Published in final edited form as:
Int J Biochem Cell Biol. 2010 January ; 42(1): 1–4. doi:10.1016/j.biocel.2009.09.002.

Clara Cell: Progenitor for the Bronchiolar Epithelium

Susan D. Reynolds, PhD[a] and **Alvin M. Malkinson, PhD**[b]

[a] Department of Pediatrics, National Jewish Health, Denver, CO

[b] Department of Pharmaceutical Sciences, University of Colorado-Denver, Aurora, CO

Abstract

Clara cells were first described as a morphologically distinct cell type by Kolliker in 1881, but take their name from the seminal study of human and rabbit bronchioles by Max Clara in 1937. Since their discovery, Clara cells have been identified as central players in protecting the airway from environmental exposures. The diverse functions of Clara cells in lung homeostasis include roles in xenobiotic metabolism, immune system regulation, and progenitor cell activity. Recent identification of a sub-population of Clara cells as a bronchiolar tissue-specific stem cell and a potential tumor initiating cell has focused the attention of cell and molecular biologists on the Clara cell and its behavior under normal and disease conditions.

Keywords

non-ciliated secretory cell; progenitor cell; stem cell; adenocarcinoma

Introduction

The Clara cell is a multifunctional cell that has been under intensive study for seventy years (Figure 1). It is a cubiodal, nonciliated cell in human and rabbit terminal bronchioles, containing a basally-situated nucleus, an apical dome extending variable distances into the airway lumen, and discrete, oval densely-staining granules (Figure 2). Ultrastructural and morphometric analysis by Plopper and colleagues provided insights into Clara cell function, and led to ongoing studies demonstrating critical roles in barrier maintenance, secretion, and metabolism (Stripp and Reynolds, 2006). Multispecies comparisons established *diversity* as a defining hallmark of Clara cell biology (Figure 3). In spite of extensive structural and functional variation, antibody reagents specific to the secretoglobin (SCGB1A1) family allowed Clara cells and their variants to be identified on the basis of secreted protein expression (Reynolds et al., 2002). Members of the SCGB protein class are referred to as uteroglobin (Ug) in humans and rabbits or Clara cell secretory protein (CCSP, CC10) in mice and rats. We will use CCSP as a catch-all phrase to indicate secretoglobin 1A1.

This review describes unique aspects of Clara cell biochemistry, cell biology, and molecular biology that define this cell as a facultative progenitor (*i.e.*, a proliferatively senescent, metabolically active cell that retains the ability to reenter the cell cycle in response to injury

Correspondence to: Susan D. Reynolds Department of Pediatrics National Jewish Health 1400 Jackson Street Denver, CO 80206 Phone: 303-270-2920 Fax: 303-398-1225 reynoldss@njhealth.org.

of the bronchiolar epithelium) and a subset of Clara cells as tissue-specific stem cells. These functional characteristics support the potential for Clara cells to serve as cancer-initiating cells (Figure 4).

Origin and plasticity: Establishment of the Clara cell pool

Embryological studies established that all airway epithelial cells, with the possible exception of neuroepithelial cells, are derived sequentially from the foregut endoderm (Rawlins et al., 2008). Subdivision of the conducting airway epithelium begins during the second trimester in human lung, and the earliest CCSP-expressing cells are positioned within the luminal aspect of neuroepithelial bodies (NEBs) (Khoor et al., 1996). Continuous labeling studies in hamsters supported the conclusion that neuroepithelial bodies serve as mitotic centers during lung development (Hoyt et al., 1991). Neural peptides secreted by pulmonary neuroendocrine cells are epithelial mitogens (Shan et al., 2004). Analyses of naphthalene-injured lungs indicated a central role for NEB in airway segmentation and the establishment of unique Clara cell pools (Stripp and Reynolds, 2006). However, normal prenatal lung development in NEB-deficient mice suggested that this structure may serve as a marker for an as yet undefined signaling center (Ito et al., 2000). Additional studies are needed to determine the functional significance of this Clara cell-NEB association.

