Stem Cell Biology and Regenerative Medicine

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Stem Cells in the Respiratory System

Humana Press

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Preface

Lungs are one of the most complex organs; mature lung is composed of at least 40 morphologically differentiated cell lineages with distinct functions. The proximal airways contain mucous, ciliated, basal, Clara, and pulmonary neuroendocrine cells, whereas the distal airways contain mainly ciliated cells and nonciliated Clara cells. Alveolar units are almost entirely composed of distinct type I and type II alveolar epithelial cells, directly exposed to the exterior and with the entire blood passing through to be oxygenated. These particular factors make the lung a susceptible organ, a target for multiple types of internal and/or external injury. The mechanisms of lung repair are complex and, depending on the type of cell affected, the repair process might have different characteristics.

Because of their multipotentiality, stem cells are considered as a novel and important alternative cell-based therapy in lung injury. To name a cell as a stem cell, it must meet two strict criteria: extended self-renewal capacity and multilineage differentiation. Progenitor cells have some but not limitless self-renewal capacity and restricted lineage differentiation potential. The most completely characterized adult stem cell is the hematopoietic stem cell, which can differentiate into all blood cells, including lymphoid, myeloid, platelet, and red blood cell lineages.

Today, the concept of plasticity and transdifferentiation of stem cells and, in particular, adult mesenchymal stem cells has engendered significant controversy regarding their use as a therapeutic agent. The benefit of stem cell therapy has been undoubtedly observed, however apparently independently of a lasting cell engraftment and differentiation. The protective effect with bone marrow cell therapy has been explained more recently by a paracrine secretion of anti-inflammatory factors that enhances the recovery from diverse acute and chronic injuries.

Lately, there has been increasing interest in local or endogenous stem cells in the lung. There is experimental evidence that the airway epithelium likely turns over every 30–50 days. Thus, resident local cells can mediate reestablishment of the airway epithelium with normal structure and function unless an injury is too severe, extensive, or chronic. Although there may be some contribution from circulating stem/progenitor cells, most evidence supports the concept that local stem/progenitor cells are the main source of new cells with the potential to differentiate into all cell types in the normal epithelium.

Taken together, these observations suggest that the process of lung repair is a very dynamic and well-coordinated set of events. In this process, external cells, preferentially bone-marrow-derived mesenchymal stem cells, are recruited into the lung after injury to downmodulate inflammatory responses. This phase of the repair will mediate a diminution of the severity of the wound, and will create an appropriate milieu for local progenitor cells and potentially some recruited bone-marrow-derived stem/progenitor cells, to regenerate the normal lung epithelium and parenchyma and restore the lung function.

In this book, the authors discuss the potential role of different types of stem cells, in the context of physiological stress and lung injury. In Chap. 1, Susan Reynolds reviews the lung structure and function and their correlation with endogenous lung stem cells. Daniel Weiss reviews in Chap. 2 the different sources of adult mesenchymal stem cells, as well as the controversial issue of cell differentiation into alveolar epithelial cells and the implications for future cell therapies in the lung. Recruitment of nonhematopoietic cells into the injured lung has not been well documented. In Chap. 3, Ellen Burnham explores the implications of mobilization and recruitment of progenitor cells, endothelial cells, and epithelial cells. In Chap. 4, Robert Strieter explains the role of another type of bone-marrow-derived progenitor cell, the fibrocytes. These cells have been implicated in pulmonary fibrosis, but as discussed by Strieter, these cells have unique properties that make them an indispensable element in the process of lung repair. An additional important factor that can determine the magnitude of cell recruitment and can have implications on the fate of the recruited cells is the type of extracellular matrix to which stem cells are exposed. In Chap. 5, Jesse Roman presents an extended review of the different proteins that form the extracellular matrix and how each of them can induce the differentiation of stem cells into fibroblasts and myofibroblasts. A novel concept for the mobilization of stem/progenitor cells is the effect of physical activity. In Chap. 6, Partick Wahl describes in detail the effect that exercise can have on the recruitment and homing of these cells into the different organs. Finally, we dedicate two chapters to discuss some clinical applications of mesenchymal stem cells. First, in Chap. 7, Micheal Matthay discusses the role of stem cells in acute lung injury and repair, and, finally, in Chap. 8, we present a complete review of the use of mesenchymal stem cells in animal models of lung diseases. These studies support the translation of mesenchymal-stem-cell-based therapy for acute lung injury, pulmonary hypertension, cystic fibrosis, and lung transplant.

