Stem cells in lung biology

At the cornerstone of contemporary biology are investigations on mechanisms of repair, remodeling, proliferation, and differentiation after injury in complex tissue such as lung. Progress in research in stem cells in lung has been remarkable since an initial National Institutes of Health conference focused on this topic in 1997 (17). Indeed, in addition to well-established paradigms of adult stem cells in lung and their transiently amplifying cell progeny (8), recent evidence suggests that stem cells may exist in other organs with multipotent capacity for transdifferentiation. Accordingly, marrow-derived stem cells appear to have a plasticity that enables them to cross lineage barriers and cause us to reevaluate concepts of lineage-restricted stem cells in adult tissue, including lung (11).

Although definitions of stem cells vary, the ability of self-renewal and differentiation are minimal consensus requisite properties. The range of this latter property defines their plasticity. Hematopoietic stem cells (HSC) have been isolated from mice and humans to varying degrees of homogeneity including: 1) immunodepletion of lineage markers (lin−); 2) exclusion of Hoechst dyes or side population (SP) cells; and 3) presence of markers such as CD34, sca1, or kit. Further enrichment based on homing to bone marrow can result in a population of HSC so enriched that a single cell can reconstitute the entire hematopoietic system of a myeloablated host (16). Mesenchymal (or marrow stromal) stem cells (MSC) are isolated from bone marrow based on their ability to adhere to tissue culture dishes. Further purification and isolation procedures vary considerably, and their differentiation potentials are reported to have a wide range depending on species and culture conditions. They are usually CD45 negative. Challenges in identification of donor-derived cells in target organs (usually via sex-mismatched strategies) and identification of differentiated cell phenotype have led to different results describing the nature of their plasticity (11). Nonetheless, the original observation of Krawe et al. (16) that male unfractonated whole bone marrow or CD34+lin− cells differentiated into bronchiolar epithelia and type II alveolar cells after transplantation into lethally irradiated female mice singularly redefined concepts of lineage-restricted stem cells in postnatal lung. MSC also appear to be able to differentiate into distal alveolar epithelial cells in bleomycin-treated mice as ascertained by colocalization with type I cell markers (16) or enrichment of donor cells in a type II cell purification (18). Undetermined aspects of the injury process appear to be critical for engraftment, and elements of cell fusion may contribute to the ultimate differentiated phenotype, as was recently shown in an ex vivo model of airway epithelium (22). Recent demonstrations of chimerism in lung of patients after bone marrow transplant (1, 13), including both an epithelial and endothelial (24) nature, underscore the importance of experimental findings in mice. Regardless of current controversies regarding the precise mechanisms underlying such phenomena, considerable interest is apparent in the use of stem cell-based therapies, alone or as a hybrid, with somatic gene transfer (10, 19).

The scarcity of potential stem cells and the requirement to produce a calibrated lung injury to establish favorable kinetics create significant challenges in the field. The heterogeneous nature of lung cell populations suggests that single cell isolation and critical molecular phenotyping of stem cells will indeed be challenging. Novel genetic markers of lineage along with pharmacologically and immunologically phenotyping of precursor cells continue to require refinement. Surprising difficulties in carefully discerning engrafted cells (sex or antigen mismatched approaches) add to ambiguities, especially as they relate to transdifferentiation vs. fusion. Nonetheless, it is encouraging that in the brief interval from the announcement of this call for papers on Stem Cells in Lung Biology, we were able to favorably peer review six outstanding manuscripts on the topic. They include information on the highlighted topics that follow.

**SP Cells**

**SP Cells** are identified via their ability to efflux Hoechst dye via ATP binding cassette half-transporter breast cancer resistance protein (Bcrp1) and are often associated with stem cell-like activity. Summer et al. (23) recently reported that SP cells comprise 0.03–0.07% of mouse lung cells and are scal antigen positive, lin−, and heterogeneous at CD45 locus and express the vascular marker CD31. Bcrp1 was immunolocalized to bronchial and vascular smooth muscle and round cells of the distal airways. This information is consistent with a unique stem cell population in lung and/or contribution of HSC stem cells to lung cell lineage.

In this issue of the American Journal of Physiology–Lung Cellular and Molecular Physiology, Giangreco and colleagues (9) indicate that a CD45−, sca1+ SP from mouse lung had a molecular phenotype similar to neuroepithelial body-associated variant Clara cells. The variant Clara cell is a label-retaining cell of multipotent differentiation capacity and is pollutant resistant. Collectively, these studies underscore unique aspects of intrapulmonary adult stem cells and support such a role for the variant Clara cell.