A stabilized form of β-catenin expressed specifically in CCSP-positive cells in mice indicated that functional maturation of Clara cells was modulated by Wnt signaling during prenatal lung development (Reynolds et al., 2008). Two populations of Clara cells may thus exist in the adult bronchiole, those “born” during lung development and those resulting from proliferation and differentiation in air-breathing post-natal animals. The functional significance of these putative “embryological” and adult Clara cells and their impact on lung injury, repair, and susceptibility to chronic lung disease are under investigation. Clara cells with tissue specific stem cell function (see below) may be analogous to canalicular stage Clara cell precursors, and constitutive β-catenin signaling in adulthood may mimic tissue specific stem cell specification. Additional studies using regulated expression of Wnt-pathway agonists and antagonists could substantiate this interpretation and define the microenvironmental influences that delineate the bronchiolar stem cell.

Individual Clara cells retain the capacity to refine their phenotype in response to alterations in the lung milieu, microenvironmental influences specific to trophic units, and exposure to environmental agents including ozone, pathogens and their byproducts, and chemotherapeutic agents. Analysis of how mouse Clara cells respond to allergic inflammation or the Th2 cytokine, IL-13, suggest a lineage relationship between Clara cells and mucus cells (Kuperman et al., 2002); (Evans et al., 2004; Larson et al., 2004). Pulse labeling studies showed that mucus metaplasia of Clara cells generates a terminally differentiated cell that can no longer enter the cell cycle. Thus, Clara cell phenotypic plasticity could be a physiological Catch-22: metaplasia to a mucus producing cell may provide critical protection of the airways but also lead to loss of reparative potential in chronic lung disease.

Functions: Clara cells as reparative cells for the airway epithelium

Within the normal lung, Clare cell proliferation maintains the facultative progenitor cell pool (self-renewal) and restores terminally differentiated cells of the conducting airway epithelium (ciliated cells). This vast reparative reservoir distinguishes lung epithelia from tissues such as the intestine that are maintained through proliferation and differentiation of tissue-specific stem cells. The unique features of lung epithelial maintenance and repair suggest that chronic lung disease could be treated through interventions that stabilize the Clara cell pool or by cell replacement strategies that restore this abundant cell type.

Studies designed to test the hypothesis that tissue-specific stem cells participate in maintenance of the bronchiolar epithelium suggest that this specialized cell is mitotically inactive in the steady state (Giangreco et al., 2009) and under conditions in which the facultative progenitor cell pool is activated by ozone-mediated injury of ciliated cells (Reynolds et al., 2000). Parenteral naphthalene exposure reduces the Clara cell population and activates a putative bronchiolar tissue-specific stem cell located within the NEB and bronchiolar-alveolar duct junction (BADJ) (Stripp and Reynolds, 2008). Both microenvironments contained naphthalene-resistant cells that could initiate and sustain repopulation of the airway epithelium. These cells divide infrequently and were distinguished from most Clara cells by less expression of the phase 1 enzyme, CYP450-2F2. Genetic sensitization of Clara cells to the anti-viral drug, gancyclovir, demonstrated that the bronchiolar stem cell expressed CCSP. These studies were the basis for naming the bronchiolar stem cell a variant CCSP-expressing cell, the vCE. vCEs are rare, sequestered within specific microenvironments (NEB or BADJ), slow-cycling (label-retaining), relatively undifferentiated (CYP450-2F2 low), and express CCSP.

