[Frontiers in Bioscience 12, 3117-3124, May 1, 2007]

Advancing the field of lung stem cell biology

Carla Bender Kim

Stem Cell Program, Children's Hospital, Harvard Stem Cell Institute, Boston, MA 02115

TABLE OF CONTENTS

1. ABSTRACT

The adult mammalian lung is a beautifully complex, integrated system of numerous types of epithelial cells, supporting cells and matrix that synergize to make gas exchange possible. Recent identification of putative endogenous and extrinsic lung stem cell populations has added to the diversity of the respiratory system. Despite these new studies and data from years of analyses of lung injury models, careful cellular and molecular analyses are needed to further define the stem and differentiated cells in the pulmonary system. New tools enabling lineage tracing or transplantation in adult lung as well as understanding the key microenvironmental clues that control the response to lung injury will be important to advance the field of lung stem cell biology.

2. ARCHITECTURE OF THE PULMONARY SYSTEM

Stem cells are the self-maintaining, or self-renewing, cells that are capable of giving rise to specialized, or differentiated, cell types of a given tissue during a particular stage of life (1-8). Progenitor cells, in contrast, do not have self-renewal capability. Stem cells may be multipotent, as is the case for hematopoietic stem cells that can give rise to every blood cell type, or unipotent, as are germline stem cells (8). Key to the identification of tissue-specific stem cells is the ability to isolate them and show that they are capable of self-renewal and differentiation. Due to the intimate association between epithelial cells and their surrounding stroma, it has been exceedingly difficult to identify solid tissue stem cells until recently (2, 9-11). This is one aspect of lung biology that has contributed to the paucity of lung stem cell biology.

The adult mammalian lung presents a complex problem to the stem cell biologist. It has been reported and frequently cited that the lung contains 40 different cell types (12, 13). Current analyses, however, suggest that this number may be a gross underestimate, as new endogenous lung cell populations and circulating transient cells found in the lung have since been identified (9, 14-17). Lineage relationships between the epithelial cells are poorly understood in adult and embryonic lung. Beyond the level of individual cells, the architecture of the lung lends itself to being considered to consist of multiple organs; the

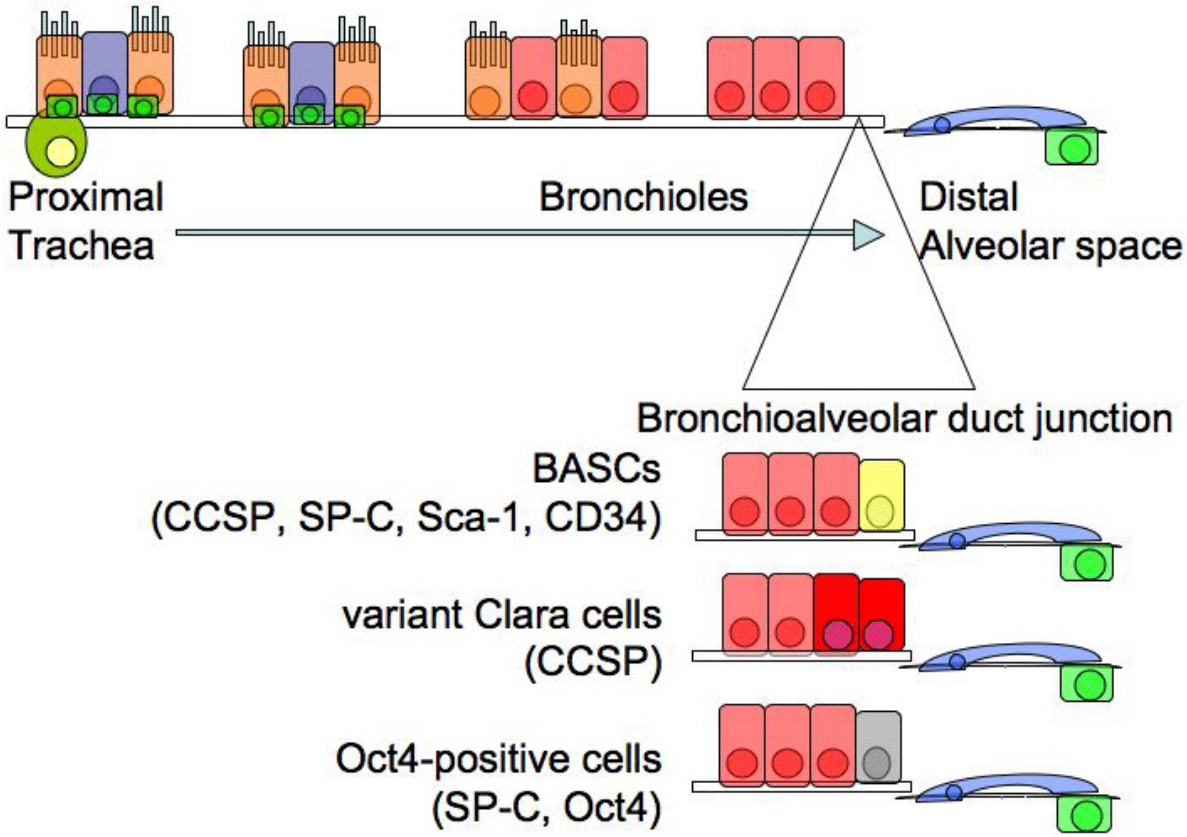

Figure 1. Diversity of epithelial cells in the respiratory system. Cartoon representations of the epithelial components from the trachea (proximal respiratory system), bronchi, bronchioles, and terminal bronchioles to the alveolar space (distal respiratory system) are shown. The bronchioalveolar duct junction, located between the terminal bronchioles (lined with Clara cells, light red) and the alveolar space (containing the squamous AT1 pneumocytes indicated in blue and the cuboidal AT2 cells indicated in light green), has been proposed to provide a distal lung stem cell niche. Putative distal lung stem cells in this region include BASCs (yellow), variant Clara cells (dark red), and Oct4-positive cells (gray). Molecular markers for each cell type are indicated in parentheses.

