Lung regeneration and translational implications of the postpneumonectomy model

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Lung regeneration research is yielding data with increasing translational value. The classical models of lung development, postnatal alveolarization, and postpneumonectomy alveolarization have contributed to a broader understanding of the cellular participants including stem-progenitor cells, cell-cell signaling pathways, and the roles of mechanical deformation and other physiologic factors that have the potential to be modulated in human and animal patients. Although recent information is available describing the lineage fate of lung fibroblasts, genetic fate mapping, and clonal studies are lacking in the study of lung regeneration and deserve further examination. In addition to increasing knowledge concerning classical alveolarization (postnatal, postpneumonectomy), there is increasing evidence for remodeling of the adult lung after partial pneumonectomy. Though limited in scope, compelling data have emerged describing restoration of lung tissue mass in the adult human and in large animal models. The basis for this long-term adaptation to pneumonectomy is poorly understood, but investigations into mechanisms of lung regeneration in older animals that have lost their capacity for rapid re-alveolarization are warranted, as there would be great translational value in modulating these mechanisms. In addition, quantitative morphometric analysis has progressed in conjunction with developments in advanced imaging, which allow for longitudinal and nonterminal evaluation of pulmonary regenerative responses in animals and humans. This review focuses on the cellular and molecular events that have been observed in animals and humans after pneumonectomy because this model is closest to classical regeneration in other mammalian systems and has revealed several new fronts of translational research that deserve consideration. (Translational Research 2014;163:363–376)

Abbreviations: AECII = alveolar epithelial type II cells; BASCs = bronchoalveolar stem cells; BrdU = bromodeoxyuridine; EGFR = epidermal growth factor receptor; EGR-1 = early growth response protein 1; EpCAM = epithelial cell adhesion molecule; FGFR = fibroblast growth factor receptor; HGF = hepatocyte growth factor; IGF-1 = insulin-like growth factor 1; KGF = keratinocyte growth factor; LR-MSCs = lung-resident mesenchymal stromal cells; PCEC = pulmonary capillary endothelial cells; PDGF = platelet-derived growth factor; PDGFR = platelet-derived growth factor receptor; proSP-C = prosurfactant protein C; Sca-1 = stem cell antigen 1; VEGF = vascular endothelial growth factor

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Regeneration of organs such as whole limbs or tails of the salamander,[1,2] the antlers of deer,[3] and the digit tips of rodents[4] is defined by the complete replacement of native structure and function at the anatomic site of excision by local cell proliferation and differentiation (epimorphosis). In contrast, the lung as a whole organ in mammals does not regenerate in this way—a new lung does not grow orthotopically from the site of bronchial excision. Thus, the lung behaves similarly to liver[5] and kidney,[6] where compensatory growth occurs by hyperplasia or hypertrophy (respectively), remodeling, and differentiation at sites within the residual organ.

Although whole lung or lobar regeneration has not yet been achieved in adult mammals, regeneration of acini embedded within lobules can be successfully provoked. *De novo* formation of new lung acini ("neo-alveolarization" or "re-alveolarization") occurs spontaneously in response to partial pneumonectomy (PNX) in pediatric humans (typically, those under 3 years of age)[7] and immature animals.[8] This process of compensatory lung growth after pneumonectomy has been defined as "lung regeneration." The span of time over which re-alveolarization is possible within the lifetime of mammals is fleeting, delimited by birth and approximately the end of post-natal alveolarization, and in some species (such as rodent species) extending into adulthood. Lung researchers have exploited this model in a variety of animal species to develop a fundamental understanding of the cellular and molecular basis for re-alveolarization, employing a framework of research principles engendered from pre- and postnatal lung development and regenerative mechanisms in other organs. Most recently, the mouse has served as a tremendous asset in this quest because of the availability of transgenic strains.[9,10]

Apart from re-alveolarization that defines lung regeneration after PNX in animals and humans, "lung regeneration" is a term that has been applied liberally in the literature to processes that do not necessarily recapitulate re-alveolarization, which are complicated by inflammation, fibrosis, and derangement of the normal ultrastructure of the lung. Examples include pulmonary responses to influenza infection,[11] bleomycin,[12] caloric restriction,[13] microcystin,[14] dexamethasone,[15] disulfiram (a retinoic acid synthesis inhibitor),[16] and elastase.[17,18] "Lung regeneration" as a term has also been applied to bioengineering of lungs: the process of seeding devitalized lung scaffolds with lung cells that has received recent interest.[19] Unlike post-PNX re-alveolarization, lung bioengineering is dependent on preformed fiber networks and basement membranes, and does not involve the generation of new acini.[19,20] Thus, it is evident that the term "lung regeneration" is used in the literature broadly, to describe diverse wound-healing processes whereby re-alveolarization is a component or is incomplete. Hence, as post-PNX lung regeneration provides the least complicated and most comprehensive model of re-alveolarization, we have focused this review article on the increasing knowledge base concerning this model, with emphasis on cellular and molecular events, and opportunities for translation of this research.

THE PARTIAL PNEUMONECTOMY MODEL

While no mammalian model perfectly captures the complexity of classic organ regeneration (epimorphosis), the PNX model has been studied as a possible analogue.[7,8,21-23] The pneumonectomized patient experiences a sudden decrease in the number of alveoli, and consequently, a reduction in surface area and gas exchange ability, prompting both immediate and longer-term responses to maintain homeostasis. PNX has been extensively described in animals and has been most thoroughly used in rodent models where genetic diversity is minimal.

The details of the experimental PNX procedure in animals vary between investigators; however, the basics of the procedure are conserved. The procedure is performed with the subject under general anesthesia (typically injectable ketamine and xylazine), utilizing mechanical ventilation with airway capture via orotracheal intubation or tracheotomy. A single incision into a superior left or right intercostal space extending to the pleura allows access to the hemithorax of interest. The pulmonary vessels and bronchi are ligated as appropriate for size to allow for lobe removal. Prior to closure of the surgical site, the remaining lung is inflated during closure of the chest to facilitate reduction of the postoperative pneumothorax; alternatively, inert material may be inserted into the hemithorax to block regrowth to varying extents (plombage).[17,24,25] Periodic refinements in technique have reduced perioperative mortality in the research setting[26,27]; in general, mortality rate after PNX in wild type mice should not exceed 10% of operated animals and may be expected to be as low as $<5\%$ with excellent technique (unpublished observations).

In comparison to animal models, humans undergoing PNX differ considerably in that the clinical patient often is affected by concurrent, complex pulmonary, or systemic disease. However, patients undergoing surgery for removal of smaller, discrete regions of the lung, as well as living lung donors, constitute a subset of human PNX patients with no, to minimal extant pulmonary disease. Human living lung donors, for instance, typically donate the left or right lower lobe, resulting in an approximate 20%–30% decrease in lung parenchyma.[28]