Lung Biology in Health and Disease Volume 240
Executive Editor: Claude Lenfant

Bronchopulmonary Dysplasia

edited by
Steven H. Abman

informa
healthcare

Bronchopulmonary Dysplasia

LUNG BIOLOGY IN HEALTH AND DISEASE

Executive Editor

Claude Lenfant
Former Director, National Heart, Lung, and Blood
Institute National Institutes of Health
Bethesda, Maryland

1. Immunologic and Infectious Reactions in the Lung, *edited by C. H. Kirkpatrick and H. Y. Reynolds*
2. The Biochemical Basis of Pulmonary Function, *edited by R. G. Crystal*
3. Bioengineering Aspects of the Lung, *edited by J. B. West*
4. Metabolic Functions of the Lung, *edited by Y. S. Bakhle and J. R. Vane*
5. Respiratory Defense Mechanisms (in two parts), *edited by J. D. Brain, D. F. Proctor, and L. M. Reid*
6. Development of the Lung, *edited by W. A. Hodson*
7. Lung Water and Solute Exchange, *edited by N. C. Staub*
8. Extrapulmonary Manifestations of Respiratory Disease, *edited by E. D. Robin*
9. Chronic Obstructive Pulmonary Disease, *edited by T. L. Petty*
10. Pathogenesis and Therapy of Lung Cancer, *edited by C. C. Harris*
11. Genetic Determinants of Pulmonary Disease, *edited by S. D. Litwin*
12. The Lung in the Transition Between Health and Disease, *edited by P. T. Macklem and S. Permutt*
13. Evolution of Respiratory Processes: A Comparative Approach, *edited by S. C. Wood and C. Lenfant*
14. Pulmonary Vascular Diseases, *edited by K. M. Moser*
15. Physiology and Pharmacology of the Airways, *edited by J. A. Nadel*
16. Diagnostic Techniques in Pulmonary Disease (in two parts), *edited by M. A. Sackner*
17. Regulation of Breathing (in two parts), *edited by T. F. Hornbein*
18. Occupational Lung Diseases: Research Approaches and Methods, *edited by H. Weill and M. Turner-Warwick*
19. Immunopharmacology of the Lung, *edited by H. H. Newball*
20. Sarcoidosis and Other Granulomatous Diseases of the Lung, *edited by B. L. Fanburg*
21. Sleep and Breathing, *edited by N. A. Saunders and C. E. Sullivan*
22. *Pneumocystis carinii* Pneumonia: Pathogenesis, Diagnosis, and Treatment, *edited by L. S. Young*
23. Pulmonary Nuclear Medicine: Techniques in Diagnosis of Lung Disease, *edited by H. L. Atkins*
24. Acute Respiratory Failure, *edited by W. M. Zapol and K. J. Falke*

For information on volumes 25–186 in the *Lung Biology in Health and Disease* series, please visit www.informahealthcare.com

187. Oxygen/Nitrogen Radicals: Lung Injury and Disease, *edited by V. Vallyathan, V. Castranova, and X. Shi*
188. Therapy for Mucus-Clearance Disorders, *edited by B. K. Rubin and C. P. van der Schans*
189. Interventional Pulmonary Medicine, *edited by J. F. Beamis, Jr., P. N. Mathur, and A. C. Mehta*
190. Lung Development and Regeneration, *edited by D. J. Massaro, G. Massaro, and P. Chambon*
191. Long-Term Intervention in Chronic Obstructive Pulmonary Disease, *edited by R. Pauwels, D. S. Postma, and S. T. Weiss*
192. Sleep Deprivation: Basic Science, Physiology, and Behavior, *edited by Clete A. Kushida*
193. Sleep Deprivation: Clinical Issues, Pharmacology, and Sleep Loss Effects, *edited by Clete A. Kushida*
194. Pneumocystis Pneumonia: Third Edition, Revised and Expanded, *edited by P. D. Walzer and M. Cushion*
195. Asthma Prevention, *edited by William W. Busse and Robert F. Lemanske, Jr.*
196. Lung Injury: Mechanisms, Pathophysiology, and Therapy, *edited by Robert H. Notter, Jacob Finkelstein, and Bruce Holm*
197. Ion Channels in the Pulmonary Vasculature, *edited by Jason X.-J. Yuan*
198. Chronic Obstructive Pulmonary Disease: Cellular and Molecular Mechanisms, *edited by Peter J. Barnes*
199. Pediatric Nasal and Sinus Disorders, *edited by Tania Sih and Peter A. R. Clement*
200. Functional Lung Imaging, *edited by David Lipson and Edwin van Beek*
201. Lung Surfactant Function and Disorder, *edited by Kaushik Nag*
202. Pharmacology and Pathophysiology of the Control of Breathing, *edited by Denham S. Ward, Albert Dahan, and Luc J. Teppema*
203. Molecular Imaging of the Lungs, *edited by Daniel Schuster and Timothy Blackwell*
204. Air Pollutants and the Respiratory Tract: Second Edition, *edited by W. Michael Foster and Daniel L. Costa*
205. Acute and Chronic Cough, *edited by Anthony E. Redington and Alyn H. Morice*
206. Severe Pneumonia, *edited by Michael S. Niederman*
207. Monitoring Asthma, *edited by Peter G. Gibson*
208. Dyspnea: Mechanisms, Measurement, and Management, Second Edition, *edited by Donald A. Mahler and Denis E. O'Donnell*
209. Childhood Asthma, *edited by Stanley J. Szefler and Søren Pedersen*
210. Sarcoidosis, *edited by Robert Baughman*
211. Tropical Lung Disease, Second Edition, *edited by Om Sharma*
212. Pharmacotherapy of Asthma, *edited by James T. Li*
213. Practical Pulmonary and Critical Care Medicine: Respiratory Failure, *edited by Zab Mosenifar and Guy W. Soo Hoo*
214. Practical Pulmonary and Critical Care Medicine: Disease Management, *edited by Zab Mosenifar and Guy W. Soo Hoo*
215. Ventilator-Induced Lung Injury, *edited by Didier Dreyfuss, Georges Saumon, and Rolf D. Hubmayr*

216. Bronchial Vascular Remodeling in Asthma and COPD, *edited by Aili Lazaar*
217. Lung and Heart–Lung Transplantation, *edited by Joseph P. Lynch III and David J. Ross*
218. Genetics of Asthma and Chronic Obstructive Pulmonary Disease, *edited by Dirkje S. Postma and Scott T. Weiss*
219. *Reichman and Hershfield's* Tuberculosis: A Comprehensive, International Approach, Third Edition (in two parts), *edited by Mario C. Raviglione*
220. Narcolepsy and Hypersomnia, *edited by Claudio Bassetti, Michel Billiard, and Emmanuel Mignot*
221. Inhalation Aerosols: Physical and Biological Basis for Therapy, Second Edition, *edited by Anthony J. Hickey*
222. Clinical Management of Chronic Obstructive Pulmonary Disease, Second Edition, *edited by Stephen I. Rennard, Roberto Rodriguez-Roisin, Gérard Huchon, and Nicolas Roche*
223. Sleep in Children, Second Edition: Developmental Changes in Sleep Patterns, *edited by Carole L. Marcus, John L. Carroll, David F. Donnelly, and Gerald M. Loughlin*
224. Sleep and Breathing in Children, Second Edition: Developmental Changes in Breathing During Sleep, *edited by Carole L. Marcus, John L. Carroll, David F. Donnelly, and Gerald M. Loughlin*
225. Ventilatory Support for Chronic Respiratory Failure, *edited by Nicolino Ambrosino and Roger S. Goldstein*
226. Diagnostic Pulmonary Pathology, Second Edition, *edited by Philip T. Cagle, Timothy C. Allen, and Mary Beth Beasley*
227. Interstitial Pulmonary and Bronchiolar Disorders, *edited by Joseph P. Lynch III*
228. Chronic Obstructive Pulmonary Disease Exacerbations, *edited by Jadwiga A. Wedzicha and Fernando J. Martinez*
229. Pleural Disease, Second Edition, *edited by Demosthenes Bouros*
230. Interventional Pulmonary Medicine, Second Edition, *edited by John F. Beamis, Jr., Praveen Mathur, and Atul C. Mehta*
231. Sleep Apnea: Implications in Cardiovascular and Cerebrovascular Disease, Second Edition, *edited by Douglas T. Bradley and John Floras*
232. Respiratory Infections, *edited by Sanjay Sethi*
233. Acute Respiratory Distress Syndrome, *edited by Augustine M. K. Choi*
234. Pharmacology and Therapeutics of Airway Disease, Second Edition, *edited by Kian Fan Chung and Peter J. Barnes*
235. Sleep Apnea: Pathogenesis, Diagnosis, and Treatment, Second Edition, *edited by Allan I. Pack*
236. Pulmonary Hypertension, *edited by Marc Humbert and Joseph P. Lynch III*
237. Tuberculosis, Fourth Edition: The Essentials, *edited by Mario C. Raviglione*
238. Asthma Infections, *edited by Richard Martin and Rand E. Sutherland*
239. Chronic Obstructive Pulmonary Disease: Outcomes and Biomarkers, *edited by Mario Cazzola, Fernando J. Martinez, and Clive P. Page*
240. Bronchopulmonary Dysplasia, *edited by Steven H. Abman*

The opinions expressed in these volumes do not necessarily represent the views of the National Institutes of Health.

Bronchopulmonary Dysplasia

edited by
Steven H. Abman
University of Colorado
The Children's Hopsital
Aurora, Colorado, USA

informa
healthcare

New York London

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Version Date: 20130118

International Standard Book Number-13: 978-1-4200-7692-9 (eBook - PDF)

Introduction

Bronchopulmonary dysplasia (BPD) is a complex and challenging disease. Since its original description by William Northway and his colleagues in 1967, many significant advances have occurred. Yet, it remains a major cause of mortality and morbidity in premature neonates, the rates of which are inversely related to the birth weight.

This new volume, *Bronchopulmonary Dysplasia*, edited by Dr Steven Abman, presents the most recent progress to its readership. The role of genetic determinants and of individual and environmental factors in the occurrence and severity of BPD are extensively addressed throughout the chapters. Of great interest is the discussion of biomarkers in BPD, as eventually they may help predict the occurrence of the disease and thus lead to early application of therapeutic options. This has the potential to reduce mortality and may limit long-term consequences of the disease.

Recent studies underscore the significance of these consequences, that is, residual lung structural changes very similar to those caused by emphysema (1–3). This concept is not new as for years neonatologists and pediatricians have called BPD a "chronic lung disease of early infancy."

Today, chronic bronchitis and emphysema (currently termed chronic obstructive pulmonary disease—COPD) are recognized as major worldwide public health problems. Although smoking is undoubtedly the main risk factor, we are well aware of the occurrence of these diseases in nonsmokers. Thus, emphysema-like lesions and bronchial obstruction that develop in survivors of BPD underscore the need for extended basic and clinical research on BPD. Undoubtedly, as stated in the Preface, this volume will "stimulate the next generation of investigators and clinicians to further improve long-term outcomes of prematurely born infants."

As the executive editor of the series of monographs Lung Biology in Health and Disease, I express my gratitude to Dr Abman for giving us the opportunity to present this volume. Both neonatologists and pulmonologists will benefit from it but, more important, their patients will as well.

Claude Lenfant, MD
Vancouver, Washington, U.S.A.

References

1. Wong PM, Lees AA, Louw J, et al. Emphysema in young adult survivors of moderate-to-severe bronchopulmonary dysplasia. Eur Respir J 2008; 32(2):321–328 [Epub April 2, 2008].
2. Askin DF, Diehl-Jones W. Pathogenesis and prevention of chronic lung disease in the neonate. Crit Care Nurs Clin North Am 2009; 21(1):11–25.
3. Bourbon JR, Boucherat O, Boczkowski J, et al. Bronchopulmonary dysplasia and emphysema: in search of common therapeutic targets. Trends Mol Med 2009; 15(4): 169–179 [Epub March 18, 2009].

Preface

Over 40 years ago, Dr William Northway and his colleagues at Stanford described the clinical, radiologic, and pathologic features of a new disorder—bronchopulmonary dysplasia (BPD). Their comprehensive phenotyping of patients with chronic lung disease following premature birth included numerous insights into the pathogenesis of the disease, including mechanistic links with hyperoxia, ventilator-induced lung injury, inflammation and infection, and its time course. This insightful, landmark study laid a strong foundation and provided the basis for subsequent work in the field, setting the stage for numerous advances in neonatal respiratory care. After all these years, many of these basic observations remain central to our current understanding of BPD.

BPD remains a disease partly defined and altered by its treatments. Despite major differences in the nature of BPD that have followed the introduction of surfactant therapy, prenatal steroids, and changes in neonatal care, BPD remains a major cause of neonatal morbidity and mortality. As survival of the tiniest of premature babies increases, many infants still develop significant impairment of lung function, leading to prolonged ventilator and NICU courses, frequent hospitalizations after NICU discharge, recurrent respiratory exacerbations, and problems with late cardiorespiratory diseases.

Nearly 10 years ago, Drs Richard Bland and Jacqueline Coalson edited an important volume that highlighted the current state of the art in our understanding of BPD (1). This book provided an important resource for basic scientists, clinician-scientists, and practicing clinicians alike. Chapters provided historical perspectives on BPD, highlighted basic mechanisms of lung development, characterized the clinical course and treatment strategies of premature infants at risk for developing BPD or with established BPD, and related topics. Their book especially highlighted the changing epidemiology and clinical course of premature newborns with BPD in the post-surfactant era, providing in-depth reviews of normal lung development, mechanisms of lung injury and repair, and clinical and pathologic features of the disease. In addition to its state-of-the-art reviews, this previous volume was especially useful for helping to define persistent questions and challenges for laboratory investigators and clinicians to better understand and treat premature newborns at risk for BPD.

Over the past decade, much work has been done to address the many challenges of premature birth and to further extend our understanding of BPD. The purpose of this current book is to simply provide an update of recent progress made in this field, and to once again raise new questions worthy of pursuit in the laboratory and clinical settings. The first section presents new advances in lung development, including recent insights into molecular pathways and the integration of growth factors, transcription factors, and cell-cell interactions in this process. The second section highlights mechanisms of lung injury and repair that disrupt normal lung airspace and vascular growth, and lead to the abnormalities of lung structure and function that characterize BPD. The third section highlights novel insights into clinical aspects of BPD, including new information on the genetic basis for BPD, its changing epidemiology, clinical course, and physiology, and updated reviews of its treatment. Finally, the fourth section presents an update on emerging therapies for the prevention of BPD, based on recent preclinical studies and multicenter randomized clinical trials.

Overall, this volume provides new insights into the pathobiology and treatment of BPD. I dedicate this book to our patients and their families, and hope that this work will help stimulate the next generation of investigators and clinicians to further improve long-term outcomes of prematurely born infants.

In addition, I would like to personally thank and acknowledge support in my career as provided by "mentors from afar," including Alan Jobe, Eduardo Bancalari, Richard Bland, and Marlene Rabinovitch, as well as local friends and colleagues who keep me in line, especially David Cornfield, John Kinsella, Vivek Balasubramaniam, Jason Gien, and Peter Mourani. Finally, I thank my family (Carolyn, Ryan, Lauren, Mark, and Megan) for their loving support.