trachea, bronchi, bronchioles and proximal lung, distal lung with terminal bronchioles, and alveolar spaces may, each, be considered an organ within the respiratory system. Each of these "organs" has been referred to as distinct anatomical locations, niches, microenvironments or sub-regions.

Unique cell types that have distinct form and function house each of the sub-regions within the lung. (12). Basal cells, secretory Goblet cells, submucosal glands, and ciliated cells line the trachea and upper airways. The non-ciliated, columnar Clara cells that line the bronchioles and terminal bronchioles secrete surfactants to aide in oxygen exchange and provide a protective epithelial barrier in the airways. The alveolar epithelium is composed of alveolar type II (AT2) cells, the cuboidal epithelial cells that produce surfactants and the resulting surface tension required for gas exchange, as well as the alveolar type I (AT1) cells, the flat epithelial cells that deliver oxygen to the blood (Figure 1). Numerous stromal cells are inter-mingled with these epithelial cells, including vasculature, fibroblasts, circulating immune cells, and resident macrophages.

3. LUNG INJURY MODELS AS A MEANS TO IDENTIFY LUNG STEM CELLS

Analyses of lung injury models have suggested that each epithelial niche contains its own stem or progenitor cell population (18). Whereas several injury models specific to the "organs" of the lung have suggested that the trachea, bronchioles, terminal bronchioles, and alveolar spaces may each harbor unique stem cell populations (19), the remainder of this review will focus on the distal lung niche. For example, naphthalene, a pollutant that specifically ablates most Clara cells, has been used to identify two subsets of "variant" Clara cells. In particular, one population of naphthalene-resistant variant Clara cells resides near neuroendocrine bodies in large airways and another is found in the bronchioalveolar duct junction in terminal bronchioles, the last portion of the airway before the alveolar space. Variant Clara cells have been observed to proliferate in response to naphthalene, and therefore they have been referred to as lung stem or progenitor cells (20, 21). Similarly, AT2 cells are often referred to in the literature as stem or progenitor cells. This nomenclature derives from studies in which AT2 cells were reported to

proliferate in response to AT1 cell injury resulting from bleomycin administration (22, 23). However, most of these conclusions were based on electron microscopy or BrdU incorporation at given time points after lung injury. Genetic evidence that new Clara cells or new alveolar cells can be derived from the variant Clara cells or AT2 cells, respectively, is lacking, as precise lineage tracing in adult lung has not been performed. Furthermore, self-renewal of Clara cells and AT2 cells in vivo has not been documented.

4. ISOLATION OF NEW PUTATIVE STEM OR PROGENITOR CELLS IN THE DISTAL LUNG

Despite the suggestion from numerous lung injury experiments that lung stem cells may exist, methods to isolate resident cellular components from putative stem cell niches in the lung had not been developed prior to our work. We recently determined that cells expressing both the AT2 cell marker, pro-surfactant protein C (SP-C), and the Clara cell marker, Clara cell antigen (CCA, also known as CC10 or Clara cell secretory protein, CCSP), are present in normal lung, and that they constitute a population in the distal lung epithelium with stem cell properties (9). These cells, named bronchioalveolar stem cells (BASCs), can be isolated using a fluorescence activated cell sorting (FACS) methodology based on the presence of the surface markers Sca-1 and CD34 and the absence of the hematopoietic and endothelial cell markers CD45 and CD31, respectively. BASCs self-renew over multiple passages and give rise to bronchiolar and alveolar cells in culture, providing evidence they are a stem cell population. In our experiments, AT2 cells did not exhibit self-renewal capacity nor were they able to differentiate into AT1 cells, indicating that AT2 cells are more likely to be differentiated cells than stem cells (9). Further supporting the hypothesis that BASCs are stem cells, they are quiescent in normal lung and proliferate or increase in number in response to bronchiolar and alveolar injury, including naphthalene treatment, bleomycin administration, and partial pneumonectomy (9) (and unpublished data, A. Hoffman and C. Kim).

