Commentary: Sca-1 and Cells of the Lung: A Matter of Different Sorts

Published in final edited form as:
Stem Cells. 2009 March ; 27(3): 606–611. doi:10.1002/stem.10.

Sca-1 and Cells of the Lung: A Matter of Different Sorts

David M. Raiser and **Carla F. Kim**
Children's Hospital Boston Stem Cell Program; Department of Genetics, Harvard Medical School; and Harvard Stem Cell Institute; Boston, MA 02115

Abstract

In two separate papers published in this issue, Teisanu et al. and McQualter et al. report the use of flow cytometry and cell sorting to identify putative bronchiolar stem cells that are low in expression for the cell surface marker Sca-1 yet negative for CD34, and a mesenchymal, fibroblastic progenitor cell population from the lung that is positive for Sca-1, respectively. At first glance, these studies may seem to suggest that Sca-1 and CD34 are not markers of an epithelial stem cell population in the lung, as we previously determined in studies that identified bronchioalveolar stem cells (BASCs), and may also appear to contradict each other. However, here we point to evidence that the findings of these three studies are not mutually exclusive, and rather, that the different cell isolation and culturing protocols used in these studies have allowed for the identification of unique pulmonary cell populations. Rather than discounting previous work on BASCs, these studies reveal the existence of new methods and new cell types which will be interesting to use in future functional tests for their importance in lung biology and lung disease.

Keywords

lung; alveolar; bronchiolar

Introduction

Flow cytometry and fluorescence activated cell sorting (FACS) are key tools that have made it possible to isolate and further purify many types of stem cells when used in combination with functional assays for stem cell properties. For instance, the ability to fractionate the hematopoietic cells into a hierarchy of well-defined subpopulations is the result of years of careful surface marker analysis and *in vitro* and *in vivo* assays. Not very long ago, the existing cocktail of selection and exclusion markers could not separate the self-renewing long-term hematopoietic stem cell (HSC) from the short-term HSC or the multipotent progenitors derived from HSCs within the bone marrow fraction positive for Sca-1 and ckit and negative for a cocktail of blood cell lineage markers (Sca-1^{pos} ckit^{pos} Lin^{neg}, or KLS). Further purification of the heterogeneous KLS population became possible with the use of Flk-2, Thy-1, and the SLAM markers, refining the definition of more purified HSCs [1, 2]. However, even now the long-term HSC pool is suspected of heterogeneity that can be further uncovered with isolation of label-retaining, infrequently proliferating HSCs, and debate continues as to the endogenous niche for HSCs [3-5]. In the mammary gland, Sca-1 has

Correspondence: 300 Longwood Ave., Karp 8-216, Boston, MA 02115, Phone: 617-919-4644, Fax: 617-730-0222, carla.kim@childrens.harvard.edu.

Author Contributions:
David M. Raiser: Conception and design, Collection and/or assembly of data, Data analysis and interpretation, Manuscript writing
Carla F. Kim: Conception and design, Financial support, Collection and/or assembly of data, Data analysis and interpretation, Manuscript writing, Final approval of manuscript

proven to be a highly controversial marker, with claims that both positive and negative populations are enriched over the other population for stem/progenitor potential [6-8]. Clearly, the use of cell surface markers has been debated in several tissues, so it is not surprising that the debate now extends to the lung as well.

Cell sorting strategies have been used to identify a number of putative stem or progenitor cell populations in the mouse lung [9-13]. Much of this work has made use of the "side population" (SP) method to identify cells with the ability to efflux the Hoechst dye, originally used to isolate HSCs [14]. Lung side population cells have been reported to include endothelial progenitors, hematopoeitic lineage cells, mesenchymal stem cells, and possible epithelial cell populations. Whereas the SP protocol allows for isolation of cells of interest without prior knowledge of cell surface markers, more recent studies have made use of candidate cell surface markers to uncover lung cells with stem or progenitor cell activity.

