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Introduction

Brigid L.M. Hogan[1] and Marko Z. Nikolić[2]

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This *Monograph* considers the different stem cells present in the developing and adult lung, how they can be derived from pluripotent cells, and the cutting-edge research underway to study them and harness their therapeutic potential https://bit.ly/30BDe7h

Almost every organ in the adult human body can maintain itself over the long term and undergo repair after injury. These properties are largely dependent on stem cells – cells that can both divide repeatedly to make more of themselves (self-renew) and generate daughters that can give rise to one or more differentiated cell type [1]. This *Monograph* brings together information about the different classes of stem cells present in both the developing and adult lung: where they are found, how they function in homeostasis and pathologic conditions, the mechanisms that regulate their behaviour, and how they may be harnessed for therapeutic purposes. The focus is on stem cells in the mouse and human lung but includes the ferret as an increasingly important new model organism. Chapters also discuss how lung tissue, including endogenous stem cells, can be generated *in vitro* from pluripotent stem cell lines. These are undifferentiated stem cells that are normally present transiently in the embryo and have the capacity to generate all the tissues of the body. Pluripotent cells can be generated from adult cells by genetic manipulation but are not present in mature organs themselves.

The stem cells of adult organs, including the lung, are laid down during development as integral components of the mature system [2, 3]. Different tissue compartments – the epithelium, stroma and vasculature – contain their own characteristic stem cells, which cannot substitute for one another and are found in characteristic locations. The immediate environment of an adult tissue stem cell is called the niche [4, 5]. In the case of epithelial stem cells, the niche may include the underlying ECM and stromal cells, as well as mechanical and other contact cues from neighbouring epithelial cells. The niche may also include blood vessels and the humoural factors (*e.g.* hormones, oxygen and nutrients) they deliver, as well as immune cells, lymphatics and nerves. Any attempts to engineer replacement organs like the lung, or to promote the survival and expansion of failing endogenous stem cells with biologics or drugs, must take into account the absolute necessity of also providing robust niches. Without a supportive environment, stem cells may function aberrantly or not at all. In many cases, stem cells can be extracted from an

[1]Dept of Cell Biology, Duke University Medical School, Durham, NC, USA. [2]UCL Respiratory, Division of Medicine, University College London, London, UK.

Correspondence: Brigid L.M. Hogan, Duke University Dept of Cell Biology, Nanaline Duke Building, Room 388, 307 Research Drive, Durham, NC 27710, USA. E-mail: brigid.hogan@duke.edu. Marko Nikolić, UCL Respiratory, Division of Medicine, Rayne Institute, 5 University Street, London, WC1E 6JF, UK. E-mail: m.nikolic@ucl.ac.uk.

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adult organ and grown in culture under different conditions. In 2D cultures at an air–liquid interface or in a microfluidics (lung-on-a-chip) device, airway stem cells self-organise into a pseudostratified mucociliary epithelium. In 3D cultures, stem cells form structures known as organoids, which differ in organisation depending on which population they are derived from [6–8]. These *ex vivo* culture systems have tremendous potential for modelling pulmonary disease and for drug screening.

Before we summarise the different stem cells found in the adult lung, some historical background is in order. Based on early studies, it was assumed that stem cells must be unspecialised and quiescent. In fact, it is now recognised that some stem cells, such as those in the crypts of the small intestine, normally proliferate quite actively, while others have specialised physiological functions [9]. For example, in the case of the lung, the type 2 alveolar stem cells, which can self-renew and differentiate into type 1 cells, are specialised to secrete surfactants, and to recruit and activate immune cells [10], while the myoepithelial stem cells of the SMGs are contractile and express smooth muscle actin. It was also assumed that undifferentiated "professional" stem cells would have a deterministic pattern of behaviour, giving rise after division to either two stem cells (symmetric behaviour) or to one stem cell and one differentiating daughter (asymmetric behaviour). It is now clear that not all stem cells follow these rules, and that alternative models are possible, even for stem cells in different regions of the same organ. Thus, in some cases, stem cells are best viewed as a heterogeneous population of cells with varying probabilities of giving rise to either two stem cells, two differentiating daughters, or one of each. Cells can transition reversibly between these states, and the probability of each decision can vary depending on the intrinsic state of the cell and signals from the local microenvironment [11, 12]. These different models mean that any new prospective stem cell type must be studied quantitatively over both the short and long term, ideally using lineage tracing, live imaging and single cell transcriptomic methods under different physiological conditions [12–14].

