Stem/Progenitor Cells in Lung Development, Injury Repair, and Regeneration

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At least two populations of epithelial stem/progenitor cells give rise to the lung anlage, comprising the laryngo-tracheal complex versus the distal lung below the first bronchial bifurcation. Amplification of the distal population requires FGF9-FGF10-FGFR2b-Sprouty signaling. Residual pools of adult stem cells are hypothesized to be the source of lung regeneration and repair. These pools have been located within the basal layer of the upper airways, within or near pulmonary neuroendocrine cell rests, at the bronchoalveolar junction as well as within the alveolar epithelial surface. Rapid repair of the denuded alveolar surface after injury is clearly key to survival. Strategies to enhance endogenous alveolar epithelial repair could include protection of epithelial progenitors from injury and/or stimulation of endogenous progenitor cell function. Protection with inosine or FGF signaling are possible small molecule therapeutic options. Alternatively, exogenous stem/progenitor cells can be delivered into the lung either intravenously, intratracheally, or by direct injection. Sources of exogenous stem/progenitor cells that are currently under evaluation in the context of acute lung injury repair include embryonic stem cells, bone marrow- or fat-derived mesenchymal stem cells, circulating endothelial progenitors, and, recently, amniotic fluid stem/progenitor cells. Further work will be needed to translate stem/progenitor cell therapy for the lung.

Keywords: stem/progenitor cell, lung

LUNG DEVELOPMENT AND STEM/PROGENITOR CELLS

The lung develops from the laryngotracheal groove, which comprises at least two populations of progenitor cells, giving rise respectively to the larynx and trachea versus the peripheral bronchi and alveolar surface. Developmental biologists have made significant progress in unraveling the molecular and genetic mechanisms directing the functional elaboration of the information stored in the genome, which, taking advantage of physical principles, forms functional structures, including as many as 40 different epithelial, mesenchymal, vascular, and lymphatic endothelial and immune cell lineages in the lung (1–3). Major instructive signaling pathway mechanisms include FGF-FGFR-Sprouty, BMP-BMPR-Gremlin, Shh-Ptc-Hip, Wnt- β -cadherin, PDGF, HGF, and VEGF signaling. Reactivation of the same or similar developmental signaling networks has been implicated in progenitor cell activation, daughter cell proliferation, and migration during repair of lung tissue after injury. Null mutation studies in mouse show that FGF10-FGFR2b-Sprouty signaling is required for amplification of epithelial progenitor

cells distal to the carina. In both FGF10- and FGFR2b-null mice, respectively, the larynx and trachea form, but the distal bronchi do not grow, whereas with misexpression of Sprouty2 under the control of the *SpC* promoter or in the *Fgf10* hypomorphic mouse, the lung is small and misshapen (1–3). This suggests that FGF10-FGFR2b-Sprouty signaling is required to amplify the peripheral progenitors, but not the proximal ones.

Stem and Progenitor Cells

Stem cells are the self-renewing, primitive, undifferentiated, multipotent source of multiple cell lineages. While such cells are critical for development and growth through childhood, residual pools of adult stem cells are hypothesized to be the source of the frequently limited tissue regeneration and repair that occurs in adults. Unlike tumor cells and embryonic stem cells, adult stem cells are not immortal, and show decreasing telomere length with increasing age. The naturally limited replacement capacity of such endogenous stem cell pools, though efficient and functional through young adult life, has been associated with the inability to repair damage that accumulates to a critical point late in life. This may occur via simple exhaustion of the stem cell pool or arise as a consequence of inherited or acquired mutations that impede proper stem cell function. It is speculated that these events could be reversed via stimulation or rehabilitation of the endogenous stem cell pool or by introduction of exogenous stem cells to the debilitated organ to reverse the effects of aging and/or disease. Thus, there has been ever-increasing recognition of the potential role stem cells could play in regenerative medicine.

Endogenous Progenitor Cells

Recent studies have shown that the failure to regenerate and repair that inevitably occurs with aging may be due to endogenous stem cell failure. Putative endogenous epithelial progenitor cells have been located within the adult lung in the basal layer of the upper airways, within or near pulmonary neuroendocrine cell rests as well as at the bronchoalveolar junction (4–11). These types of progenitor cells are discussed in greater detail in other papers in this issue.

