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REVIEW

Pulmonary epithelial stem cells

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Abstract. Classically, the stem/progenitor cells of the pulmonary epithelium are considered to be the basal and mucous cells of the proximal airways, Clara cells in the bronchioles and type II pneumocytes in the alveoli. Recent data suggest that there is a variant of Clara cells, lying in pulmonary neuroendocrine bodies, that meets several stem cell criteria and that type II pneumocytes exist in at least two populations, one of which is more resistant to injury. However, a complete revision of our understanding of pulmonary stem cell biology is underway as a result of the discovery of pulmonary epithelium derived from blood-borne cells. In addition, the existence in the lung of a 'universal' pluripotent cell has long been speculated upon and now some initial evidence has emerged with the identification of a spore-like cell that can differentiate *in vitro* to bronchiolar tissue.

INTRODUCTION

The average, normal adult lung contains around 70 m^2 of gas diffusion surface. The lining in many areas comprises only a monolayer of cells and, in the case of alveolar type I pneumocytes, can be as thin as 0.1 μm. This epithelial surface is constantly open to potential injury and so, to maintain that protective lining, rapid response mechanisms are in place that lead to epithelial renewal. Key amongst these are the stem and progenitor cells. Although the exact nature of stem cells has been the subject of considerable debate (Morrison 1997; Potten 1997), a generally accepted definition is that they are clonogenic cells that are capable of self-renewal and multi-lineage differentiation (Till & McCulloch 1961; Metcalf & Moore 1971; Fuchs & Serge 2000; Weissman 2000; Blau *et al*. 2001). This stable population of undifferentiated cells gives rise to progenitors that have little or no capacity to self-renew and that show signs of differentiation. As far as the lung is concerned, research into stem cells has been hindered by the structural and functional complexities of the organ. For several years, the consensus has been that there are various types of stem cells, differing according to their position within the pulmonary tree, and that they often form pools, ready to proliferate in response to injury and effect local repair (Magdeleno *et al*. 1998; Engelhardt 2001; Emura 2002; Otto 2002). However, the recent finding

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of blood-borne cells in the lung and the characterization of a potential universal stem cell, giving rise to intact lung tissue, are challenging some of the long-held views about the nature of the pulmonary epithelium and its capacity to renew.

DEVELOPMENT AND ORGANIZATION OF PULMONARY EPITHELIUM

The mammalian lung develops as an outgrowth of the embryonic gut. In humans, it originates from a diverticulum of the ventral wall of the primitive oesophagus somewhere between 4 and 5 weeks of gestation. From then on, the endoderm/epithelium undergoes dichotomous branching into the splanchnic mesenchyme that surrounds it. This highly ordered patterning process of repeated bud outgrowth and division of terminal units (Hogan 1999) is known as branching morphogenesis and gives rise to the pulmonary tree and defines the proximal-distal axis of the lung. The development of the mammalian lung has been divided into four phases and, in humans, the timings of these are; embryonic 0–5 weeks, glandular 5–16 weeks, canalicular 16–26 weeks and saccular 26 weeks to term (Adamson 1991). The primordial lining that forms in the embryonic stage develops into pseudostratified epithelium during the early glandular phase and, as branching progresses, columnar epithelium forms. During the glandular and into the canalicular phase, the initial thick layer of stratified epithelial cells starts to thin and shows gradation, becoming thinner along the length of the tree. Submucosal glands first appear at around 10 weeks in the trachea but not until 16 weeks in the bronchi. Bronchioles appear during the canalicular stage, marking the start of the formation of gas exchange units. A lumen is present at this stage and the epithelium starts to look more cuboidal, an appearance that remains into the final saccular phase where alveolar ducts and air sacs begin to open. The final formation of alveoli takes place post-natally. Thus, the mature lung has distinct anatomical regions lined by different types of epithelial cells. In the mature lung, the trachea and major bronchi are lined by pseudostratified epithelium. The major phenotypes in the proximal airways are ciliated and mucous secretory (or goblet) cells, with the more infrequent neuroendocrine cells and the less well-differentiated basal cells lying in a basal position. The bronchioles are also lined by ciliated cells, but possess a separate phenotype known as the Clara cells that are non-ciliated. The alveoli are lined by flattened squamous (type I pneumocytes) and cuboidal (type II pneumocytes) cells. A further class of epithelial cells that populates the lung is the neuroendocrine cells that first appear around 8 weeks of gestation. These contain biogenic amines, commonly serotonin (Lauweryns *et al*. 1986), and/or peptides, including bombesin (Wharton *et al*. 1978) and calcitonin gene-related peptide (Johnson & Wobken 1987). They are relatively frequent in the developing lung, where they play a major role in airway growth and development, but form > 1% of epithelial cells in adult lung, where they are seen as scattered elements in the epithelium or in innervated epithelial corpuscles, so-called neuroepithelial bodies (Lauweryns & Cokelaere 1973; Cutz & Orange 1977).