**Tracheal Epithelial Basal Cells**

Current dogma suggests that the normal quiescent airway epithelium can respond to injury via a reparative process involving label-retaining stem cell-like precursors, highly proliferative transiently amplifying cells, and ultimate terminal differentiation. With the use of a combination of bromodeoxyuridine retention after airway injury in mice and heterotopic tracheal grafts after surface epithelial removal, Borthwick and colleagues (3) suggested that stem cell niches were apparent in glandular ducts and at more distal foci near cartilage-intercartilage junctions in the pseudostratiﬁed airway segment. Subsequently, Grove et al. (10) indicated that label-retaining cells in the lower trachea were basal cells enriched in cytokinin 5. In their current study, Schoch and colleagues (21) created bitransgenic mice via breeding of K5 promoter-GFP and Rosa26 (β-galactosidase in all cells) as starting cells for a colony-forming efficiency assay in an air-liquid interface culture system and concluded that adult mouse proximal tracheal...
surface epithelial stem-like cells reside in basal cell compartment. Support for this concept is apparent in work by Hong and colleagues (13). These authors noted an increase in GSI-B4-reactive/K14-immunoreactive basal cells after secretory cell ablation with naphthalene in intact mice. These investigators then unambiguously defined the differentiation and clonogenic potential of this population by engineering a bitransgenic ligand-regulated Cre-lox P reporter mouse. The ubiquitous reporter (LacZ) was activated within K14-expressing progenitor cells during airway repair from naphthalene, and tagged clusters included basal, ciliated, and secretory cell types consistent with multipotent potential of K14-expressing basal cells. Heterogeneity of stem cell niches (8) is indeed apparent, and the evidence is quite apparent that Clara cells have stem-like cell characteristics in the more distal conducting airway (12).

In addition to profound gaps in our knowledge regarding progenitor cells in upper airway epithelium, the molecular determinants of differentiation are also unclear. Motile ciliated cells represent critical components of the differentiated airway epithelium. Several studies have demonstrated a critical role for Foxj1 forkhead box transcription factor in contributing to elements of terminal differentiation in the upper airway. In particular, foxj1 has a temporal and spatial expression pattern consistent with a role in ciliated epithelium (2), and ectopic expression via surfactant protein C promoter of foxj1 in a transgenic mouse results in cilia in alveolar epithelium (25). Targeted deletion of Foxj1 results in mice with absent cilia (4, 6). In the current study of You and colleagues (26), complementation of Foxj1 null airway cells promotes differentiation only in late-stage ciliogenesis, suggesting a role for this transcription factor in postcentriologenesis (rather than commitment) stage. Although this careful morphological and air-liquid interface model cannot totally rule out a role for Foxj1 in earlier aspects of ciliogenesis, it does suggest that other factors may be involved.

Alveolar Epithelial Progenitor Cells and Lung Injury

A significant body of information is consistent with the notion that alveolar type II cells are progenitor cells responsible for repair of injured distal (17). In contrast to the uninjured quiescent lung, acute lung injury enhances proliferation in the distal airway epithelium and results in a heterogeneous subpopulation of alveolar type II cells (5). In their current study, Reddy et al. (20) speculated that a proliferative group of type II cells that were resistant to damage could be isolated from hypoxic rats and that new phenotypic information on this potential transiently amplifying, pluripotent group of cells could be obtained. From in situ analysis of a number of surface markers, a strategy was developed using E-cadherin antibody-coated magnetic beads, and cells of the E-cadherin-negative subpopulation were damage resistant, proliferative, and had elevated telomerase activity. Presumably, these E-cadherin-negative cells were resistant to hypoxic damage in situ or were capable of rapidly repairing such damage. Although additional complexities of subculture may have confounded some of the observations, it is indeed apparent that the injured lung reveals a heterogenous group of cells, and presumably, pluripotent cell(s) reside within the proliferative, damage-resistant fraction.

Precursor Cells in Vascular Remodeling in Experimental Pulmonary Hypertension

In contrast to insight regarding progenitor:progeny lineage in respiratory epithelium, considerably less information is available regarding the vascular compartment of lung. In systemic vasculature, roles for residential vascular cells and bone marrow-derived hematopoietic stem cells and circulating endothelial progenitor cells are apparent. Like airway epithelium, pulmonary endothelium and the vascular wall are normally quiescent, and, accordingly, appropriate stimuli are necessary to reveal the nature of progenitor:progeny lineages. Accordingly, Davie and colleagues (7) have utilized a well-calibrated model of chronic hypobaric hypoxic-induced pulmonary hypertension in newborn calves to reveal: 1) novel pulmonary arterial remodeling in adventitial segment of the vasculature and 2) potential contributions of bone marrow-derived HSC and/or circulating endothelial progenitor cells in such remodeling. In this latter regard, the investigators demonstrated a hypoxic-induced increase in c-kit+ circulating mononuclear cells as well as an increase in immunoreactive c-kit+ cells in the remodeling pulmonary arterial wall. Extracellular molecules consistent with concepts of vessel growth including vascular endothelial growth factor, fibronectin, and thrombin were present in the vascular wall, and, in addition, the mononuclear cell fraction noted above was capable of differentiating into phenotypically positive endothelial or smooth muscle cells in culture. Although markers for this type of study are not as well advanced as those of epithelial lineage, potential is apparent for both bone marrow-derived stem cells and resident stem cells in affecting vascular remodeling after hypoxic stimuli in the lung.

Insight into the molecular basis of repair and remodeling in lung is critical to essential elements of pulmonary pathobiology. Information derived from studies on progenitor:progeny lineages in airways and vasculature in embryonic and postnatal lung is critical toward understanding the pathogenesis of acute and chronic lung disease. The characterization and phenotyping of resident stem cells in lung as well as progress in contributory roles of bone marrow-derived stem cells will provide new insight into malignant changes in pulmonary neoplastic or hypertensive disorders and pathologies associated with asthma and chronic obstructive lung disease. The sensitivity and/or resistance of these cells to various environmental agents and/or drugs will help focus concerns on relevant compartments associated with environmental and drug-induced lung disease. The promise of novel therapeutic approaches, including somatic gene transfer and stem cell-based therapies, requires new information on potential lung stem cells.

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