Maintenance and Repair of the Bronchiolar Epithelium

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Bronchioles of the distal conducting airway are lined by a simple epithelium composed primarily of nonciliated secretory (Clara) cells and ciliated cells. These cells are long-lived in the normal lung; renewal is mediated by cells that constitute a nonclassical stem cell hierarchy. Within this type of hierarchy, facultative progenitor cells are responsible for normal epithelial maintenance and rare adult tissue-specific stem cells are activated only in response to depletion of the facultative progenitor cell pool. This organizational structure is a departure from the classical stem cell hierarchies that maintain rapidly renewing tissues such as the epithelium of the small intestine. This article compares cellular and molecular mechanisms of epithelial renewal in the relatively quiescent bronchiolar epithelium and in the mitotically active intestinal epithelium. Fundamental distinctions between stem cell hierarchies of slowly and rapidly renewing epithelia are highlighted and may provide insight into tissue-specific interpretation of signals that mediate repair in some tissues but lead to remodeling and chronic disease in other organ systems.

Keywords: stem cell; progenitor; bronchiole; repair

Mechanisms regulating tissue maintenance in the steady state and repair after injury vary considerably between organs. In this context, the rate of cell replacement in the normal condition is a distinctive functional property that allows classification of epithelia into two broad subsets: rapidly renewing and slowly renewing. Clear distinctions in both the cellular organization and molecular regulation of tissue replacement can be made between these classes of tissues. The epithelium lining the gut turns over rapidly. Within the small intestine, specialized cell types lining villi are postmitotic and these differentiated cell types are replaced every 3 to 5 days (1). Constant renewal of villus epithelial cells requires frequent stem cell proliferation for the generation of large numbers of transit-amplifying cells (2). The transit-amplifying cell, like the tissue stem cell, proliferates frequently but eventually commits to the differentiation pathway. Rapid cellular renewal serves to maintain epithelial function and barrier properties in the harsh environment of the intestinal lumen. The epidermis and esophagus are other examples of rapidly renewing tissues whose rate of turnover serves to maintain an intact barrier against the external environment (3). However, the rate of differentiated cell turnover is considerably slower in many other tissues, yet can increase dramatically after injury. These include internal organs such as the pancreas, liver, and thymus, which have no direct exposure

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GLOSSARY

Progenitor cell: A collective term used to describe any cell that has the capacity to proliferate. The term progenitor cell is commonly used to indicate a cell that is in the process of cell division or has the potential to enter the cell cycle. Terminology that takes into account the functional distinctions among progenitor cells is suggested below.

Adult tissue stem cell: A relatively undifferentiated cell that has the capacity for unlimited self-renewal through stable maintenance within a stem cell niche. Adult tissue stem cells have a differentiation potential equivalent to the cellular diversity of the tissue in which they reside. The hematopoietic stem cell is a prototypical adult tissue stem cell.

Transit-amplifying cell: The progeny of a tissue stem cell that retain relatively undifferentiated character and have a finite capacity for proliferation. The sole function of transit-amplifying cells is generation of sufficient specialized progeny for tissue maintenance.

Obligate progenitor: A cell that loses its ability to proliferate once it commits to a differentiation pathway. Intestinal transit-amplifying cells are obligate progenitor cells.

Facultative progenitor: A cell that exhibits differentiated features when in the quiescent state yet has the capacity to proliferate for normal tissue maintenance and in response to injury. Bronchiolar Clara cells are an example of this cell type.

Classical stem cell hierarchy: A stem cell hierarchy in which the adult tissue stem cell actively participates in normal tissue maintenance and gives rise to a transit-amplifying cell. Within this type of hierarchy, renewal potential resides in cells at the top of the hierarchy (i.e., the stem and transit-amplifying cell).

Nonclassical stem cell hierarchy: A stem cell hierarchy in which the adult tissue stem cell does not typically participate in normal tissue maintenance but can be activated to participate in repair after progenitor cell depletion.

Rapidly renewing tissue: Tissues in which homeostasis is dependent on maintenance of an active mitotic compartment. Specialized cell types are located in a spatially distinct compartment and have a short half-life. Rapid turnover of differentiated cell types requires continuous proliferation of stem and/or transit-amplifying cells. The prototypical rapidly renewing tissue is the intestinal epithelium.