STEM CELL NICHES

Proximal airways

In the trachea and bronchi, the basal and mucous secretory cells are widely believed to be stem cells (Breeze & Wheeldon 1977; Reid & Jones 1979; Kauffman 1980; Donnelly *et al*. 1982).

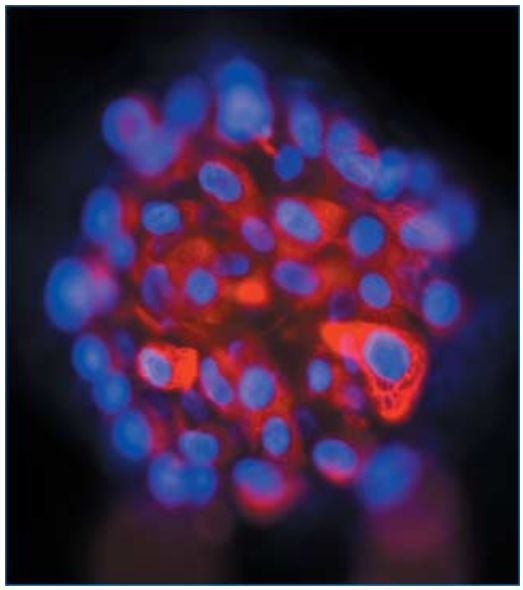

Figure 1. Progenitor cells freshly isolated from the epithelium of human fetal lung (gestational age 12 weeks). The epithelial phenotype is shown by immunostaining for cytokeratin (red). These cells were maintained *in vitro* and, after two weeks, were found to express surfactant protein C, a specific marker of type II pneumocytes (nuclei counterstained blue with DAPI). Photograph courtesy of Dr HM Romanska.

The basal cells, and the parabasal cells that lie just above them, certainly form a pluripotential reserve cell that, unlike the surrounding epithelium, usually survives injury (Emura 1997). The basal cell appears at around 10 weeks of gestation in the human trachea, is roughly triangular and lies under the columnar epithelial layer, with one edge firmly anchoring the epithelium to the basement membrane. One of the first suggestions that basal cells may be precursors of airway epithelium came with the observation that, in rat lung, they take up [^{3}H]thymidine that is later found in ciliated and goblet cells (Bindreiter *et al*. 1967). In addition, they accumulate at the sites of injury (Lane & Gordon 1974) and their histological appearance is intermediate, sharing characteristics with ciliated cells and goblet cells (Breeze & Wheeldon 1997). *In vitro* procedures that involve denuding the trachea have demonstrated the capacity of basal cells to produce all the major cell phenotypes found in the trachea, including basal, ciliated, goblet and granular secretory cells (Inayama *et al*. 1988, 1989; Nettesheim *et al*. 1990; Liu *et al*. 1994). Evidence that the basal and parabasal cells are stem cells in human lung includes the demonstration, using a proliferation marker (MIB-1), that they form a disproportionate fraction of the proliferative compartment of the epithelium (Boers *et al*. 1998).

The evidence that mucous cells are stem cells is not so abundant, but particular interest has been paid to this possibility in view of their potential as targets for gene therapy in cystic fibrosis (Engelhardt *et al*. 1995; Duan *et al*. 1998). Bromodeoxyuridine (BrdU) label-retaining cells (LRCs) are considered to be stem cells (Engelhardt 2001) and such cells have been found in gland ducts of the upper trachea following injury (Borthwick *et al*. 2001). The same group also showed, by heterotopic tracheal grafting on denuded trachea, that the surface epithelium was reconstituted by cells from the glands. In a model of tracheal epithelial regeneration, secretory, and basal, cells were found to dedifferentiate into a highly proliferative phenotype which gave rise to mucociliary epithelium (Liu *et al*. 1994).

Bronchioles

Clara cells were first described in 1937 (Clara 1937) and have since been shown to be progenitors of themselves and of ciliated cells in the bronchioles (Evans *et al*. 1976, 1978; Plopper & Dungworth 1987; Boers *et al*. 1999). However, more recent research has established that a subset of Clara cells fulfils the criteria of adult, niche-specific stem cells. Pools of stem cells have been discovered that express Clara cell secretory protein (CCSP) but are not typical Clara cells as they are resistant to airway pollutants such as naphthalene (Mahvi *et al*. 1977; Plopper *et al*. 1992; Stripp *et al*. 1995; Reynolds *et al*. 2000a,b; Hong *et al*. 2001; Giangreco *et al*. 2002). Generally, Clara cells are enriched with cytochrome P-450 enzymes that would make them vulnerable to naphthelene (Devereux *et al*. 1981). In addition, these variant CCSP-expressing (or vCE) cells show multipotent differentiation. The vCE cells are located in discrete pools in neuroepithlial bodies and at the broncho-alveolar duct junction.