Steven H. Abman, MD

Reference

1. Bland RD, Coalson JJ. Chronic Lung Disease in Early Infancy. New York: Marcel Dekker, 2000.

Contributors

Steven H. Abman Pediatric Heart Lung Center, Sections of Critical Care and Pulmonary Medicine, Department of Pediatrics, University of Colorado Denver, School of Medicine and The Children's Hospital, Aurora, Colorado, U.S.A.

Namasivayam Ambalavanan University of Alabama at Birmingham, Birmingham, Alabama, U.S.A.

Richard L. Auten Duke University Medical Center, Durham, North Carolina, U.S.A.

Philip L. Ballard University of California at San Francisco, San Francisco, California, U.S.A.

Roberta A. Ballard University of California at San Francisco, San Francisco, California, U.S.A.

Eduardo Bancalari University of Miami Miller School of Medicine, Miami, Florida, U.S.A.

Eugenio Baraldi University of Padua, School of Medicine, Padua, Italy

Vineet Bhandari Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Olivier Boucherat Centre de Recherche en Cancérologie de l'Université Laval, Centre Hospitalier Universitaire de Québec, L'Hôtel-Dieu de Québec, Québec, Canada

Jacques R. Bourbon INSERM, Unité 955-Institut Mondor de Recherche Biomédicale, Faculté de Médecine, Université Paris 12, Créteil, France

Joy V. Browne University of Colorado Denver School of Medicine and The Children's Hospital, Aurora, Colorado, U.S.A.

Waldemar Carlo University of Alabama at Birmingham, Birmingham, Alabama, U.S.A.

Robert G. Castile The Ohio State University, Columbus, Ohio, U.S.A.

Nelson Claure University of Miami Miller School of Medicine, Miami, Florida, U.S.A.

Jonathan M. Davis Tufts University School of Medicine, Boston, Massachusetts, U.S.A.

Christophe Delacourt INSERM, Unité 955-Institut Mondor de Recherche Biomédicale, Faculté de Médecine, Université Paris 12, Créteil, France

Marco Filippone University of Padua, School of Medicine, Padua, Italy

Neil N. Finer University of California, San Diego, San Diego, California, U.S.A.

Emily Fox Hospital for Sick Children, Toronto, Ontario, Canada

Anne Greenough Division of Asthma, Allergy & Lung Biology, MRC-Asthma U.K. Centre in Allergic Mechanisms of Asthma, King's College London, London, U.K.

Henry L. Halliday Queen's University Belfast and Royal Maternity Hospital, Belfast, Northern Ireland, U.K.

Alison A. Hislop Institute of Child Health, University College London, London, U.K.

David A. Ingram Herman B Wells Center for Pediatric Research, Indianapolis, Indiana, U.S.A.

Robert P. Jankov University of Toronto, Toronto, Ontario, Canada

Alan H. Jobe University of Cincinnati, Cincinnati, Ohio, U.S.A.

Suhas Kallapur University of Cincinnati, Cincinnati, Ohio, U.S.A.

John P. Kinsella The Children's Hospital and University of Colorado School of Medicine, Aurora, Colorado, U.S.A.

Stella Kourembanas Harvard Medical School, Boston, Massachusetts, U.S.A.

Boris W. Kramer Academisch Ziekenhuis Maastricht, Maastricht, The Netherlands

Tina A. Leone University of California, San Diego, San Diego, California, U.S.A.

William M. Maniscalco University of Rochester School of Medicine, Rochester, New York, U.S.A.

Peter M. Mourani Pediatric Heart Lung Center, Sections of Critical Care and Pulmonary Medicine, Department of Pediatrics, University of Colorado Denver, School of Medicine and The Children's Hospital, Aurora, Colorado, U.S.A.

Leif D. Nelin The Ohio State University, Columbus, Ohio, U.S.A.

Sanna Padela University of Toronto, Toronto, Ontario, Canada

Richard Parad Harvard Medical School, Boston, Massachusetts, U.S.A.

Martin Post Hospital for Sick Children, Toronto, Ontario, Canada

Rebecca S. Rose Indiana University, Indianapolis, Indiana, U.S.A.

Ola Didrik Saugstad University of Oslo, Oslo, Norway

Michael D. Schreiber University of Chicago, Chicago, Illinois, U.S.A.

Wei Shi Developmental Biology and Regenerative Medicine Program, Saban Research Institute, Children's Hospital Los Angeles, Keck School of Medicine and School of Dentistry, University of Southern California, Los Angeles, California, U.S.A.

Christian P. Speer University Children's Hospital, Würzburg, Germany

A. Keith Tanswell University of Toronto, Toronto, Ontario, Canada

Bernard Thébaud University of Alberta, Edmonton, Alberta, Canada

Linda J. Van Marter Harvard Medical School, Boston, Massachusetts, U.S.A.

Maximo Vento Hospital Universitario Materno Infantil La Fe, Valencia, Spain

Michele C. Walsh Rainbow Babies and Children's Hospital, Case Western Reserve University School of Medicine, Cleveland, Ohio, U.S.A.

David Warburton Developmental Biology and Regenerative Medicine Program, Saban Research Institute, Children's Hospital Los Angeles, Keck School of Medicine and School of Dentistry, University of Southern California, Los Angeles, California, U.S.A.

Kristi Watterberg University of New Mexico, Albuquerque, New Mexico, U.S.A.

Carl White National Jewish Medical and Research Center, Denver, Colorado, U.S.A.

Mervin C. Yoder Herman B Wells Center for Pediatric Research, Indianapolis, Indiana, U.S.A.

Contents

1
Genetic Mechanisms of Lung Development and Bronchopulmonary Dysplasia: An Integrative View

DAVID WARBURTON and WEI SHI
Developmental Biology and Regenerative Medicine Program, Saban Research Institute, Children's Hospital Los Angeles, Keck School of Medicine and School of Dentistry, University of Southern California, Los Angeles, California, U.S.A.

I. Lung Development and Bronchopulmonary Dysplasia

The major function of the human neonatal lung is to rapidly clear fluid from the airways and to begin to exchange gas. The gas diffusion surface of the mature human neonatal lung has a honeycomb-like structure, comprising extensively branched, perfectly matched ducts for air and blood. This configuration maximizes the gas exchange surface area between air and blood, and facilitates maximally efficient packing within the chest cavity. In humans, the gas exchange membrane, which is about 1-μm thick, consists of type I alveolar epithelial cells (AECI), basement membrane, and endothelial cells, with a total surface area that increases in the adult to about 70 m^2. This vast and complex structure is developed sequentially by early epithelial tube branching and late septation of terminal air sacs. Perturbation of this developmental process results in abnormal lung structure and, hence, deficiency of the gas exchange function. Thus, premature human delivery interrupts this developmental process, resulting in an injury response phenotype that depends on the stage of lung maturity at the time of delivery. Northway et al. (1) coined the term *bronchopulmonary dysplasia (BPD)* to label the clinical, radiographic, and pathological features of chronic airway obstruction (broncho) and interstitial lung disease (pulmonary) with emphysema-like alveolar destruction and abnormal peripheral lung development (dysplasia). In those now far-off days respiratory distress syndrome (RDS) due to a combination of delayed fluid clearance, structural immaturity, and surfactant deficiency of the premature lung with the radiographic appearance of hyaline membrane disease were recognized as the major etiological factors from about 32 up to 36 weeks' gestation. Supportive treatment (oxygen plus pressure plus time) (2) plus fluid overload and left-to-right shunting through a patent ductus arteriosus (3) were recognized early on as the key postnatal, mostly iatrogenic, factors. With the widespread implementation of prophylactic artificial surfactant therapy, coupled with improvements in neonatal care including control of thermoregulation, judicious fluid therapy, gentler ventilation, and so on, the threshold for survival in human prematurity moved steadily downward toward 24 weeks' gestation. In these extremely premature infants, alveolarization has barely started, and accordingly, the critical feature of the "new BPD" was recognized to be alveolar hypoplasia (4). This chapter provides an overview of the

integrated genetic and molecular processes that drive lung development and discusses concisely how lung injury, repair, and regeneration impact them in the context of BPD.

II. Early Lung Development: The Bauplan of the Lung

The lung originates from the ventral surface of the primitive foregut at five weeks' gestation in human. The lung anlage emerges as the laryngotracheal groove, located in the ventral foregut endoderm, which invaginates into the surrounding splanchnic mesenchyme (Fig. 1). Then a pair of primary buds evaginate from the laryngotracheal groove. The respiratory tree then develops by branching morphogenesis, in which reiterated outgrowth, elongation, and subdivision of these epithelial buds occur in a bilaterally asymmetrical pattern. Three lobes on the right side and two lobes on the left side are formed in human lung. There are 23 generations of airway branching in human. The first 16 generations branch stereotypically and are thus highly reproducible, and this is completed by 16 weeks' gestation, whereas the remaining 7 generations are random and are completed by about 20 to 24 weeks' gestation. Alveolarization begins around 20 weeks' gestation in human and continues postnatally at least until 7 years of age.

A. Genes Controlling Induction of the Early Lung

Early lung induction is under control of many gene products that act cooperatively to precisely define the location of laryngotracheal groove formation and specify proximal-distal, dorsal-ventral, and left-right axes of the developing lung. The earliest endodermal signals essential for gut morphogenesis and gut tube closure are the GATA (zinc finger proteins that recognize GATA DNA sequence) and hepatocyte nuclear factor (HNF/Fox) transcription factors. Foxa2 is required for gut tube closure, whereas GATA-6 is required for activation of the lung developmental program in the foregut endoderm. *Hnf-3/Foxa2β* is a survival factor for the endoderm, and its expression is induced by Sonic hedgehog (*Shh*) signaling. Also, *Tbx4* can induce ectopic bud formation in the esophagus by activating the expression of *Fgf10* (5). In addition, left-right asymmetry is controlled by several gene products including *nodal*, *Lefty-1,2*, and *Pitx-2*. For example, single-lobed lungs are found bilaterally in *Lefty-1*$^{-/-}$ mice, and isomerism of lung is found in Pitx2 null mutants. Retinoids and their transcriptional factor receptors also play key roles in induction of the early lung branching process.

B. Complexities of Distal Airway Branching: Some Simplifying Concepts

At first sight, intrapulmonary airway branching in the developing lung distal to the primary bronchi appears to become increasingly complicated as it proceeds distally and the number of individual branches increases into the millions. But, once the laryngotracheal complex and left-right laterality are established, distal airway branching is thought to be driven by a master branch generator routine, with three slave subroutines instructing a periodicity clock that times the appearance of subsequent branches, a rotational orientation subroutine that determines the orientation of the branches around the axis of the airway, and finally, a bifurcation subroutine (6,7). Thus, branching morphogenesis of the bronchi in early mouse embryo lung can be parsed anatomically into three simple geometric forms, termed domain branching, planar, and orthogonal

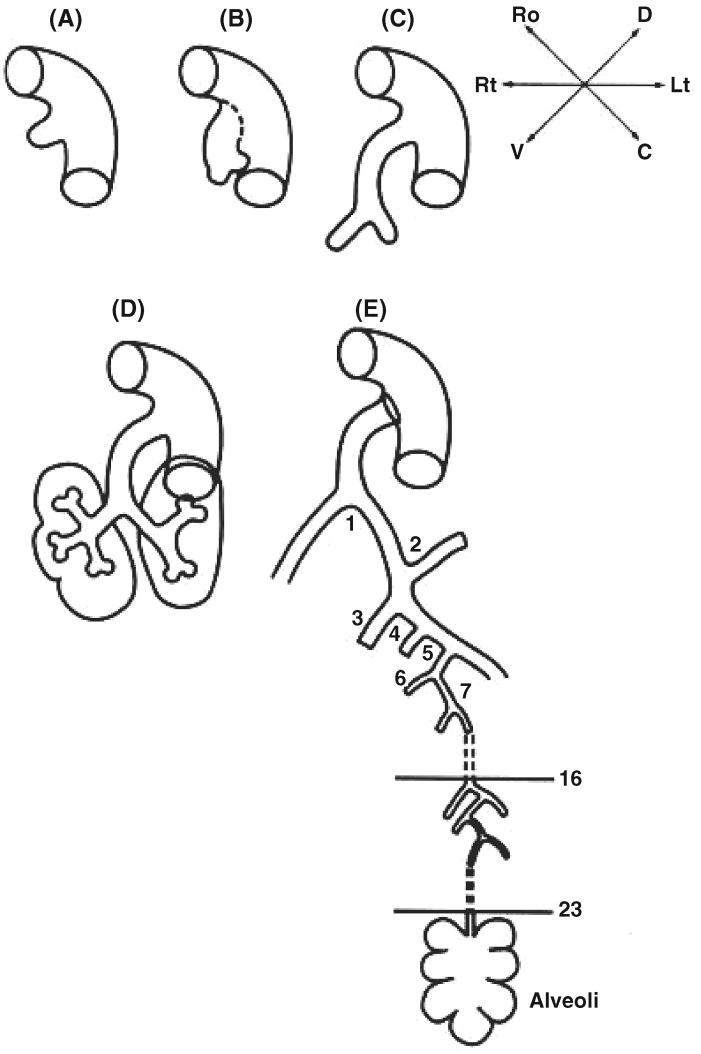

Figure 1 Diagrams showing key events in human lung morphogenesis. (**A**) The primitive lung anlage emerging as the laryngotracheal groove from the ventral surface of the primitive foregut at five weeks' gestation in human. (**B**) The primitive trachea separating dorsoventrally from the primitive esophagus as the two primary bronchial branches arise from the lateral aspects of the laryngotracheal groove at 5 ± 6 weeks' gestation in human. (**C**) The embryonic larynx and trachea with the two primary bronchial branches separated dorsoventrally from the embryonic esophagus at six weeks in human. (**D**) The primitive lobar bronchi branching from the primary bronchi at seven weeks in human. (**E**) A schematic rendering of the term fetal airway in human. The stereotypically reproducible, first 16 airway generations are complete by 16 weeks in human. Between 16 and 23 weeks, the branching pattern is random and is completed by about 24 weeks in human. Alveolarization begins after about 20 weeks in human and is complete by 7 years of age at the earliest.

bifurcation. These basic forms are repeated iteratively to form different arrangements of branches. These arrangements have been termed bottlebrush array, planar array, and rosette array. The bottlebrush array describes the sequential proximal to distal emergence of secondary branches along the airway. The bottlebrush mechanism can be reoriented to form a second row of branches at right angles to the first row. The planar and rosette arrays describe the patterns formed by sequential bifurcation of the tip of secondary, tertiary, and subsequent buds at right angles to each other. Repetition of these simple branching modules together with the hierarchical control and coupling of them may therefore explain how the genome could encode the highly complex yet stereotypic pattern of early bronchial branch formation using a relatively simple toolbox of genetic modules. Genes that drive these specific processes are discussed later on in this chapter.