Lacking precise lineage tracing, currently published data leave open the possibility that either variant Clara cells, AT2 cells, BASCs, or other cells are the functional bronchioalveolar stem cells that maintain distal lung homeostasis. Interestingly, BASCs were identified in the bronchioalveolar duct junction, the region previously described as a putative lung stem cell niche and the location of one set of variant Clara cells (9, 20). Importantly, both cell populations are resistant to naphthalene. It is likely that at least a subset of variant Clara cells are BASCs, but variant Clara cells have not been tested for expression of BASC markers such as SP-C, Sca-1 or CD34. It is also conceivable that BASCs give rise to variant Clara cells, which go on to produce more differentiated Clara cells that line the majority of the airways (9). In CCSP-HSVtk mice, which express the thymidine kinase gene only in cells expressing the Clara cell secretory protein (24), administration of ganciclovir resulted in ablation of Clara cells and death due to pulmonary failure (24). These data indicate at least two possibilities: (1) that the stem cells required for repair of Clara cell injury express CCSP, suggesting that either the variant Clara cells or BASCs are stem cells, or (2) that CCSP-expressing cells are required as a support (physical or signaling in nature, see below) for the stem cells that give rise to new Clara cells. Interestingly, Aso *et. al.*, who originally reported the division of AT2 cells after bleomycin treatment, also noted a population of cuboidal epithelial cells that were continuous with bronchiolar epithelium and which exhibited "downgrowth" after alveolar injury (22). The authors hypothesized that these cells were undifferentiated, because their structural features were "strikingly similar to fetal epithelium." Given the location of these undifferentiated cells as well as their response to lung injury, they are likely to be BASCs; thus, this study supports the possibility that both AT2 cells and BASCs could be involved in repair of alveolar epithelium.

Recently, a new population of putative lung stem or progenitor cells was also described. Ling *et. al.* isolated cells from the neonatal lung that were able to form epithelial colonies and differentiate into AT2- and ATI-like cells (14). The colonies that these cells gave rise to were positive for Oct4, a known marker of ES cells and germ cell tumors (25, 26). Interestingly, the authors showed that the postnatal cells could be infected with SARS virus, raising the possibility that stem cells in the lung are the specific targets of viruses that cause respiratory problems (14).

The relationship of these Oct4-positive cells to BASCs is unclear. Although the cultured Oct4-positive cells did exhibit Sca-1-staining, Sca-1 was only assessed after colony formation in culture rather than as a means of cell isolation. Whereas BASCs exhibited self-renewal in appropriate culture conditions, self-renewal capacity of the Oct4-positive cells has not been demonstrated to date. In addition, the methods used to culture BASCs and these cells varied; growth-arrested mouse embryonic fibroblasts were used to support undifferentiated growth of BASCs (1), whereas the Oct4-positive cells were initially cultured together with their resident stromal component cells in a serum-free environment. BASCs exhibited ability to give rise to Clara-, AT2- and AT1-like cells in the presence of Matrigel. In contrast, the neonatal Oct4-positive cells appeared to differentiate sequentially into AT2-like and then AT1-like cells on collagen-coated plates. Distinct from BASCs, the neonatal Oct4-positive cells stained for CCSP and not SP-C, yet were also reported to be present in the BADJ. The Oct4-positive cells reported were from neonatal lung, but the precise age of isolation of these cells was not clear; BASCs were identified in adult mice six to eights weeks of age. As the developmental origins of BASCs have yet to be identified, it is a formal possibility that BASCs derive from the neonatal cells. Clearly, direct comparison of BASCs, variant Clara cells, and Oct4-positive lung cells will be needed to determine their functional overlap, if any.

Aside from endogenous epithelial stem cells, a number of other cells have recently been identified in the lung that may also contribute to differentiated epithelial

