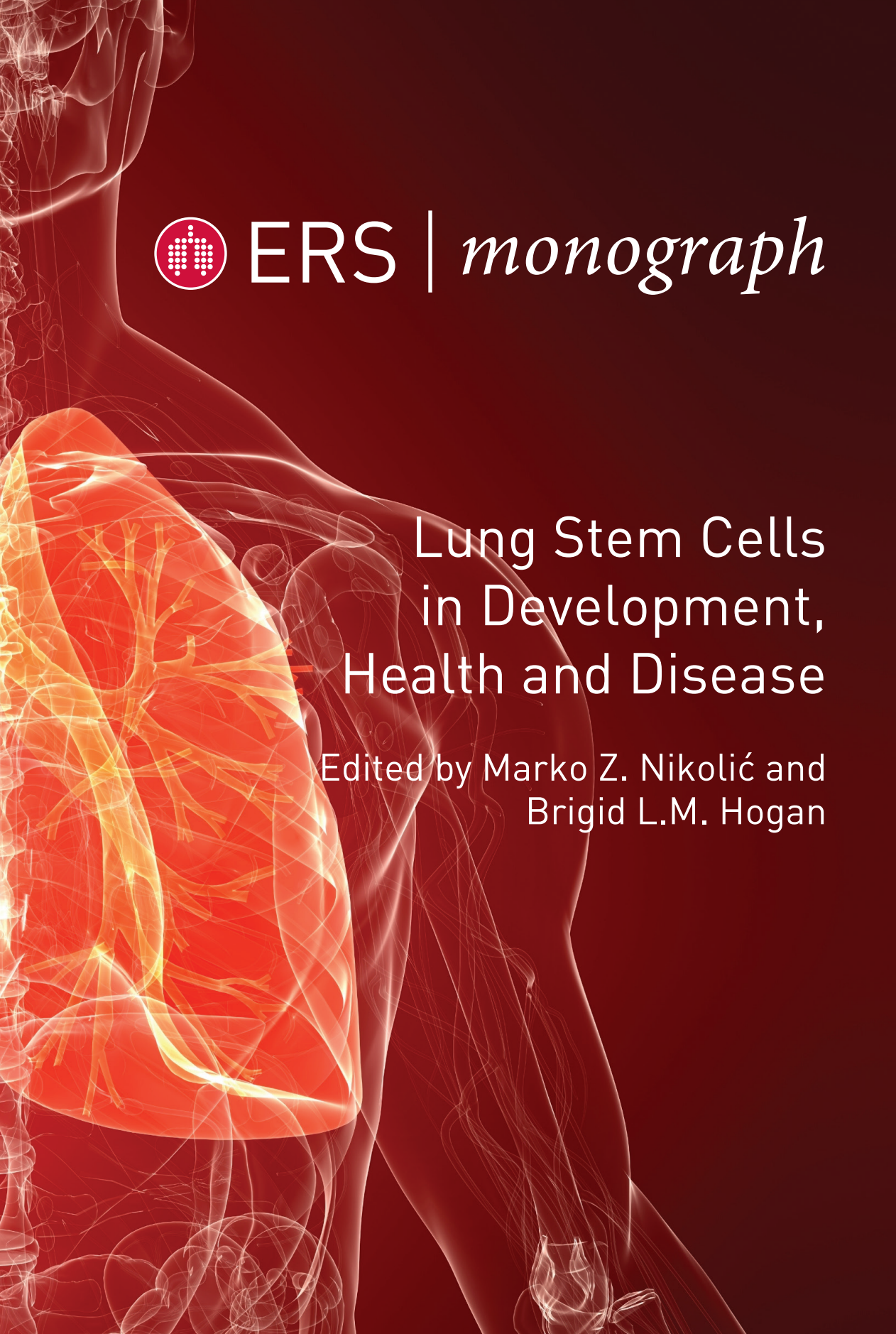

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Lung Stem Cells in Development, Health and Disease

Edited by Marko Z. Nikolić and Brigid L.M. Hogan

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Editor in Chief
John R. Hurst

This book is one in a series of *ERS Monographs*. Each individual issue provides a comprehensive overview of one specific clinical area of respiratory health, communicating information about the most advanced techniques and systems required for its investigation. It provides factual and useful scientific detail, drawing on specific case studies and looking into the diagnosis and management of individual patients. Previously published titles in this series are listed at the back of this *Monograph*.