C. The Impact of Hydraulic Pressure and Airway Peristalsis

The embryonic lung is filled with liquid. Active chloride secretion through the cystic fibrosis transmembrane conductance regulator (CFTR) and other chloride ion channels attracts sodium and thus creates an osmotic gradient, which draws water into the lumen. Lung liquid is produced from the earliest stages of embryonic lung development up to delivery. The hydraulic pressure within the lung lumen is determined by the rate of production of liquid together with the pinch-cock valve function of the primitive larynx. Obstruction of fluid outflow by clipping or cauterization of the embryonic trachea increases the intraluminal pressure by about two- to threefold. This is accompanied by a threefold increase in branching of the airway. Most notably, the rate of bud extension increases by about twofold whereas the interbud distance is halved. These effects of increased intraluminal pressure depend on FGF10-FGFR2b-Sprouty signaling (8). Several clinical trials have been made to determine whether tracheal obstruction to increase intraluminal pressure can accelerate fetal lung maturation in human fetuses with congenital diaphragmatic hernia. However, clinical opinion remains divided on whether there is any therapeutic role for this highly risky intervention. Nevertheless, the connection between physical force, morphogenetic signaling, and lung development is clearly important, and nowhere this is more evident than in BPD where barotrauma clearly plays a major role in the etiology. The discovery that waves of airway peristalsis that are calcium-driven and originate in a rhythm generator in the proximal airways also play a key role in embryonic lung development suggests that airway smooth muscle has an essential function even very early in lung development.

D. Coupling of Endothelial (Blood and Lymphatic Capillaries) with Epithelial Morphogenesis

Tight coupling of endothelial with epithelial development is also clearly important for efficient gas transport and fluid clearance at birth. Capillary plexi surround the primitive airway from the earliest stages of development. Vascular endothelial growth factor (VEGF) signaling from the epithelium to the developing capillary endothelium is essential for the development of the primitive capillary hemangioblasts into mature networks of capillary endothelium. Likewise, the endothelium signals back to the epithelium to coordinate morphogenesis of these tissue compartments. There is a constant stereotypic anatomical relationship between the developing pulmonary capillaries, arteries, and veins (Fig. 2).

The arteries run along the superior surface of the developing lobules, whereas the veins run along the interior surface. The lymphatics also develop under the control of the

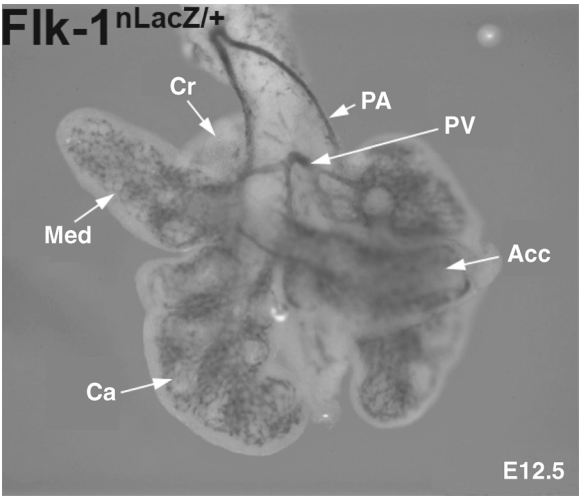

Figure 2 Blood vessels of the developing lung are shown at E12.5 in a special transgenic mouse. The blue signal is generated by staining for LacZ, driven in endothelial cells by the Flk-1 (VEGF receptor) promoter. *Source*: Artwork by Pierre del Moral. *Abbreviations*: PA, pulmonary artery; PV, pulmonary vein; Cr, cranial lobe; Med, median lobe; Ca, caudal lobe; Acc, accessory lobe of right lung.

VEGFR3 isoform of VEGF receptor and play a key role in regulating lung interstitial liquid content. Null mutation of VEGR3 markedly retards mobilization of fetal lung liquid at birth because of lymphatic hypoplasia, leading to failure of lymphatic drainage of interstitial fluid.

III. Histological Stages in Lung Development

Histologically, lung development and maturation has been divided into four stages: the pseudoglandular stage, the canalicular stage, the terminal sac stage, and the alveolar stage (Fig. 3).

A. The Pseudoglandular Stage (5–17 Weeks of Human Pregnancy, Embryonic E9.5–16.6 Days in Mouse Embryo)

This is the earliest lung development stage in which the embryonic lung undergoes branching morphogenesis developing epithelial tubular structures with lining cuboidal epithelial cells that resemble an exocrine gland. However, this fluid-filled respiratory tree structure is too immature to perform efficient gas exchange.

B. The Canalicular Stage (16–25 Weeks of Human Pregnancy, E16.6–E17.4 Days in Mouse Embryo)

The cranial part of the lung develops relatively faster than the caudal part, resulting in partial overlap between this stage and the previous stage. During the canalicular stage,

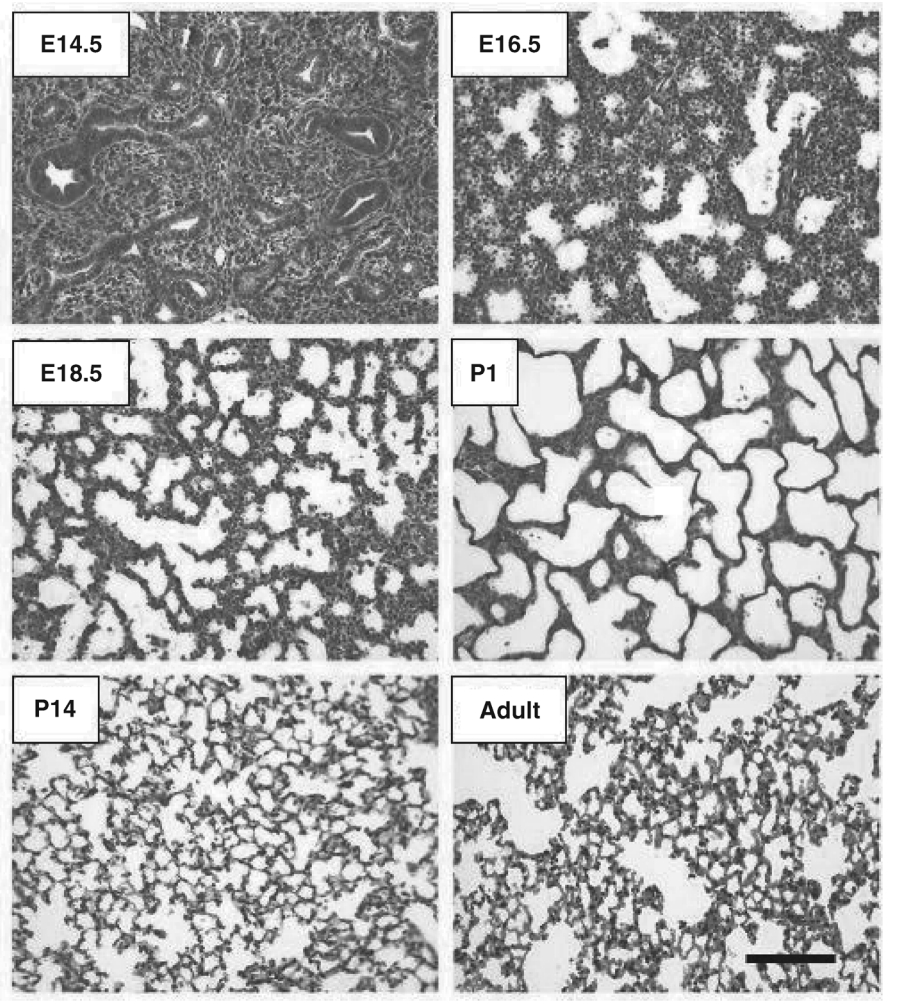

Figure 3 Histology of mouse lungs at various stages of development. Embryonic mouse lung develops from early pseudoglandular stage (E14.5) to canalicular stage (E16.5) and further terminal sac stage (E18.5 and P1). Neonatal lungs undergo further alveolarization, resulting in many septa formation (P14). Finally, a mature honeycomb-like structure with normal respiratory function is formed, as observed in adult. Scale bar: 100 μm.

the respiratory tree is further expanded in diameter and length, accompanied by vascularization and angiogenesis along the airway. A massive increase in the number of capillaries occurs. The terminal bronchioles are then divided into respiratory bronchioles and alveolar ducts, and the airway epithelial cells are differentiated into peripheral squamous cells and proximal cuboidal cells.

C. The Terminal Sac Stage [24 Weeks to Late Fetal Period in Human, E17.4 to Postnatal Day 5 (P5) in Mouse]

There is substantial thinning of the interstitium during the terminal sac stage. This results from apoptosis as well as ongoing differentiation of mesenchymal cells (9,10). Additionally, at this stage, the AEC are more clearly differentiated into mature squamous type I pneumocytes and secretory rounded type II pneumocytes. The capillaries also grow rapidly in the mesenchyme surrounding the saccules to form a complex network. In addition, the lymphatic network in lung tissue becomes well developed. The thick wall of these saccules, also called primary septae, comprises lining epithelial cells on both sides of a connective tissue core, within which there is a double parallel network of capillaries. Toward the end of this stage, the fetal lung can support air exchange in prematurely born human neonates. Although human premature infants can breathe with the lung that has developed to the end of terminal sac stage, the immature lung is nevertheless vulnerable to hyperoxic injury and barotrauma, resulting in the alveolar hypoplasia phenotypes described as new BPD. Maturation of surfactant synthesis and secretion is a key factor in determining whether the newborn lung can sustain gas exchange without collapsing. Another key factor is the rapid switch from chloride ion-driven fluid secretion into the airway to sodium-driven uptake of fluid out of the airway. This latter is driven by the birth response of the adrenergic system to cord cutting at birth.

How lung development is controlled at this stage is still incompletely known. The hydrostatic pressure inside the lumen of the airway, which is due to chloride ion and hence liquid secretion from epithelium in the developing lung (11–13), integrated with chemotactic signals from the mesenchyme such as FGF10 play important roles in forming terminal sacs. Mechanical factors also play an important role. Diaphragm muscle in $MyoD^{-/-}$ mice is significantly thinned and cannot support fetal breathing movements. As a result, lung is hypoplastic, and the number of proliferating lung cells is decreased in $MyoD^{-/-}$ lungs at E18.5. Therefore, mechanical forces generated by contractile activity of the diaphragm muscle play an important role in normal lung growth at this stage (14).

D. The Alveolar Stage (Late Fetal Period to Childhood in Human, P5–P30 in Mouse)

Alveolization is the last stage of lung development. The majority of the gas exchange surface is formed during this stage. Alveolarization can be positively and negatively influenced by many exogenous factors including oxygen concentration, stretch in fetal airway, dexamethasone, and retinoic acid.

Forming new septa within terminal sacs is the key step for differentiation of the saccule into alveoli. This involves a complex interaction between myofibroblasts in the mesenchyme, adjacent airway epithelial cells, and vascular endothelial cells. Controlled multiplication and differentiation as well as migration of the myofibroblast progenitor cells within terminal sac walls are important for new septa formation. Myofibroblasts, smooth muscle precursor cells having the morphology of fibroblasts, migrate to the proper position within nascent alveolar septa, and synthesize and deposit elastin (15,16). This is the first step of new secondary septa development (16). Platelet-derived growth factor-A (PDGF-A) and its receptor PDGF-α play important roles in forming new septa.

Pdgf-a$^{-/-}$ or *Pdgf-*$\alpha^{-/-}$ shows a phenotype comprising loss of alveolar myofibroblasts and elastin, failure of alveolar septation, and this develops into emphysema due to alveolar hypoplasia (15,17). Besides PDGF-A, there are some other key proteins that mediate cell-cell interaction within terminal sac walls. For example, Roundabout (ROBO) is a receptor known to be involved in repellent signaling controlling axonal extension in the developing neuronal system. ROBO and its ligand SLIT are also involved in the regulation of nonneuronal cell migration (18). In E18, one day before birth, *Slit-2* is expressed in the saccular mesenchyme surrounding the airways. At the same time, *Robo* is expressed on the apical aspects of the airway epithelium adjacent to the ligand *Slit-2*, suggesting interactive roles in pulmonary bronchiolar development (19). A *Robo* knockout mouse has been shown to have loss of septation and thickened mesenchyme (20). In addition, transforming growth factor β (TGF-β)-Smad3 signaling in peripheral lung epithelial cells is also essential for secondary alveolar septa formation (21). Abrogation of TGF-β type II receptor in lung epithelial cells results in reduction of AECI and alveolar formation (22).

E. Alveolar Septum Formation

Two additional processes are necessary in septum differentiation to form a septum with final mature morphology and function. One is the thinning out of the septal mesenchyme and the other is the maturation of the double capillary network into a single capillary bed. Thinning of the mesenchymal tissue involves apoptosis of "unwanted" cells in the postnatal lung mesenchyme. There is a substantial reduction in the number of interstitial myofibroblasts resulting from increased apoptosis during this phase of rapid alveolarization (23,24). The immature lung contains at least two morphologically distinct fibroblast populations, lipid-filled interstitial fibroblasts (LFIF) and non-LFIF (NLFIF). After alveolarization, apoptosis occurs preferentially in only one of these fibroblast populations, the LFIF. Apoptosis was correlated with downregulation of insulin-like growth factor I receptor (*Igf-IR*) mRNA and cell surface protein expression (25). This thinning of the previously thickened immature interstitium occurs simultaneously with the ongoing expansion of the epithelial, blood vessel, and airspace compartments in the rapidly developing septa. A mature capillary bed is also vital for the proper function of alveoli, but the mechanism is still incompletely known. In the developing lungs, VEGF isoforms and their receptors have been identified as being important for endothelial survival and proliferation in the alveolar wall. Inhibition of VEGF signaling results in abnormal lung vascular growth and reduced alveolarization (26). Finally, the new septum differentiates into a functional respiratory membrane that consists of AECI, basement membrane, and capillary endothelial cells. The respiratory membrane provides a short distance for gases diffusion and thus facilitates optimal gas exchange. It is estimated that about 50 million alveoli are present in neonatal lung at term. However, by age seven to eight years, when the alveolarization is substantially complete, the number of alveolar units in the lung has grown about six times, to about 300 million alveoli.

Retinoid acid (RA) has been shown to increase the number of alveoli (27) and can partially rescue a block in alveolar formation induced by dexamethasone (28). In adult rats, RA has also been reported to reverse the anatomical features of elastase-induced emphysema, in which there is destruction of septa (27). In the *RAR-*γ gene deletion mouse, there is a developmental defect in alveolar formation most consistent with a

defect in elastin deposition. The additional deletion of one retinoid X receptor (*RXR*α) allele results in a decrease in alveolar surface area and alveolar number (29). Retinoids affect multiple cellular functions that are involved in alveolar septal formation such as proliferation, migration, and temporal differentiation of cells (30). Retinoic acid is an active metabolite of vitamin A. Vitamin A deficiency has long been known to injure lungs and impairs function of rat type 2 pneumocytes (29). Taken together, the evidence suggests that RA may play an important role in alveolar development. For more on RA signaling, see following text.

IV. Specific Cell Types in the Lung

More than 40 specific types of cells are differentiated during embryonic lung development. The epithelial cell lineages are arranged in a distinct proximal-distal spatial pattern in the airways.

