ELSEVIER

EXPERIMENTAL HEMATOLOGY

Experimental Hematology 32 (2004) 340–343

Lung stem cells: New paradigms

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The intrinsic anatomical complexity of the lung, its slow cell turnover, and the lack of regenerative models are among the factors that have complicated the study and isolation of adult lung stem cells. Despite this, several endogenous lung progenitor cells have been identified in the proximal and distal lung. However, there is limited data regarding the lineage relationships, self-renewal properties, and clonality of these specific lung cell progenitors. More recent work showing that marrow cells can engraft as differentiated cells of solid organs has suggested new stem cell paradigms for the lung. In this review, we explore the implications of these new studies for lung stem cell biology. We also summarize and discuss the ongoing controversies that these studies have generated.

The lung is an extremely complex three-diminensional structure composed of numerous morphologically distinct epithelial cell types arrayed along bifurcating tubes. These tubes serve to conduct inspired and expired gas to and from distal alveolar-capillary units. This intrinsic complexity along with several unique aspects of lung structure and biology has complicated the study and identification of lung stem cells.

Unlike other epithelial surfaces (i.e., skin, gastrointestinal tract), airway and alveolar epithelia have very slow cell turnover and minimal regenerative capacity. This overall tissue quiescence has significantly impeded the identification of lung stem cells. This is further compounded by the technical complexities associated with isolating pure populations of lung cells for in vitro study. One additional issue is the technical difficulties associated with the histological evaluation of an organ that contains a gas-tissue interface.

Despite these limitations, several types of endogenous lung progenitor cells have been identified in the proximal and distal lung. There is, however, a paucity of information regarding the lineage relationships, self-renewal properties, and clonality of these currently identified lung progenitors. In the proximal conducting airways, basal cells, Clara cells, and cells that reside in submucosal glands have been shown to function as progenitors [1–4]. Most recently, variant-Clara cells residing within neuroepithelial bodies [5–8] or bronchoalveolar duct junctions [9] have been shown to contribute to airway epithelial repair after naphthalene injury.

In the gas exchange distal air sacs (alveoli), the cuboidal type II cell is thought to function as the progenitor of the alveolar epithelium based on a capacity to replenish itself and to give rise to terminally differentiated flat type I cells [10,11]. This model has evolved from in vitro data showing that cells with a type I cell phenotype arise during culture of primary type II cells. Furthermore, classic in vivo thymidine incorporation studies have shown serial progression of labeling from type II into type I cell nuclei after lung injury [12–15]. To date, a single lung stem cell that can give rise to multiple epithelial lineages in the proximal and distal lung has not been identified.

New paradigms

A variety of recent studies in mice have suggested that marrow stem cells can serve as progenitors of differentiated cells of solid organs; these findings have challenged long-held views regarding the fixed nature of adult stem cell potential and suggest the possibility of circulating tissue stem cells. The data supporting a new stem cell paradigm are from experiments in which irradiated mice have undergone bone marrow transplantation with donor cells that express a tracking marker (i.e., green fluorescent protein [GFP]; β-galactosidase; lacZ; Y-chromosome) [16–21]. In these studies, marrow-derived organ parenchymal cells were subsequently identified through the histological colocalization

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0301-472X/04 $–see front matter.
doi: 10.1016/j.exphem.2004.01.009

of the tracking marker along with selective differentiation markers. Based on this experimental approach, marrow-derived cells have been proposed to contribute to a variety of epithelial cell types in various organs.

Supporting this new paradigm are data from human transplant studies. Using the Y-chromosome as a tracking marker, analyses of sex-mismatched allografts have suggested the presence of parenchymal cells of purported marrow origin [22–24]. Similar to the findings in mice, marrow-derived cells have been suggested to engraft in multiple types of human epithelial tissues, such as skin, intestine, kidney, liver, and lung [23,25–28].