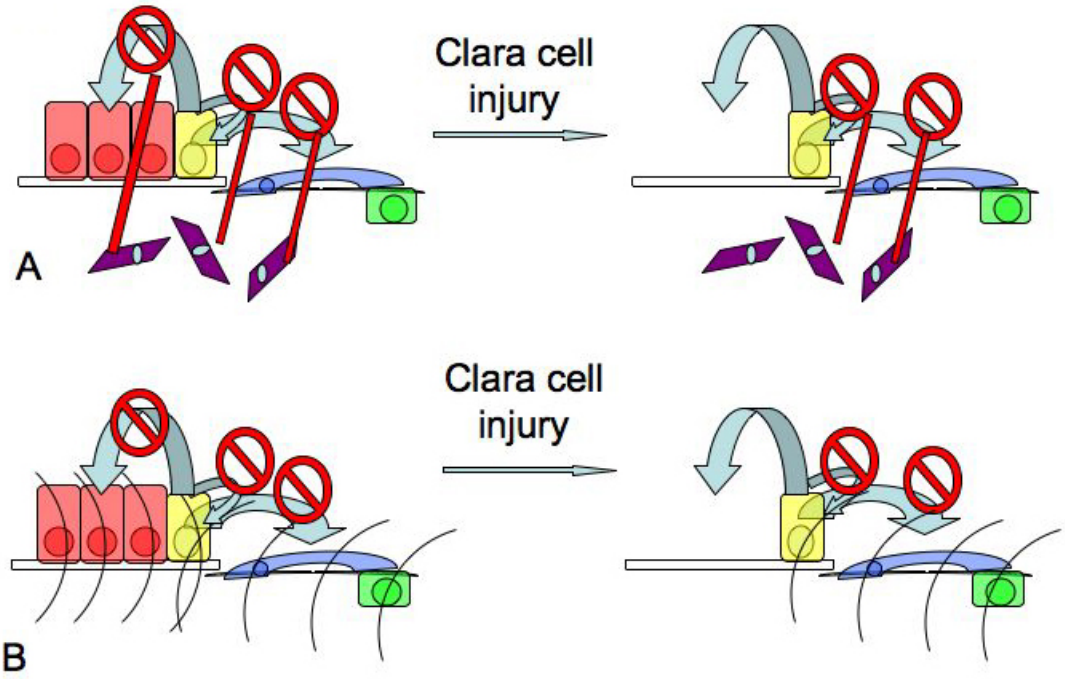

Figure 2. Two models for regulation of distal lung stem cell self-renewal and differentiation. A. Stromal cells sense and regulate the activity of lung stem cells. Left, in normal lung, lung stem cells (e.g. BASCs, in yellow), remain quiescent (self-renewal and differentiation arrows are blocked) due to inhibitory signals directly from nearby stromal or supportive niche cells in the bronchioalveolar duct junction. Upon lung injury (right), stromal cells may detect the lack of Clara cells and reduce the signal inhibiting differentiation to Clara cells, allowing lung stem cells to produce new epithelial cells to repair damage. Importantly, stromal cells may also provide positive, stimulatory signals for lung stem cell self-renewal or differentiation, but this possibility was not illustrated for simplicity. B. Overlapping signaling gradients from nearby specialized lung epithelial cells, rather than particular stromal cells, regulate distal lung stem cell function. In this scenario, Clara cells and alveolar cells directly release signaling gradients that uniquely overlap in the location of the distal lung stem cell. These signals may be inhibitory, as illustrated, or positive regulatory signals (not shown) that prevent lung stem cell renewal or differentiation in the context of normal homeostasis. After lung injury, loss of a particular epithelial cell type (as illustrated, Clara cells) results in the loss of that cell-specific signal and thus stimulation of lung stem cells to produce new epithelial cells. The signals in either model would be restored to baseline levels after epithelial repair.

cells or aide the function of lung stem cells. Circulating epithelial cells have been identified as a potential source for lung epithelial repair (17). Bone marrow cells and fibrocytes are also found in the lung under certain conditions (15, 16, 27). These cell types will not be discussed further here, but it remains important to consider the evolving complexity of the pulmonary system, and to consider the possibility that stem cells within the lung as well as cells in the surrounding tissue influence lung epithelial homeostasis.

5. DEFINING THE LUNG STEM CELL MICROENVIRONMENT

Just as the individual stem cell types within the lung need to be identified and characterized, the lung stem cell microenvironment also needs to be defined. Other tissue stem cells have been shown to be surrounded by discreet cell populations that provide signaling to inhibit stem cell proliferation or differentiation during normal maintenance or to stimulate stem cell division and/or differentiation in times of needed tissue repair (28-30). It is possible that distal lung stem cells are similarly regulated by surrounding supportive cells (Figure 2A). However, despite the diversity of epithelial cell types along the airways, the surrounding stromal cell types seem to be quite uniform in type (e.g. ciliated cells, fibroblasts) proximally to distally. The alveolar space, with its combination of capillaries, macrophages, AT1 cells and AT2 cells, certainly contains different supportive cells (e.g. endothelial cells, macrophages) than do bronchioles, but the cellular components of the alveolar space do not appear to be different whether the alveolar space is near terminal bronchioles versus an alveolar space in a more distal region. Therefore, it is unlikely that the bronchioalveolar duct junction where BASCs, variant Clara cells and Oct4-positive cells reside is housed by a unique supporting cell type not found in any other lung sub-region.

Rather than being supported by specific stromal cell types, distal lung stem cells in the bronchioalveolar duct junction may be regulated by signals that result as a consequence of the juxtaposition between Clara cells and AT2 cells (Figure 2B). In this model, Clara cells in the

bronchioles and AT2 cells in the alveolar space may produce distinct regulatory factors that overlap only in the BADJ to regulate lung stem cells and inhibit differentiation or proliferation. Depletion of Clara cells or AT2 cells would result in a lack of the signal inhibiting differentiation toward the Clara cell lineage or the AT2 cell lineage, respectively. This would then stimulate lung stem cells to produce new epithelial cells until the corresponding inhibitory signal is sufficiently restored. There is a precedent for regulation of cell fate by morphogen gradients in developmental biology, perhaps best examined in the Drosophila embryo (31). Interestingly, cell surface molecules, which are frequently used in identification of stem cell populations, may be key players in establishment of regulatory gradients (31). Signaling that influences lung stem cells and other adult stem cells may or may not be concentration-dependent, but regardless may involve overlapping regulation from many neighboring cells rather than just one stromal niche cell type. Clearly, more careful descriptive molecular and cellular studies of putative lung stem cell niches will be needed to test these ideas regarding the lung stem cell microenvironment.

6. DEFINING LUNG CELL RELATIONSHIPS *IN VIVO*

We proposed that BASCs are the stem cells that maintain bronchiolar and alveolar cell homeostasis (9). However, it is possible that BASCs will exhibit properties of progenitors, rather than stem cells, *in vivo*. It is also possible that, rather than a standard hierarchy of stem, progenitor, and differentiated cells as is described for the hematopoietic and other systems (32), that the lung exhibits a different lineage relationship structure. For example, there may yet be distinct populations of Clara cells and AT2 cells that harbor limited self-renewal activity or differentiation capacity; a subset of Clara and AT2 cells may either be stem cells or progenitor cells. This possibility may explain the previous data showing the proliferation of these cells after lung injury, even if they are not the definitive regional stem cells. Of note, in the absence of injury, mouse lung epithelial cells rarely exhibit cellular turnover, perhaps necessitating longevity and self-renewal for most of its resident epithelial cells.