Slowly renewing tissue: Tissues in which the steady-state mitotic index is very low. Specialized cell types are broadly distributed and long-lived, and a subset of these cells, the facultative progenitor cell, retains the ability to enter the cell cycle. The relative stability of the differentiated cell pool is paralleled by infrequent proliferation of stem and/or transit-amplifying cells. The lung is an example of a slowly renewing tissue.

to the external environment, as well as the lung, which has elaborate defense mechanisms to avoid injury by inhaled environmental agents.

Here we discuss mechanisms of epithelial maintenance within the slowly renewing bronchiole and relate this to what is known in the rapidly renewing epithelium of the small intestine. We suggest that the multifunctional role of Clara cells in lung homeostasis defines this cell population as a facultative progenitor cell. Clara cells perform specialized functions necessary for host defense in the normal state but retain the ability to proliferate in response to injury. Facultative progenitor cells are also found in other slowly renewing tissues, such as the liver (hepatocyte), pancreas (β cell), and thymus (cortical and medullary epithelial cells). These cells may represent a previously unrecognized tier of stem cell hierarchies specific to slowly regenerating epithelia. The broad distribution of the facultative progenitor cells within these tissues suggests that the organization of such nonclassical stem cell hierarchies is distinct from that of rapidly renewing tissues and that novel signaling mechanisms regulate proliferation, differentiation, and migration within these tissues. This discussion was presented at the Aspen Lung Conference on Lung Injury and Repair in June 2007.

EPITHELIAL RENEWAL IN THE NORMAL AND REPAIRING BRONCHIOLE

Epithelial maintenance as well as renewal after injury to ciliated cells is associated with proliferation of endogenous nonciliated epithelial cells. The steady-state proliferative index of the airway epithelium is low, with approximately 1% of bronchiolar epithelial cells entering the S-phase of the cell cycle within a 24-hour period (4). This rate increases by greater than 10-fold after injury. Cells that incorporate [^{3}H]-thymidine in the steady-state epithelium or after NO_2-induced depletion of ciliated cells were shown to be a subpopulation of bronchiolar nonciliated cells that lacked the typical features of differentiated cells, such as secretory granules and smooth endoplasmic reticulum (5). This cell type, termed the "type A cell," was shown by pulse-chase methods to be a derivative of the Clara cell (6). These studies demonstrated that Clara cells of the normal adult bronchiolar epithelium exhibit differentiated character, yet retained the capacity to proliferate in response to normal cell attrition or injury.

We suggest that the abundant Clara cells of mammalian bronchiolar airways constitute a large pool of broadly distributed facultative progenitor cells that are capable of effecting repair of a normally quiescent epithelium. After restoration of normal physiologic conditions, the mitotic type A cell returns to the quiescent Clara cell state. As such, the reparative capacity of bronchiolar airways is conditional rather than obligate. Thus, in stark contrast with the intestine, which is reliant on a rare stem cell and spatially restricted stem/transit-amplifying cell compartment, maintenance and renewal of the bronchiolar epithelium is dependent on transient activation of a facultative progenitor to its proliferative form. Within this tissue, adult tissue stem cells are activated only in response to depletion of the facultative progenitor cell pool. Thus, reparative mechanisms are highly specialized in slowly and rapidly renewing epithelia and these adaptations are likely to be a reflection of anatomic, physiologic, and functional constraints of the organs in which they reside.

SPATIAL ORGANIZATION OF FUNCTIONALLY DISTINCT CELL TYPES WITHIN SLOWLY AND RAPIDLY RENEWING EPITHELIA

Utilization of an abundant, broadly distributed facultative progenitor cell for bronchiolar homeostasis and repair meets the functional constraints of this organ. The slow replacement kinetics observed for the conducting airway epithelium results from the effective integration of functions contributing to host defense with those contributing to maintenance of reparative capacity. In contrast, the harsh environment within the intestinal lumen requires rapid replacement of the intestinal epithelium for maintenance of function. The requirement for rapid cell replacement is met through continuous proliferation of tissue stem cells and their descendents, transit-amplifying cells. These highly mitotic cell types lack differentiated characteristics seen among epithleial cells lining the villus and are sequestered within protective microenvironments termed the "crypts of Leiberkuhn." This anatomic organization results in protection of the proliferative compartment responsible for maintenance of the epithelium without compromising absorptive and protective functions of epithelium in direct contact with the intestinal lumen. Thus, architectural adaptations that suit the distinct functional characteristics of the bronchiole and intestine are tightly coupled with the unique characteristics of progenitor cell types within these organs.