Caveats to this “stemness” claim include identification of multiple label-retaining cell types (neuroepithelial and differentiated Clara cells) in naphthalene injured bronchioles, identical differentiation potential of vCE and Clara cells, and failure to fulfill Koch's postulates through isolation and functional analysis of putative stem cells *in vivo*. One study employed the cell surface markers, stem cell antigen 1 (Sca1) and CD34, to identify a population of CCSP-pro surfactant protein C (proSPC) dual positive cells (Kim et al., 2005). Although this enriched cell population was contaminated with ciliated cells, *in vitro* analysis indicated that these cells could generate daughters that expressed markers associated with terminally differentiated bronchiolar and alveolar cell types. The validity of Sca1 and CD34 as markers for the bronchoalveolar stem cell has been questioned (McQualter et al., 2009; Teisanu et al., 2009). A sharp border is apparent between lineage-traced cells of the terminal bronchiole and the alveolar duct of steady state mice (Rawlins et al., 2009; Reynolds et al., 2008). These data, in addition to demonstrations that Clara cells alter their phenotype in response to injury, suggest that *in vitro* culture induces a third level of Clara cell plasticity, the ability to assume phenotypic characteristics of secretory cells from adjacent compartments. The functional significance of this phenotypic plasticity and its contribution to lung health and disease is unexplored.

Associated pathologies: Clara cells and cancer

Inflammation encourages neoplasia, as shown by elevated cancer risk in patients with chronic obstructive pulmonary disease (COPD) and the inverse correlation between lung tumor macrophage content and patient survival (Malkinson, 2005). Clara cells may regulate inflammation through secretion of CCSP and consequent regulation of eicsoanioid production and the clotting cascade, immune effecter cell chemotaxis and phagocytosis. In human adenocarcinomas, most tumor cells are CCSP-negative even if they have morphologic or other biochemical characteristics of Clara cells (Linnoila et al., 2000). Transfection of CCSP-negative lung cancer cell lines with the CCSP gene diminished their invasiveness and anchorage-independence. CCSP −/− mice are more susceptible to chemically-induced lung tumorigenesis than wild type controls (Linnoila et al., 2000). Thus, CCSP may have tumor suppressor activity that is due in part to its anti-inflammatory function.

The cellular heterogeneity within a tumor makes it difficult to assign a cell of origin, particularly if a characteristic cell marker such as CCSP is down-modulated upon neoplastic conversion and the cell of interest is phenotypically plastic. Pro-SPC mRNA, a marker of alveolar type 2 cells, is often expressed in lung tumors in mice. Whether this is because tumors arise from type 2 cells or because cancer cells gain this expression is unclear. The presence of both of these cell-specific markers, along stem cell markers such as Sca1 and CD34, led Kim et al (Kim et al., 2005) to propose a subset of tissue stem cells as the cells of lung tumor origin.

Figure 4A shows a fascinating example of the cellular heterogeneity in a spontaneous mouse lung tumor found in a FVB/N mouse, a strain susceptible to chemically-induced lung tumorogenesis. Most cells within the lung tumor express proSPC exclusively, whereas rare cells, including highly aggressive cells invading a bronchiole, express both SPC and CCSP. Figure 4B illustrates label-retention in uninvolved and tumor cells, indicating that a subpopulation of tumor cells is long-lived and cycles infrequently. Identification of a rare population of such “label-retaining” cells supports the concept of a cancer stem cell.

Cell Facts

- Clara cells are non-ciliated secretory cells in the small airways and trachea. Their morphology and biochemical composition display amazing heterogeneity within the airway epithelium of a single species, among different species, and in response to injury.
- Clara cells have several lung protective functions. They detoxify xenobiotics and oxidant gasses, control the extent of inflammation, participate in mucociliary clearance of environmental agents, and proliferate / differentiate to maintain the ciliated cell population.
- Clara cells are secretory and the source of Clara cell secretory protein (CCSP) and contribute surfactant apoproteins A, B, and D, proteases, antimicrobial peptides, several cytokines and chemokines, and mucins to the extracellular fluid lining the airspaces.
- In humans, many forms of lung cancer may originate from Clara cells, including adenocarcinoma, the most frequently diagnosed form of lung cancer. Whether Clara cells have a similar etiologic function in mouse models of adenocarcinoma is more controversial.