Bronchioalveolar stem cells (BASCs) were initially identified based on their residence in the region between the bronchiolar and alveolar cells in terminal bronchioles, known as the bronchio-alveolar duct junction (BADJ), and distinct co-expression of the bronchiolar Clara cell marker CCSP and the alveolar type II (AT2) cell marker SPC [12]. BASCs can be isolated from dissociated murine lung using a FACS-based protocol wherein cells are sorted positively for expression of the cell surface marker Sca-1 and negatively for the endothelial marker CD31 and the hematopoietic marker CD45, and further purification of BASCs was achieved by sorting rare CD34^{pos} cells from within the Sca-1^{pos} population. Isolated BASCs have the key stem cell properties of self-renewal and multipotency in that they can be passaged multiple times in culture on feeders and, in clonal assays, they can differentiate into CCSP^{pos} cells or SPC^{pos} cells (singly positive for each) or even cells positive for the alveolar type I marker aquaporin 5 when grown on Matrigel, a basement membrane matrix preparation [12, 15]. Additionally, BASCs are among the first cells to proliferate *in vivo* in response to naphthalene injury, bleomycin injury, and induction of oncogenic K-ras. Since their initial characterization in 2005, several other groups have studied BASCs, or at least cells that resemble BASCs based on marker expression, *in vivo*[16-21]. For a more detailed description of our current model of BASC function and others' studies of BASCs, please see Raiser et.al. for review [22].

In their studies, Teisanu et al. [23] and McQualter et al. [24] suggest that the combination of Sca-1 and CD34 does not mark an epithelial stem cell population in the lung. What follows is a relatively detailed analysis of the work presented by these two groups. In many cases we agree with their conclusions, while in others we offer an alternative interpretation. In response, we offer that the data presented in these papers is not inconsistent with the observations made by our lab and others regarding the Sca-1 expression status of the BASC population, and it is plausible that variations in isolation and digestion methods, sorting antibodies, and culturing conditions are responsible for different results when using Sca-1 for prospective lung cell isolation.

Sca-1 as a prospective lung mesenchymal cell marker

McQualter et al. used FACS analysis during different developmental stages, cell culturing, microarray analysis of sorted cells, and immunofluorescence to conclude that Sca-1 serves as a mesenchymal lung cell marker, rather than an epithelial stem cell marker. They found that Sca-1^{pos} cells first emerge in the neonatal lung, and they report Sca-1 protein to be restricted to the parenchyma of the distal lung in tissue sections. A gene expression study presented by the authors also shows that cells lacking Sca-1 exhibit an epithelial profile, whereas the Sca-1^{pos} population (excluding hematopoietic and endothelial cells) appears to have a more mesenchymal-like profile. Lastly, in comparative growth and differentiation assays, Sca-1^{pos} cells appeared to predominantly exhibit fibroblast-like morphologies, with

rare epithelial colonies also present, and Sca-1^{pos} cells were capable of differentiating into "lipofibroblastic," osteogenic and chondrogenic cell lineages.

Importantly, many of the studies presented by McQualter et. al. are in agreement with ours and others' findings regarding BASCs. First, this group notes that Sca-1 expression is not detectable before birth but increases postnatally during alveolar expansion in the mouse neonate. Interestingly, the appearance of the BASC population – as defined by the emergence of SPC and CCSP dual positive cells – also occurs almost exclusively after birth; these cells are not present in the normal embryonic lung [18, 21, 25] (Kim et al. unpubl.). The developmental timing of Sca-1 expression thus coincides nicely with the appearance of SPC^{pos} CCSP^{pos} BASCs in the neonatal lung and the significant alveolar expansion that occurs in the period following birth [26]. We hypothesize that BASCs do not directly play a role in the embryonic development of the lung, an idea supported by this group's additional data. Second, McQualter et. al. reported epithelial morphology resulting from the culture of Sca-1^{neg} cells and more rarely from Sca-1^{pos} cells. We completely agree that the Sca-1^{neg} population contains epithelial cells, and we posit that this population is largely AT2 cells [12]. We also agree that the Sca-1^{pos} population remains heterogeneous; by using GFP-transgenic mice, we have recently found that the Sca-1^{pos} population is indeed more heterogeneous than we previously appreciated, and cells with mesenchymal morphologies can be observed in culture when this entire population is plated on mouse embryonic fibroblasts (MEFs) (Figure 1); however, epithelial colonies are more frequently observed in our cell culture conditions [12, 15]. Third, McQualter et al. show that Sca-1^{pos} cells more highly express genes associated with mesenchymal cells in comparison to the Sca-1^{neg} cells, which express epithelial cell markers. It is notable that the gene expression data presented in this study was not accompanied by validation from real-time PCR or other methods, nor was it clear from the report what were the precise cell populations being used as comparative cells from other tissues. Aside from these caveats, a careful look at the gene expression data shown reveals that SPC and CCSP (Scgb1a1), markers of BASCs, were substantially expressed in the Sca-1^{pos} fraction as well as the negative fraction. As above, we would also expect that the Sca-1^{neg} cells should retain epithelial cell gene expression. It is also important to consider that many of the genes that the authors assign to a mesenchymal signature are known markers of rare stem cell populations from other tissues, and known expression in mesenchymal cells does not preclude a marker from being an identifier of a rare epithelial stem cell. For example, CD34 is a known marker of skin epithelial stem cells [27], Gli2 expression has been shown in mammary epithelial stem cells, neuroepithelial cells, and neural retinal cells [28-30], and numerous procollagens have been found to be highly expressed in stem cell populations, such as Col15a1 which is highly expressed in ES cells [31]. Collectively, these data most likely indicate that the Sca-1^{pos} population isolated by McQualter et. al. is heterogeneous, and may include both epithelial lung stem cells as well as mesenchymal stem cells; further purification of each stem cell pool will allow relevant gene expression differences to become more apparent.