Another feature of adult stem cells that has emerged from recent studies is the fact that the fate of the differentiating daughter cells is not invariant but can change with signals from the environment. Abnormal conditions can also trigger some differentiated cells to "dedifferentiate" or "transdifferentiate" back into stem cells [4, 15, 16]. This "cell plasticity" is particularly evident in response to injury or inflammation. Such conditions are frequently encountered in studies on the adult lung because cell turnover is normally very slow; in order for the full potential of stem cells to be realised, or for new reserves to be revealed, it is necessary to experimentally damage the tissue and to follow repair and remodelling over time. As described in several chapters, a wide range of injury/repair models are typically used in the mouse lung, most of them affecting the epithelium. They include exposure to detergent (polidocanol), acid (or sulfur dioxide), ozone, naphthalene, elastase and bleomycin, as well as virus infection and cell-specific conditional deletion using diphtheria toxin. What these studies have revealed is that the identity and fate of activated stem cells can vary depending on the severity and nature of the injury and the age of the animal. Therefore, to understand the *in vivo* functional role of any stem cell population, it is important to use as many different experimental variables as possible, and to follow the fate of stem cell descendants quantitatively over long periods of time.

Figure 1 summarises the epithelial/endothelial stem cells that have been identified to date in the adult mouse lung; as yet, very little is known about the lineage relationship among adult mesenchymal cells. The figure indicates whether similar stem cells have been identified in the human lung, but here our knowledge base is also very limited, and

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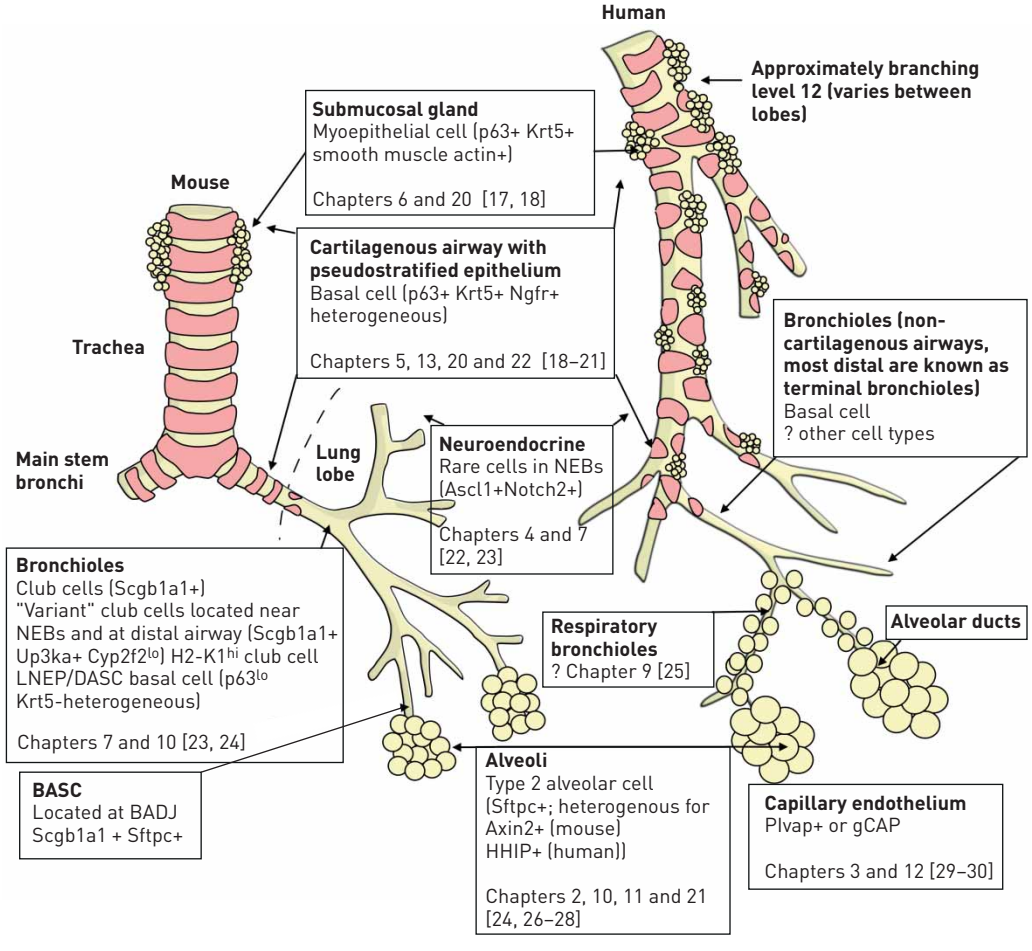