Cartilage lies outside the submucosa and decreases in amount as the caliber of the bronchi decreases. Cartilage is present in the bronchi, but not in the bronchioles. Two major cell components of the proximal bronchial epithelium are identified: pseudostratified ciliated columnar cells and mucous (goblet) cells. Both of them are derived from basal cells, but ciliated cells predominate in number. Goblet cells release mucus granules into the bronchial lumen to prevent drying of the walls and traps particulate matter. Mucous cells begin to mature around 13 weeks' gestation in humans, when the mature ciliated columnar cells are already present. The molecular markers for mucous cells are mucins (MUC5B, 5A, 5C). The beating of cilia results in a cephalad movement of the mucus blanket, thereby cleaning and protecting the airway. In the case of cystic fibrosis, cilia movement is disabled due to the thick mucous layer that is caused by mutation of the *Cftr* gene, which encodes a transmembrane Na^+ ion transporter protein. This phenotype also makes the airway surface vulnerable to microbial infection. In chronic airway injury, both repair and fully experimental exposure of the epithelium to IL-9 before it becomes fully differentiated result in goblet cell hyperplasia. Exposure to IL-9 resulted in increased lysozyme and mucus production by the epithelia (31). IL-4, IL-13, and allergens enhance the release of TGF-α, which is a ligand for the epidermal growth factor receptor (EGFR) that also stimulates fibroblast proliferation and goblet cell differentiation (32).

There are three different types of cells in bronchial submucosal glands: myoepithelial cells surround the gland, whereas mucous cells (pale cytoplasm) and serous cells (basophilic cytoplasm) produce mucins. These secreted mucins mix with lysozyme and IgA on airway surface.

Kulchitsky cells are also found on the airway surface next to bronchial glands. Their precise function is unclear. It is believed that they are pulmonary neuroendocrine cells that produce a variety of peptide hormones such as serotonin and calcitonin. Their fingerlike cytoplasmic extensions usually reach the airway lumen. Kulchitsky cells expressing the markers gastrin-releasing peptide, calcitonin gene-related peptide, and chromogranin may be related to certain lung neoplasms (i.e., small cell carcinoma and carcinoid tumors). However, pulmonary neuroendocrine cells differentiate earlier by 10 weeks of gestation in humans and are also the first airway epithelial cells to be fully differentiated in mouse.

Clara cells are found in the distal bronchiolar airway epithelium that normally lacks mucous cells. They produce a mucus-poor, watery proteinaceous secretion. They assist with clearance and detoxification, as well as reduction of surface tension in small airways. The most important cellular marker of Clara cell is Clara cell–specific protein (CC10, CCSP, or uteroglobin). Cytochrome P450 reductase and CC10 can also be used as cellular markers for Clara cells. Clara cells begin to mature during the 19th week in humans. In normal mice, only a small number of mucin-positive cells are present in the airway. However, numerous mucous cells that are derived from Clara cells with excessive mucin production or reduced mucin secretion can be detected during mucous metaplasia.

The majority of the alveolar surface is normally covered by type I epithelial cells. These flat cells are believed to be terminal differentiated cells, expressing several specific molecular markers such as T1α and aquaporin 5. T1α is a differentiation marker gene of lung alveolar epithelial type I cells. It is developmentally regulated and encodes an apical membrane protein of unknown function. In the absence of T1α protein, type I cell differentiation is blocked. Homozygous T1α null mice die at birth of respiratory failure, and their lungs cannot be inflated to normal volumes (33). Aquaporin 5 is a water channel in type I epithelial cells.

Type I epithelial cells only account for 40% of the total airway epithelial cells, even though 95% of the surface area of the alveolar wall is covered by this type of cells. The remaining 60% of the epithelial cells are rounded cells that cover only 3% of the alveolar surface, named type II pneumocytes.

Type II pneumocytes are plump or cuboidal and have a finely stippled cytoplasm and surface microvilli. They manufacture surfactant phospholipids and proteins that reduce the surface tension in the lung. This equalizes pressures, stabilizes and maintains alveoli in an open position despite the variation in alveolar size, and prevents atelectasis at end-expiration. Four surfactant proteins, SP-A, -B, -C, and -D, also play critical roles in maintaining lung function. SP-A and -D participate in host defense in the lung, whereas SP-B and -C contribute to the surface tension–lowering properties of the lipoprotein complex termed pulmonary surfactant (34). Type II cells are capable of regeneration and replacement of type I cells after injury. A commonly used cellular marker of type II cell is SP-C (*SftpC*).

Alveolar macrophages constitute a small percentage of the cells in alveoli, but they represent a major cellular sentinel of the host defense mechanism in the alveolar space. They are part of the mononuclear phagocyte system and are derived primarily from blood monocytes. However, once they get into the lung, their turnover rate is extremely slow.

V. Stem and Progenitor Cells in the Respiratory System

Respiratory stem and progenitor cells have important functions in repairing damaged trachea, bronchi, bronchioles, and alveoli. However, the precise identification of lung stem/progenitor cells remains uncertain. The large surface area and highly branched and folded geography of the lung dictates that there must be several kinds of stem or progenitor cells in the respiratory system. In the trachea and bronchi, certain basal cells and mucus-gland duct cells are believed to be stem/progenitor cells. Clara-like cells and

type II pneumocytes are also thought to function as stem/progenitor cells in bronchioles and alveoli, respectively. Another population of stem/progenitor cells lies at the bronchoalveolar duct junction (35). They can function as bipotential precursors of both the SP-C and CC10-expressing cell lineages.

It has recently been reported that bone marrow–derived mesenchymal stem cells can differentiate into airway epithelial cells in airway and type I pneumocytes in alveoli, particularly by injury. In contrast, in vitro cell culture indicates that Syrian hamster fetal lung epithelial M3E3/C3 cells can differentiate into Clara cells and type II pneumocytes under different culture conditions. Whether CCSP-expressing cells with pre-Clara cell phenotypes are stem cells for the entire respiratory tract remains to be determined. In addition, the concept of a pluripotent stem cell for the whole lung needs to be further investigated due to the great differences between identified stem cell and progenitor candidates in proximal bronchi and distal alveoli. Recently, we have discovered that lung contains populations of cells with stem or progenitor cell characteristics that can be sorted by FACS from adult rat and also mouse lung. This population is relatively resistant to apoptosis and may possibly be responsible for repopulation of the damaged alveolar surface. Another such population of stem or progenitor cells sorted as "side cells" on FACS has been identified to possibly repopulate in several different tissues, including the bone marrow.

Airway smooth muscle is derived from at least two distinct progenitor cell populations. One population comes from the periphery of the embryonic lung and is derived from the *Fgf10*-expressing progenitor cells that lie in the submesothelial mesenchyme. This population of cells at first plays a key role in mediating branching morphogenesis of the peripheral airway by virtue of expression of FGF10 as a chemotactic and proproliferative growth factor. However, as the airway extends outward into the peripheral mesenchyme, these progenitor cells relocate to lie along the more proximal stalk portion of the distal bud. In this location, they differentiate into smooth muscle cells (SMCs), most probably under the paracrine inductive influence of bone morphogenetic protein 4 (BMP4) and SHH, which are secreted by the underlying airway epithelium (36). On the contrary, another population of smooth muscle progenitor cells arises around the upper airway and proximal bronchi (37). The two populations of smooth muscle progenitor cells appear to meet distal to the major lobar and segmental branches. It is speculated that the size of these smooth muscle progenitor populations that are laid down during airway branching in the embryonic lung may determine the eventual propensity of the airway to undergo obstructive disease processes later in life such as BPD and asthma. Moreover, these progenitor pools may be targets for nicotine derived from maternal smoking.

VI. Molecular Mechanisms of Lung Development

Normal lung development is controlled by many genes as well as physical and chemical factors including intraluminal hydraulic pressure and relative hypoxia. Genetic factors responsible for lung development include (*i*) transcription factors that directly modulate gene expression in the cell nucleus, (*ii*) peptide growth factors and cytokines as well as their related intracellular signaling components that mediate cell-cell interaction, and (*iii*) extracellular matrix (ECM) that provides important environmental cues for

developing lung cells to differentiate. The specification of all these integrated regulatory mechanisms are still being explored, but the interaction between epithelium and mesenchyme compartments has long been known to play a critical role during airway branching morphogenesis and lung maturation.

A. Transcription Factors

Lung growth is initiated and developed through changes in specific gene expression. The activity and expression level of relevant transcription factors determine gene expression profiles in the developing lung, and consequently, the morphogenetic process in a particular temporospatial order. Recent advances in mouse genetic technology allow us to evaluate each factor by either overexpressing or knocking out a specific gene. Three major groups of transcription factors such as forkhead box transcription factors, Nkx homeodomain transcription factors, and *Gli* play important roles in lung development.

Forkhead Box Transcription Factor Family

Many members of the forkhead box family transcription factors, such as Foxa1, Foxa2, HFH8, and HFH4, are important regulatory factors involved in lung development. These transcription factors share homology in the winged helix DNA-binding domain and play important roles in pulmonary cellular proliferation and differentiation.

HNF-3α (Foxa1) and HNF-3β (Foxa2) share 93% homology in their amino acid sequences and were first identified as essential factors in hepatocyte differentiation (38). However, *Hnf-3β* is also expressed in developing lung, with higher levels in proximal airway-lining epithelial cells and lower levels in the distal type II epithelial cells (39). Overexpression of *Hnf-3β* under the control of the lung epithelial−specific *SP-C* promoter in vivo inhibits lung branching morphogenesis and vasculogenesis (40). Also, HNF-3α and HNF-3β have important functions in regulating expression of CCSP as well as SPs in both bronchiolar and type II epithelial cells (41–43). *Hnf-3β* is inducible by interferon and, in turn, regulates the expression of the *Nkx* homeodomain transcription factor *Nkx2.1* (also termed *Ttf-1* and *CebpI*), which in turn regulates transcription of the SP genes in lung peripheral epithelium (44,45).

HFH8 is another important member of this family of proteins that contribute to lung development. At E9.5, *Hfh-8* expression is restricted to the splanchnic mesoderm contacting the embryonic gut and presumptive lung bud, suggesting that *Hfh-8* may participate in the mesenchymal-epithelial induction of lung and gut morphogenesis. HFH-8 expression continues in lateral mesoderm-derived tissue during development. By day E18.5, *Hfh-8* expression is restricted to the distal lung mesenchyme and the muscular layer of the bronchi (46). One important regulated target of HFH8 is *Pdgf* receptor that is also expressed in mesenchyme (15,47,48). The level of *Hfh-8* expression is important for normal lung development, as an alveolar hemorrhage phenotype is observed in *Hfh8* (+/−) mice, while *Hfh8* (−/−) mice died in utero. In addition, reduction of *Hfh-8* expression in *Hfh-8*$^{+/-}$ mutants is accompanied by decreased expression of VEGF and its receptor 2 (Flk-1), BMP4, and the transcription factors of the Brachyury T-Box family (Tbx2–Tbx5) and lung Kruppel-like factor (49). HFH8-binding sites are also found in the promoter region of genes such as *Bmp4*, *Hgf*, and *Hoxa5* that are very important in controlling lung morphogenesis (50,51).

Hfh4 (*Foxj1*) is the key factor in controlling ciliated epithelial cell differentiation. *Hfh4* is expressed in E15.5 airway epithelium just before the appearance of ciliated epithelial cells (52). Defective ciliogenesis in airway epithelial cells and randomized left-right asymmetry are observed in $Hfh4^{-/-}$ null mutant mice, mimicking Kartagener syndrome in humans. This congenital syndrome can result in perinatal lethality, but in low penetrance gives rise to situs inversus, sinusitis, bronchoectasis, and sterility, all caused by defects in ciliary beat (53,54). Interestingly, in mesenchyme-free airway epithelial culture, inhibition of endogenous BMP4 signaling by adding exogenous BMP antagonist Noggin results in increased expression of the proximal lung markers CCSP and HFH4 (55).

Foxp1, *Foxp2*, and *Foxp3* are newly discovered members of the forkhead box family of transcription factors that are expressed at a high level in mouse lung and gut tissues. All three proteins are expressed in lung epithelium. *Foxp1* and *Foxp4* are expressed in both proximal and distal airway epithelium, whereas Foxp2 is expressed primarily in distal epithelium. Foxp1 protein expression is also observed in the mesenchyme and vascular endothelial cells of the lung (10).

Nkx and Hox Homeodomain Transcription Factors

One of the most important homeodomain transcription factors in lung development is NKX2.1, also called TTF-1 (thyroid-specific transcription factor) or CEBP-1. *Nkx2.1* is expressed in foregut endoderm-derived epithelial cells including developing lungs, thyroid, and pituitary, as well as in some restricted regions of fetal brain (56,57). $Nkx2.1^{-/-}$ mice suffer severe impairment in branching morphogenesis of the lung and tracheoesophageal septum formation. The distal airway branches are totally absent, while only the two main bronchial stems are formed in *Nkx2.1* knockout mice, which indicates that lung development is arrested at a very early stage (58,59). In developing mouse lung, *Nkx2.1* is expressed in the proximal and distal airway epithelia and, at later stages of lung development, in the distal AEC (39). *Nkx2.1* expression is strictly controlled, and increased expression of *Nkx2.1* causes dose-dependent morphological alterations in postnatal lung. Modest overexpression of *Nkx2.1* causes type II pneumocyte hyperplasia and increased levels of SP-B. Higher expression level of *Nkx2.1* disrupts alveolar septation, causing emphysema due to alveolar hypoplasia. The highest overexpression of *Nkx2.1* in transgenic mice causes severe pulmonary inflammation, fibrosis, and respiratory failure, associated with eosinophil infiltration as well as increased expression of eotaxin and IL-6 (60). *Nkx2.1* is critical for SP, *T1*α, and *Ccsp* gene expression (56,61–66). *Nkx2.1*-deficient pulmonary epithelial cells fail to express nonciliated marker genes, including differentiated *Sp-B*, *Sp-C*, and *Ccsp*. *Bmp4* expression in these cells is also reduced. Phosphorylation of NKX2.1 is important. Mice with point mutation of seven serine phosphorylation sites of NKX2.1 died immediately following birth with malformation of acinar tubules and pulmonary hypoplasia. Meanwhile, expression of SPs, secretoglobulin 1A, and *Vegf* was decreased (67). *Nkx2.1* expression can be activated by HNF-3β (44) and GATA-6 (68) transcription factors during lung morphogenesis.

The expression of Hox transcription factors shows a proximal to distal polarity in developing lung. *Hoxa5*, *Hoxb2*, and *Hoxb5* expression are restricted to distal lung mesenchyme. *Hoxb3* and *Hoxb4* genes are expressed in the mesenchyme of both

proximal airway and distal lung (69–71). The importance of these genes during lung development is well illustrated in gene-targeting experiments in mice. *Hoxa5*$^{-/-}$ null mutant mice display defects of tracheal formation and impaired lung branching morphogenesis, with tracheal occlusions, diminished SP expression, and thickening of alveolar walls (69).

GLI Family of Zinc Finger Transcription Factors

GLI1, -2, and -3 are very important zinc-finger transcription factors, which are activated by the SHH pathway. All of them are expressed in lung mesoderm rather than endoderm, particularly in the distal portion (72). Null mutation of *Gli2* plus *Gli3* genes results in total absence of lung. Mice with *Gli3* single deficiency are viable, but the size of the lung is smaller and the shape of the lung is also altered (72). In *Gli2*$^{-/-}$ null mutant mice, the right and left lungs are not separated but exist as a single lobe with a reduced size, and the primary branching in right lung is defective. Also, both trachea and esophagus are hypoplastic, though separated from each other. However, proximal-distal differentiation is normal (73). Therefore, *Gli2* plays an important role in the asymmetric patterning of the lung.