Marked, deleted and expanded bronchiolar cells for cell surface comparisons

Teisanu et al. used a different approach to examine cell surface marker expression on putative lung stem cell populations, utilizing lung cell autofluorescence properties and transgenic mouse models to visualize changes in the relative size of the bronchiolar stem cell compartment. First, they used a transgenic mouse strain in which CCSP-expressing cells are ablated following ganciclovir treatment (the CCTK model)[32], and showed that there is no observed decrease in the size of the Sca-1^{pos} CD34^{pos} fraction (thought to contain BASCs) when compared to a wild-type situation. Second, in a differentiation-deficient model in which potentiated β-catenin results in an increase in the SPC^{pos}CCSP^{pos} cell pool [21], there was also no corresponding increase observed in the Sca-1^{pos} CD34^{pos} fraction.

Lastly, use of a CCSP-driven Cre recombinase lineage-tagging system led the authors to claim Sca-1^{pos} CD34^{pos} cells are not derived from a CCSP^{pos} population. Changes in the relative abundance of the highly autofluorescent (AF^{hi}) and dimly autofluorescent (AF^{low}) populations in these scenarios led the authors to the conclude that bronchiolar stem cells can be identified by a CD34^{neg} Sca-1^{low} AF^{low} cell surface phenotype (after CD45 and CD31 exclusion). The use of genetic models of cell ablation, cell tagging, and cell expansion by these authors offers an interesting approach to the identification of the cell surface phenotype of distal lung epithelial cells, complementing the gene expression and culture data of McQualter and colleagues. While models such as these come with caveats of their own, using genetic models such as these provides the opportunity to perturb the normal situation and visualize resultant changes in various populations.

Alternative interpretations can reconcile the findings of Teisanu and colleagues with our previous studies of BASCs. First, both studies have shown that Sca-1 is found on the surface of a subset of bronchiolar epithelial cells. The Stripp group makes the distinction that Sca-1^{low} cells within the Sca-1^{pos} population are a bronchiolar stem cell pool. We similarly believe that the Sca-1^{low} portion of the Sca-1^{pos} cells in our studies may indeed be enriched for BASC activity, as we observe better epithelial cell growth when Sca-1^{low} cells are included in cell sort collections for culture (data not shown, Roach and Kim, unpublished data); it will likely be informative to carefully compare Sca-1^{high} and Sca-1^{low} cells in our own self-renewal and differentiation assays. Also, when thinking about the comparisons made in situations of genetic abrogation by Teisanu et. al., the changes in the total number of cells present in a cellular pool must be considered when comparing the size of a FACS-sorted cell population based on percentages. In other words, ablation of a cell type can alter the percentage of the total population comprised of other cell types, and it is difficult to assign meaning to subtle changes, or lack of changes, in population percentages without more information about total cell numbers. Next, in the lineage-tagging model used to mark CCSP-expressing cells, the authors claim that Sca-1^{pos} and CD34^{pos} populations are not of an epithelial, bronchiolar lineage; however, this interpretation is in disagreement with the FACS data presented, wherein both of these populations appear to contain a small percentage of YFP-tagged cells. Thus, the data of Teisanu et. al. could also be interpreted as showing evidence that a rare fraction of Sca-1^{pos} and CD34 ^{pos} cells are in the bronchiolar cell lineage, consistent with our hypothesis of the lineage relationships in the distal lung [22]. Together with the inability of the CCSP-driven Cre used to mark every CCSP-expressing cell (90% of bronchiolar cells were labeled without a report of the fraction of BASCs labeled), the possibility that unmarked BASCs are present in the assayed populations cannot be excluded. Additionally, issues with detection of CD34 must be considered here as well (see below). Teisanu et al. also discuss the possibility that SPC expression is upregulated during the cell isolation process, and this accounts for the SPC^{pos} CCSP^{pos} cells seen in the Sca-1^{pos} CD34^{neg} sorted cell fractions that they conclude exclusively contain bronchiolar cells. It should be noted that in our studies we find SPC^{pos} CCSP^{pos} cells in both the Sca-1^{pos} CD34^{neg} and Sca-1^{pos} CD34^{pos} populations (data not shown). However, the Sca-1^{pos} CD34^{pos} fraction allowed us to exclude ciliated cells and isolate the Sca-1^{pos} cells with the most proliferative potential in culture. The notion that gene expression could be altered in the preparation of cells for FACS sorting is worth exploring further, and it will be important to see the results of gene expression studies on freshly sorted cell populations to confirm the cytospin immunostaining results. Finally, the authors conclude that, based on the data discussed above in conjunction with observations of the autofluorescent populations in the models tested, the Sca-1^{low}, CD34^{neg}, AF^{low} population harbors bronchiolar stem cells. Without subjecting these cells to any functional assays of stem cell potential, it is difficult to accept the stem cell identity of this population.