Endogenous Alveolar Epithelial Progenitors

Rapid repair of the denuded alveolar surface after injury is clearly key to survival. The alveolar surface is very large (i.e., 70 m²) in adult humans. The size and spatial restrictions of the alveolar surface therefore suggest that at least one progenitor cell per alveolus must be required to achieve rapid coverage and repair of alveolar epithelial leak. Thus, a large number of cells must function as a “ready reserve” to repair damaged alveolar surface. Driscoll and coworkers (2000) (12) showed that after acute oxygen injury, expression of telomerase, a stem/progenitor cell marker, is widely up-regulated in alveolar epithelial cells (AEC) during the recovery phase. This suggests that alveolar epithelial cells either contain a subpopulation of progenitor cells, or that the majority of alveolar epithelial cells can undergo reactivation into a progenitor-like state in response to

(Received in original form January 25, 2008; accepted in final form June 23, 2008)

Supported by NIH grants HL60231, 44060, 44977, 75773 to D.W. Travel grant from Astra Zeneca to D.W.

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Proc Am Thorac Soc Vol 5, pp 703–706, 2008

DOI: 10.1513/pats.200801-012AW

Internet address: www.atsjournals.org

injury cues. The putative AEC progenitor cells can be sorted out by fluorescence-activated cell sorter (FACS) from primary AEC populations isolated from rat lungs during the recovery phase from sublethal hyperoxia, using the criteria of surface E-cadherin expression as well as relative resistance to apoptosis (9). The telomerase-positive and E-cadherin-reduced AEC obtained from this FACS sort are also relatively more proliferative and relatively resistant to injury-induced apoptosis. Thus we speculate that this subpopulation may be responsible for the proliferative phase of repopulation of the injured alveolar epithelium (13). Our recent studies show that without telomerase expression, resistance to injury and repopulation of damaged alveoli are both compromised, indicating that this pathway is critical for alveolar progenitor cell activity. The pattern of telomerase immunohistochemistry in hyperoxia-injured lungs support the concept that if alveolar stem/progenitor cells do in fact exist, they must be widely distributed over the alveolar surface (12). This hypothesis was strengthened by the discovery of bronchoalveolar stem cells (BASC), by Kim and colleagues (10). These cells appear to reside at the alveolar-airway junction and express both alveolar (Sp-C) and airway (CC10) epithelial cell markers. They are resistant to damage and proliferate after injury *in vivo* and are also multipotent in clonal assays *in vitro*. The existence of these two putative endogenous alveolar stem cell populations may thus provide a target for directed regenerative therapies in the lung.

Taking acute oxygen injury as an example, AEC undergo DNA as well as other forms of damage such as glutathione depletion, mitochondrial failure, and apoptosis (13–15). We have shown that FGF7 and inosine treatment can ameliorate DNA damage in alveolar epithelial cells as well as enhancing mitochondrial protection and the ability of AEC to migrate and repair in an *in vitro* scratch assay (16). FGF7 has also been evaluated by others *in vivo* as a treatment to enhance resistance to alveolar injury in animal models (17, 18). In recent work we also find that FGF10 has a protective effect against lung injury. We have also recently shown (19) that inosine has protective properties against oxygen injury, including glutathione repletion, mitochondrial protection, decreased apoptosis, and increased VEGF expression. The latter finding of increased VEGF expression suggests that inosine may also be useful indirectly for protection or repair of alveolar capillary endothelium. Thus, it appears that protection or enhancement of alveolar progenitor cell function may be a viable therapeutic option that could possibly be evaluated in clinical trials using small molecules such as inosine.

Endogenous Mesenchymal Progenitors

Until recently, the early lung mesenchyme was recognized to exert interesting inductive properties on the early lung epithelium to initiate branching morphogenesis. That FGF9 in the mesothelium activates and controls FGF10 signaling from the peripheral mesenchyme via FGFR2b, SHP2, Grb2, Sos, and Ras in the epithelium, and that Sprouty2 is an inducible modulator of this signaling pathway was also recognized (20–23).