There seems to be some controversy as to whether pulmonary neuroenodrine cells (PNEC) can proliferate. The classical view is that they cannot (Hoyt *et al*. 1990; Montengua *et al*. 1992; McDowell *et al*. 1993; Gosney 1997) and it has been suggested that PNEC populations may be maintained (Linnoila 1982) and become hyperplastic (Sunday and Willett 1992, 1994) from a multipotent progenitor. Such a progenitor has been described in the mouse lung where Clara cell protein and a product of PNECs, calcitonin gene-related peptide (CGRP) were co-expressed in most epithelial cells of the distal airways of the murine lung at E13-15 (Wuenschell *et al*. 1996), although this evidence is based solely on immunocytochemistry. Certainly, PNECs secrete regulatory factors that play roles in the regulation of epithelial cell renewal, for example gastrin-releasing peptide (or mammlian bombesin) that is a potent epithelial cell mitogen (Wharton *et al*. 1978; Sunday *et al*. 1990, 1991; Cutz *et al*. 1995). A study designed to test whether vCE and/or CGRP-expressing PNECS have pluripotent capacity for epithelial renewal used a transgenic model with thymidine kinase gene under the direction of the CCSP promoter (Hong *et al*. 2001). Following temporary ablation of the entire CCSP-expressing population, hyperplasia of CGRP-expressing PNECs occurred, but these cells did not repopulate the epithelium, supporting the view that they are not stem cells.

Alveoli

The first evidence that type II pneumocytes (Fig. 1) are stem cells emerged 50 years ago when they were shown to proliferate following injury (Mackin 1954). It was shown that they restore the alveolar epithelium following generalized damage by oxidants, e.g. oxygen and nitrogen dioxide, by giving rise to either new type II cells or the squamous type I pneumocytes, the latter being destroyed following most types of lung injury (Evans *et al*. 1971; Adamson & Bowden 1974a; Evans *et al*. 1975; Witschi 1978; Kauffman 1980; Bowden 1981). Injury to type I cells is not the only stimulus for proliferation of type II pneumocytes. They respond in this way when damaged selectively by butylated hydroxytoluene (Adamson *et al*. 1977) and bleomycin (Adamson & Bowden 1974b, 1979) or as a consequence of inflammatory infiltration, e.g. following particle inhalation (Shami *et al*. 1986). It has been shown that alveolar type II cell injury and apoptosis may be an important early feature in the pathogenesis of pulmonary fibrosis (Haschek & Witschi 1979; Katzenstein 1985; Maeyama *et al*. 2001).

It has emerged recently that there may be two subpopulations of type II pneumocytes, one of which comes closer to fitting the definition of a stem cell (Reddy *et al*. 2003). Type II cells isolated from rats following exposure to hyperoxia could be selected according to their immunoreactivity for E-cadherin into damaged, quiescent cells with low telomerase activity and an E-cadherin negative population that was undamaged, proliferative and expressed high levels of telomerase activity.

When grown *in vitro*, alveolar type II cells soon lose their cuboidal shape, becoming flattened, and cease to express phenotypic markers while taking on those characteristic of type I cells (Dobbs *et al*. 1985; Shannon *et al*. 1987; Dobbs *et al*. 1988; Danto *et al*. 1992). Although, type I cells *in vivo* appear to be terminally differentiated (Adamson & Bowden 1974a), following differentiation from type II cells *in vitro*, they have been found to re-express markers of type II pneumocytes (Leslie *et al*. 1993).

STEM/PROGENITOR CELLS FROM OUTSIDE THE LUNG

A growing body of evidence has shown that tissues can be repaired by cells acquired via the circulation (for review see Forbes *et al*. 2002). It has been shown in experimental models that bone marrow-derived stem cells can engraft in the lung and differentiate into mature epithelial phenotypes (Kotton *et al*. 2001; Krause *et al*. 2001) and that this process increases in response to injury (Theise *et al*. 2002). For the human lung, chimerism has been demonstrated in pulmonary epithelium, including that of the alveoli, following transplantation of haematopoietic stem cells (Kleeberger *et al*. 2003) or lung (Surratt *et al*. 2003), although neither study found evidence for engraftment of bone marrow cells specifically. There is some debate as to whether the blood-borne cells that embed in the lung actually differentiate to lung epithelium or fuse with cells *in situ*, as has been shown to occur *in vitro* (Spees *et al*. 2003). The presence in the lung epithelium of cells recruited from the circulation could provide new therapeutic opportunities for a range of pulmonary diseases by providing means to repair the lung and a novel route for gene therapy.

UNIVERSAL STEM/PROGENITOR CELL

There is some evidence that a pluripotent stem cell exists in the lungs of adult sheep and rats that can generate lung-like tissue *in vitro*, specifically of the alveolar (Cortiella *et al*. 2000) and bronchiolar regions (Vacanti *et al*. 2001). The isolated cells were extremely small and had a very low demand for oxygen, leading the researchers to term them ‘spore-like’ (Vacanti *et al*. 2001). Similar cells were found throughout the body, in each case giving rise *in vitro* to the tissue from which they were isolated. It is thought that they lie dormant until activated by injury or disease.

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