Marrow to lung

Krause et al. found widespread engraftment as alveolar type II cells and airway epithelial cells after transplantation of a single marrow stem cell into mice [17]. In this study, single male donor cells selected on the basis of size fractionation, lineage depletion, and rapid marrow homing capacity were used for transplantation into marrow-ablated female mice. Engrafted cells were identified on the basis of colocalization of the Y-chromosome and selective lung epithelial markers. Levels of type II cell engraftment reached 20% at 11 months after transplantation. Follow-up studies by these investigators using donor whole marrow or $CD34^+/Lin^-$ cells found that engraftment in the lung as alveolar type II cells was detectable at 5 days after bone marrow transplantation, and was robust by 2 months [29,30].

In another study, Kotton et al. injected 1 to 2 million plastic-adherent $lacZ^+$ marrow cells into mice that did not receive prior marrow ablation [31]. In this study, the marrow cells were not purified beyond plastic adherence, and were cultured for 1 week in basic serum-containing media prior to administration. At 5 to 30 days post–direct intravenous injection into uninjured and bleomycin-injured recipients, a small number of engrafted cells with the characteristic flattened morphology of type I cells were found. In addition, these cells appeared to assume the molecular phenotype of type I alveolar cells as they expressed the type I cell surface marker, T1α, and bound the lectin, *Lycopersicon esculentum*. Despite careful analysis, type II cell engraftment was not observed in this study, even at time points as early as 24 hours after cell injection. Engraftment was significantly more robust following induction of bleomycin injury. Similarly, Ortiz et al. found engraftment of marrow-derived mesenchymal stem cells in the lung after bleomycin injury [32]; these cells were localized to areas of injury and exhibited an epithelial morphology.

In more recent work, a marrow cell derivative obtained after prolonged culturing in a defined growth medium was found to engraft as multiple epithelial cell types after injection into nonirradiated or irradiated adult nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice [19]. These cells, termed multipotent adult progenitor cells (MAPC), adhered to plastic, could be serially passaged, and expressed primitive stem cell markers in vitro [33–36]. Injection of single MAPCs into blastocysts further demonstrated the ability of these cells to contribute to cell lineages from multiple tissues including blood, liver, intestine, and lung [19]. In these studies β-galactosidase-labeled MAPCs was found to engraft in lung tissue as $cytokeratin^+/CD45^-$ cells residing within the alveolar wall. Whether MAPCs normally participate in tissue homeostasis is not yet clear.

Despite these findings, studies showing marrow-to-organ engraftment remain a source of ongoing controversy. One overriding issue relates to their reproducibility. For example, Wagers et al. extensively analyzed tissues from multiple mice transplanted with a single GFP^+ hematopoietic stem cell (HSC) and observed almost a complete lack of non-hematopoietic engraftment except for a single GFP^+ cerebellar Purkinje cell and 7 GFP^+ hepatocytes [37]. Some have argued that these types of observations can be accounted for by major variations in experimental design [38]. These variations include differing cell purification methods, application of tissue injury, and the types of histological techniques employed to evaluate engraftment.

One recurrent concern relates to the ability to distinguish by immunohistochemical methods differentiated organ cells from hematopoietic marrow-derived cells contained within juxtaposed capillaries and tissue spaces [37,38]. This is a significant issue for the lung because of the high number of resident hematopoietic cells and the extensive capillary network. It has been argued that deconvolution or confocal microscopy, proof of absent CD45 expression in engrafted cells, and the rigorous use of isotype control antibodies may minimize these issues. Ultimately, the development of transgenic mice that express fluorescent markers under the control of lung cell–specific promoters may be necessary to clarify these issues.

Importantly, several laboratories demonstrated that fusion of marrow-derived cells with recipient cells explains the colocalization of tracking and differentiation markers, rather than true stem cell plasticity [39–43]. Fusion of marrow-derived cells with organ cells has been documented in recipient liver, heart, and brain [39,44]. On the other hand, the functional implications of fusion remain unclear and may conceivably be a potential mechanism for injury repair [16,44]. Fusion events, however, may not mediate all cell engraftment events [39,45].