To elucidate the biology of lung stem cells, and to determine the lineage relationships between BASCs, Clara cells, AT2 cells, and other putative distal lung stem cells, two key developments are required. First, a transplantation assay is needed to test the function of isolated putative lung stem cells. For example, although we have demonstrated the multipotency of BASCs in culture, it will be important to define their potential *in vivo* (9). Therefore, transplantation methods to introduce genetically marked lung stem cells into the respiratory system of mice after lung cell damage will need to be established. Second, tools for lineage tracing in adult lung are required. A number of genetic marking tools could be employed for lineage tracing, including doxycyclin or tamoxifen-inducible alleles (33), Cre/loxP or Flp/Frt (34) recombination, and reporters such as LacZ, luciferase, GFP, YFP, or DsRed, given that they can work with high efficiency in the lung.

Transplantation of isolated hematopoietic stem cells into lethally irradiated hosts to reconstitute all hematopoietic lineages has served as a gold standard for defining hematopoietic stem cells (35), but there is currently no comparable system available to test lung stem cells *in vivo*. One assay has been established for reconstitution of epithelial cells in denuded trachea (36), but this system provides a test for putative stem cells for ciliated, submucosal, and Goblet cells rather than distal Clara cells or alveolar cells. In addition, the denuded trachea assay relies on subcutaneous implantation of the tracheal graft (36), rather than mimicking the distal lung region that is relevant for testing the functions of BASCs, other putative distal lung stem cells, and differentiated cells. There is an established protocol to deplete the mammary gland of epithelial cells and orthotopically inject putative stem cell populations to replace mammary epithelial tissue, suggesting that a similar approach is feasible in the lung (10, 37-39). In addition, FACS isolated skin stem cells are capable of giving rise to new, functional hair follicles when injected into nude mice (2). The lessons learned from these transplantation studies in non-essential tissues, combined with more sophisticated genetic strategies, should be incorporated into studies designed to test lung stem cell function *in vivo*.

Development of a transplantation assay for lung stem cells *in vivo* would most likely involve injuring the lung of a recipient animal as a means to ablate endogenous lung stem cells, followed by introduction of genetically marked, freshly isolated lung stem cells (Figure 3). It is conceivable that bleomycin or naphthalene treatment would provide a stimulus for engrafted lung stem cells. Genetic means to eliminate endogenous lung stem cells may be necessary to make a sufficiently empty niche for the introduced stem cells to engraft and give rise to differentiated cell types (40).

Addition of growth factors or other supportive cell types may be needed to assist in an *in vivo* lung stem cell transplantation assay (2), and so it may be necessary to optimize transplantation conditions first using an *ex vivo* lung culture system. Isolated and cultured lung buds at embryonic day 11.5 have been shown to undergo differentiation that parallels development *in utero* for up to one week (41). In addition, lung explants placed under the kidney capsule of immunocompromised mice displayed lung cell differentiation beyond the cultured explants and more accurately recapitulated alveolar differentiation *in vivo* (42). These systems may prove useful in defining the cell types and factors required for lung stem cell function. Finally, it may be useful to inject putative lung stem cells into chick embryos to determine if they contribute to lung development *in vivo*, as has been previously performed to test the differentiation potential of neural stem cells (43).

New tools for lineage tracing experiments will be crucial for testing the function of lung stem cells without removing them from their natural surroundings. Currently available transgenic mice have provided tools to begin to understand lung cell lineages in development (44, 45). However, these strains have not proven useful in adult lung