Acknowledgments

The scanning electron micrograph presented in Figure 2A was provided by Dr. David Dinsdale, University of Leicester, UK. The transmission electron micrograph shown in Figure 2B was generated in the Center for Biologic Imaging, University of Pittsburgh, Pittsburgh PA, USA and was generated in collaboration with Dr. Barry R. Stripp while at the University of Pittsburgh. The authors thank the National Institutes of Health CA33497 (AMM), CA132552 (AMM), HL075585 (SDR) and the Cystic Fibrosis Foundation for Pilot (SDR) and Research Grant (SDR) support.

Literature Cited

Evans CM, Williams OW, Tuvim MJ, Nigam R, Mixides GP, Blackburn MR, DeMayo FJ, Burns AR, Smith C, Reynolds SD, et al. Mucin is produced by clara cells in the proximal airways of antigen-challenged mice. Am J Respir Cell Mol Biol 2004;31:382–394. [PubMed: 15191915]

Giangreco A, Arwert EN, Rosewell IR, Snyder J, Watt FM, Stripp BR. Stem cells are dispensable for lung homeostasis but restore airways after injury. Proc Natl Acad Sci U S A 2009;106:9286–9291. [PubMed: 19478060]

Hoyt RF Jr. McNelly NA, McDowell EM, Sorokin SP. Neuroepithelial bodies stimulate proliferation of airway epithelium in fetal hamster lung. Am J Physiol 1991;260:L234–240. [PubMed: 2018146]

Ito T, Udaka N, Yazawa T, Okudela K, Hayashi H, Sudo T, Guillemot F, Kageyama R, Kitamura H. Basic helix-loop-helix transcription factors regulate the neuroendocrine differentiation of fetal mouse pulmonary epithelium. Development 2000;127:3913–3921. [PubMed: 10952889]

Khoor A, Gray ME, Singh G, Stahlman MT. Ontogeny of Clara cell-specific protein and its mRNA: their association with neuroepithelial bodies in human fetal lung and in bronchopulmonary dysplasia. J Histochem Cytochem 1996;44:1429–1438. [PubMed: 8985135]

Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 2005;121:823–835. [PubMed: 15960971]

Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM, Zhu Z, Elias JA, Sheppard D, Erle DJ. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. Nat Med 2002;8:885–889. [PubMed: 12091879]

Larson SD, Plopper CG, Baker G, Tarkington BK, Decile KC, Pinkerton K, Mansoor JK, Hyde DM, Schelegle ES. Proximal airway mucous cells of ovalbumin-sensitized and -challenged Brown Norway rats accumulate the neuropeptide calcitonin gene-related peptide. Am J Physiol Lung Cell Mol Physiol 2004;287:L286–295. [PubMed: 15064227]

Linnoila RI, Szabo E, DeMayo F, Witschi H, Sabourin C, Malkinson A. The role of CC10 in pulmonary carcinogenesis: from a marker to tumor suppression. Ann N Y Acad Sci 2000;923:249–267. [PubMed: 11193761]

Malkinson AM. Role of inflammation in mouse lung tumorigenesis: a review. Exp Lung Res 2005;31:57–82. [PubMed: 15765919]

McQualter JL, Brouard N, Williams B, Baird BN, Sims-Lucas S, Yuen K, Nilsson SK, Simmons PJ, Bertoncello I. Endogenous fibroblastic progenitor cells in the adult mouse lung are highly enriched in the sca-1 positive cell fraction. Stem Cells 2009;27:623–633. [PubMed: 19074419]

Rawlins EL, Okubo T, Que J, Xue Y, Clark C, Luo X, Hogan BL. Epithelial stem/progenitor cells in lung postnatal growth, maintenance, and repair. Cold Spring Harb Symp Quant Biol 2008;73:291–295. [PubMed: 19028985]