A Matter of Different Sorts: Distinguishing features of each study

Whereas numerous aspects of the work presented by these papers are consistent with our own findings, it is also important to consider the distinct findings of each study by taking into account the sharp contrasts in the isolation and culture protocols used by each group (Table 1). For example, McQualter et. al. used C57Bl6 mice as a cell source, collagenase type I digestion and density gradient for single cell isolations, and laminin-coated plates, 10% CO2 and low oxygen in their culture conditions. In contrast, we previously used 129SvEJ mice, collagenase and dispase digestion, and feeder layers in 5% CO2 and standard tissue culture oxygen content in our cultures. These distinctions alone could dramatically alter the cell surface phenotype of freshly isolated lung cells (e.g., via variable antigen cleavage by enzyme activity) or result in completely different populations of cells being collected and/or propagated by each group. To test this possibility, we have recently subjected our passaged BASCs to a standard mesenchymal stem cell (MSC) differentiation assay, and in contrast to control MSCs, BASCs failed to produce any cells positive for adipogenic differentiation assessed by Oil Red O staining or osteogenic differentiation after three weeks in appropriate media (M. Aslam, S. Kourembanas, C. Kim, unpublished data); these data support our view that Sca-1^{pos} BASCs and McQualter's Sca-1^{pos} mesenchymal cells are distinct cell types. Teisanu et.al. used yet another method for cell isolation, which is optimized for retrieving bronchiolar epithelial cells, in contrast to our protocol that yields very few bronchiolar cells and was adapted from an AT2 cell preparation method.

Our most recent data examining the overlap of Sca-1 and CD34 in lung cell isolations further illustrate two key points in the distinctions in each study: first, that variability in staining and sorting ability exists even between differently-conjugated versions of the same antibody clone, and second, that our consistently lower percentages of both total Sca-1^{pos} cells and Sca-1^{pos} CD34^{pos} cells (as compared to those reported by McQualter et al. and Teisanu et al.) implies a significant consequence of variations in cell isolation and enrichment protocols. The McQualter Sca-1^{pos} fraction is entirely CD34^{pos} (within the CD45^{neg} CD31^{neg} fraction) when using an antibody from BD Pharmingen, whereas only a fraction of Sca-1^{pos} BASCs (2-20%) are CD34^{pos} ([12] and Figure 2). Using a CD34 antibody from eBioscience, Teisanu et al. report contributions of CD34^{pos} cells to the Sca-1^{pos} fraction greater than what we have observed (~34-36%, as approximated from reported numbers in wild-type mice), but a complete overlap is not reported. In previously published work, we used a CD34-PE antibody from BD Pharmingen for FACS isolation of BASCs [12], but this antibody is no longer commercially available. We more recently observed varying amounts of Sca-1 and CD34 overlap depending on the color conjugate used for CD34 detection with an eBioscience antibody, and importantly the currently available CD34-FITC antibody provides results most similar to our previous work, in which we found that the CD34^{pos} cells were dim and found mostly in the Sca-1^{low} population (Figure 2). In light of these findings, we recommend careful testing of reagents when wishing to use CD34 as a marker of BASCs in FACS-based isolation protocols. From this information, it is clear that CD34 ^{pos} cells in all studies are not necessarily the same population. As a final comment on differences in technique, it is important to keep in mind that McQualter and Teisanu did not use the same staining protocol we use to detect Sca-1^{pos} cells in tissue sections, nor did they report careful multi-color labeling to search for rare CCSP^{pos} SPC^{pos} Sca-1^{pos} cells at the BADJ, which we did previously detect in sections [12].