Because bronchiolar facultative progenitor cells are abundant and broadly distributed, they are uniquely positioned to effectively coordinate critical aspects of the epithelial response to environmental exposures. In the steady state, these cells secrete low-molecular-weight proteins and chemical mediators important in host defense. Apically secreted proteins function in immunoregulation (7–9), in protection from environmental agents (10, 11), and potentially in coordination of mechanical activities such as mucociliary clearance (12). Facultative progenitor cells of the airway are also a source of mucus proteins important for bacterial clearance (13, 14). As the preferred progenitor cell within airways and in the alveolar epithelium (6, 15), the facultative progenitor cell population represents a vast repository of cells that can respond on a cell-by-cell basis to environmental exposures. Consequently, dual functionality of the bronchiolar facultative progenitor allows coordination of the response to acute stress or overt injury. Importantly, the response to environmental insult can be graded, allowing appropriate and site-specific regulation of magnitude and duration, and synchronization of the acute response with reparative mechanisms.

Properties of progenitor cells in slowly renewing organs vary between normal and injured conditions. In the steady state, facultative bronchiolar progenitor cells fulfill functions analogous to postmitotic secretory and absorptive cells of the villus epithelium in the steady state. Airway injury results in a transient change in the facultative progenitor, leading to loss of "differentiated" ultrastructural character and assumption of a less differentiated phenotype that is analogous to that of the obligate progenitor of the intestinal crypt. This "undifferentiated" state is maintained for a period of days after acute airway injury and is followed by differentiation of the nascent epithelium to reestablish mucociliary functions typical of the quiescent state (6). Thus, the abundance and broad distribution of the facultative progenitor endow the bronchiolar epithelium with a high degree of functional flexibility and the capacity to respond rapidly to depletion of long-lived cell types.

Unlike the slow replacement kinetics of the normal bronchiolar epithelium, turnover of the intestinal epithelium is a continuous and rapid process. This disparity in the normal replacement kinetics of the lung and intestinal epithelium is associated with fundamental differences in the distribution of available progenitor cells (16). The intestinal epithelium is partitioned into mitotic and differentiation compartments. The mitotic zone, the crypt of Lieberkuhn, is largely protected from the luminal contents. Within the small intestine, this epithelium

is largely composed of undifferentiated cells that proliferate continuously and a less abundant population of differentiated Paneth cells residing at the base of the crypt. The progeny of mitotic crypt cells replace the transient population of postmitotic differentiated cells. The latter cell types are limited to fingerlike villi that project into the lumen of the small intestine or line a flat tubular lumen within the colon. Partitioning of the epithelium in such a manner provides the necessary differentiated cell types for absorptive and secretory functions that are critical at the villus–lumen interface, yet maintains a highly proliferative population of undifferentiated progenitor cells that are "protected" from the harsh environment of the intestinal lumen.

We suggest that the rate of epithelial renewal in the normal state dictates not only temporal partitioning of the functional states but also the need for spatial segregation of mitotic and differentiated epithelial cell types. The latter property is in turn related to the availability of sequestering microenvironments in obligate versus facultative states. Anatomic constraints of the airway preclude the existence of a distinct protective compartment necessary for protection of obligate progenitor cells, such as those of the intestinal crypt. Airways can accommodate the absence of a highly mitotic progenitor due to the slow rate of epithelial turnover and a sporadic requirement for activation of the facultative progenitor.