The objective of this book is to review the most relevant and recent concepts for the use of local, endogenous, or exogenous progenitor/stem cells in the prevention and repair of the lung after injury. This is a very dynamic field, currently in constant evolution. The authors presenting their work here are indisputable leaders in their field, making this book an exciting collection of reviews by an outstanding group of investigators.

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Chapter 1
Stem and Progenitor Cells of the Airway Epithelium

Susan D. Reynolds, Moumita Ghosh, Heather M. Brechbuhl, Shama Ahmad, and Carl W. White

1 Introduction

1.1 Tissue-Specific Stem Cells

A tissue-specific stem cell is defined as a cell that self-renews and has a differentiation potential equivalent to the cellular diversity of its resident tissue [1]; thus, proliferation and differentiation are the two parameters that are most commonly used to identify a tissue-specific stem cell. Still, these are relative terms rather than hard and fast definitions. For instance, a tissue-specific stem cell has a greater mitotic potential than other progenitor cells. It is thought that the stem cell spreads its allotted number of cell divisions over a long period, potentially the lifespan of the animal. In a similar vein, the tissue-specific stem cell has a greater differentiation potential than other progenitor cells. In a diverse tissue such as the hematopoietic system, differences in differentiation potential are easily discerned. However, in a simple tissue such as the airway epithelium, a single differentiated cell type may exist. Thus, the differentiation potential of the tissue-specific stem cell could be equivalent to that of a simple progenitor cell. The nuanced definition of potential, be it proliferation or differentiation, makes definitive identification of tissue-specific stem cells a difficult goal.

1.2 Lung-Epithelial-Tissue-Specific Stem Cells

Lung epithelial cells that fit the definition of a tissue-specific stem cell have been identified by their resistance to various chemical injuries and by their sequestration

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in specialized microenvironments [2]. However, the standard definition cannot distinguish between a lung-tissue-specific stem cell and another progenitor cell type, the facultative progenitor (see below). Difficulty distinguishing lung facultative progenitor cells from lung-tissue-specific stem cells is a consequence of cell lifespan, which is long, and cellular diversity, which is low.

1.3 Facultative Progenitor Cell

Facultative progenitor cells fulfill essential cellular and biochemical functions in their quiescent state; however, facultative progenitor cells retain the ability to alter cellular structure and mitotic status in response to cellular damage. These injury-induced changes can be quite dramatic and result in loss of differentiation markers, biochemical functions, and a 10–20-fold increase in mitotic activity. Proliferation of facultative progenitor cells and regionally appropriate differentiation of their daughter cells results in maintenance of the facultative progenitor cell pool (self-renewal) and restoration of terminally differentiated cells. The abundance and broad distribution of facultative progenitor cells make them a critical component of epithelial defense against environmental challenge.

1.4 Lung Facultative Progenitor Cells

The major differences between lung facultative progenitor cells and the tissue-specific stem cells is differentiation status and abundance. Lung facultative progenitor cells are responsible for secretion, absorption, metabolism, immunomodulation, mucocillary clearance, and barrier maintenance. These cells fall into two major categories, basal cells and secretory cells. Secretory cells are further subdivided into several subclasses: Clara-like, Clara, alveolar type 2 cells. The various facultative progenitor cell types inhabit specific compartments of the normal lung airway: the tracheobronchial (basal and Clara-like cells), bronchiolar (Clara cells), and alveolar (alveolar type 2 cells) epithelia. Clara-like and Clara cells are the progenitors for terminally differentiated cells, ciliated cells. Alveolar type 2 cells are the progenitor for terminally differentiated alveolar type 1 cells. The presence of this vast reparative reservoir distinguishes the lung epithelium from tissues such as the intestine which are maintained exclusively through proliferation and differentiation of the tissue-specific stem cell.

1.5 Questions Relevant to Lung Stem Cells

The structural and functional diversity of the lung epithelium begs several questions regarding tissue-specific stem cells and their attributes. First, should the definition of a stem cell, as presented above, be refined to reflect specifics of the lung epithelium? Second, given the nuances of the lung epithelium, what attributes should a

lung stem cell exhibit? Third, given the species-specific differences in airway structure, do findings in mice relate directly to the human lung and visa versa? These questions shape the existing lung stem and progenitor cell literature as well as ongoing research. By keeping these questions in mind, the reader will be able to critically evaluate the data presented below.