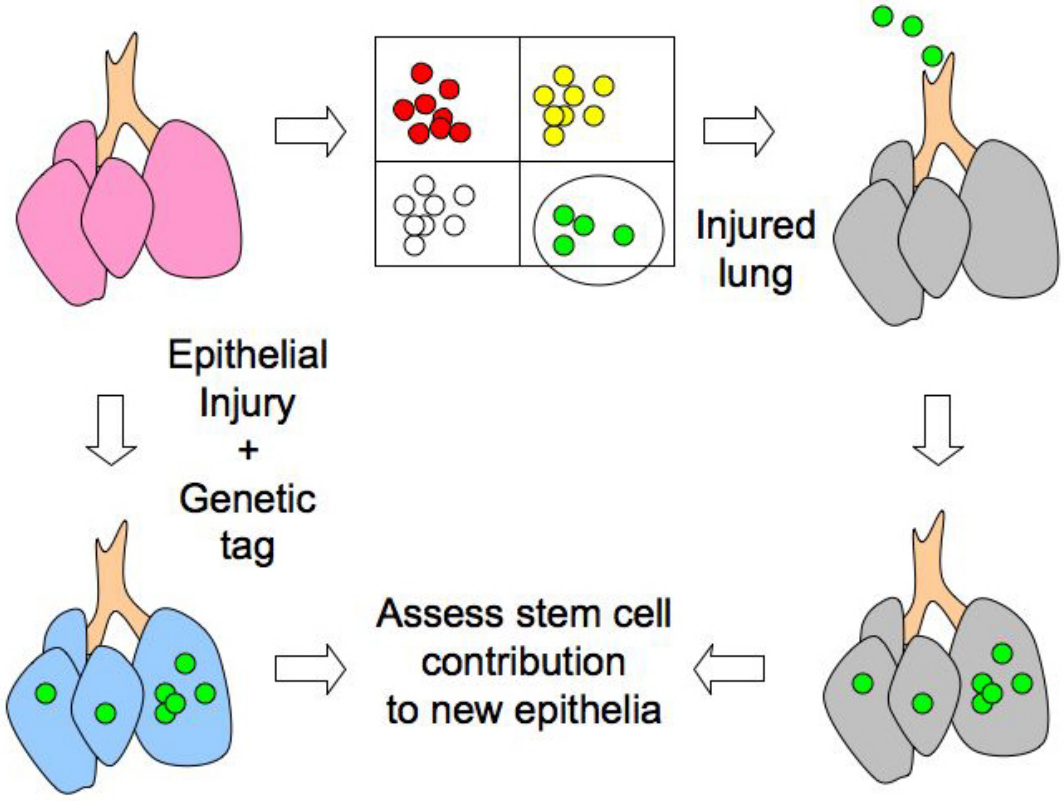

Figure 3. Approaches to testing the potential of lung stem cells *in vivo*. Top left, normal lung (pink) could be used for isolation of lung stem cells by FACS. Subsequent injection of isolated, labeled lung stem cells into injured lungs (gray) of a recipient animal followed by time for engraftment and repair of injured epithelia would provide a method to test the potential of isolated lung stem cells to contribute to lung cells *in vivo*. A parallel approach would use genetic tagging methods provided at the same time as lung epithelial injury, time for repair, and thus assessment of endogenous lung stem cell contribution to epithelial cells.

studies. For example, SP-C-rtTA; tetO-Cre mice are a valuable tool for marking embryonic lung precursor cells *in utero*, but limited Cre-mediated recombination has been observed in these mice in adult lung (44, 46). In addition, studies in our laboratory have indicated doxyclycline-independent Cre expression in Clara cells in adult SP-C-rtTA; tetO-Cre mice (C Kim, unpublished data). Our observations suggest that the SP-C-rtTA transgene is constitutively active in adult Clara cells, likely due to aberrant effects on SP-C transcription at the genomic site of transgene insertion. This finding underscores the importance of using a knock-in approach that relies on endogenous transcriptional regulation rather than using transgenic strains for lineage tracing experiments, and supports the need for generating new strains of Cre mice.

Lung stem cell-specific Cre-ER^{T2} mice, in which Cre is fused to a tamoxifen-inducible estrogen receptor (33), would allow for spatial and temporal (in adult vs. all of development) regulation of Cre to be used in combination with Cre-dependent reporter alleles for lineage tracing (47). This work would involve providing a genetic mark to lung stem cells only after lung injury, followed by repair time and analysis of the genetic tag in new epithelial cells (Figure 3). Lung stem cell-specific Cre mice would also be useful for studying the genetic networks that control lung stem cell self-renewal and differentiation, as well as the mechanisms of lung disease and tumorigenesis.

In order to specifically manipulate BASCs, and other lung stem cells, it will be crucial to identify novel lung stem-cell specific markers. This may be achieved by gene expression or proteomic analyses of highly purified lung stem cell populations. This type of study would also make it possible to compare the possible control mechanisms that regulate lung stem cells and other adult stem cells. Lung stem cells may share more common features with stem/progenitor cells of infrequently dividing tissues such as the mammary gland than with embryonic, hematopoietic or skin stem cells. It remains to be determined if stem cell signatures will be more reflective of the developmental relationship of tissues, common among all stem cells, or shared only between stem cell populations undergoing similar proliferative demands.

7. CONCLUSION

Lack of knowledge of the potential of pulmonary stem cells precludes understanding their role in the mechanisms of lung disease as well as their potential beneficial uses in treating disorders that affect lung epithelia. It is conceivable that distal lung stem cell

function is critically affected in diseases such as cystic fibrosis, congenital lung hypoplasia, neonatal respiratory distress syndrome, emphysema and chronic obstructive pulmonary disorder (COPD), since these disorders all involve injured or depleted bronchiolar or alveolar epithelium (48). In terms of possible therapeutic uses, transplanted lung stem cells might be useful in replacing damaged lung cells in patients, or it may be useful to apply stem cells to generate functional alveolar structures or enhanced surfactant (48). Perhaps even more feasible, it may be possible to design drugs that stimulate a patient's own endogenous stem cells. To make these possible therapeutic advances a reality, however, it will be key to directly test the potential of isolated lung stem cells in animals or to have a system to assess endogenous lung stem cell function.

The combination of creation of a lung stem cell transplantation assay and endogenous lung stem cell lineage tracing tools are essential to move the field of lung stem cell biology forward. Once these systems are established, it will be possible to compare the potency of lung stem cells from diseased lung to that of stem cells from normal lung. These advances will bring us closer to understanding the intricacies of lung biology.

8. ACKNOWLEDGEMENTS

The author thanks those who have contributed to the field of lung biology and lung injury over numerous years, and in particular, those whose work was not cited due to space constraints or inadvertent omission.