Conclusion

It is unclear exactly which methodical differences are responsible for distinct results, but what can be gleaned is that, when comparing the results of these and previous studies, it is essential to consider the cell isolation methods used to obtain various lung cell populations. Similar to the history of the hematopoietic stem cell, studies of stem cells of the lung are

subject to a learning process that will evolve over time. The work presented by McQualter et al. and Teisanu et al. contribute additional, useful information to the search for lung stem cell phenotypes. The data underscore the possibility that many stem cell populations exist in the lung with distinct lineage potential (Figure 3), and the ability to purify and functionally assay these populations will require consistent use of well-defined protocols for isolation, culturing, and functional assays. In the future, it may be important to compare these protocols as more markers to fractionate and purify subpopulations of Sca-1^{pos} lung cells and other lung stem cells become known. Most importantly, future studies to define lung stem cells should include multiple, combined approaches: gene expression studies, cultures to assess self-renewal and differentiation, and genetically engineered alleles, and an important goal is to couple these techniques with *in vivo* functional assays.

Acknowledgments

We thank Barry Stripp and Roxana Teisanu for sharing data in advance of publication, Rebecca Roach for technical assistance, Muhammad Aslam and Stella Kourembanas for collaborative work for mesenchymal cell differentiation, and Kerstin Sinkevicius and Sima Zacharek for critical reading of the manuscript. This work was supported with funds from a Harvard Stem Cell Institute Seed Grant and NHLBI RO1HL090136 (to CFK).

References

1. Christensen JL, Weissman IL. Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells. Proc Natl Acad Sci U S A. Dec 4; 2001 98(25):14541–14546. [PubMed: 11724967]
2. Kiel MJ, Yilmaz OH, Iwashita T, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell. Jul 1; 2005 121(7):1109–1121. [PubMed: 15989959]
3. Wilson A, Oser GM, Jaworski M, et al. Dormant and self-renewing hematopoietic stem cells and their niches. Ann N Y Acad Sci. Jun.2007 1106:64–75. [PubMed: 17442778]
4. Wilson A, Laurenti E, Oser G, et al. Hematopoietic Stem Cells Reversibly Switch from Dormancy to Self-Renewal during Homeostasis and Repair. Cell. 2008 Online before print.
5. Foudi A, Hochedlinger K, Van Buren D, et al. Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. Nat Biotech. 2008 Online before print.
6. Shackleton M, Vaillant F, Simpson KJ, et al. Generation of a functional mammary gland from a single stem cell. Nature. Jan 5; 2006 439(7072):84–88. [PubMed: 16397499]
7. Welm B, Behbod F, Goodell MA, Rosen JM. Isolation and characterization of functional mammary gland stem cells. Cell Prolif. Oct; 2003 36(Suppl 1):17–32. [PubMed: 14521513]
8. Welm BE, Tepera SB, Venezia T, Graubert TA, Rosen JM, Goodell MA. Sca-1(pos) cells in the mouse mammary gland represent an enriched progenitor cell population. Dev Biol. May 1; 2002 245(1):42–56. [PubMed: 11969254]
9. Summer R, Fitzsimmons K, Dwyer D, Murphy J, Fine A. Isolation of an adult mouse lung mesenchymal progenitor cell population. Am J Respir Cell Mol Biol. Aug; 2007 37(2):152–159. [PubMed: 17395889]
10. Summer R, Kotton DN, Sun X, Ma B, Fitzsimmons K, Fine A. Side population cells and Bcrp1 expression in lung. Am J Physiol Lung Cell Mol Physiol. Jul; 2003 285(1):L97–104. [PubMed: 12626330]
11. Reynolds SD, Shen H, Reynolds PR, et al. Molecular and functional properties of lung SP cells. Am J Physiol Lung Cell Mol Physiol. Apr; 2007 292(4):L972–983. [PubMed: 17142352]
12. Kim CF, Jackson EL, Woolfenden AE, et al. Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell. Jun 17; 2005 121(6):823–835. [PubMed: 15960971]
13. Giangreco A, Shen H, Reynolds SD, Stripp BR. Molecular phenotype of airway side population cells. Am J Physiol Lung Cell Mol Physiol. Apr; 2004 286(4):L624–630. [PubMed: 12909587]

14. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med. Apr 1; 1996 183(4):1797–1806. [PubMed: 8666936]
15. Dovey JS, Zacharek SJ, Kim CF, Lees JA. Bmi1 is critical for lung tumorigenesis and bronchioalveolar stem cell expansion. Proc Natl Acad Sci U S A. Aug 19; 2008 105(33):11857–11862. [PubMed: 18697930]
16. Nolen-Walston RD, Kim CF, Mazan MR, et al. Cellular kinetics and modeling of bronchioalveolar stem cell response during lung regeneration. Am J Physiol Lung Cell Mol Physiol. Jun; 2008 294(6):L1158–1165. [PubMed: 18375744]
17. Yanagi S, Kishimoto H, Kawahara K, et al. Pten controls lung morphogenesis, bronchioalveolar stem cells, and onset of lung adenocarcinomas in mice. J Clin Invest. Oct; 2007 117(10):2929–2940. [PubMed: 17909629]
18. Zhang Y, Goss AM, Cohen ED, et al. A Gata6-Wnt pathway required for epithelial stem cell development and airway regeneration. Nat Genet. Jul; 2008 40(7):862–870. [PubMed: 18536717]
19. Qian S, Ding JY, Xie R, et al. MicroRNA expression profile of bronchioalveolar stem cells from mouse lung. Biochem Biophys Res Commun. Dec 12; 2008 377(2):668–673. [PubMed: 18948085]
20. Yang Y, Iwanaga K, Raso MG, et al. Phosphatidylinositol 3-kinase mediates bronchioalveolar stem cell expansion in mouse models of oncogenic K-ras-induced lung cancer. PLoS ONE. 2008; 3(5):e2220. [PubMed: 18493606]
21. Reynolds SD, Zemke AC, Giangreco A, et al. Conditional stabilization of beta-catenin expands the pool of lung stem cells. Stem Cells. May; 2008 26(5):1337–1346. [PubMed: 18356571]
22. Raiser DM, Zacharek SJ, Roach RR, et al. Stem Cell Biology in the Lung and Lung Cancers: Employing Pulmonary Context and Classic Approaches. Cold Spring Harb Symp Quant Biol. Nov 26.2008
23. Teisanu R, Lagasse E, Whitesides J, Stripp BR. Prospective isolation of bronchiolar stem cells based upon immunophenotypic and autofluorescence characteristics. Stem Cells. 2008
24. McQualter J, Brouard N, Williams B, et al. Endogenous fibroblastic progenitor cells in the adult mouse lung are highly enriched in the Sca-1 positive fraction. Stem Cells. 2008
25. Tyagi S, Srisuma S, Bhattacharya S, Mariani T. Developmental ontogeny of bronchio-alveolar stem cells. FASEB J. 2007; 21:975–979.
26. Mund SI, Stampanoni M, Schittny JC. Developmental alveolarization of the mouse lung. Dev Dyn. Aug; 2008 237(8):2108–2116. [PubMed: 18651668]
27. Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E. Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. Cell. Sep 3; 2004 118(5): 635–648. [PubMed: 15339667]
28. Liu S, Dontu G, Mantle ID, et al. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. Cancer Res. Jun 15; 2006 66(12):6063–6071. [PubMed: 16778178]
29. Takanaga H, Tsuchida-Straeten N, Nishide K, Watanabe A, Aburatani H, Kondo T. Gli2 Is A Novel Regulator of Sox2 Expression In Telencephalic Neuroepithelial Cells. Stem Cells. Oct 16.2008
30. Perron M, Boy S, Amato MA, et al. A novel function for Hedgehog signalling in retinal pigment epithelium differentiation. Development. Apr; 2003 130(8):1565–1577. [PubMed: 12620982]
31. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. Stemness": transcriptional profiling of embryonic and adult stem cells. Science. Oct 18; 2002 298(5593):597–600. [PubMed: 12228720]
32. Reynolds SD, Hong KU, Giangreco A, et al. Conditional clara cell ablation reveals a self-renewing progenitor function of pulmonary neuroendocrine cells. Am J Physiol Lung Cell Mol Physiol. Jun; 2000 278(6):L1256–1263. [PubMed: 10835332]