2 Conducting Airway Structure and Function

2.1 Functional Domains

The conducting airway is a set of tubular structures that decrease in caliber from proximal to distal. For the purposes of this chapter, only the region extending from the trachea (proximal) through the terminal bronchiole (distal) will be discussed. Between these extremes are the bronchial, and bronchiolar regions.

2.2 Tracheobronchial Domain

The proximal portion of the conducting airway epithelium is termed the "tracheobronchial epithelium." This region contains two functionally distinct epithelia, the submucosal glands and the surface epithelium. The submucosal glands consist of acini that are linked by ducts to the lumen of the trachea (mouse) and to the trachea, bronchi, and bronchioles of the human airway [3]. The submucosal glands are further specialized into mucus and serous domains that secrete biochemically distinct proteins [4].

The tracheobronchial surface epithelium is pseudostratified, with each cell being in contact with the basement membrane. Basal cells are typically located adjacent to the basement membrane and contact it through hemidesmosomes. Basal cells have limited exposure to the lumen under normal conditions. Secretory and ciliated cells are the other major cell types in this region. They are linked to each other and to basal cells through desmosomes. Gap junctions serve as portals for movement of small molecules between secretory and ciliated cells. The human trachea, bronchi, and the first six generations of the bronchiolar epithelium are supported by cartilage. The synonymous region of the mouse airway is the trachea and bronchi.

2.3 Bronchiolar and Terminal Bronchiolar Domain

The distal portion of the conducting airway is termed the "bronchiolar epithelium." This region is a simple columnar or cuboidal epithelium. Secretory and ciliated cells are the main cellular constituents. Minor cell types include pulmonary neuroendocrine cells (PNECs). In the human bronchiole, basal cells are a rare cell type.

However, basal cells are not detected in the lower airways of mice. As a consequence of these anatomical distinctions, the mouse bronchiolar epithelium is most similar to the terminal bronchiolar epithelium of the human airway.

2.4 Origin of Airway Domains

Embryology studies in mice established that all airway epithelial cells, with the possible exception of neuroepithelial cells, are derived from the foregut endoderm [5]. In mice, airway and alveolar progenitors are specified very early in development, between embryonic days 2 and 4. Interestingly, this specification occurs prior to identification of the lung anlagen [6]. The trachea and esophagus begin to separate on embryonic day 9.5 [7, 8]. The process of airway tube formation is termed "branching morphogenesis." It is completed by the pseudoglandular stage of lung development [9].

Continuous labeling studies in hamsters supported the conclusion that neuroepithelial bodies (NEBs) serve as mitotic centers that promote airway segment lengthening [10]. NEBs are structurally similar to carotid bodies and are composed of PNECs. Neural peptides secreted by PNECs are epithelial mitogens [11]. These analyses indicated a central role for NEBs in airway segmentation and the establishment of unique secretory cell pools [12]. 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 [13]. Additional studies are needed to determine the functional significance of this secretory cell–NEB association.

Submucosal glands are formed in the postnatal period. These structures were not tagged in mice using a surfactant protein C promoter regulated system even when recombination was induced from embryonic day 0.5 through postnatal day 7 [6]. These data may indicate that mouse submucosal glands are derived from a different set of progenitor cells than those that form the surface epithelium. However, a cautious interpretation of these data is warranted considering that lineage tracing studies strongly support a lineage relationship between bronchial and glandular lineages in the mouse and human [3, 14, 15].

2.5 Birth Date of Airway Epithelial Cells

Lineage tracing analysis demonstrated that cells "born" during lung development persist into adulthood [16]; thus, two populations of epithelial cells may exist in the adult airway, those "born" during lung development and those resulting from proliferation and differentiation in air-breathing postnatal animals. Functional maturation of epithelial cells, particularly airway secretory cells, may be modulated by Wnt signaling during prenatal lung development [17]. The functional significance of "embryological" and adult cells and their impact on lung injury, repair, and susceptibility to chronic lung disease are under investigation.

3 Conducting Airway Progenitor Cell Types

3.1 Tracheobronchial Epithelium

Cellular mechanisms regulating replacement of terminally differentiated ciliated cells were the focus of early injury-repair studies. These studies demonstrated that the tracheobronchial epithelium is populated by two progenitor cell pools, the aforementioned basal cell and a specialized secretory cell, the Clara-like cell [12]. Histological and pulse–chase analysis of tracheobronchial repair after NO_2 or ozone exposure identified the Clara-like cell as the progenitor for ciliated cells [18].