PARTICIPATION OF STEM CELLS IN TISSUE MAINTENANCE VARIES BETWEEN ORGANS: CLASSICAL AND NONCLASSICAL HIERARCHIES

The definition of an adult tissue stem cell reflects the context in which it is viewed. The classical definition of an adult tissue stem cell is based on studies investigating the regenerative capacity of rapidly renewing tissues or cell types. Accordingly, a tissue-specific stem cell is one that self-renews and gives rise to each of the differentiated cell types within its native tissue. Availability of cell-type–specific markers and a robust functional assay allowed isolation of rare bone marrow–derived cells that fit this definition and hence definitive identification of a hematopoietic stem cell (17). However, cell selection and tissue reconstitution techniques are difficult to apply to solid tissues. As a consequence, *in situ* techniques were developed to segregate reparative cells on the basis of cell cycle frequency (18–21) and differentiation potential (22, 23). Application of DNA pulse-chase labeling methods to rapidly renewing epithelia led to the designation of tissue-specific stem cells as a reparative cell that proliferates less frequently than its daughter cells, the transit-amplifying pool (otherwise referred to as the progenitor cell). As such, stem cells retain labeled DNA, whereas highly mitotic transit-amplifying cells dilute the marked DNA. A mechanistic explanation for the property of DNA label retention within the stem cell versus depletion within the transit-amplifying cell relates to the constant turnover of the differentiated cell pool. The need for constant replenishment of differentiated cell types, such as those of the villus epithelium, requires the continuous proliferation of the transit-amplifying pool. Because transit-amplifying cells have limited capacity for self-renewal, periodic stem cell activation is required to maintain the regenerative capacity of the epithelium. On the basis of this premise, stem and transit-amplifying cells should be functionally distinguished according to their proliferative frequency within a defined time period. However, recent studies investigating properties of the intestinal stem cell hierarchy argue that frequent proliferation may be a shared functional property of the stem and transit-amplifying cell populations (2). If validated, these studies suggest that alternative mechanisms are responsible for preservation of stem cell genomic integrity.

In contrast to the short half-life of differentiated cells within rapidly renewing epithelia, differentiated cells of the slowly renewing lung, liver, and pancreas epithelium are a relatively stable population (24). This distinction in the rate of epithelial turnover impacts the longevity of the facultative progenitor cell. Consequently, facultative progenitor cells that are specified during development may be maintained for a considerable fraction of the natural life of an organism and may constitute the dominant source of renewing cells in the adult tissue (25). Thus, longevity and cell cycle frequency do not readily distinguish stem, transit-amplifying, and facultative progenitor cells of these tissues in the normal state. Identification of stem cells in these tissues is highly dependent on effective depletion of the facultative progenitor. In these tissues, extensive injury results in limited proliferation of a spatially restricted cell and focal regeneration of the epithelium (26–28). Thus, reparative cells of relatively quiescent epithelia must meet a broad set of phenotypic and functional criteria to be considered tissue stem cells. Importantly, such cells are defined as a rare cell type that is sequestered in a specialized microenvironment, lacks functional attributes that sensitize the facultative progenitor to environmental agents, and proliferates incrementally in response to injury. Within these nonclassical stem cell hierarchies, differentiation status rather than cell cycle frequency seems to be the critical distinction between the stem cell and other cells with mitotic potential. Moreover, the contribution made by stem cells to maintenance of the tissue is highly dependent on the lifespan of the facultative progenitor cell pool. Although tissue stem cells actively participate in the normal maintenance of rapidly renewing tissues, this is not the case for slowly renewing tissues (29). In slowly renewing organs, stem cell activation is part of an adaptive response to an increase in the rate at which differentiated cells and facultative progenitor cells are depleted.

Within the bronchiole, tissue-specific stem cells have been identified within the neuroepithelial body and bronchiolar duct junction microenvironments (30, 31). These cells are resistant to the Clara cell–specific toxicant naphthalene (4, 32). Pulse-chase studies in which the DNA of proliferating cells is labeled with either tritiated thymidine or bromodeoxyuridine indicate that this population gives rise to nascent secretory and ciliated cells and represents a long-term label-retaining population. Genetic ablation studies demonstrate that bronchiolar tissue–specific stem cells express the marker Clara cell secretory protein (CCSP) and that these cells are necessary for repair of the injured epithelium (30, 31, 33). *In vitro* analysis suggested the existence of a bronchoalveolar stem cell (34). However, lineage tracing analysis *in vivo* demonstrated a clear demarcation between the airway and alveolar compartments (S. D. Reynolds and colleagues, unpublished manuscript) and suggested segregation of these compartments from Embryonic Day 16.5 onward. The relationship between extensive depletion of the airway facultative progenitor cell and airway stem cell activation defines the Clara cell and the bronchiolar stem cell as a member of a nonclassical stem cell hierarchy.