9. REFERENCES

1. Chiasson, B.J., V. Tropepe, C.M. Morshead, and D. van der Kooy: Adult mammalian forebrain ependymal and subependymal cells demonstrate proliferative potential, but only subependymal cells have neural stem cell characteristics. J Neurosci, 19, 4462-71 (1999)
2. Blanpain, C., W.E. Lowry, A. Geoghegan, L. Polak, and E. Fuchs: Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. Cell, 118, 635-48 (2004)
3. Johansson, C.B., S. Momma, D.L. Clarke, M. Risling, U. Lendahl, and J. Frisen: Identification of a neural stem cell in the adult mammalian central nervous system. Cell, 96, 25-34 (1999)
4. Doetsch, F., I. Caille, D.A. Lim, J.M. Garcia-Verdugo, and A. Alvarez-Buylla: Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell, 97, 703-16 (1999)
5. Morris, R.J., Y. Liu, L. Marles, Z. Yang, C. Trempus, S. Li, J.S. Lin, J.A. Sawicki, and G. Cotsarelis: Capturing and profiling adult hair follicle stem cells. Nat Biotechnol, 22, 411-7 (2004)
6. Kruger, G.M., J.T. Mosher, S. Bixby, N. Joseph, T. Iwashita, and S.J. Morrison: Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neuronal subtype potential, and factor responsiveness. Neuron, 35, 657-69 (2002)
7. Clarke, M.F. and M. Fuller: Stem cells and cancer: two faces of eve. Cell, 124, 1111-5 (2006)
8. Yamashita, Y.M., M.T. Fuller, and D.L. Jones: Signaling in stem cell niches: lessons from the Drosophila germline. J Cell Sci, 118, 665-72 (2005)
9. Kim, C.F., E.L. Jackson, A.E. Woolfenden, S. Lawrence, I. Babar, S. Vogel, D. Crowley, R.T. Bronson, and T. Jacks: Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell, 121, 823-35 (2005)
10. Shackleton, M., F. Vaillant, K.J. Simpson, J. Stingl, G.K. Smyth, M.L. Asselin-Labat, L. Wu, G.J. Lindeman, and J.E. Visvader: Generation of a functional mammary gland from a single stem cell. Nature, 439, 84-8 (2006)
11. Wang, S., A.J. Garcia, M. Wu, D.A. Lawson, O.N. Witte, and H. Wu: Pten deletion leads to the expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. Proc Natl Acad Sci U S A, 103, 1480-5 (2006)
12. Bannister, L., ed. *Respiratory System*. Thirty-eighth ed. 1999, Churchill Livingstone: New York. Chapter 11.
13. Weiss, D.J., M.A. Berberich, Z. Borok, D.B. Gail, J.K. Kolls, C. Penland, and D.J. Prockop: Adult stem cells, lung biology, and lung disease. NHLBI/Cystic Fibrosis Foundation Workshop. Proc Am Thorac Soc, 3, 193-207 (2006)
14. Ling, T.Y., M.D. Kuo, C.L. Li, A.L. Yu, Y.H. Huang, T.J. Wu, Y.C. Lin, S.H. Chen, and J. Yu: Identification of pulmonary Oct-4+ stem/progenitor cells and demonstration of their susceptibility to SARS coronavirus (SARS-CoV) infection in vitro. Proc Natl Acad Sci U S A, 103, 9530-5 (2006)
15. Phillips, R.J., M.D. Burdick, K. Hong, M.A. Lutz, L.A. Murray, Y.Y. Xue, J.A. Belperio, M.P. Keane, and R.M. Strieter: Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. J Clin Invest, 114, 438-46 (2004)
16. Schmidt, M., G. Sun, M.A. Stacey, L. Mori, and S. Mattoli: Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. J Immunol, 171, 380-9 (2003)
17. Gomperts, B.N., J.A. Belperio, P.N. Rao, S.H. Randell, M.C. Fishbein, M.D. Burdick, and R.M. Strieter: Circulating progenitor epithelial cells traffic via CXCR4/CXCL12 in response to airway injury. J Immunol, 176, 1916-27 (2006)
18. Otto, W.R.: Lung epithelial stem cells. J Pathol, 197, 527-35 (2002)
19. Bortnick, A.E., E. Favari, J.Q. Tao, O.L. Francone, M. Reilly, Y. Zhang, G.H. Rothblat, and S.R. Bates: Identification and characterization of rodent ABCA1 in isolated type II pneumocytes. Am J Physiol Lung Cell Mol Physiol, 285, L869-78 (2003)
20. Giangreco, A., S.D. Reynolds, and B.R. Stripp: Terminal bronchioles harbor a unique airway stem cell population that localizes to the bronchoalveolar duct junction. Am J Pathol, 161, 173-82 (2002)
21. Hong, K.U., S.D. Reynolds, A. Giangreco, C.M. Hurley, and B.R. Stripp: 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 (2001)
22. Aso, Y., K. Yoneda, and Y. Kikkawa: Morphologic and biochemical study of pulmonary changes induced by bleomycin in mice. Lab Invest, 35, 558-68 (1976)