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Preface

John R. Hurst

It is a privilege to introduce this latest *ERS Monograph*, on Lung Stem Cells in Development, Health and Disease.

The mission of the European Respiratory Society is to promote lung health in order to alleviate suffering from disease and drive standards for respiratory medicine globally. Science, education and advocacy are at the core of that mission. But it is only through development in basic science that we will make transformational discoveries to improve lung health. It has been an ambition of the Editorial Board to commission *Monographs* to address topics in respiratory science, not just clinical reviews, and this is the first of a new series.

I am particularly pleased, therefore, to see publication of a *Monograph* that will educate and inform you about one of the most exciting areas of respiratory science: lung stem cells. I warmly congratulate the Guest Editors, Marko Z. Nikolić and Brigid L.M. Hogan on commissioning and collating a comprehensive, state-of-the-art series of review articles from a diverse group of authors. It has been a pleasure to work with them both, and to see their proposal through to publication. Credit too, to the chapter authors: not just for the quality of their reviews, but also for sharing their enthusiasm and passion for respiratory science, which I hope will inspire others to pursue such avenues of research.

What will you find here? I can do no better than quote the Guest Editors' introduction: "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." Please, read on, I assure you that you will not be disappointed.

Disclosures: J.R. Hurst reports receiving grants, personal fees and non-financial support from pharmaceutical companies that make medicines to treat respiratory disease. This includes reimbursement for educational activities and advisory work, and support to attend meetings.

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Guest Editors

Marko Z. Nikolić

Marko Z. Nikolić is a UKRI (UK Research and Innovation) Innovation/Rutherford Fellow at University College London (London, UK) as part of the UK Regenerative Medicine Platform. After completing a Wellcome PhD Programme for Clinicians and a Clinical Lectureship at the Gurdon Institute in Cambridge (UK) with Emma Rawlins, he moved to University College London to set up his independent research group.

He is interested in developmental and stem cell biology in the context of lung regeneration, while also contributing to the Human Cell Atlas as a member of the HCA Lung Biological Network. He continues his clinical commitments as an Honorary Consultant in Respiratory Medicine at Royal Papworth (Cambridge, UK), Cambridge University Hospitals and University College London Hospitals Foundation Trusts.

Brigid L.M. Hogan

Brigid L.M. Hogan began her career in the UK as a developmental biologist where she made seminal discoveries about the genetic and cellular mechanisms controlling the formation of organ systems in the mammalian embryo. After moving to the USA in 1988, she began to focus more on the lung after initially being captivated by its branching morphogenesis. Her lab was among the first to use genetically engineered mice to identify and study stem and progenitor cells in the adult lung and their role in tissue maintenance and repair after injury. Her lab also developed some of the first lung organoids. Many of her trainees are now conducting innovative and translational research in the field of lung biology. From 2002 to 2019, Brigid was George Barth Geller Professor and Chair of the Department of Cell Biology at Duke University (Durham, NC, USA). She is a member of the National Academy of Medicine and the National Academy of Sciences in the USA and a Fellow of the Royal Society of London.