Rawlins EL, Okubo T, Xue Y, Brass DM, Auten RL, Hasegawa H, Wang F, Hogan BL. The role of Scgb1a1+ Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. Cell Stem Cell 2009;4:525–534. [PubMed: 19497281]

Reynolds SD, Giangreco A, Power JH, Stripp BR. Neuroepithelial bodies of pulmonary airways serve as a reservoir of progenitor cells capable of epithelial regeneration. Am J Pathol 2000;156:269–278. [PubMed: 10623675]

Reynolds SD, Reynolds PR, Pryhuber GS, Finder JD, Stripp BR. Secretoglobins SCGB3A1 and SCGB3A2 define secretory cell subsets in mouse and human airways. Am J Respir Crit Care Med 2002;166:1498–1509. [PubMed: 12406855]

Reynolds SD, Zemke AC, Giangreco A, Brockway BL, Teisanu RM, Drake JA, Mariani T, Di PY, Taketo MM, Stripp BR. Conditional stabilization of beta-catenin expands the pool of lung stem cells. Stem Cells 2008;26:1337–1346. [PubMed: 18356571]

Shan L, Emanuel RL, Dewald D, Torday JS, Asokanathan N, Wada K, Wada E, Sunday ME. Bombesin-like peptide receptor gene expression, regulation, and function in fetal murine lung. Am J Physiol Lung Cell Mol Physiol 2004;286:L165–173. [PubMed: 12959933]

Stripp, BR.; Reynolds, SD. Clara Cells. In: Shapiro, S.; Laurent, G., editors. Encyclopedia of Respiratory Medicine. Elsevier; St. Louis, MO: 2006. p. 471-477.

Stripp BR, Reynolds SD. Maintenance and repair of the bronchiolar epithelium. Proc Am Thorac Soc 2008;5:328–333. [PubMed: 18403328]

Teisanu RM, Lagasse E, Whitesides JF, Stripp BR. Prospective isolation of bronchiolar stem cells based upon immunophenotypic and autofluorescence characteristics. Stem Cells 2009;27:612–622. [PubMed: 19056905]