B. Peptide Growth Factors That Mediate Lung Morphogenesis

E11 mouse embryo lung can grow and branch spontaneously in serum-free medium in vitro. A variety of growth factors added into the culture medium can influence lung growth in the culture system (74,75). Such experiments indicate that the embryonic lung mesenchymal and epithelial cells can communicate through autocrine or paracrine factors. In this way, different signaling pathways are coordinated to control lung growth at the right time and right place. Many of those factors are peptide growth factors, including FGF, EGF, TGFβ, IGF, PDGF, SHH, etc. The expression and modification of these proteins and their downstream signaling components are strictly controlled during normal lung development. Loss of function of many of these genes perturbs normal lung development and function in mice.

FGF Family

FGF family members can be found in all vertebrate and invertebrate animals. Their regulatory functions during respiratory organogenesis are very well conserved from drosophila (76,77) to mammals. On the basis of their protein sequence homology, FGFs have been divided into several subgroups. Similarly, their cognate transmembrane protein tyrosine kinase receptors are classified into several different types, contributing to the specificity of FGF ligand binding (78). Heparin or heparan sulfate proteoglycan, an ECM protein, has been reported to be essential for FGF ligand–receptor binding and activation (79,80). FGFs play important roles in cell proliferation, migration, and differentiation during embryo development. Inhibition of fibroblast growth factor receptor signaling at different stages of embryo development shows that FGF signaling is required for branching morphogenesis early in lung development. Later inhibition of FGFR signaling in E14.5 lung decreased lung tubule formation before birth and caused severe emphysema at maturity. In E16.5, FGFR inhibition caused mild focal emphysema. Inhibition of FGFR signaling after birth did not alter alveolarization (81).

One of the best-studied FGF family members during embryonic lung development is FGF10. Despite the formation of larynx and trachea, the distal embryonic lung is completely missing in *Fgf10*$^{-/-}$ null mice (82). *Fgf10* is expressed in the mesenchyme of E11 to E12 mouse lungs, adjacent to distal epithelial tubules. These sites of expression change dynamically in a pattern that is compatible with the idea that FGF-10 appears in the mesenchyme at prospective sites of bud formation (83). Culture experiments have shown that FGF10 has a chemotactic effect on nearby epithelium, so that the nearby epithelial tips proliferate and migrate toward FGF10-expressing mesenchyme or FGF10 beads (84,85). FGF10 also controls the differentiation of the epithelium by inducing *Sp-C* expression and by downregulating the expression of *Bmp4* (55). Several other regulatory molecules such as SHH, BMPs, and TGF-βs may cross talk with FGF10 to coordinate control of embryonic lung morphogenesis. These interactions will be further discussed later in this chapter.

FGF7 (KGF) is found in the developing lung mesenchyme during late stages (86). In early cultured mouse embryonic lung, addition of FGF7 promotes epithelium growth and formation of a cyst-like structure with extensive cell proliferation. FGF7 can also contribute to distal airway epithelial cell differentiation (87,88). *Erm* and *Pea3* are ETS domain transcription factors known to be downstream of FGF signaling. FGF7 can induce *Erm/Pea3* expression more effectively than FGF10. *Erm* is transcribed exclusively in the epithelium, whereas *Pea3* is expressed in both epithelium and mesenchyme. When examined at E18.5, transgenic expression of a repressor form of *Erm*, specifically in the embryonic lung epithelium, shows that the distal epithelium of *Sp-C-Erm* transgenic lungs is composed predominantly of immature type II cells, while no mature type I cells are observed. In contrast, the differentiation of proximal epithelial cells, including ciliated cells and Clara cells, appears to be unaffected (89,90). FGF7 does not seem to protect against hyperoxic inhibition of normal postnatal alveoli formation and early pulmonary fibrosis, but FGF7 consistently had a significant protective/preventive effect against the development of pulmonary hypertension during hyperoxia (91). However, *Fgf7*$^{-/-}$ mutant mice have apparently no gross abnormalities in the lung (92), suggesting a redundant function of FGF7 with other factors during lung development.

Another FGF family member FGF9 also regulates branching morphogenesis. In E10.5 lung, *Fgf9* is expressed in the visceral pleura lining the outside of the lung bud as well as in the epithelium of the developing bronchi. At E12.5 and E14.5, *Fgf9* expression persists in the mesothelium of the visceral pleura, but is no longer detected in airway epithelium (93). *Fgf9* null mice exhibit reduced mesenchyme and decreased branching of the airways, but show significant distal airspace formation and pneumocyte differentiation. The reduction in the amount of mesenchyme in *Fgf9*$^{-/-}$ lungs limits the expression of mesenchymal *Fgf10* (94). Recombinant FGF9 protein inhibits the differentiation response of the mesenchyme to N-SHH, but does not affect proliferation (95).

The signaling cascade activated by FGF-10 and -9 involves Raf, MAP ERK kinase, and extracellular-regulated kinases (ERK) 1 and 2 as signal transducers. MAP ERK kinase inhibition has been shown to reduce lung branching and epithelium cell proliferation, but increase mesenchyme cell apoptosis in fetal lung explants (13). FGF signaling is regulated at several levels. One of the key negative regulators is the Sprouty family. There are four sprouty (Spry) genes in mouse (*mSpry1–4*) and human (*hSpry1–4*). Murine *Spry2* is expressed in the distal tip of embryonic lung epithelial branches, but is downregulated between the sites of new bud formation. Murine *Spry4* is predominantly

expressed in the distal mesenchyme of the embryonic lung (96) and may play roles in branching morphogenesis. Sprouties (SPRY1, -2, and -4) act as suppressors of Ras-MAP kinase signaling (97–99). Overexpression of *mSpry2* or *mSpry4* can inhibit lung branching morphogenesis through reducing epithelium cell proliferation (100–102). SPRED-1 and -2 are two sprouty-related proteins, which contain EVH-1 domains. *Spreds* are predominantly expressed in mesenchymal cells. Expression of *Spreds* is especially strong in the peripheral mesenchyme and epithelium of new bud formation. After birth, *Spreds* expression decreases, while the expression of *Sprouties* expression is still high. Both *Sprouties* and *Spreds* play important roles in mesenchyme-epithelium interaction during lung development (9).

TGF-β/BMP Family

The TGF-β superfamily comprises a large number of structurally related polypeptide growth factors including TGF-β, BMP, and activin subfamilies. TGF-β ligands bind to their cognate receptors on cell surface and activate downstream Smad proteins, which translocate into the nucleus and modulate target gene expression (103).

TGF-β Subfamily

TGF-βs are well known for their inhibitory effects on embryonic lung branching morphogenesis. There are distinct expression patterns for the three isoforms of TGF-βs, TGF-β1, -β2, and -β3. In early mouse embryonic lung (E11.5), TGF-β1 is expressed in the mesenchyme, particularly in the mesenchyme underlying distal epithelial branching points, whereas TGF-β2 is localized in distal epithelium. TGF-β3 is mainly expressed in proximal mesenchyme and mesothelium. Each isoform of TGF-βs plays a unique and nonredundant role during embryonic development. Mice lacking TGF-β1 develop normally but die within the first two months of life as a result of aggressive pulmonary inflammation (104). A TGF-$\beta2^{-/-}$ null mutation results in embryonic lethality around E14.5 in mice, and one of the abnormally developed organs is lung (105). TGF-$\beta3^{-/-}$ null mutant mice display cleft palate, retarded lung development, and neonatal lethality (106,107). Misexpression of TGF-β1, leading to excessive TGF-β1 activation, always results in an adverse phenotype that depends on the developmental stages at which TGF-β1 is expressed. Overexpression of TGF-β1 in early mouse embryonic lung epithelium inhibits lung branching morphogenesis in vitro (108), while misexpression of *Sp-C* promoter-controlled TGF-β1 in embryonic lung epithelium results in arrest of embryonic lung growth and epithelial cell differentiation as well as inhibition of pulmonary vasculogenesis (109,110). Clinically, the presence of excess TGF-β1 activity in tracheal aspirates of human premature infants who develop more severe BPD suggests a crucial role for TGF-β1 in lung maturation (111,112). On the other hand, misexpression of TGF-β1 in adult rats results in a chronic, progressive interstitial pulmonary fibrosis with increased proliferation and matrix secretion by the mesenchyme (113,114). Misexpression of TGF-β1 in neonatal rat lung using recombinant adenoviral vectors results in neonatal alveolar hypoplasia and interstitial fibrosis that phenocopies BPD (115). In addition, TGF-β1 may be one of the most important factors that are involved in the pulmonary inflammation response to exogenous factors, such as infection, bleomycin, or endotoxin. Blockade of the TGF-β-Smad3 pathway in $Smad3^{-/-}$ null mutant mice

strongly attenuates bleomycin-induced pulmonary fibrosis (114). TGF-β-activated kinase-1-binding protein-1 (TAB1) was identified as a molecule that activates TGF-β-activated kinase-1 (TAK1). *Tab1* mutant embryonic fibroblast cells displayed drastically reduced TAK1 kinase activities and decreased sensitivity to TGF-β stimulation. *Tab1* mutant mice died of cardiovascular and lung dysmorphogenesis (116).

The activity of TGF-β signaling is regulated precisely at multiple levels. For example, β6 integrin, LTBPs, and thrombospondin are involved in regulating the release of TGF-β mature peptide, whereas betaglycan, endoglin, or decorin influences the affinity of TGF-β receptor binding. Mutation of the above genes display phenotypes related to malfunction of TGF-βs. For example, loss-of-function mutation both in the human and mouse endoglin gene, whose protein product binds to both TGF-β ligand and its type I receptor (Alk1), causes hereditary hemorrhagic telangiectasia (117). Null mutation of LTBP-3 or -4 causes profound defects in elastin fiber structure and lung alveolarization, which is similar to the phenotypic changes observed in Smad3 knockout mouse lung (118,119). In addition, TGF-β signaling in epithelial cells versus mesenchymal cells of developing mouse lung has distinct regulatory impacts on lung branching morphogenesis and alveolarization in vivo. Selective blockade of endogenous TGF-β signaling in embryonic lung mesenchymal cells results in retarded lung branching after midgestation, while abrogation of epithelial cell–specific TGF-β signaling only causes abnormal postnatal lung alveolarization, but does not have significant impact on prenatal lung development (22).

BMP Subfamily

BMPs, with more than 20 ligand family members, have been shown to regulate many developmental processes, including development of the lung. Expression of *Bmp3*, *-4*, *-5,* and *-7* are detected in embryonic lung. BMP4 is an important BMP member that plays a key role in normal lung development. Addition of exogenous BMP4 to intact embryonic lung explant culture stimulates lung branching, as reported by us and other groups (120,121). However, in isolated E11.5 mouse lung endoderm cultured in Matrigel, addition of BMP4 inhibited epithelial growth induced by the morphogen FGF10 (85). On the other hand, transgenic overexpression of BMP4 in the distal endoderm of fetal mouse lung, driven by a 3.7-kb human SP-C promoter, causes abnormal lung morphogenesis with cystic terminal sacs (122). In contrast, SP-C promoter-driven overexpression of either the BMP antagonist *Xnoggin* or the Gremlin to block BMP signaling results in severely reduced distal epithelial cell phenotypes and increased proximal cell phenotypes in the lungs of transgenic mice (51). Interestingly, blockade of endogenous BMP4 in embryonic mouse lung epithelial cells using a conditional gene knockout approach results in abnormal lung development with similar dilated terminal sacs as seen in BMP4 transgenic mouse lung (123), suggesting that appropriate level of BMP4 is essential for normal lung development. As extracellular growth factors, BMPs bind to heteromeric complexes of BMP serine/threonine kinase type I and type II receptors to activate intracellular signal pathway. Three cognate BMP type I receptors (Alk2, Alk3, and Alk6) have been identified. Among them, Alk3 expresses predominantly in distal airway epithelial cells during mouse lung development. Abrogation of Alk3 in mouse lung epithelia either from early lung organogenesis or from late gestation resulted in similar neonatal respiratory distress phenotypes,

accompanied with collapsed lungs (124). Early induction of Alk3 knockout in lung epithelial cells causes retardation of early lung branching morphogenesis and reduces cell proliferation and differentiation. But late gestation induction of Alk3 knockout also causes significant epithelial apoptosis accompanied by lack of surfactant secretion (124). Furthermore, canonical Wnt signaling was perturbed, possibly through reduced WIF-1 expression in Alk3 knockout lungs (124). Therefore, deficiency of appropriate BMP signaling in lung epithelial cells results in prenatal lung malformation, neonatal atelectasis, and respiratory failure.

In addition, BMP signaling is also important in lung vasculogenesis and angiogenesis. Mutations of BMP type II receptor (BMPRII) and change in expression level of BMP antagonist Gremlin are associated with primary pulmonary hypertension (125,126). Moreover, upregulation of Gremlin is also associated with pulmonary fibrosis and the severity of the pathology (127,128).

SHH Pathway

Sonic hedgehog is a vertebrate homologue of *hedgehog* (Hh) that patterns the segment, leg, wing, eye, and brain in *Drosophila*. Hh binds to patched (Ptc), a transmembrane protein, and releases its inhibitory effect on downstream smoothened (Smo), which is a G-protein-coupled seven-span transmembrane protein. This leads to the activation of cubitus interruptus (Ci), a 155-kDa transcription factor that is usually cleaved to form a 75-kDa transcription inhibitor in cytosol. Elements of the *Drosophila* Hh signaling pathway and their general functions in the pathway are highly conserved in vertebrates, albeit with increased levels of complexity. Gli1, -2, and -3 are the three vertebrate Ci gene orthologues (129).

The SHH signal transduction pathway plays important roles in mesenchyme-epithelium interaction, which is very important in morphogenesis. In developing mouse lung, *Shh* is detected in the tracheal diverticulum, the esophagus, and later in the trachea and lung endoderm. *Shh* is expressed at low levels throughout the epithelium, whereas at higher level in the growing distal buds (130,131). Null mutation of *Shh* produces profound hypoplasia of the lung and failure of trachea-esophageal septation. Mesenchymal expression of *Ptc*, *Gli1*, and *Gli3* are all downregulated in the *Shh* knockout lung. However, proximal-distal differentiation of epithelial airway is preserved (132,133). Also, *Fgf10* expression is widespread in the epithelium in *Shh* null mutant lung, instead of the precisely location-restricted expression seen in wild-type control. Lung-specific *Shh* overexpression results in severe alveolar hypoplasia and a significant increase in interstitial tissue caused by an increased proliferation of epithelium and mesenchyme (130). Defective hedgehog signaling may lead to esophageal atresia and tracheoesophageal fistula (134). Gli1, -2, and -3 are three zinc-finger transcription factors activated by SHH signaling. Their functions in embryonic lung development have been discussed earlier.

HIP1, a membrane-bound protein, directly binds all mammalian hedgehog (HH) proteins and attenuates HH signaling (135). *Hip1* is transcriptionally activated in response to Hh signaling, overlapping the expression domains of *Ptch1* (135,136). Targeted disruption of *Hip1* results in neonatal lethality with respiratory failure. Although asymmetry in their growth was conserved, the initial stereotyped branching from the two primary buds was absent in $Hip1^{-/-}$ lungs. Hedgehog signaling is

upregulated in *Hip1* mutants. *Fgf10* expression was slightly downregulated at the distal tips of the primary lung buds in *Hip1*$^{-/-}$ lungs at E10.5, but completely absent from the mesenchyme where secondary branching normally initiates (136). Attenuated PTCH1 activity in a *Hip1*$^{-/-}$ mutant lungs leads to an accelerated lethality. *Hip1* and *Ptch1* have redundant roles in lung branching control (136). Both of them can attenuate SHH signal in lung development and pancreas development (136,137).