23. Mason, R.J. and M.C. Williams: Type II alveolar cell. Defender of the alveolus. Am Rev Respir Dis, 115, 81-91 (1977)
24. Reynolds, S.D., K.U. Hong, A. Giangreco, G.W. Mango, C. Guron, Y. Morimoto, and B.R. Stripp: Conditional clara cell ablation reveals a self-renewing progenitor function of pulmonary neuroendocrine cells. Am J Physiol Lung Cell Mol Physiol, 278, L1256-63 (2000)
25. Nichols, J., B. Zevnik, K. Anastassiadis, H. Niwa, D. Klewe-Nebenius, I. Chambers, H. Scholer, and A. Smith: Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell, 95, 379-91 (1998)
26. Gidekel, S., G. Pizov, Y. Bergman, and E. Pikarsky: Oct-3/4 is a dose-dependent oncogenic fate determinant. Cancer Cell, 4, 361-70 (2003)
27. Wang, G., B.A. Bunnell, R.G. Painter, B.C. Quiniones, S. Tom, N.A. Lanson, Jr., J.L. Spees, D. Bertucci, A. Peister, D.J. Weiss, V.G. Valentine, D.J. Prockop, and J.K. Kolls: Adult stem cells from bone marrow stroma differentiate into airway epithelial cells: potential therapy for cystic fibrosis. Proc Natl Acad Sci U S A, 102, 186-91 (2005)
28. Tumbar, T., G. Guasch, V. Greco, C. Blanpain, W.E. Lowry, M. Rendl, and E. Fuchs: Defining the epithelial stem cell niche in skin. Science, 303, 359-63 (2004)
29. Calvi, L.M., G.B. Adams, K.W. Weibrecht, J.M. Weber, D.P. Olson, M.C. Knight, R.P. Martin, E. Schipani, Divieti, F.R. Bringhurst, L.A. Milner, H.M. Kronenberg, and D.T. Scadden: Osteoblastic cells regulate the haematopoietic stem cell niche. Nature, 425, 841-6 (2003)
30. Zhang, J., C. Niu, L. Ye, H. Huang, X. He, W.G. Tong, J. Ross, J. Haug, T. Johnson, J.Q. Feng, S. Harris, L.M. Wiedemann, Y. Mishina, and L. Li: Identification of the haematopoietic stem cell niche and control of the niche size. Nature, 425, 836-41 (2003)
31. Tabata, T. and Y. Takei: Morphogens, their identification and regulation. Development, 131, 703-12 (2004)
32. Reya, T., S.J. Morrison, M.F. Clarke, and I.L. Weissman: Stem cells, cancer, and cancer stem cells. Nature, 414, 105-11 (2001)
33. Indra, A.K., X. Warot, J. Brocard, J.M. Bornert, J.H. Xiao, Chambon, and D. Metzger: Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: comparison of the recombinase activity of the tamoxifen-inducible Cre-ER(T) and Cre-ER(T2) recombinases. Nucleic Acids Res, 27, 4324-7 (1999)
34. Rodriguez, C.I., F. Buchholz, J. Galloway, R. Sequerra, J. Kasper, R. Ayala, A.F. Stewart, and S.M. Dymecki: High-efficiency deleter mice show that FLPe is an alternative to Cre-loxP. Nat Genet, 25, 139-40 (2000)
35. Seaberg, R.M. and D. van der Kooy: Stem and progenitor cells: the premature desertion of rigorous definitions. Trends Neurosci, 26, 125-31 (2003)
36. Delplanque, A., C. Coraux, R. Tirouvanziam, I. Khazaal, E. Puchelle, Ambros, D. Gaillard, and B. Peault: Epithelial stem cell-mediated development of the human respiratory mucosa in SCID mice. J Cell Sci, 113 (Pt 5), 767-78 (2000)
37. Deome, K.B., L.J. Faulkin, Jr., H.A. Bern, and P.B. Blair: Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. Cancer Res, 19, 515-20 (1959)
38. Hoshino K, G.W.: Transplantability and life span of mammary gland during serial transplantation in mice. Nature, 213, 193-4 (1967)
39. Welm, B.E., S.B. Tepera, T. Venezia, T.A. Graubert, J.M. Rosen, and M.A. Goodell: Sca-1(pos) cells in the mouse mammary gland represent an enriched progenitor cell population. Dev Biol, 245, 42-56 (2002)
40. Eggan, K., S. Jurga, R. Gosden, I.M. Min, and A.J. Wagers: Ovulated oocytes in adult mice derive from non-circulating germ cells. Nature, 441, 1109-14 (2006)
41. Wuenschell, C.W., M.E. Sunday, G. Singh, Minoo, H.C. Slavkin, and D. Warburton: Embryonic mouse lung epithelial progenitor cells co-express immunohistochemical markers of diverse mature cell lineages. J Histochem Cytochem, 44, 113-23 (1996)
42. Vu, T.H., Y. Alemayehu, and Z. Werb: New insights into saccular development and vascular formation in lung allografts under the renal capsule. Mech Dev, 120, 305-13 (2003)
43. Morrison, S.J., P.M. White, C. Zock, and D.J. Anderson: Prospective identification, isolation by flow cytometry, and in vivo self-renewal of multipotent mammalian neural crest stem cells. Cell, 96, 737-49 (1999)
44. Perl, A.K., S.E. Wert, A. Nagy, C.G. Lobe, and J.A. Whitsett: Early restriction of peripheral and proximal cell lineages during formation of the lung. Proc Natl Acad Sci U S A, 99, 10482-7 (2002)
45. Perl, A.K., S.E. Wert, D.E. Loudy, Z. Shan, P.A. Blair, and J.A. Whitsett: Conditional recombination reveals distinct subsets of epithelial cells in trachea, bronchi, and alveoli. Am J Respir Cell Mol Biol, 33, 455-62 (2005)
46. Whitsett, J.A. and A.K. Perl: Conditional control of gene expression in the respiratory epithelium: A cautionary note. Am J Respir Cell Mol Biol, 34, 519-20 (2006)
47. Ahn, S. and A.L. Joyner: In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog. Nature, 437, 894-7 (2005)
48. Neuringer, I.P. and S.H. Randell: Lung stem cell update: promise and controversy. Monaldi Arch Chest Dis, 65, 47-51 (2006)

Key Words: Lung, stem cells, bronchiolar, alveolar, BASC, Review,

Send correspondence to: Dr Carla Bender Kim, Children's Hospital , 300 Longwood, Ave., Karp 8-216 Boston, MA 02115, Tel: 617-919-4644 Fax: 617-730-0222, E-mail: carla.kim@childrens.harvard.edu

http://www.bioscience.org/current/vol12.htm