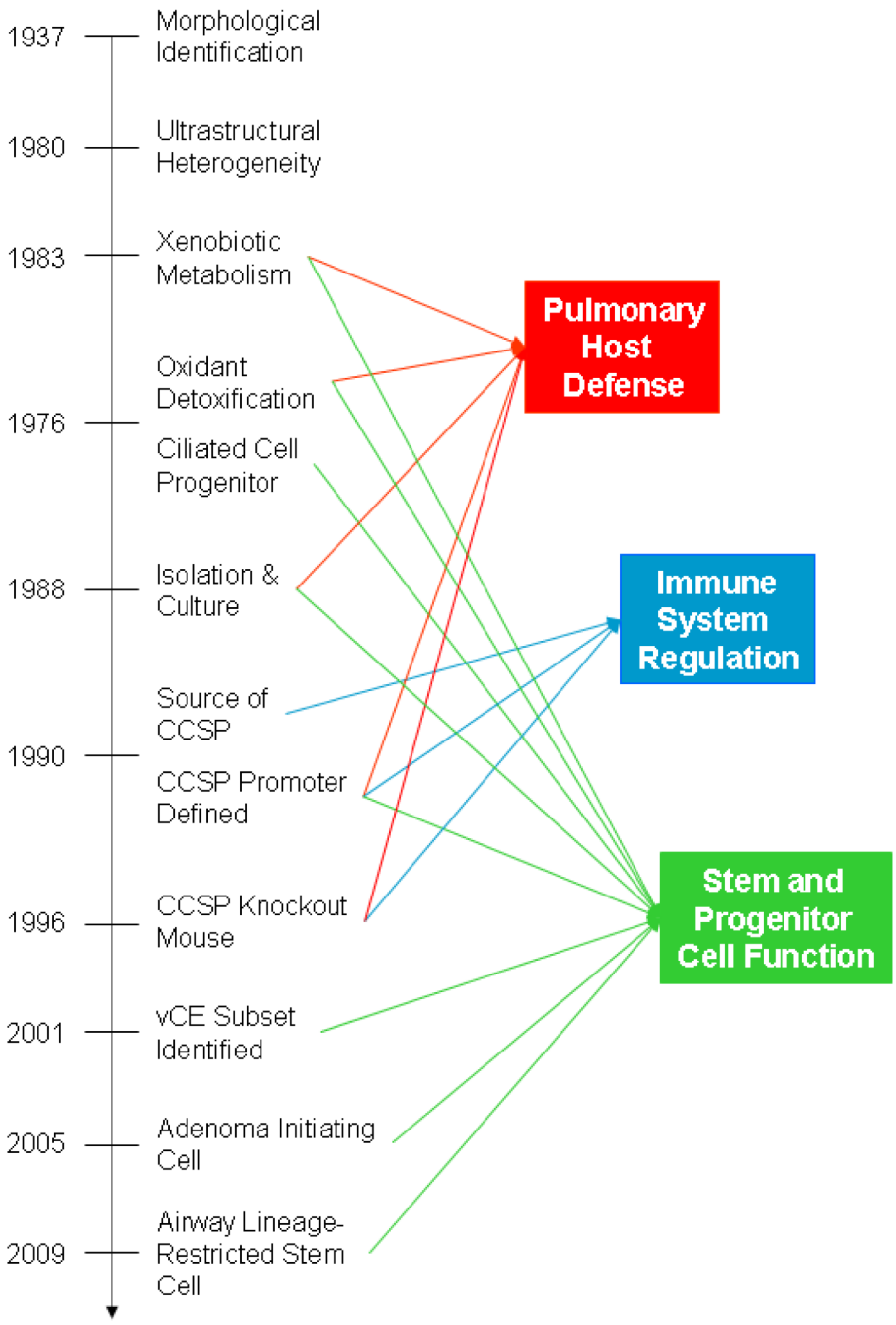

Figure 1.
Clara cells were discovered in the early twentieth century. Structural and functional analyses revealed roles in pulmonary host defense, immune system regulation, and epithelial repair.