Wnt/β-Catenin Pathway

Wnt signals are transduced through seven-transmembrane-type Wnt receptors encoded by *Frizzled* (*Fzd*) genes to activate the β-catenin–TCF pathway, the JNK pathway, or the Ca^{2+}-releasing pathway. The Wnt/β-catenin pathway plays a critical role in many developmental and tumorigenesis processes. Following Wnt binding to the receptor, β-catenin is dephosphorylated and translocates to the nucleus to activate downstream gene expression (138).

Interestingly, all members of the *Fzd* gene family are expressed in embryonic and neonatal lung, albeit at different levels. *Fzd* genes are differentially expressed in the epithelium and mesenchyme. Expression of the *Fzd2*, *Fzd5*, *Fzd6*, and *Fzd8* was observed predominantly in the epithelium, while *Fzd4* and *Fzd10* were expressed in the mesenchyme. Expression of *Fzd1* and *Fzd7* was observed both in the epithelium and the mesenchyme, while *Fzd3* and *Fzd*9 were only marginally expressed. This spatial distribution suggests differential roles for different *Fzd* receptor genes in the Wnt signaling pathway during the development of the lung (139).

In mouse lung development, between embryonic days 10.5 and 17.5 (E10.5–E17.5), β-catenin was localized in the cytoplasm, and often also in the nucleus of the undifferentiated primordial epithelium, differentiating alveolar epithelium, and adjacent mesenchyme. Other Wnt/β-catenin pathway members, *Tcf1*, *Lef1*, *Tcf3*, *Tcf4*, *sFrp1*, *sFrp2*, and *sFrp4*, are also expressed in the primordial epithelium, alveolar epithelium, and adjacent mesenchyme in specific spatiotemporal patterns (140). In human fetal lung, nuclear β-catenin is present in pulmonary acinar buds (141). Null mutation of β-catenin in mice results in abnormal cystic structure formation in the lung and prenatal lethality. On the basis of molecular marker detection, the lungs are composed primarily of proximal airways, suggesting that β-catenin is one of the essential components to specify proximal-distal axis of the lung (139).

EGF Family Growth Factors

EGF, TGF-α, and amphiregulin are all EGFR ligands. Loss or gain of function experiments in mice, rat, or other animal models proves that EGF ligands can positively modulate early mouse embryonic lung branching morphogenesis and cytodifferentiation through EGFR (75,142,143). EGF is also expressed in mature AEC and regulates type 2 cell proliferation through an autocrine mechanism both in culture and in vivo (144). However, respiratory epithelial cell overexpression of TGF-α under the control of the *Sp-C* promoter of transgenic mice induces postnatal lung fibrosis (145). Overexpression of TGF-α caused severe pulmonary vascular disease, which was mediated through EGFR signaling in distal epithelial cells. Reductions in VEGF may contribute to the pathogenesis of pulmonary vascular disease in TGF-α mice (146).

EGFR is a tyrosine kinase receptor that transfers EGF signals into the cell. Abnormal branching and poor alveolization are observed in mice deficient in *Egfr*$^{-/-}$. Mechanical stretch–stimulated EGFR phosphorylation, at least in part, induces differentiation of fetal epithelial cells via EGFR activation of the ERK pathway. Blockade of the EGFR or ERK pathway by specific inhibitors decreased stretch-inducible *Sp-C* mRNA expression. Maybe EGFR is part of a mechanic stimulus signal sensor during fetal lung development (147). Aberrant expression of matrix metaloprotease proteins (MMPs) is also detected in *Egfr*$^{-/-}$ null mutant mice, which suggests that MMPs may be involved in EGFR-regulated lung growth (148).

TNF-α-converting enzyme (TACE) is a transmembrane metalloprotease-disintegrin that functions as a membrane sheddase to release the ectodomain portions of many transmembrane proteins, including the precursors of TNFα and several other cytokines, as well as the receptors for TNFα, and neuregulin (ErbB4) (149). Neonatal TACE-deficient mice had visible respiratory distress, and their lungs failed to form normal saccular structures, resulting in a reduction of normal air-exchange surface. Mouse embryonic lung explant cultures show that TGF-α and EGF can rescue the inhibition of TACE activity (150).

Platelet-Derived Growth Factors

There are four types of PDGF peptides. The PDGF-A and PDGF-B can form homodimers (AA or BB) or heterodimers (AB). Two types of PDGF receptors, α and β, are present in embryonic mouse lung and are differentially regulated in fetal rat lung epithelial cells and fibroblasts (151). PDGF-A regulates both DNA synthesis and early branching in early mouse embryonic lung epithelium in culture (152). *Pdgf-A* homozygous null mutant mice are perinatally lethal. The pulmonary phenotypes include lack of lung alveolar SMCs, reduced deposition of elastin fibers in the lung parenchyma, and developing lung emphysema due to complete failure of alveogenesis (15,17). Abrogation of PDGF-B chain expression with antisense oligodeoxynucleotides reduces the size of the epithelial component of early embryonic mouse lung explants, but does not reduce the number of branches (153). PDGF-B and its receptor are crucial for vascular growth and integrity during the alveolar phase (16). PDGF-C and -D also dimerize and bind to PDGF-α or -β receptor (154,155). PDGF-C mRNA expression shows a significant increase in lung fibrosis induced by bleomycin (156).

Insulin-like Growth Factors

The IGFs and their receptors are expressed in both rodent and human fetal lung (157–161). Null mutant mice for the cognate type 1 IGF receptor (*Igf1r*) gene always die at birth with respiratory failure and severe growth deficiency (45% of normal birth weight). Dwarfism is further exacerbated (70% of size reduction) in either *Igf1* and *Igf2* double null mutants or *Igf1r* and *Igf2* double null mutants. There does not appear to be a gross defect in primary branching morphogenesis per se; the lungs merely appear hypoplastic (162). IGF signaling may play a role in facilitating other peptide growth factor pathways during lung morphogenesis. IGF1R signaling function is required for both the mitogenic and transforming activities of the EGF receptor (163). The lungs displayed reduced airspace in the *Igf1*-deficient embryos and neonates, and the phenotype was exacerbated

in additionally leukemia inhibitory factor (*Lif*) null mutant mice, which showed abnormal epithelial cells and decreased Sp3 expression. In addition, *Nkx2.1* and *Sp-B* expression are reduced in the lung of these double null mutant neonates. Thus, LIF and IGF-I have cooperative and distinct tissue functions during lung development (164). IGF1 is also a potent trophic factor for fetal lung endothelial cells. In human fetal lung explants, inactivation of IGF-IR results in a loss of endothelial cells, attenuates time-dependent increase in budding of distal airway, and increases mesenchymal cell apoptosis (165).

VEGF Isoforms and Cognate Receptors

Lung development must form a fine alignment between the alveolar surface and the surrounding pulmonary capillary system for effective gas exchange. VEGF are potent effectors of vascular development in lung morphogenesis. VEGF signals through the cognate receptors fetal liver kinase-1 (Flk-1, VEGFR2) and fetal liver tyrosinase-1 (Flt-1, VEGFR1) (166). VEGF is regulated by hypoxia-inducible transcription factor-2α (167). During lung organogenesis in mouse, VEGF120, -164, and -188 isoforms are expressed in pulmonary epithelial and mesenchymal cells around E12.5 and play a role in regulating endothelial cell proliferation and maintaining microvascular structure (168,169). Further, as epithelial branching progresses, *Vegf-A* expression becomes restricted to the distal lung (170), which may be partly due to the high affinity of VEGF-A for matrix components that concentrate VEGF-A around the branching tips (171,169). Thus, leading to the speculation that VEGF signaling may play a critical role in lung vascular development and diseases.

Vasculogenesis is initiated as soon as the lung evaginates from the foregut epithelium (172). Development of the vascular system influences branching morphogenesis of the airway as well as alveolarization. In transgenic mice, where the *Vegf* transgene is misexpressed under the control of SP-C promoter, gross abnormalities in lung morphogenesis are associated with a decrease in acinar tubules and mesenchyme (173); thus, suggesting that excessive VEGF signaling may disrupt both vascular and epithelial morphogenesis in the lung. Additionally, VEGF-A signaling through Flk-1 plays a key functional role in mediating cross talk between the epithelial, mesenchymal, and endothelial compartments during epithelial and vascular branching morphogenesis of the early mouse embryonic lung in explant culture (174). VEGF has also been demonstrated to play a role in maintaining alveolar structure (26). Lungs from newborn mice treated with antibodies to Flt-1 were reduced in size and displayed significant immaturity with a less complex alveolar pattern (175) (Fig. 4).

C. ECM and Lung Development

The protein components of extracellular basement membrane, laminins (LNs), entactin/nidogen, type IV collagen, perlecan, SPARC, and Fibromodulin, are important in mediating cell-cell and cell-ECM interaction during fetal lung morphogenesis. Basement membrane components are differentially expressed and have a specific cell distribution during lung morphogenesis. ECM components not only provide the support for tissue architecture but also play an active role in modulation of cell proliferation and differentiation (176). For example, basement membrane components may serve as a barrier

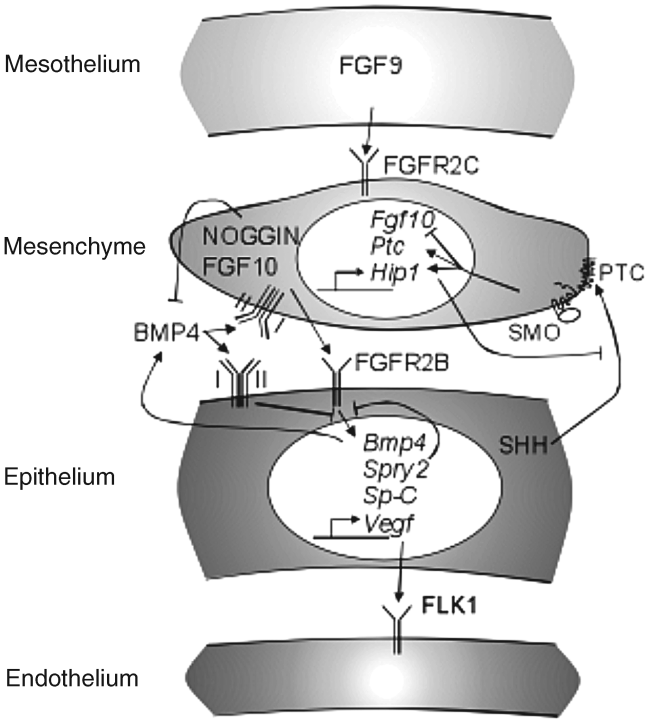

Figure 4 Integrative summary of selected growth factor signaling events during early lung morphogenesis. FGF9 is produced by the mesothelium, which signals through FGFR2c on the underlying mesenchyme. This stimulates *Fgf10, Ptc, and Hip* gene transcription. FGF10 in turn signals from the mesenchyme to FGFR2b on the epithelium. This signaling event is chemotactic for the epithelium, in addition to stimulating *Bmp4, Spry2, Vegf, and Sp-C* transcription. SPROUTY2 is an inducible negative downstream modulator of FGFR signaling. VEGF, produced by the epithelium, signals through FLK1 on the endothelium. BMP4, produced by the epithelium, signals back to the mesenchyme through its cognate receptors to modulate mesenchymal differentiation, particularly into smooth muscle. BMP4 ligand bioavailability is in turn negatively modulated by NOGGIN, which is produced in the mesenchyme. SHH, produced by the epithelium, signals through its cognate receptors Ptc and Smo located on the mesenchyme to both negatively modulate *Fgf10* transcription, but also to positively regulate mesenchyme differentiation into smooth muscle. This network diagram is highly simplified and does not include other key signaling mechanisms including Wnt ligands, Wnt antagonists such as DKK1, and their role in controlling fibronectin deposition at branch points. While all members of the network must interact correctly to control morphogenesis, the ratio of FGF10 to SPROUTY2 is a major factor that determines the periodicity and geometry of branching morphogenesis (7). *Source*: From Stijn de Langhe and Pierre del Moral.

and reservoir of growth factors, which in turn regulate epithelial and mesenchymal cell proliferation. Absence or inhibition of the interaction of epithelial cells with the basement membrane results in failure of normal lung development (177,178).

LNs are glycoproteins involved in cell adhesion, migration, proliferation, and differentiation during tissue development and remodeling. LNs are composed of three chains, one central (α) and two laterals (β and γ), that are linked by disulfide bonds to form a cross-shaped molecule (179). To date, five α, three β, and three γ chain isoforms have been identified, which suggests that their combination can lead to approximately 30 variants of LN (180–189). The α1 chain has been found principally localized in the basement membrane at the epithelial-mesenchymal interface, with a predominant distribution in specific zones. The LN α1 chain has also been identified around some mesenchymal cells. Molecular analysis dissecting the α1 chain isoform has shown that a domain in the cross-region of the α1 chain is involved in the regulation of lung epithelial cell proliferation (190). The α4 chain, found in LN8 and LN9 variants, has been reported to highly express in lung and heart tissues during mouse development (191,183–185). The LN α4 chain, localized principally around vessels in fetal lung, may play a role principally in the organization of lung mesenchyme (188,192). The α5 chain, found in LN10 and LN11, has been indicated abundantly expressed during fetal lung morphogenesis (188,193,194). Mouse embryos bearing mutated LN α5 chain isoform display a poor lobe septation and bronchiolar branching, suggesting that the LN α5 chain isoform might be the most indispensable LN variant for lung branching morphogenesis.

A constant expression of β1 and γ1 is observed during fetal lung development (195). These two chains also have a role in cell adhesion. The globular domains near the N-terminal of β1 and γ1 chains participate in the regulation of cell polarization (196,197). Immunohistochemistry studies have demonstrated that the LN β2 chain isoform is localized in the basement membrane of prealveolar ducts, airways, SMCs of airways, and arterial blood vessels, as well as type II pneumocytes.

Nidogen (150 kDa) is a constituent of the basement membranes. Nidogen binds to the γ1 and γ3 chains of LN and forms a link between LN and collagen IV (187,198,199). Nidogen is actively synthesized by mesenchymal cell during fetal lung development, which suggests that it has a key role in the organization of the basement membrane during lung morphogenesis (200). Blocking the interaction of nidogen with LN affects the progression of lung development (198,200,201). Susceptibility of nidogen to degradation by matrix metalloproteinases may contribute in the remodeling and degradation of the basement membrane (202).

Proteoglycans comprise a core protein with sulfated carbohydrate side chains. They function as flexible structures in the organization of the basement membrane and may also play an important role as a reservoir for growth factors, water, and ions. Perlecan is a predominant proteoglycan in the basement membrane. It is composed of an approximately 450-kDa core protein with three heparan sulfate chains. Perlecan is involved in the control of smooth cell proliferation and differentiation since increased cell proliferation of fetal lung SMCs is accompanied by a highly increased synthesis of perlecan (203). Growth and branching of E13 mouse lung explants can be disrupted by inhibiting PG sulfation. The migration of epithelial cells toward invaded lung mesenchyme as well as toward beads soaked in FGF10 is inhibited when PG sulfation fails; chlorate severely decreased branching morphogenesis in lung mesenchymal and epithelial tissue recombinants (204).