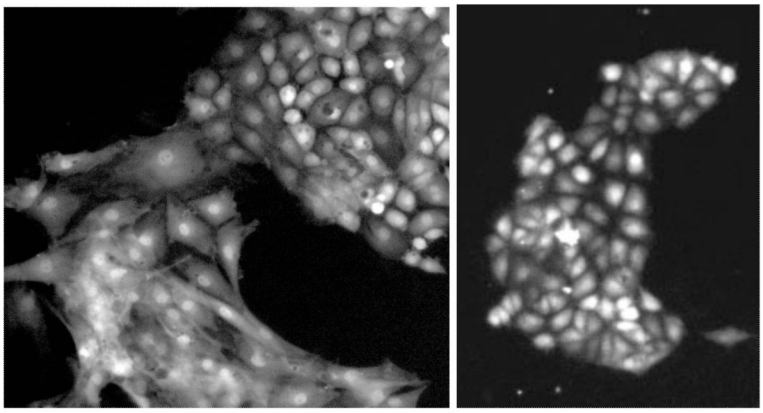

FIGURE 1. Colony morphology of sorted Sca-1^{pos} and Sca-1^{neg} cell populations

Cells were isolated from *β-actin-GFP* mouse lungs as described [12], and the viable CD45^{neg}CD31^{neg} population was divided into Sca-1^{pos} (left panel) and Sca-1^{neg} (right panel) fractions and plated on irradiated MEF feeders. Colonies of epithelial (upper right) and mesenchymal (lower left) morphologies were observed in the Sca-1^{pos} fraction, whereas the Sca-1^{neg} fraction yielded mostly epithelial colonies.

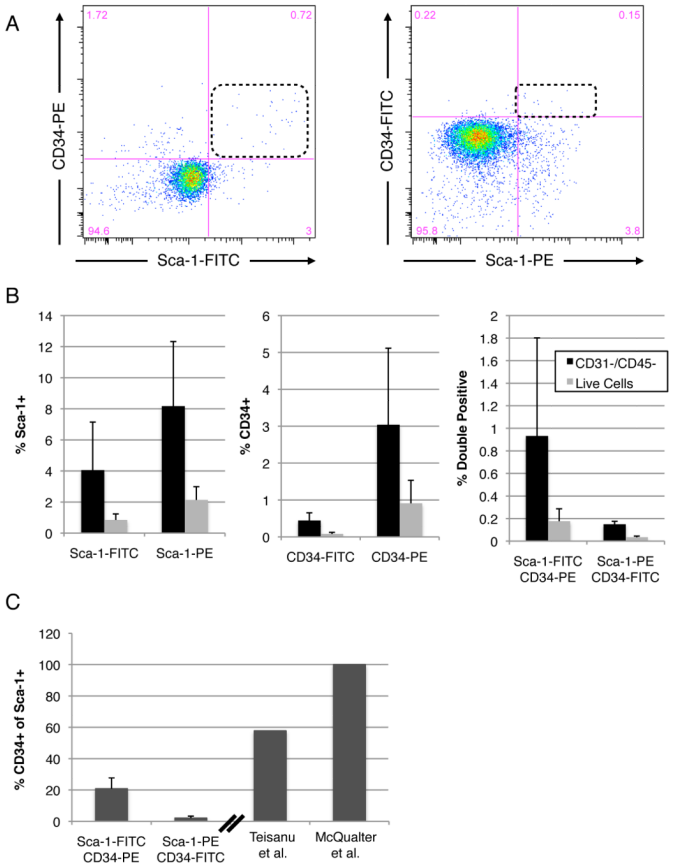

FIGURE 2. Choice of antibody conjugates affects apparent size of Sca-1^{pos} and CD34^{pos} populations

(A) Lungs from C57Bl/6 mice were processed as described [12]. Aliquots from each of four individual mice were stained with antibodies against CD45, CD31, and a combination of either Sca-1-FITC/CD34-PE or Sca-1-PE/CD34-FITC; dead cells were excluded with 7AAD. (Sca-1 antibodies were purchased from BD Pharmingen, and CD34 antibodies were purchased from eBioscience.) Representative plots of the viable CD45^{neg}CD31^{neg} populations are shown. Note the high autofluorescence in the FITC channel, a characteristic trait of lung cells and notably AT2 cells as in our previous studies. (B) Total Sca-1^{pos} (left), total CD34^{pos} (middle), and Sca-1^{pos} CD34^{pos} double positive populations as percentages of the parent CD45^{neg}CD31^{neg} population (black bars) or all viable cells (gray bars). (C) Percent of Sca-1^{pos} cells also positive for CD34; right two bar values were calculated or taken directly from data presented in the publications listed.