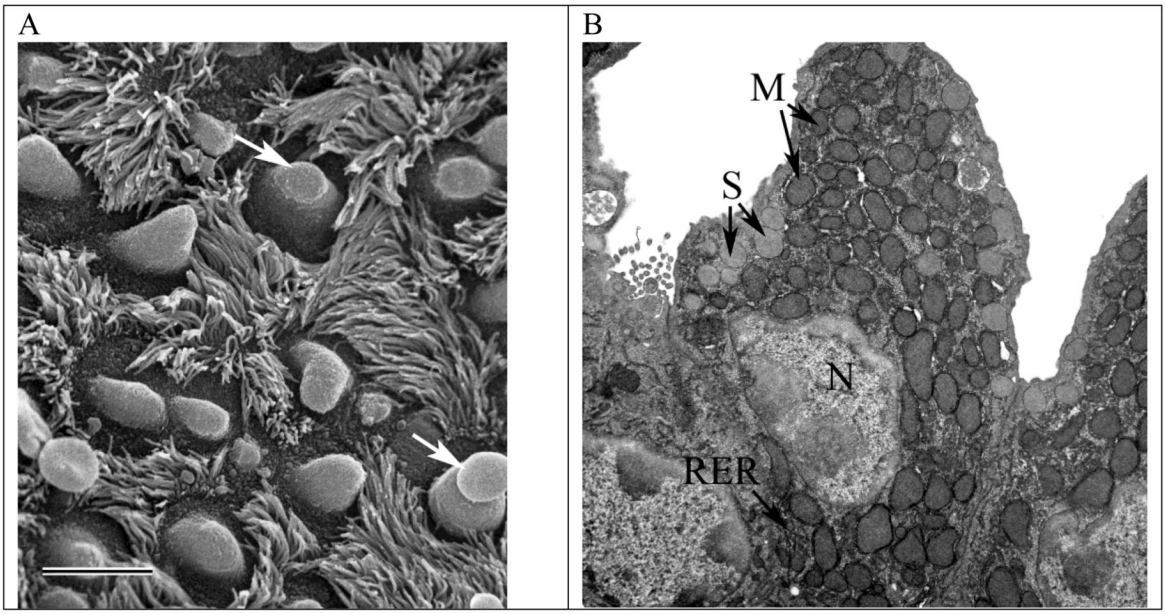

Figure 2.

A. Scanning electron micrograph of the lining of the proximal bronchiole of a rat showing Clara cells, some of which are undergoing apocrine secretion (arrows), surrounded by ciliated cells. (Bar = 10um). B. Transmission electron micrograph of a terminal bronchiolar Clara cell. Numerous mitochondria (M), secretory granules (S), rough endoplasmic reticulum (RER), and the basal nucleus (N) are indicated.