REGULATION OF STEM CELL HIERARCHIES BY Wnt/β-CATENIN

The initial description of adult tissue stem cell hierarchies within rapidly renewing tissues led to use of these systems to define signaling pathways critical for regulation of proliferation, differentiation, and migration. Within these tissues, initial clues into regulatory mechanisms were provided through studies investigating molecular mechanisms of epithelial hyperplasia and tumorigenesis. Genetic perturbations involving components of

the β-catenin destruction complex lead to uncontrolled proliferation of intestinal stem/transit cells, loss of their capacity for appropriate lineage specification and differentiation, and migratory defects (37). Direct roles for Wnt/β-catenin in mediating these outcomes have been implied from studies involving genetically modified mouse models, for which potentiation or inhibition of β-catenin signaling leads to either expansion or regression of the proliferative compartment within intestinal crypts, respectively, and loss of differentiated intestinal epithelial cells (37, 38). Potentiation of the Wnt/β-catenin pathway has similarly profound influences on the self-renewal capacity and differentiation potential of other stem cell hierarchies, such as those of the hematopoietic system (39–42) or the epidermis (43–46), leading in each case to expansion of the stem/progenitor cell pool and either arrested or altered differentiation. However, even though the Wnt/β-catenin pathway plays a critical role in regulation of lung development (47–49), it is unclear if it fulfills similar roles in the maintenance of adult lung stem cells. We have used mouse models allowing conditional manipulation of β-catenin to reveal roles for this pathway in regulation of the bronchiolar stem cell hierarchy. We have established that potentiation of β-catenin signaling leads to the arrested differentiation of immature bronchiolar epithelial cells and stabilization in a state that closely resembles the bronchiolar stem cell (S. D. Reynolds, unpublished manuscript). However, we have also demonstrated that β-catenin signaling is not necessary for maintenance of the adult bronchiolar stem cell or regulation of other cellular components of the bronchiolar stem cell hierarchy (manuscript in preparation).

Further distinctions between classical and nonclassical stem cell hierarchies may derive from extrinsic factors that regulate the key events of tissue maintenance: proliferation, differentiation, and migration. Within the close confines of the intestinal crypt or the bulge region of the hair follicle, these processes may be coordinately regulated through extrinsic regulatory cues. By analogy with germline stem cells (35), the ability of a classical niche to harbor tissue stem cells may be conferred through integrin-mediated contacts with the extracellular matrix (17). Migration of cells away from the niche would attenuate this signaling process and render cells susceptible to alternative regulatory influences. Numerous paracrine factors impinge on mitotic cells of the crypt, including the multifunctional Wnt/β-catenin signaling pathway (36). Although this pathway is believed to act as a critical regulator of proliferation and differentiation, downstream effectors of migration may in fact function in establishment of the domain structure of the crypt–villus axis. Similar processes may be in play within nonclassical hierarchies and could account for spatial segregation of the stem cell and the facultative progenitor. However, the unique capacity for incremental proliferation by the broadly distributed facultative progenitor cell pool predicts utilization of signaling pathways distinct from those critical to organization of rapidly renewing tissues.

Even though our studies question the importance of the Wnt/β-catenin signaling pathway in regulation of the adult bronchiolar stem cell hierarchy, they suggest an important role for β-catenin signaling in modulating differentiation of bronchiolar epithelial cells and establishment of the stem cell hierarchy during late lung development. The apparent paradox in which active β-catenin signaling is sufficient to preserve the stem cell phenotype but is unnecessary for adult stem cell maintenance suggests that other signaling pathways regulate stem cell activity within the adult niche. Studies in epidermis suggest that distinct signals may regulate steady-state proliferation of the stem cell compartment and stem cell maintenance (50, 51). However, it is likely that many signaling pathways interact to mediate normal regulation of the proliferative and differentiation responses of a tissue stem cell hierarchy, and roles for these signals in regulation of the reparative characteristics of different organs are likely to be highly tissue specific.