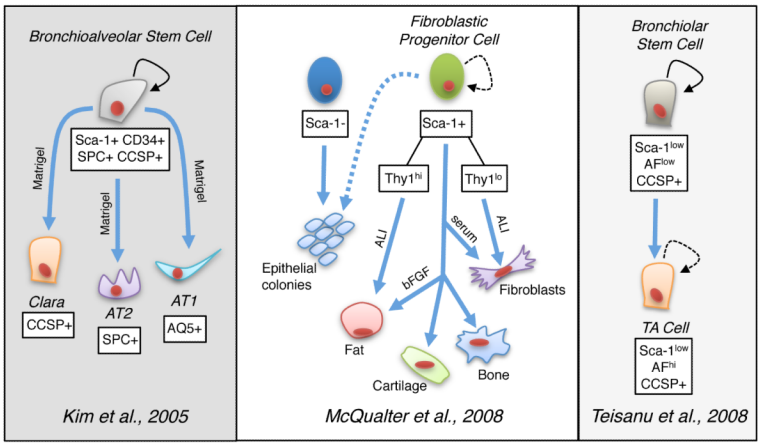

FIGURE 3. Proposed cell lineage relationships of lung stem/progenitor cells to differentiated progeny

(Left) Bronchioalveolar stem cells are Sca-1^{pos} CD34^{pos} cells that can self-renew on feeder cells and can differentiate into three distinct epithelial cell types on Matrigel. (Center) McQualter et al. demonstrate predominant mesenchymal potential and rare epithelial potential of Sca-1^{pos} cells under various *in vitro* culture conditions. (Right) Teisanu et al. use FACS data to infer a stem (AF^{low}) to progenitor (AF^{hi}) lineage from Sca-1^{low} lung cells. All cells depicted here are also CD45^{neg} and CD31^{neg}. Abbreviations: SPC, pro-surfactant protein C; CCSP, Clara cell secretory protein; AT1, alveolar type I cell; AT2, alveolar type II cell; AQ5, aquaporin 5; ALI, air-liquid interface culture; AF, autofluorescent in the AlexaFluor 647 channel; TA cell, transit-amplifying cell. Hatched lines indicate rare events and/or limited potential.

Table 1

Reference	**McQualter et al.; *Stem Cells* 2008**	**Teisanu et al.; *Stem Cells* 2008**	**Kim et al.; *Cell*2005**	**Summer et al.; *AJRCMB* 2007**
Isolation Optimized For:	--	Bronchiolar Cells	Alveolar Cells	Epithelial Cell Exclusion
Digestive Enzyme(s) / Enrichment Strategies	Collagenase Density gradient centrifugation	PBS/EGTA Elastase DNase	Dispase Collagenase Agarose DNase	Collagenase Dispase
Stem / Progenitor Phenotype	CD45- CD31- Sca-1+ CD34+[a] Thy-1[b]	CD45- CD31- **Sca-1^{low}** CD34-A plow/hi [d]	CD45- CD31- **Sca-1+** CD34+	SP CD45- CD31- **Sca-1+**[e] (others)[e]
Stem / Progenitor Cell Type	Mesenchymal	Epithelial (Bronchiolar)	Epithelial	Mesenchymal
Primary Culture Media / Conditions	Low Oxygen; 20% serum OR defined serum-free media	ND	10% serum	20% serum (SP); 10% serum (non-SP)
Primary Culture Substrate	Laminin-coated plates	ND	On MEFs	Plastic
In vitro Differentiation Capacity	Cartilage, Bone[c]; Fat (*bFGF* supplement); Fat/ Fibroblastic Cells (*ALI culture*)	ND	Alveolar and Bronchiolar Lineages (*on Matrigel*)	Smooth Muscle, Cartilage, Bone, Fat (*published differentiation protocols*)
In vitro Self-renewal Potential	Yes (clonogenic assays, LD)	ND	Yes (serial passaging, clonogenic assays, LD)	Yes (serial passaging)
Sca-1 In situ Expression	Endothelium, Distal parenchyma	Endothelium, Proximal airway epithelium	Endothelium, Ciliated Cells, BASCs	Endothelium (Summer et al., 2003)

Abbreviations: ALI = air-liquid interface; LD = limiting dilution; ND = not determined; MEFs = mouse embryonic fibroblasts

[a] analysis only; not used to sort populations for *in vitro* assays

[b] heterogeneous expression; Thy-1^{hi} and Thy-1^{low} fractions show distinct potential in culture assays

[c] data not published

[d] AF = autofluorescence in the AlexaFluor 647 and PE channels; low = stem cells, high = facultative transit-amplifying cells

[e] expression determined after long-term culturing