Figure 1 Schematic representation showing location of currently known stem cells in mouse and human adult lung. The intralobar human lung is shown from branch generation 12. A question mark indicates that it is currently not known whether such a stem cell type exists in the human lung. References can be found in the chapters cited. Additional references: for basal cells [31, 32, 33]; for rare neuroendocrine cells [34]; for AT2 cells [33]; for capillary endothelium [35]. NEB: neuroendocrine body; LNEP: lineage-negative epithelial progenitor; DASC: distal airway stem cell.

nothing is known about potential stem cells in the simple columnar epithelium lining the respiratory bronchioles. Importantly, recent single cell transcriptomic studies have revealed significant heterogeneity within the stem cell populations, both at steady state and during repair. For example, the basal cells of the pseudostratified epithelium include a subpopulation that appears biased towards differentiating into luminal or secretory cells [31, 36, 37]. Currently, it is not clear whether these subpopulations represent transitional components of a lineage hierarchy or distinct stem cells. Moreover, functionally, we do not know whether the different subpopulations differ in their ability to engraft into a damaged lung and behave as stem cells over the long term. This information is potentially important for cell replacement therapies, for which the reparative capacity of the donor cells should be optimal. To experimentally test different populations, we need to develop robust stem cell engraftment assays for different regions of the adult mouse lung, equivalent to bone marrow transplantation for HSCs. The gold standard definition of a stem cell is a cell that

can engraft into damaged tissue and replace lost cells over the long term; but until efficient and quantitative assays are available for the lung, this criterion will be hard to apply. From another point of view, there is growing evidence that cells in "transitional states" between different cell types, including stem cells, may, under certain conditions, accumulate and potentially promote pathological changes in damaged tissue [38, 39].

The chapters in this *Monograph* contain many examples of how technical and conceptual breakthroughs over the past decade have advanced our understanding of lung stem cells and the mechanisms that control their proliferation and differentiation. It is almost certain that similar innovations over the next 10 years will have an enormous impact on our use of lung stem cells for therapeutic purposes. Some of the ways in which this may happen are discussed in several chapters as well as the final chapter.

References

1. Cable J, Fuchs E, Weissman I, *et al.* Adult stem cells and regenerative medicine – a symposium report. *Ann N Y Acad Sci* 2020; 1462: 27–36.
2. Herriges M, Morrisey EE. Lung development: orchestrating the generation and regeneration of a complex organ. *Development* 2014; 141: 502–513.
3. Morrisey EE, Hogan BLM. Preparing for the first breath: genetic and cellular mechanisms in lung development. *Dev Cell* 2010; 18: 8–23.
4. Chacón-Martínez CA, Koester J, Wickström SA. Signaling in the stem cell niche: regulating cell fate, function and plasticity. *Development* 2018; 145: dev165399.
5. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* 1978; 4: 7–25.
6. Barkauskas CE, Chung M-I, Fioret B, *et al.* Lung organoids: current uses and future promise. *Development* 2017; 144: 986–997.
7. Kim J, Koo B-K, Knoblich JA. Human organoids: model systems for human biology and medicine. *Nat Rev Mol Cell Biol* 2020; 21: 571–584.
8. Sellgren KL, Butala EJ, Gilmour BP, *et al.* A biomimetic multicellular model of the airways using primary human cells. *Lab Chip* 2014; 14: 3349–3358.
9. Clevers H, Watt FM. Defining adult stem cells by function, not by phenotype. *Annu Rev Biochem* 2018; 87: 1015–1027.
10. Whitsett JA, Alenghat T. Respiratory epithelial cells orchestrate pulmonary innate immunity. *Nat Immunol* 2015; 16: 27–35.
11. Simons BD, Clevers H. Strategies for homeostatic stem cell self-renewal in adult tissues. *Cell* 2011; 145: 851–862.
12. McKinley KL, Castillo-Azofeifa D, Klein OD. Tools and concepts for interrogating and defining cellular identity. *Cell Stem Cell* 2020; 26: 632–656.
13. Mesa KR, Kawaguchi K, Cockburn K, *et al.* Homeostatic epidermal stem cell self-renewal is driven by local differentiation. *Cell Stem Cell* 2018; 23: 677–686.
14. Rompolas P, Mesa KR, Kawaguchi K, *et al.* Spatiotemporal coordination of stem cell commitment during epidermal homeostasis. *Science* 2016; 352: 1471–1474.
15. Ge Y, Fuchs E. Stretching the limits: from homeostasis to stem cell plasticity in wound healing and cancer. *Nat Rev Genet* 2018; 19: 311–325.
16. Tata PR, Rajagopal J. Cellular plasticity: 1712 to the present day. *Curr Opin Cell Biol* 2016; 43: 46–54.
17. Tata A. Stem cells of submucosal glands: their function as tissue stem cells and a reserve population for airway repair. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 70–83.
18. Pai AC, Parekh KR, Engelhardt JF, *et al.* Ferret respiratory disease models for the study of lung stem cells. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 273–289.
19. Lin B, Sun J, Mou H, *et al.* Adult mouse and human airway epithelial basal stem cells. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 56–69.
20. Meyer KB, Wilbrey-Clark A, Nawijn M, *et al.* The Human Lung Cell Atlas: a transformatioinal resource for cells of the respiratory system. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 158–174.