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Introduction

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

@ERSpublications
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

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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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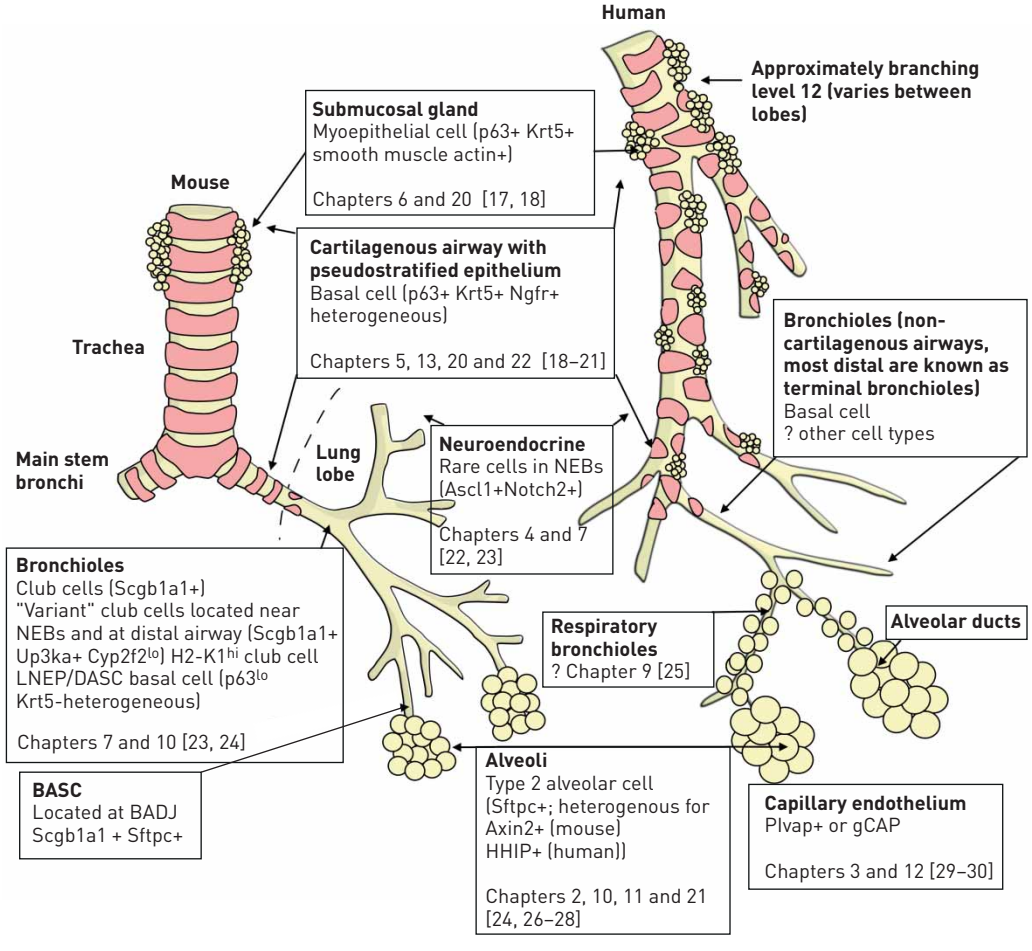

Figure 1 Schematic representation showing location of currently known stem cells in mouse and human adult lung. The intralobar human lung is shown from branch generation 12. A question mark indicates that it is currently not known whether such a stem cell type exists in the human lung. References can 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.

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Disclosures: None declared.

List of abbreviations

AEC1, AEC2	alveolar epithelial cell 1 and 2
ARDS	acute respiratory distress syndrome
ASM	airway smooth muscle
AT1, AT2	alveolar type 1, alveolar type 2
BADJ	bronchoalveolar duct junction
BASC	bronchoalveolar stem cell
BMP	bone morphogenetic protein
BPD	bronchopulmonary dysplasia
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CGRP	calcitonin gene-related peptide
COVID-19	coronavirus disease 2019
ECM	extracellular matrix
EGF	epidermal growth factor
FACS	fluorescence-activated cell sorting
FGF	fibroblast growth factor
HGF	hepatocyte growth factor
HSC	haematopoietic stem cell
IL	interleukin
IPF	idiopathic pulmonary fibrosis
iPSC	induced pluripotent stem cell
LPS	lipopolysaccharide
MSC	mesenchymal stromal cell
NEB	neuroepithelial body
NGFR	nerve growth factor receptor
PDGFR	platelet-derived growth factor receptor
PNEC	pulmonary neuroendocrine cell
RSV	respiratory syncytial virus
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
scRNA-seq	single-cell RNA sequencing
SDF	stromal cell-derived factor
SMG	submucosal gland
TASC	type 2-associated stromal cell
TGF	transforming growth factor
TNF	tumour necrosis factor
VEGF	vascular endothelial growth factor

Throughout the *Monograph* the term "bronchus" refers to an airway with cartilage support, while a "bronchiole" is a smaller diameter airway, without cartilage support.