The appropriateness of the experimental models employed to detect engraftment in lung tissue of bone marrow–derived cells also remains at issue. Models that have employed GFP or lacZ labels assume that detectable transgene expression is maintained in all cell types after transplantation. Models that track the Y-chromosome are not susceptible to problems of transgene expression; however, the Y-chromosome cannot be detected unless the plane of tissue section being analyzed passes through the correct portion of the cell nucleus. Even if a particular model

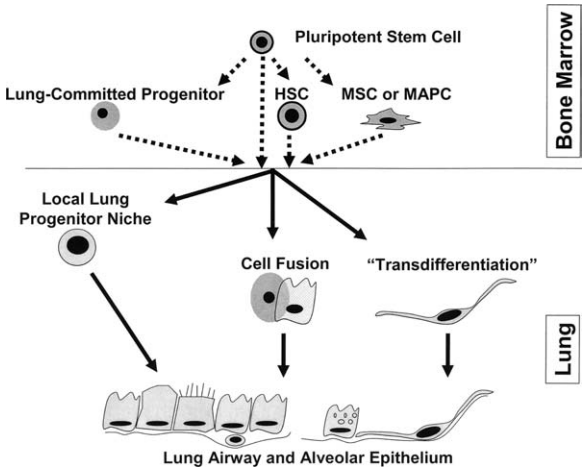

Figure 1. Models of lung epithelial derivation from bone marrow cells. Several possible marrow cell types may serve as the source of lung epithelial cells. These include: 1) a pluripotent marrow cell, 2) hematopoietic stem cells (HSC), 3) mesenchymal stem cells (MSC), 4) multipotent adult progenitor cells (MAPC), or 5) a lung-committed progenitor cell. Three possible biological mechanisms may mediate lung epithelial engraftment. These include: 1) trafficking of marrow cells to a local progenitor niche in the lung, 2) fusion of bone marrow–derived cells with differentiated epithelial cells in the lung, or 3) direct "transdifferentiation" into lung epithelial cells.

is proven reliable, a difficult challenge remains to clarify whether engraftment is only a feature of experimental conditions or can occur in a more physiologic state in the intact organism.

New strategies to identify resident lung stem cells

Recent work has shown that small populations of resident cells within solid organs display phenotypic features of known marrow stem cells. Taking this into account, investigators have attempted to isolate and characterize tissue stem cells by identifying organ cells that display these specific stem cell phenotypes.

One such strategy is to identify organ cells that express stem cell antigens, such as Sca-1 or c-kit [24,45–49]. This approach is limited by the relative lack of cellular specificity for antigens expressed by stem cells. For example, in lung tissue, Sca-1 is expressed throughout the endothelium of arteries, veins, and capillaries [50]. Another strategy is based upon the observed capacity of HSCs to efflux Hoechst dye, a process mediated by the ABC half transporter Bcrp1 (breast cancer resistance protein) [51]. Such cells, termed side population (SP) cells, can be isolated by dual-wavelength flow cytometry due to an absence of staining with Hoechst dye [52,53].

Marrow SP cells are $CD45^+/Sca\text{-}1^+/c\text{-}kit^+/Lin^-$ and are highly enriched for hematopoietic stem cell activity. In addition to the bone marrow, SP cells have been identified in various solid organs [21,46,54–56]. In the adult lung, Summer et al. showed that these cells comprise 0.03% of total cell suspensions [55]. In contrast to the marrow, two subtypes of lung SP cells can be identified based on the presence or absence of the hematopoietic marker CD45. Notably, both lung SP sub-populations are $Sca\text{-}1^+$, Lin^-, and express Bcrp1 [55]. Recently, cell suspensions prepared from lung airway digests were also found to contain SP cells, some of which appeared to express the phenotype of variant-Clara cells of the neuroepithelial body [57]. While the SP phenotype is a powerful tool for the identification of stem cells, there are no functional studies to show that these cells may represent lung stem cells. The role of lung SP cells in tissue reconstitution as well as their relationship to defined marrow stem cell populations is currently under study.

In conclusion, there is accumulating evidence supporting the possibility that marrow-derived cells can engraft as differentiated epithelial cells of the lung (Fig. 1). Furthermore, resident lung cells with features of marrow stem cell populations have been identified. Although controversial, these observations challenge fundamental concepts regarding the origin and repertoire of adult stem cells and suggest dramatic new cell-based therapies for lung disease.

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