3.2 Basal Cells

The basal cell was identified as a supportive cell type that anchored the epithelium to the basement membrane [19]. Consequently, the basal cell has been referred to as a "reserve" cell in the literature [19]. However, recent studies indicate that basal cells proliferate actively in the steady-state mouse trachea and bronchi. These cells increase their mitotic rate dramatically in response to Clara cell depletion [20].

3.2.1 Basal Cells – Surface Epithelium

Basal cells are distinguished from other epithelial cell types by their pyramidal shape and by their distinct keratin expression profile. In the steady state, basal cells express primarily keratins 5 and 14. Basal cells are distributed throughout the human airway. They are abundant in the trachea and the first six generations of the respiratory track. This region is pseudostratified and is supported by cartilage. Basal cells are also found in the bronchiolar epithelium of the human lung. In this region, the epithelium is columnar and basal cells are rare. In rodent lungs, basal cells are located primarily in the trachea and bronchi. Rare basal cells, one cell per high-powered field, are found in the mouse bronchial epithelium. As a consequence of these species-specific differences in airway structure, care must be taken to ensure that similar regions are compared.

3.2.2 Basal Cells – Submucosal Glands

Basal cells are also located in the glandular epithelium. Here, basal cells are found along the basement membrane of the gland ducts and the acini. These cells are thought to be contractile and as a consequence are referred to as "myoepithelial cells." In humans and mice, these cells express keratins 5 and 14. The mitotic index in the glandular epithelium is very low in the adult and may reflect the fact that this region is relatively protected from the environmental exposures that drive proliferation in the surface epithelium.

3.2.3 Basal Cells – Plural Membrane

The plural membrane is a final location of lung basal cells. This region is characterized by low cell density and basal cells are a rare cell type. Consequently, little is known of basal cell function within the visceral lining of the lung. These cells have been lineage-traced using the Wilm's tumor 1 gene (Wt1) promoter and several recombination substrates [21]. Interestingly, these cells served as progenitors for mesenchymal cells within the vascular walls. These studies raise the possibility that markers associated with basal cells of the epithelium are utilized, potentially in a different functional role, in cells of the plural membrane. These cells have not been characterized in the context of lung injury and repair.

3.3 Secretory Cells

Airway secretory cells are a specialized cell type that were first defined morphologically as a nonciliated cell. Subsequent ultrastructural analysis identified abundant rough endoplasmic reticulum and secretory granules as unique subcellular organelles [22]. Such cells secrete proteins into the luminal space, however, they are also a source of antioxidant compounds. Genetic alterations to airway secretory cells are associated with direct changes in cell function [23] as well as alterations to adjacent ciliated cells and to more distant inflammatory cells such as the alveoalar macrophage [24, 25].

3.3.1 Clara-Like and Clara Cells

Clara cells are defined structurally as nonciliated cells (reviewed in [26, 12]). The Clara cell is a multifunctional cell type that has been studied for nearly a century. These cells were originally described as cuboidal, nonciliated cells in human and rabbit terminal bronchioles. They contain a basally situated nucleus, an apical dome that extends variable distances into the airway lumen, and discrete, oval densely staining granules. These cells constitute approximately 50% of cells in the bronchial and bronchiolar epithelium and 70% of cells in the terminal bronchiolar epithelium. Their shape varies from columnar to cuboidal along the proximal to distal axis.

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 [22]. Multispecies comparisons demonstrated that Clara cell structure varies among species and along the proximal to distal axis of the airway epithelium. Despite this heterogeneity, studies employing oxidant gas exposure and pulse–chase strategies indicated that most if not all rabbit [27] or rat [28, 29] Clara-like cells have the ability to proliferate in response to injury. The ultrastructural differences between proximal and distal airway secretory cells led to the designation of upper airway secretory cells as Clara-like cells [12].