Smooth muscle. It has recently emerged that the peripheral mesenchyme that expresses Fgf10 also serves as a progenitor cell population for peripheral airway smooth muscle (24–26). Thus, lineage tracing studies with Fgf10-lacZ reveal that airway smooth muscle progenitors begin as Fgf10-expressing cells that, as the airway grows outwards, become distributed along the elongating peripheral airway, much as a sock goes up the leg as one puts it on. Transdifferentiation of these progenitors to express α -smooth muscle actin fibers occurs under the control of SHH and BMP4, which are expressed proximal to the very tip of the airway. Thus, the population size and placement of

peripheral airway smooth muscle progenitors appears to occur very early in development. Another population of airway smooth muscle progenitors was recently shown to arise in the proximal mesenchyme and advance peripherally.

Vascular progenitors. A primitive capillary plexus surrounds the laryngotracheal groove as it buds from the foregut at Embryonic Days 9 to 10 in mouse and at 4 to 5 weeks in human. At this very early stage, the primitive capillaries can be visualized by the expression of β -galactosidase under the control of the *Flk1* promoter. This promoter is active and is the earliest marker of hemangioblasts. Under the stimulation of VEGF, which is secreted mainly by the primitive epithelium, these hemangioblasts differentiate into a stereotypic capillary network that surrounds the bronchial, lobar, and segmental branches of the airway (26, 27). Correct organization of this vasculature plexus appears to be essential for correct airway branching as well as tissue perfusion. Correct matching between capillary endothelium and the gas diffusing surface of the lung determines the eventual maximal gas-diffusing capacity of the lung. Thus, mesothelial-mesenchymal-epithelial-endothelial cross-talk matches epithelial and vascular progenitor function and will likely be essential if lung regeneration using endogenous or exogenous stem/progenitor cells is to succeed.

Prospects for Exogenous Stem/Progenitor Cells

Considerable excitement and hope has been generated by the possibility that exogenous stem/progenitor cells could be used to enhance lung repair or regeneration, particularly in patients with respiratory failure due to severe alveolar hypoplasia or destruction, such as bronchopulmonary dysplasia or chronic obstructive pulmonary disease (COPD). Exogenous stem/progenitor cells are operationally defined as multipotent cells with the capacity to maintain themselves indefinitely as stem cells, while simultaneously dividing to give rise to daughter progenitor cells. Progenitor cells in turn continue to divide, but give rise to differentiated cell lineages. These differentiated cell lineages in turn give rise to tissues and organs, in a temporospatial collaboration with other differentiated cell lineages. Thus, knowledge and understanding of endogenous stem cell properties informs the use of exogenous populations, and historically have defined how they may be used successfully. However, recent studies using exogenous populations have shown that strict adherence to the role of the endogenous stem cell, via engraftment in appropriate numbers and correct lineage differentiation and function, may not be the only mechanism for achieving success with exogenous stem cell therapy.

Exogenous stem/progenitor cells may be delivered into the lung either intravenously, intratracheally, or by direct injection. Since the alveolar capillary bed is perfused by the entire right cardiac output and is very narrow (5–7 μ m), it serves as a sieve to trap larger, more rigid cells than the standard red or white blood cell. Thus, after intravenous administration the immediate efficiency of exogenous stem/progenitor cell arrival and trapping in the lung is very high. However, diapedesis of these cells into the lung parenchyma and eventual integration into lung cell lineages is extremely inefficient in the absence of lung injury. Even in the presence of injury, the efficiency of integration relative to the entire lung cell population is quite low (< 2%). Therefore, improving efficiency of uptake and integration appears to be a major prerequisite before clinical trials for lung regeneration using exogenous stem/progenitor cells can be contemplated. As for internally generated, versus externally delivered exogenous lung stem cells, circulating signals from the damaged lung such as GM-CSF may stimulate the bone marrow to release increased numbers of mesenchymal stem cells. However, it has recently been shown that mesen-

chymal stem cells from the bone marrow may make a substantial contribution to bleomycin-induced fibrosis in mice, so this may be a major caveat (28).