CHRONIC LUNG DISEASE: ROLES FOR STEM CELLS IN INITIATION AND PERPETUATION

Facultative progenitor cells exhibit profound phenotypic plasticity that facilitates protection of the epithelium but may also hinder its progenitor function (13, 14). Terminal airways of patients with asthma and patients with chronic obstructive pulmonary disease undergo metaplastic transition to a mucosecretory phenotype, a cellular response that does not alter cell density (52). Thus, chronic mucosecretory disease may represent a situation in which epithelial regeneration is compromised but not severe enough to activate latent tissue-specific stem cells. In contrast, depletion of normal and mucosecretory cell types has been reported in severe lung disease, particularly end-stage asthma and bronchiolitus obliterans–associated lung transplantation (53–55). These circumstances mimic those associated with tissue-specific stem cell activation in animal models but do not lead to epithelial regeneration. Clearly, additional studies in mice are needed to determine the impact of ongoing disease on stem cell activation. In particular, functionality of the stem cell population in the context of inflammatory mediators central to progression of these chronic lung diseases is of critical import.

Transition of lung progenitor cells from a facultative to an obligate progenitor state may contribute to initiation or perpetuation of chronic lung disease. As indicated above, participation of the Clara cell population in epithelial regeneration is an acute response to cell attrition or injury. Entry of the Clara cell into the cell cycle is accompanied by loss of differentiated functions, such as secretion of the major Clara cell product, CCSP. Extensive analysis of CCSP protein levels in acute and chronic lung disease suggests that differentiated functions of the facultative progenitor are compromised (56), potentially as a consequence of continuous proliferation. If confirmed, conversion of the facultative progenitor to an obligate progenitor may also exhaust the proliferative potential of this cell type. Senescence of the Clara cell would leave the epithelium deficient in both differentiated and regenerative functions. Thus, transition from a facultative to an obligate progenitor may result in a cascade of changes that contribute to epithelial fragility through loss of cellular autocrine/paracrine-protective mechanisms, epithelial hypoplasia, and dysregulation of interactions between the epithelial, mesenchymal, and vascular compartments.

Tumor initiation within various epithelia has been attributed to mitotic activation of a postmitotic cell type or transformation of a tissue stem cell (57). Either mechanism may result in establishment of a cancer stem cell that is relatively quiescent and resistant to chemotherapeutic drugs that target actively cycling cells. Within the lung, facultative progenitor cells of the airway and alveolar epithelium have been implicated as precursors to non–small cell lung carcinoma. Because these cells have a native capacity to proliferate, mechanisms regulating their initial transition to a tumorigenic phenotype are likely to be distinct from those regulating formation of precancerous lesions by postmitotic cells of the intestine. Importantly, tumor initiation may be the rate-limiting step in transformation of differentiated intestinal cells, whereas tumor progression may be a key regulator in lung cancer. Bronchiolar stem cells may also function as tumor-initiating cells (34, 58), and mechanisms regulating their contribution to lung cancer may be more similar to those governing tumors derived from facultative progenitor cells than those from obligate stem cells of the gut. Thus, com-

parative analysis of molecular mechanisms regulating stem cell activity in classical and nonclassical stem cell hierarchies may lend itself to a better understanding of organ-specific aspects of oncogenesis and development of treatment strategies that take into account these differences.

FUTURE DIRECTIONS

The long-standing interests of many laboratories in understanding the process of airway injury and repair have provided important insights into both cellular and molecular mechanisms. These studies define components of the airway stem cell hierarchy and how cell types of this hierarchy participate in steady-state maintenance of the epithelium in addition to repair after injury. Critical gaps in our knowledge relate to the precise molecular regulation of the reparative process and long-term maintenance of reparative capacity. Further insights into these processes will require development of improved methods for identification and manipulation of distinct cell types within the lung. Ultimately, the capacity to recapitulate aspects of the biology *in vitro* and subsequently validate findings by reconstituting and/or precisely manipulating the *in vivo* system will be key to future progress. These studies will provide the foundation on which translational studies aimed at modulating tissue reparative capacity may be performed, with the goal of developing novel interventions for debilitating lung diseases in humans.

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