3.3.2 Secretory Cell Molecular Markers

All mouse airway secretory cells, from the trachea to the terminal bronchioles, express a low molecular weight protein, Clara cell secretory protein (CCSP). Thus, CCSP expression in the mouse is synonymous with the Clara-like and Clara cell types. However, human proximal airway secretory cells are more readily recognized by expression of mucins such as Muc5Ac. In adult human airways, expression of CCSP is restricted to the terminal bronchioles. These "differences" in expression of CCSP in the adult human and the mouse have led to the conclusion that human airways do not have a constitutive population of non-mucus-secreting secretory cells. However, studies in mice suggest that mucus cells are derived from CCSP-expressing cells through a metaplastic transition [30, 31]. These mucus cells may be postmitotic, although there is controversy regarding this point. These studies suggest that the human airway does have secretory cells that are functionally similar, if not molecularly identical, to the CCSP-positive mouse Clara-like and Clara cells. However, the lineage relationship has not been evaluated in the human.

3.3.3 Secretory Cells – Cellular Specialization

Biochemical specialization of the airway is recognized by the establishment of molecularly distinct airway secretory cell types. These specialized cells are established during the middle stage of lung development, between embryonic days 12 and 14, in the mouse. Subdivision of the human conducting airway epithelium begins during the second trimester in human lung, and the earliest secretory cells are positioned within the luminal aspect of NEBs [32]. These spatially-restricted secretory cells are CCSP-positive. In the early postnatal period, secretory cell specialization can be identified by regionally specific expression of secretory protein messenger RNAs [33].

3.3.4 Secretory Cells – Submucosal Gland

Secretory cells are also located in the submucosal glands. As indicated above, the glands form in the postnatal period, suggesting a distinct molecular plan for this region. Glandular secretory cells of the adult human and mouse do not express CCSP. Rather they express a distinct repertoire of host defense proteins [34]. Molecular analysis of mechanisms regulating submucosal gland development revealed a complex role for the Wnt–β-catenin signaling pathway in bud formation and elongation [35, 36]. These studies demonstrated a clear role for β-catenin-dependent gene expression. However, the DNA binding cofactors for β-catenin, Lef1 and TCF4, were differentially regulated as a function of gland development. Gene deletion studies suggest a compensatory role for TCF family members in implementation of Wnt ligand signaling. These cofactors may be part of a positive–negative regulatory circuit that is regulated by the Wnt ligand, Wnt 3a [14].

3.4 Bronchiolar Epithelium

The bronchiolar epithelium contains two progenitor cell pools, Clara cells and PNECs.

3.4.1 Clara Cells

Clara cells are the most prevalent progenitor cells within the distal airways. These cells respond to ciliated cell injury by alterations in their differentiated functions and proliferation [29]. These changes are described further in Sect. 4. As indicated for Clara-like cells, Clara cells of the mouse are most readily recognized by expression of CCSP. These cells also express other secreted proteins, including SCGB3A2 [33] and enzymes involved in phase I and II metabolism [37].

A unique characteristic of mouse Clara cells has been exploited to evaluate the stem cell hypothesis. In this species, Clara cells express the monooxygenase cytochrome P450 2F2 [38]. This enzyme metabolized the xenobiotic agent naphthalene to a cytotoxic epoxide. Under conditions where the epoxide cannot be detoxified, Clara cells die via necrosis. This cellular toxicity initiates within 6 h of parenteral exposure and dead and dying cells slough between 24 and 48 h [39]. Similar methods cannot be used to evaluate stem cells in the human or cultures of human cells, as this species does not express cytochrome P450 2F2 in the secretory cell population. However, alternative agents that exploit the unique phase I and II metabolism of human secretory cells may exist and could be used to test the stem cell hypothesis in vitro.

3.4.2 Pulmonary Neuroendocrine Cells

PNECs are found as isolated cells or in clusters termed “neuroepithelial bodies” (NEBs). Human and mouse PNECs are recognized by dense core granules on transmission electron micrographs or by expression of neural peptides, such as calcitonin gene related peptide and chromogranin A, on paraffin sections. PNECs proliferate in response to various forms of epithelial injury in humans and mice. This results in an increase in the number of NEBs (hypertrophy) and in an increased number of cells per NEB (hyperplasia) [40]. Proliferation of PNECs is limited to one or two cycles as indicated by retention of ^{3}H-thymine deoxyribose by PNECs after naphthalene injury [41, 42].