Fibronectin also plays important roles in lung development. In branching morphogenesis, repetitive epithelial cleft and bud formation create the complex three-dimensional branching structures characteristic of many organs. Fibronectin is essential for cleft formation during the initiation of epithelial branching in salivary gland. Immunofluorescence comparisons of fibronectin localization during early branching of lung and kidney also showed an accumulation of fibronectin at sites of epithelial constriction and indentation (205), supporting possible roles for fibronectin in branching morphogenesis of the lung (206). Direct tests for roles of fibronectin, by treatment of developing lung rudiments with antifibronectin antibody or siRNA, inhibited branching morphogenesis, while fibronectin supplementation promoted branching of lung (205). The EIIIA segment of fibronectin is one of the major alternatively spliced segments and modulates the cell proliferative potential of fibronectin in vitro. The EIIIA-containing fibronectin isoform localized in both the epithelial cells and the mesenchyme. Its expression gradually decreased from the pseudoglandular stage to the saccular stage and then slightly increased from the saccular stage to the alveolar stage. This change in expression pattern of EIIIA-containing fibronectin seemed to be in accord with the change in the number of PCNA-positive cells in the distal pulmonary cells throughout lung development (207).

Extracellular matrix is under dynamic control during lung development. The MMPs are a large family of ECM-degrading enzymes. MMPs are inhibited by the family of tissue inhibitors of metalloproteinases, termed TIMPs. Activity of MMPs may be required during development and normal physiology in several ways: (*i*) to degrade ECM molecules and allow cell migration, (*ii*) to alter the ECM microenvironment and result in alteration in cellular behavior, and (*iii*) to modulate the activity of biologically active molecules by direct cleavage, release from bound stores, or modulating of activity of their inhibitors (208). In *Timp3* null mutant mouse, lung airway branching is inhibited. Compared with wild type, the number of bronchioles is reduced and alveologenesis was attenuated in the *Timp3* null (209). The *Timp3* null animals spontaneously develop progressive alveolar air space enlargement similar to that seen in human emphysema (210). Early postnatal exposure to dexamethasone (Dex) influences MMP2 and MMP9, as well as their tissue inhibitors (TIMP1 and TIMP2) in the developing rat lung: the expression of *Timp2* is reduced and that of *Mmp9* increases. These changes may be responsible, in part, for some of the known adverse maturational effects of steroids on lung structure in the newborn (211). MT1-MMP, which acts as a potent activator of MMP2, is a major downstream target of EGFR signaling in lung. $Egfr^{-/-}$ mice had low expression of *MT1-Mmp*. Extracts from lungs of $Egfr^{-/-}$ mice showed a tenfold reduction in active MMP-2. At birth, the abnormal lung alveolization phenotype of $Mmp2^{-/-}$ mice is similar to that of $Egfr^{-/-}$ mice, albeit somewhat less severe (148). The balance between the activity of MMPs and TIMPs is important to normal lung development.

Retinoic Acid and Lung Morphogenesis

Retinoids (all-*trans*, 9-*cis*, and 13-*cis*) are fundamental for normal development and homeostasis of a number of biological systems including the lung. There is a precisely controlled RA synthesis and degradation system in mammals. Retinaldehyde dehydrogenase-2 (RALDH-2) plays a prominent role in generating RA during organogenesis

(212–214). RA signaling is mediated by its nuclear receptors of the steroid hormone receptor superfamily: RAR (α, β, γ) and retinoid RXR (α, β, γ). RAR/RXR heterodimers have also been shown to transduce RA signaling in vivo (215). Within the E13.5 lung, *Rar-β* isoform transcripts are specifically localized to the proximal airway epithelium and immediately adjacent mesenchyme, whereas *Rarα1*, *Rarα2*, and *Rarγ2* isoforms are ubiquitously expressed (216).

RA signaling is required for lung bud initiation. Acute vitamin A deprivation in pregnant rats at the onset of lung development results in blunt-end tracheae and lung agenesis in some embryos, which is similar to $Fgf10^{-/-}$ null mutant mice (217,218). Disruption of RA signaling in *Rarα/β2* knockout mice leads to agenesis of the left lung and hypoplasia of the right lung (219). Interestingly, lung branching morphogenesis is characterized by a dramatic downregulation of RA signaling in the lung. Prevention of downregulating RA signal by treating embryonic lung explants with high concentrations of RA (10^{-6} to 10^{-5} M) results in dramatic disruption of distal budding and formation of proximal-like immature airways (220,221). Continued RA activation by overexpression of constitutively activated *Rarα* chimeric receptors also resulted in lung immaturity. Lungs did not expand to form saccules or morphologically identifiable type I cells. High levels of *Sp-C*, *Nkx2.1*, and *Gata6* but not *Sp-A* or *Sp-B* in the epithelium at birth suggested that in these lungs differentiation was arrested at an early stage. Down-regulation of RA signaling, however, is required to allow completion of later steps of this differentiation program that ultimately form mature type I and II cells (222). RA inhibits expression and alters distribution of *Fgf10* and *Bmp4*, which are required for distal lung formation (220,221). Pan-RAR antagonism alters the expression of *Tgf-β3*, *Hnf-3β*, and *Cftr* in proximal tubules and that of *Bmp4*, *Fgf10*, and *Shh* within the distal buds (216).

It is also noteworthy that during early stages of lung branching (day 11–12.5), *Raldh-2* expression is concentrated in trachea (mesenchyme) and proximal lung (mesothelium) at sites of low branching activity. The *Raldh-2* pattern is not overlapping with that of *Fgf-10*, supporting the idea that RA signaling restricts *Fgf-10* expression and helps to define the proximal-distal axis of the developing lung. However, during later postnatal stages of lung development, RA has been shown to increase the number of alveoli, and therefore, partially rescue dexamethasone-induced suppression of alveolarization. In adult rats, RA has also been reported to reverse the anatomical features of elastase-induced emphysema in which there is destruction of septal structures (27,28,223). In *Rarγ* gene deletion mouse, there is a developmental defect in alveolar formation, consistent with a defect in elastin deposition (30). This combined evidence suggests that RA may play an important but rather complex role in alveolar development during late lung development.

Fetal Lung Surfactant Maturation, Lung Liquid Absorption, and the Transition to Air Breathing

Maturation of the fetal lung surfactant system is one of the two key steps to prepare the lung for air breathing. During the last eight weeks of human gestation, fetal lung glycogen is broken down and converted into surfactant phospholipids, the most important of which is disaturated phosphatidylcholine. This maturation is under the control of and can be stimulated by corticosteroids. Null mutation of the glucocorticoid receptors and of corticotrophin-releasing hormone block this maturation in mice. Human mutations in

various components of the surfactant system have been found such as SP-B that adversely affect stability of pulmonary surfactant and hence the ability to maintain lung inflation.

The transition to air breathing occurs rapidly in the mature neonatal lung. Immediately following severance of the umbilical circulation, a significant spike in catecholamine levels switches off chloride secretion and stimulates sodium/potassium ATPase (224–226). This reverses the production of tracheal fluid and leads to its rapid absorption into the lung interstitium and thence into the lymphatic and pulmonary capillary circulation. Null mutation of Na/K ATPase in mice leads to failure to absorb fetal lung liquid, which causes significant respiratory distress and even neonatal lethality (227). In human infants, delayed lung liquid absorption can manifest as transient tachypnea of the newborn.

Impact of Lung Injury on the Developmental Program

The lung has a relatively limited genetic repertoire to respond to injury. Initial injury to the epithelium results in an outpouring of cyotokines and chemoattractants including IL1, IL6, IL8, etc. Macrophages are recruited into the lung and enter the airways together with neutrophils and lymphocytes. Physiological levels of TGFβ peptides are actually protective of the airway and prevent inflammation, as revealed by null mutation in mice. Yet excessive amounts of TGFβ1 activity have been detected in the tracheal fluid of human premature infants who go on to get severe forms of BPD and hence have a worse pulmonary prognosis (228). As discussed earlier, transgenic misexpression of TGFβ1 in mice substantially phenocopies BPD (115). Another important feature of the airway cytokine milieu in human prematures who do badly with BPD is deficiency of IL10 (229,230). IL10 is an important anti-inflammatory cytokine, leading to the hypothesis that the airway of the human premature may be particularly prone to acute and eventually chronic inflammation, which in turn leads to production of TGFβ1 by inflammatory cells in the airway. Targets for suppression by excessive TGFβ1 signaling include epithelial and endothelial proliferation, while mesenchymal lineages may be stimulated. Likewise suppressive interactions of TGFβ signaling with morphogenetic pathways such as VEGF, FGF, PDGF, and SHH signaling may also play an important role in mediating the alveolar hypoplasia and airway obstruction phenotype characteristic of BPD.

The Hope of Recovery and Regeneration

Clinical experience in the special care nursery demonstrates that in most cases BPD is apparently at least somewhat reversible, that airway inflammation eventually resolves, and that lung alveolar growth can sometimes resume. This gives hope that with supportive care, even severe cases of BPD may eventually move toward resolution. However, one current view of BPD is that it is a disease in which damage to progenitor cells in the peripheral airway may be compounded by failure to resolve inflammation within a reasonable period. Thus, full recovery may be marred by failure to continue to grow alveoli. Survivors of extreme prematurity are now beginning to appear in adult pulmonary practice with reduced diffusion capacity, airways obstruction, and an “empty chest” on CT radiography as a result of BPD in infancy. Some of this is due to airway

epithelial damage and smooth muscle hypertrophy and some due to endothelial vascular damage, while some may be due to subsequent intercurrent infections, particularly RSV. Nevertheless, the neonatal lung is reassuringly relatively resilient. Newer studies in mice show that oxygen-induced BPD models can be ameliorated in terms of alveolarization both by VEGF therapy and by various kinds of exogenous stem cells. Thus, hope springs eternal for improved postnatal lung recovery from the ravages of prematurity, oxygen plus pressure plus time. Translation of this hope into practices that can prevent or improve BPD outcomes may depend on an increasingly thorough integrative understanding of the genetics of lung development, injury, repair, and regeneration in the human premature lung.

References

1. Northway WH Jr., Rosan HC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. N Engl J Med 1967; 276:357–368.
2. Philip AG. Oxygen plus pressure plus time: the etiology of bronchopulmonary dysplasia. Pediatrics 1975; 55:44–50.
3. Bell EF, Warburton D, Stonestreet BD, et al. Effect of fluid administration on the development of symptomatic patent ductus arteriosus and congestive heart failure in premature infants. N Engl J Med 1980; 302:589–604.
4. Jobe A H, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care 2001; 163:1723–1729.
5. Sakiyama J, Yamagishi A, Kuroiwa A. Tbx4-Fgf10 system controls lung bud formation during chicken embryonic development. Development 2003; 130:1225–1234.
6. Metzger RJ, Klein OD, Martin GR, et al. The branching program of mouse lung development. Nature 2008; 453:745–750.
7. Warburton D. Developmental biology: order in the lung. Nature 2008; 453:733–735.
8. Unbekandt M, del Moral PM, Sala FG, et al. Tracheal occlusion increases the rate of epithelial branching of embryonic mouse lung via the FGF10-FGFR2b-Sprouty2 pathway. Mech Dev 2008; 125:314–324.
9. Hashimoto S, Nakano H, Singh G, et al. Expression of Spred and Sprouty in developing rat lung. Mech Dev 2002; 119(suppl 1):S303–S309.
10. Lu MM, Li S, Yang H, et al. Foxp4: a novel member of the Foxp subfamily of winged-helix genes co-expressed with Foxp1 and Foxp2 in pulmonary and gut tissues. Mech Dev 2002; 119(suppl 1):S197–S202.
11. Blewett CJ, Zgleszewski SE, Chinoy MR, et al. Bronchial ligation enhances murine fetal lung development in whole-organ culture. J Pediatr Surg 1996; 31:869–877.
12. Kitano Y, Yang EY, von Allmen D, et al. Tracheal occlusion in the fetal rat: a new experimental model for the study of accelerated lung growth. J Pediatr Surg 1998; 33:1741–1744.
13. Papadakis K, Luks FI, De Paepe ME, et al. Fetal lung growth after tracheal ligation is not solely a pressure phenomenon. J Pediatr Surg 1997; 32:347–351.
14. Inanlou MR, Kablar B. Abnormal development of the diaphragm in mdx:MyoD-/-(9th) embryos leads to pulmonary hypoplasia. Int J Dev Biol 2003; 47:363–371.
15. Bostrom H, Willetts K, Pekny M, et al. PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. Cell 1996; 85:863–873.
16. Lindahl P, Karlsson L, Hellstrom M, et al. Alveogenesis failure in PDGF-A-deficient mice is coupled to lack of distal spreading of alveolar smooth muscle cell progenitors during lung development. Development 1997; 124:3943–3953.
17. Bostrom H, Gritli-Linde A, Betsholtz C. PDGF-A/PDGF alpha-receptor signaling is required for lung growth and the formation of alveoli but not for early lung branching morphogenesis. Dev Dyn 2002; 223:155–162.

18. Wu JY, Feng L, Park HT, et al. The neuronal repellent Slit inhibits leukocyte chemotaxis induced by chemotactic factors. Nature 2001; 410:948–952.
19. Anselmo MA, Dalvin S, Prodhan P, et al. Slit and Robo: expression patterns in lung development. Gene Expr Patterns 2003; 3:13–19.
20. Xian J, Clark KJ, Fordham R, et al. Inadequate lung development and bronchial hyperplasia in mice with a targeted deletion in the Dutt1/Robo1 gene. Proc Natl Acad Sci U S A 2001; 98:15062–15066.
21. Chen C, Chen H, Sun J, et al. Smad1 expression and function during mouse embryonic lung morphogenesis. Am J Physiol Lung Cell Mol Physiol 2005; 288:L1033–L1039.
22. Chen H, Zhuang F, Liu YH, et al. TGF-beta receptor II in epithelia versus mesenchyme plays distinct role in developing lung. Eur Respir J 2008; 32:285–295.
23. Awonusonu F, Srinivasan S, Strange J, et al. Developmental shift in the relative percentages of lung fibroblast subsets: role of apoptosis postseptation. Am J Physiol 1999; 277:L848–L859.
24. Schittny JC, Djonov V, Fine A, et al. Programmed cell death contributes to postnatal lung development. Am J Respir Cell Mol Biol 1998; 18:786–793.
25. Srinivasan S, Strange J, Awonusonu F, et al. Insulin-like growth factor I receptor is downregulated after alveolarization in an apoptotic fibroblast subset. Am J Physiol Lung Cell Mol Physiol 2002; 282:L457–L467.
26. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, et al. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. J Clin Invest 2000; 106:1311–1319.
27. Massaro GD, Massaro D. Postnatal treatment with retinoic acid increases the number of pulmonary alveoli in rats. Am J Physiol 1996; 270:L305–L310.
28. Massaro GD, Massaro D. Retinoic acid treatment partially rescues failed septation in rats and in mice. Am J Physiol Lung Cell Mol Physiol 2000; 278:L955–L960.
29. McGowan S, Jackson SK, Jenkins-Moore M, et al. Mice bearing deletions of retinoic acid receptors demonstrate reduced lung elastin and alveolar numbers. Am J Respir Cell Mol Biol 2000; 23:162–167.
30. Chytil F. Retinoids in lung development. FASEB J 1996; 10:986–992.
31. Vermeer PD, Harson R, Einwalter LA, et al. Interleukin-9 induces goblet cell hyperplasia during repair of human airway epithelia. Am J Respir Cell Mol Biol 2003; 28:286–295.
32. Lordan JL, Bucchieri F, Richter A, et al. Cooperative effects of Th2 cytokines and allergen on normal and asthmatic bronchial epithelial cells. J Immunol 2002; 169:407–414.
33. Ramirez MI, Millien G, Hinds A, et al. T1alpha, a lung type I cell differentiation gene, is required for normal lung cell proliferation and alveolus formation at birth. Dev Biol 2003; 256:61–72.
34. Whitsett JA, Weaver TE. Hydrophobic surfactant proteins in lung function and disease. N Engl J Med 2002; 347:2141–2148.
35. Kim CF, Jackson EL, Woolfenden AE, et al. Identification of bronchoalveolar stem cells in normal lung and lung cancer. Cell 2005; 121:823–835.
36. DeLanghe S, Carraro G, Warburton D, et al. Levels of mesenchymal FGFR2 signaling modulate smooth muscle progenitor cell commitment in the lung. Dev Biol 2006; 299:52–62.
37. Shan L, Subramanian M, Emanuel RL, et al. Centrifugal migration of mesenchymal cells in embryonic lung. Dev Dyn 2008; 237:750–757.
38. Qian X, Costa RH. Analysis of hepatocyte nuclear factor-3 beta protein domains required for transcriptional activation and nuclear targeting. Nucleic Acids Res 1995; 23:1184–1191.
39. Zhou L, Lim L, Costa RH, et al. Thyroid transcription factor-1, hepatocyte nuclear factor-3beta, surfactant protein B, C, and Clara cell secretory protein in developing mouse lung. J Histochem Cytochem 1996; 44:1183–1193.
40. Zhou L, Dey CR, Wert SE, et al. Hepatocyte nuclear factor-3beta limits cellular diversity in the developing respiratory epithelium and alters lung morphogenesis in vivo. Dev Dyn 1997; 210:305–314.