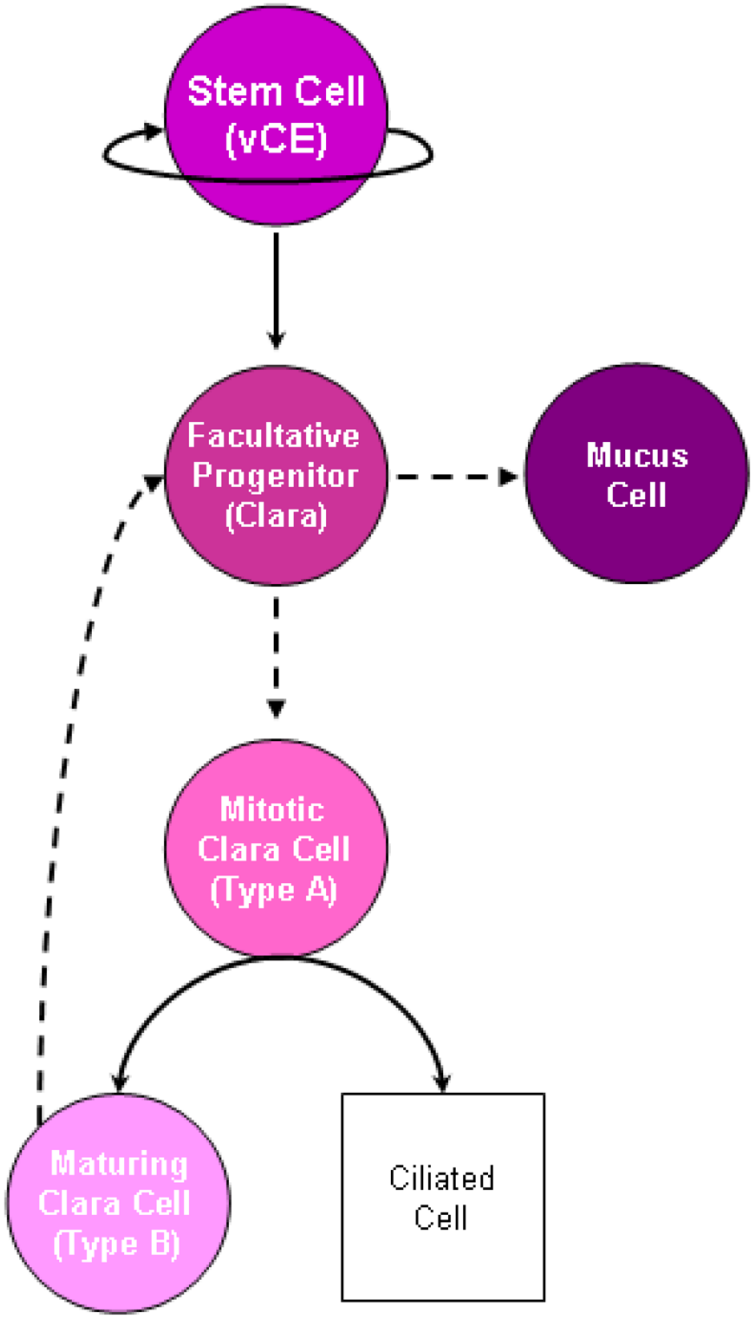

Figure 3.
Clara cell diversity: Clara cell structure and function varies as the lung milieu changes. In the normal adult lung, Clara cells are non-mitotic and perform essential homeostatic functions. Epithelial injury activates quiescent stem cells (vCE) resulting in self-renewal (elliptical line) and generation of facultative progenitor cells (Clara). Injury can also initiate dedifferentiation of Clara cells (dashed arrow) to a Type A intermediate. Type A cells divide (double-headed arrow) and generate two daughter cell types, ciliated cells and maturing (slightly differentiated) cells (Type B). Type B cells can differentiate (dashed arrow) into Clara cells. Some injuries stimulate mucus secretion by Clara cells (Mucus cell).

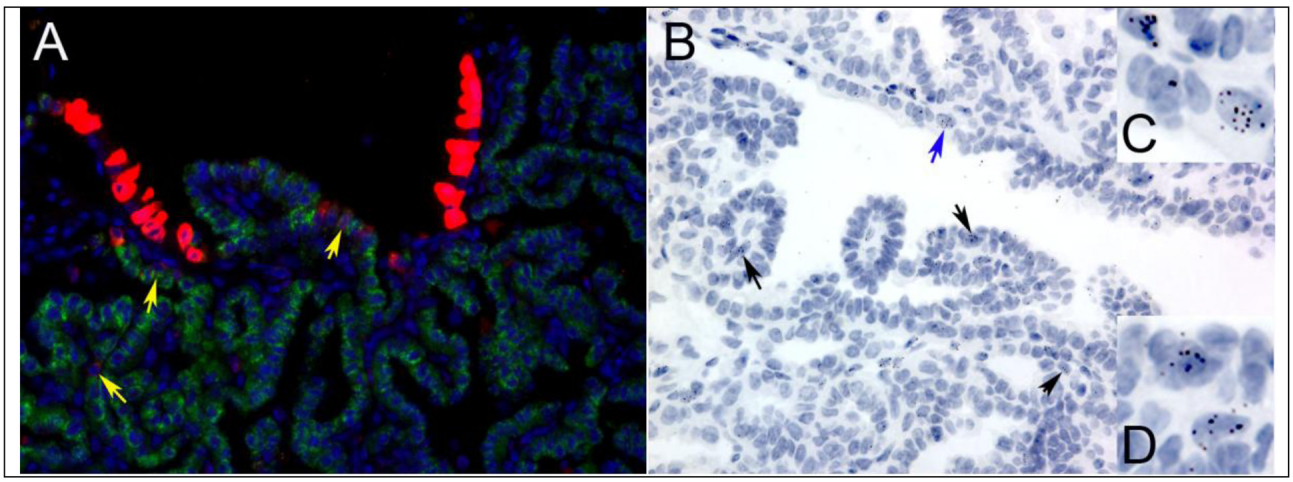

Figure 4.

Biomarker expression in a spontaneous lung adenocarcinoma. A. Triple color image in which CCSP (red), proSPC (green), and nuclei (blue) are detected by dual immunofluorescence. CCSP-proSPC dual positive cells are indicated by yellow arrows. Airway CCSP+ cells are intentionally overstained to allow detection of CCSP+ tumor cells. B. Analysis of label-retaining cells. Cells "born" 120 days prior to analysis are indicated by autoradiographic grains (black dots). Cells of similar age in unaffected airway (blue arrow) and in tumor tissue (black arrows) are shown at higher magnification in insets C and D.