Dual immunofluorescence analysis suggested a lineage relationship between PNECs and Clara cells [42]; however, formal lineage tracing has not been used to critically test this point in the adult mouse. Chimera studies and lineage tracing in utero suggest that PNECs are a distinct lineage [43]. Several studies identified the NEB as a potential stem cell microenvironment [2]. Interestingly, NEB structure changes with injury [44]. Alterations in cellular and cell–basement membrane interactions were observed but functional consequences were not investigated. Owing to the paucity of data regarding progenitor cell activity of PNECs, these cells will not be discussed further.

4 Facultative Progenitor Cell Pools

4.1 Basal Cells

Steady-state basal cells exhibit two molecular phenotypes, keratin 5+/14– and keratin 5+/14+. These two subsets were 80 and 20% of the steady-state basal cell population, respectively. Although the steady-state mitotic index of the trachea is low, about 10% using Ki67 as a mitotic marker, the basal cell subsets comprised approximately half of all mitotic cells in the mouse trachea (Cole et al. in press). Lineage tracing indicated that these steady-state basal cells were responsible for maintenance of the basal cell population. Contribution to the secretory and ciliated cell pools was not detected over a 40-day window, suggesting that the basal and secretory/ciliated lineages were distinct (Ghosh, M & Reynolds, S.D. unpublished).

4.1.1 Steady-State and Reparative Basal Cells

Basal cell phenotype and function varied in the context of secretory cell injury. Naphthalene-mediated depletion of the tracheal Clara-like cell pool initiated an epithelial repair process that was driven by the abundant and broadly distributed basal cell population. These progenitor cells were uniformly keratin 14 positive and were derived from the keratin 5+/14– and keratin 5+/14+ basal cell pools (Cole, in press). Increased keratin 14 gene expression was responsible for this altered molecular phenotype. The keratin 14+ basal cell population represented at least 80% of mitotic cells on recovery days 3 and 6. These cells were highly proliferative, with approximately 40% of cells cycling at a given time point. The unbiased distribution of keratin 14+ reparative cells along the proximal–distal axis and parallel restitution of the secretory cell population indicated that epithelial repair was mediated primarily by a broadly distributed population of basal cell progenitors rather than through activation of a proximally restricted tissue-specific stem cell.

4.2 Clara-Like and Clara Cells

Clara-like and Clara cells respond to ciliated cell depletion through dedifferentiation and proliferation. Pulse–chase studies in combination with ultrastructural analysis demonstrated that the initial event was a morphological change [28, 29, 18] (Fig. 1.1). Loss of secretory granules and endoplasmic reticulum resulted in the generation of a transient cell type, the type A cell. This cell entered the cell cycle. One of the two daughter cells redifferentiated through a type B intermediate to restore the Clara cell population. The other daughter differentiated into a nascent ciliated cell. Clara cells located throughout the bronchiolar epithelium had the capacity to undergo these morphological alterations and to proliferate. Mechanisms regulating differentiation of daughter cells have not been delineated. However, the fact that

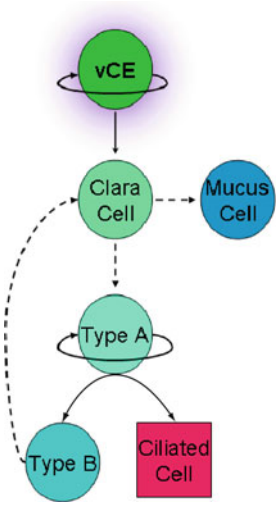

Fig. 1.1 The bronchiolar stem cell hierarchy. See the text for details

cellular representation varies in the bronchial and bronchiolar epithelium suggests that regionally specific signals regulate cell fate decisions.

4.2.1 Phenotypic Plasticity Is a Hallmark of Clara-Like and Clara Cells

Individual Clara cells 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 by-products, and chemotherapeutic agents. In response to injury, reparative Clara cells express surfactant protein B, potentially to maintain patency of the small airways during repair [45]. Analysis of the response of mouse Clara cells to allergic inflammation or the Th2 cytokine, interleukin-13 [46], suggest a lineage relationship between Clara cells and mucus cells [30, 31]. Pulse-labeling studies showed that mucus metaplasia of Clara cells generates a terminally differentiated cell that can no longer enter the cell cycle. Thus, 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.

5 Evidence in Support of Lung Stem Cells

5.1 Classic Stem Cell Methods

A clear understanding of the assays used to identify a tissue-specific stem cell is critical to the interpretation of the studies that attempt to identify lung stem cells. Label retention has been used as a functional measure of stem-cell-like behavior