Sources of exogenous stem/progenitor cells that are currently available include embryonic stem cells, bone marrow- or fat-derived mesenchymal stem cells, and recently amniotic fluid stem/progenitor cells. Embryonic stem cells (ES), derived from blastocysts, propagate readily and are capable of forming aggregates (embryoid bodies) that generate a variety of specialized cell types, including neural, cardiac, and pancreatic cells. However, obtaining these cells has historically involved the destruction of embryos for their retrieval. To avoid the obvious ethical issues with human ESC, scientists have looked at other potential sources for pluripotent cells. Bone marrow and fat-derived stem populations appear to be enriched for mesenchymal stem cells. While recent studies have shown that these cells may ameliorate injury, it appears that they may do so via a pseudo-pharmaceutical effect, with the transient release of anti-inflammatory cytokines and possible stimulation of endogenous repair, rather than by engraftment and tissue regeneration *per se*. However, as noted, these populations also have the potential to contribute to the development of fibrosis, so much work is still required to understand the mechanism underlying the effects of these cells to manipulate them toward an efficacious therapy.

Human amniotic fluid stem cells (hAFSC) are derived from discarded amniocentesis specimens and are therefore ethically neutral. They are pluripotent, giving rise to all three germ layers in mouse chimeras as well as *in vitro* under the correct conditions (29). They also have the great advantage of not giving rise to tumors in contrast to human embryonic stem cells, which give rise to teratomas when injected *in vivo*. hAFSC can integrate and differentiate into specific tissue cell lineages when placed in the correct niche environment, such as the embryonic kidney (30).

Within the embryonic kidney, hAFSC can incorporate into tubular and glomerular structures and express the appropriate cell lineage marker genes. We have likewise found in preliminary studies that hAFSC will incorporate into mouse embryonic lung and express human epithelial cell markers. Following intravenous injection, hAFSC become trapped in the lung as expected on the first pass with high efficiency as detected by bioluminescence. However, diapycnosis into the lung tissue, as remarked above, is still highly inefficient in the absence of lung injury. Nevertheless, we find that after lung injury in nude mice *in vivo*, the efficiency of hAFSC integration and expression into upper versus lower airway epithelia is increased, depending upon the type of injury. Thus, after oxygen injury, hAFSC are taken up into the Sp-C-positive alveolar epithelial lineage, whereas after naphthalene injury, hAFSC are taken up into the CC10-positive Clara cell lineage. These cells then persist in the lung and are detectable for a long period of time (months). However, the number of persisting cells is not great. Thus, the utility of stem cell therapy with hAFSC for lung repair and regeneration remains to be seen, particularly in diseases such as COPD in which major destruction of cell differentiation niches has occurred.

Thus, in summary, the early embryonic lung epithelium comprises at least two populations of stem/progenitors that give rise respectively to the airway proximal to the lung versus the distal bronchi, bronchioles, and alveoli. The distal population is amplified by FGF9-FGF10-FGFR2b-Sprouty signaling. The epithelium is an important source of VEGF signaling, which is key to matching endothelial with epithelial progenitor cell function in the peripheral lung. The mesenchyme of the lung also gives rise to two distinct populations of peripheral and proximal airway smooth muscle progenitors. The latter arise

from the same population of cells that express *Fgf10*. Exogenous progenitor cells may exert more of a pseudo-pharmacologic effect on lung inflammatory homeostasis than any major effect on lung regeneration *per se*. However, this idea remains to be fully evaluated.

Conflict of Interest Statement: D.W. has been reimbursed by AstraZeneca for attending the Lund COPD Conference and by Boehringer Ingelheim for attending the TAC Conference. He and W.S. have received an equipment grant from Philip Morris to purchase a smoking machine. L.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. R.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. W.S. received \$200,000 during 01/01/2008 to 12/31/2008 from Philip Morris External Research Program as a research grant for studying genetic factors and susceptibility to smoke-induced emphysema. B.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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