasts in emphysema are limited to the observations by Sirianni and coworkers (Sirianni et al 2006). These authors showed that direct intercellular contacts between alveolar fibroblasts and alveolar epithelial cells or capillary endothelial cells are greatly reduced in the emphysematous lung. Those results suggest that pulmonary emphysema fibroblasts may lack the capacity to participate in the direct intercellular communications which play an essential role in fetal lung development and probably during lung repair in the lung (Warburton et al 2001; Parker et al 2004; Warburton and Bellusci 2004).

The abnormal phenotype of alveolar fibroblasts in pulmonary emphysema was described more extensively in vitro on primary lines of pulmonary fibroblasts obtained following the explant culture technique. First, pulmonary emphysema fibroblasts show signs of premature senescence. When compared to fibroblasts obtained from normal human lung, fibroblasts obtained from emphysematous lung have a markedly reduced proliferation rate. Indeed, emphysema fibroblast proliferation was described as 50%–60% of controls (Nobukuni et al 2002; Noordhoek et al 2003; Holz et al 2004). In one study, those cells were shown to express senescence-associated beta-galactosidase (SA-beta-Gal), a marker of cellular senescence (Muller et al 2006). Moreover, in some studies emphysema fibroblasts did not exhibit the typical spindle-shaped microscopic appearance of fibroblasts and expressed smooth-muscle actin, indicating a degree of differentiation towards the myofibroblast phenotype. Secondly, in addition to premature senescence of pulmonary emphysema fibroblasts precluding an elevation of their number, those cells seem to secrete low levels of growth factors. In particular, HGF secretion by those cells is sharply reduced compared to normal lung fibroblasts and they poorly respond to exogenously added KGF (Plantier et al 2005). Given the

potential regenerative effect of HGF and KGF in the lung, a low secretion of this factor by lung fibroblasts may play a role in the pathogenesis of emphysema. Altogether, those results indicate an altered phenotype of pulmonary fibroblasts which may partly contribute to the absence of alveolar regeneration observed in emphysema.

Alveolar regeneration systems are therapeutic targets for emphysema

The therapeutic manipulation of alveolar regeneration systems would represent a tremendous advance for the treatment of emphysema as well as other diseases characterized by the destruction of alveoli. Such results were obtained in animal models of emphysema through the administration of pharmacological agents, gene therapy or the implantation of mesenchymal stem cells, raising hope that such an effect may be attainable in human patients.

The proof of concept that alveolar regeneration was a possible therapeutical goal in emphysema was brought by the pioneering work by Donald and Gloria Massaro (Massaro and Massaro 1997). In this study, all-trans retinoic acid induced partial alveolar regeneration 20 days after emphysema was induced in rats with the tracheal instillation of elastase. This result was reproduced by some groups (Ishizawa et al 2004), while others did not (Tepper et al 2000; Lucey et al 2003; Fujita et al 2004; March et al 2004).

The strong regenerative effect of exogenous HGF was shown in the elastase-induced emphysema model in mice. Its effect was associated to the engraftment into the lung of cells derived from the circulation (Ishizawa et al 2004). This effect was reproduced in rats transfected intravenously with the human HGF gene seven days after the instillation of elastase (Shigemura et al 2005). Adrenomedullin, an angiogenic factor, has been shown to possess alveolar regenerative properties in the same model, albeit at a lesser degree than HGF (Murakami et al 2005). Interestingly, an angiogenic stimulus seems necessary for the induction of alveolar regeneration in the emphysematous lung as KGF, which targets mainly epithelial cells, was not found to exert such an effect, even though its cytoprotective properties allowed it to fully prevent the constitution of emphysema in mice instilled with elastase (Plantier et al 2006).

All of the studies cited so far relied on the administration of soluble factors, either directly or by gene transfection. Another approach to the development of alveolar regenerative therapeutics has been the transplantation into the lung of mesenchymal stem cells obtained from adipose tissue (Shigemura et al 2006a, 2006b). Adipose-tissue derived stromal cells were obtained from inguinal subcutaneous fat and applicated to the elastase-injured lung, resulting in alveolar regeneration and higher levels of PaO_2 and maximal oxygen consumption. Interestingly, adipose-tissue derived stromal cells secrete particularly high levels of HGF. Their regenerative effect in the emphysematous lung may be related to this characteristic as their engraftment into the lung was not reported. Overall, the feasibility of inducing alveolar regeneration in rodents with elastase-induced emphysema has been thoroughly demonstrated.

Alveolar regeneration in human emphysema?

Whether alveolar regeneration may be obtained in pulmonary emphysema patients in the near future remains unsure. To this day, a single study has been devoted to the exploration of the effect of a regenerative agent in this disease, namely retinoic acid (Roth et al 2006). In this crossover study, all-trans retinoic acid, 13-cis retinoic acid or placebo were administered for 6 months to 148 patients with moderate-to-severe COPD and a predominant component of emphysema. Despite the fact that high plasma levels of retinoic acid were obtained in treated patients, no difference in pulmonary function or quality of life was observed between groups in this study.

Among the candidate drugs for regeneration therapy in the lung, HGF seems to be of particular interest. While the use of this factor for the treatment of lung disease has not been reported to date, encouraging preliminary results have been obtained in the treatment of peripheral arterial disease where HGF is well tolerated in the short term and induces an increase in distal perfusion (Morishita et al 2004). However, HGF has been reported to facilitate the growth of tumors in various organs, including the lung (Stabile et al 2006). Since patients with pulmonary emphysema are most frequently smokers or ex-smokers at a high risk of lung cancer, whether HGF itself is a good candidate for the long-term treatment of this disease remains to be evaluated.

Conclusion

Alveolar regeneration can be induced in animal models of diffuse or localized alveolar loss and can be repressed by exposure to cigarette smoke. Defects in alveolar regeneration systems have been identified in the human emphysematous lung and may play an important role in the course of this disease which is characterized by alveolar loss. Restoration and/or induction of these systems in emphysematous patients may represent a major advance in the treatment of pulmonary emphysema, a disease characterized by the loss of pulmonary alveoli.

Encouraging results have been obtained in elastase-induced emphysema in rodents, and agents known to induce alveolar regeneration in this model are currently under evaluation for the treatment of emphysema and other diseases.

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