 https://doi.org/10.1183/2312508X.10002321

21. Vaidyanathan S, McCarra M, Desai TJ. Lung stem cells and therapy for cystic fibrosis. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 306–321.
22. Dash B, Kim E, Sun X. Neuroendocrine cells in lung development and disease. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 44–55.
23. Dabrowska C, Li J, Mulay A, *et al.* Adult mouse intralobar airway stem cells. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 84–98.
24. Toth A, Zhao B, Zacharias WJ. Alveolar epithelial stem cells in homeostasis and repair. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 122–133.
25. Basil MC, Morrisey EE. Respiratory bronchioles: a unique structure in the human lung. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 114–121.
26. Li J, Tang N. Alveolar stem cells in lung development and regrowth. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 17–30.
27. Thorley AJ, Rock JR. Mesenchymal cells, immune cells and the lung stem cell niche. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 134–143.
28. Platé M, Kobayashi Y, Chambers RC, *et al.* Epithelial stem cells at the intersection of tissue regeneration and pulmonary fibrosis. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 290–305.
29. Stoilova T, Ruhrberg C. Lung blood and lymphatic vascular development. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 31–43.
30. Vila Ellis L, Shuet Lin Kong C, Chen J. Endothelial cells in the lung. *In*: Nikolić MZ, Hogan BLM, eds. Lung Stem Cells in Development, Health and Disease (ERS Monograph). Sheffield, European Respiratory Society, 2021; pp. 144–157.
31. Carraro G, Mulay A, Yao C, *et al.* Single-cell reconstruction of human basal cell diversity in normal and idiopathic pulmonary fibrosis lungs. *Am J Respir Crit Care Med* 2020; 202: 1540–1550.
32. Goldfarbmuren KC, Jackson ND, Sajuthi SP, *et al.* Dissecting the cellular specificity of smoking effects and reconstructing lineages in the human airway epithelium. *Nat Commun* 2020; 11: 2485.
33. Travaglini KJ, Nabhan AN, Penland L, *et al.* A molecular cell atlas of the human lung from single-cell RNA sequencing. *Nature* 2020; 587: 619–625.
34. Ouadah Y, Rojas ER, Riordan DP, *et al.* Rare pulmonary neuroendocrine cells are stem cells regulated by Rb, p53, and Notch. *Cell* 2019; 179: 403–416.
35. Gillich A, Zhang F, Farmer CG, *et al.* Capillary cell-type specialization in the alveolus. *Nature* 2020; 586: 785–789.
36. Plasschaert LW, Žilionis R, Choo-Wing R, *et al.* A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature* 2018; 560: 377–381.
37. Watson JK, Rulands S, Wilkinson AC, *et al.* Clonal dynamics reveal two distinct populations of basal cells in slow-turnover airway epithelium. *Cell Rep* 2015; 12: 90–101.
38. Kobayashi Y, Tata A, Konkimalla A, *et al.* Persistence of a regeneration-associated, transitional alveolar epithelial cell state in pulmonary fibrosis. *Nat Cell Biol* 2020; 22: 934–946.
39. Vieira Braga FA, Kar G, Berg M, *et al.* A cellular census of human lungs identifies novel cell states in health and in asthma. *Nat Med* 2019; 25: 1153–1163.

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