41. Bingle CD, Hackett BP, Moxley M, et al. Role of hepatocyte nuclear factor-3 alpha and hepatocyte nuclear factor-3 beta in Clara cell secretory protein gene expression in the bronchiolar epithelium. Biochem J 1995; 308(pt 1):197–202.
42. Bohinski RJ, Di Lauro R, Whitsett JA. The lung-specific surfactant protein B gene promoter is a target for thyroid transcription factor 1 and hepatocyte nuclear factor 3, indicating common factors for organ-specific gene expression along the foregut axis. Mol Cell Biol 1994; 14:5671–5681.
43. He Y, Crouch EC, Rust K, et al. Proximal promoter of the surfactant protein D gene: regulatory roles of AP-1, forkhead box, and GT box binding proteins. J Biol Chem 2000; 275:31051–31060.
44. Ikeda K, Shaw-White JR, Wert SE, et al. Hepatocyte nuclear factor 3 activates transcription of thyroid transcription factor 1 in respiratory epithelial cells. Mol Cell Biol 1996; 16:3626–3636.
45. Samadani U, Porcella A, Pani L, et al. Cytokine regulation of the liver transcription factor hepatocyte nuclear factor-3 beta is mediated by the C/EBP family and interferon regulatory factor 1. Cell Growth Differ 1995; 6:879–890.
46. Peterson RS, Lim L, Ye H, et al. The winged helix transcriptional activator HFH-8 is expressed in the mesoderm of the primitive streak stage of mouse embryos and its cellular derivatives. Mech Dev 1997; 69:53–69.
47. Shinbrot E, Peters KG, Williams LT. Expression of the platelet-derived growth factor beta receptor during organogenesis and tissue differentiation in the mouse embryo. Dev Dyn 1994; 199:169–175.
48. Souza P, Tanswell AK, Post M. Different roles for PDGF-alpha and -beta receptors in embryonic lung development. Am J Respir Cell Mol Biol 1996; 15:551–562.
49. Kalinichenko VV, Lim L, Stolz DB, et al. Defects in pulmonary vasculature and perinatal lung hemorrhage in mice heterozygous null for the Forkhead Box f1 transcription factor. Dev Biol 2001; 235:489–506.
50. Ohmichi H, Koshimizu U, Matsumoto K, et al. Hepatocyte growth factor (HGF) acts as a mesenchyme-derived morphogenic factor during fetal lung development. Development 1998; 125:1315–1324.
51. Weaver M, Yingling JM, Dunn NR, et al. Bmp signaling regulates proximal-distal differentiation of endoderm in mouse lung development. Development 1999; 126:4005–4015.
52. Hackett BP, Brody SL, Liang M, et al. Primary structure of hepatocyte nuclear factor/forkhead homologue 4 and characterization of gene expression in the developing respiratory and reproductive epithelium. Proc Natl Acad Sci U S A 1995; 92:4249–4253.
53. Brody SL, Yan XH, Wuerffel MK, et al. Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice. Am J Respir Cell Mol Biol 2000; 23:45–51.
54. Chen J, Knowles HJ, Hebert JL, et al. Mutation of the mouse hepatocyte nuclear factor/forkhead homologue 4 gene results in an absence of cilia and random left-right asymmetry. J Clin Invest 1998; 102:1077–1082.
55. Hyatt BA, Shangguan X, Shannon JM. BMP4 modulates fibroblast growth factor-mediated induction of proximal and distal lung differentiation in mouse embryonic tracheal epithelium in mesenchyme-free culture. Dev Dyn 2002; 225:153–165.
56. Guazzi S, Price M, De Felice M, et al. Thyroid nuclear factor 1 (TTF-1) contains a homeodomain and displays a novel DNA binding specificity. EMBO J 1990; 9:3631–3639.
57. Lazzaro D, Price M, De Felice M, et al. The transcription factor TTF-1 is expressed at the onset of thyroid and lung morphogenesis and in restricted regions of the foetal brain. Development 1991; 113:1093–1104.
58. Kimura S, Hara Y, Pineau T, et al. The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. Genes Dev 1996; 10:60–69.

59. Minoo P, Su G, Drum H, et al. Defects in tracheoesophageal and lung morphogenesis in Nkx2.1(-/-) mouse embryos. Dev Biol 1999; 209:60–71.
60. Wert SE, Dey CR, Blair PA, et al. Increased expression of thyroid transcription factor-1 (TTF-1) in respiratory epithelial cells inhibits alveolarization and causes pulmonary inflammation. Dev Biol 2002; 242:75–87.
61. Boggaram V. Regulation of lung surfactant protein gene expression. Front Biosci 2003; 8:d751–d764.
62. Bruno MD, Bohinski RJ, Huelsman KM, et al. Lung cell-specific expression of the murine surfactant protein A (SP-A) gene is mediated by interactions between the SP-A promoter and thyroid transcription factor-1. J Biol Chem 1995; 270:6531–6536.
63. Ramirez MI, Rishi AK, Cao YX, et al. TGT3, thyroid transcription factor I, and Sp1 elements regulate transcriptional activity of the 1.3-kilobase pair promoter of T1alpha, a lung alveolar type I cell gene. J Biol Chem 1997; 272:26285–26294.
64. Whitsett JA, Glasser SW. Regulation of surfactant protein gene transcription. Biochim Biophys Acta 1998; 1408:303–311.
65. Yan C, Sever Z, Whitsett JA. Upstream enhancer activity in the human surfactant protein B gene is mediated by thyroid transcription factor 1. J Biol Chem 1995; 270:24852–24857.
66. Zhang L, Whitsett JA, Stripp BR. Regulation of Clara cell secretory protein gene transcription by thyroid transcription factor-1. Biochim Biophys Acta 1997; 1350:359–367.
67. DeFelice M, Silberschmidt D, DiLauro R, et al. TTF-1 phosphorylation is required for peripheral lung morphogenesis, perinatal survival, and tissue-specific gene expression. J Biol Chem 2003; 278:35574–35583.
68. Shaw-White JR, Bruno MD, Whitsett JA. GATA-6 activates transcription of thyroid transcription factor-1. J Biol Chem 1999; 274:2658–2664.
69. Aubin J, Lemieux M, Tremblay M, et al. Early postnatal lethality in Hoxa-5 mutant mice is attributable to respiratory tract defects. Dev Biol 1997; 192:432–445.
70. Bogue CW, Lou LJ, Vasavada H, et al. Expression of Hoxb genes in the developing mouse foregut and lung. Am J Respir Cell Mol Biol 1996; 15:163–171.
71. Volpe MV, Martin A, Vosatka RJ, et al. Hoxb-5 expression in the developing mouse lung suggests a role in branching morphogenesis and epithelial cell fate. Histochem Cell Biol 1997; 108:495–504.
72. Grindley JC, Bellusci S, Perkins D, et al. Evidence for the involvement of the Gli gene family in embryonic mouse lung development. Dev Biol 1997; 188:337–348.
73. Motoyama J, Liu J, Mo R, et al. Essential function of Gli2 and Gli3 in the formation of lung, trachea and oesophagus. Nat Genet 1998; 20:54–57.
74. Jaskoll TF, Don-Wheeler G, Johnson R, et al. Embryonic mouse lung morphogenesis and type II cytodifferentiation in serumless, chemically defined medium using prolonged in vitro cultures. Cell Differ 1988; 24:105–117.
75. Warburton D, Seth R, Shum L, et al. Epigenetic role of epidermal growth factor expression and signalling in embryonic mouse lung morphogenesis. Dev Biol 1992; 149:123–133.
76. Glazer L, Shilo BZ. The Drosophila FGF-R homolog is expressed in the embryonic tracheal system and appears to be required for directed tracheal cell extension. Genes Dev 1991; 5:697–705.
77. Sutherland D, Samakovlis C, Krasnow MA. Branchless encodes a Drosophila FGF homolog that controls tracheal cell migration and the pattern of branching. Cell 1996; 87:1091–1101.
78. Ornitz DM, Itoh N. Fibroblast growth factors. Genome Biol 2001; 2:REVIEWS3005.
79. Izvolsky KI, Zhong L, Wei L, et al. Heparan sulfates expressed in the distal lung are required for Fgf10 binding to the epithelium and for airway branching. Am J Physiol Lung Cell Mol Physiol 2003; 285:L838–L846.
80. Izvolsky KI, Shoykhet D, Yang Y, et al. Heparan sulfate-FGF10 interactions during lung morphogenesis. Dev Biol 2003; 258:185–200.

81. Hokuto I, Perl AK, Whitsett JA. Prenatal, but not postnatal, inhibition of fibroblast growth factor receptor signaling causes emphysema. J Biol Chem 2003; 278:415–421.
82. Min H, Danilenko DM, Scully SA, et al. Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless. Genes Dev 1998; 12:3156–3161.
83. Bellusci S, Grindley J, Emoto H, et al. Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung. Development 1997; 124:4867–4878.
84. Park WY, Miranda B, Lebeche D, et al. FGF-10 is a chemotactic factor for distal epithelial buds during lung development. Dev Biol 1998; 201:125–134.
85. Weaver M, Dunn NR, Hogan BL. Bmp4 and Fgf10 play opposing roles during lung bud morphogenesis. Development 2000; 127:2695–2704.
86. Post M, Souza P, Liu J, et al. Keratinocyte growth factor and its receptor are involved in regulating early lung branching. Development 1996; 122:3107–3115.
87. Cardoso WV, Itoh A, Nogawa H, et al. FGF-1 and FGF-7 induce distinct patterns of growth and differentiation in embryonic lung epithelium. Dev Dyn 1997; 208:398–405.
88. Deterding RR, Jacoby CR, Shannon JM. Acidic fibroblast growth factor and keratinocyte growth factor stimulate fetal rat pulmonary epithelial growth. Am J Physiol 1996; 271: L495–L505.
89. Liu Y, Jiang H, Crawford HC, et al. Role for ETS domain transcription factors Pea3/Erm in mouse lung development. Dev Biol 2003; 261:10–24.
90. Liu Y, Hogan BL. Differential gene expression in the distal tip endoderm of the embryonic mouse lung. Gene Expr Patterns 2002; 2:229–233.
91. Frank L. Protective effect of keratinocyte growth factor against lung abnormalities associated with hyperoxia in prematurely born Rats. Biol Neonate 2003; 83:263–272.
92. Guo L, Degenstein L, Fuchs E. Keratinocyte growth factor is required for hair development but not for wound healing. Genes Dev 1996; 10:165–175.
93. Colvin JS, Feldman B, Nadeau JH, et al. Genomic organization and embryonic expression of the mouse fibroblast growth factor 9 gene. Dev Dyn 1999; 216:72–88.
94. Colvin JS, White AC, Pratt SJ, et al. Lung hypoplasia and neonatal death in Fgf9-null mice identify this gene as an essential regulator of lung mesenchyme. Development 2001; 128:2095–2106.
95. Weaver M, Batts L, Hogan BL. Tissue interactions pattern the mesenchyme of the embryonic mouse lung. Dev Biol 2003; 258:169–184.
96. Mailleux AA, Tefft D, Ndiaye D, et al. Evidence that SPROUTY2 functions as an inhibitor of mouse embryonic lung growth and morphogenesis. Mech Dev 2001; 102:81–94.
97. Hacohen N, Kramer S, Sutherland D, et al. Sprouty encodes a novel antagonist of FGF signaling that patterns apical branching of the Drosophila airways. Cell 1998; 92:253–263.
98. Kramer S, Okabe M, Hacohen N, et al. Sprouty: a common antagonist of FGF and EGF signaling pathways in Drosophila. Development 1999; 126:2515–2525.
99. Reich A, Sapir A, Shilo B. Sprouty is a general inhibitor of receptor tyrosine kinase signaling. Development 1999; 126:4139–4147.
100. Hadari YR, Kouhara H, Lax I, et al. Binding of Shp2 tyrosine phosphatase to FRS2 is essential for fibroblast growth factor-induced PC12 cell differentiation. Mol Cell Biol 1998; 18:3966–3973.
101. Perl AK, Hokuto I, Impagnatiello MA, et al. Temporal effects of Sprouty on lung morphogenesis. Dev Biol 2003; 258:154–168.
102. Tefft D, Lee M, Smith S, et al. mSprouty2 inhibits FGF10-activated MAP kinase by differentially binding to upstream target proteins. Am J Physiol Lung Cell Mol Physiol 2002; 283:L700–L706.
103. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signaling. Nature 2003; 425:577–584.

104. McLennan IS, Poussart Y, Koishi K. Development of skeletal muscles in transforming growth factor-beta 1 (TGF-beta1) null-mutant mice. Dev Dyn 2000; 217:250–256.
105. Bartram U, Molin DG, Wisse LJ, et al. Double-outlet right ventricle and overriding tricuspid valve reflect disturbances of looping, myocardialization, endocardial cushion differentiation, and apoptosis in TGF-beta(2)-knockout mice. Circulation 2001; 103:2745–2752.
106. Kaartinen V, Voncken JW, Shuler C, et al. Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction. Nat Genet 1995; 11:415–421.
107. Shi W, Heisterkamp N, Groffen J, et al. TGF-beta3-null mutation does not abrogate fetal lung maturation in vivo by glucocorticoids. Am J Physiol 1999; 277:L1205–L1213.
108. Zhao J, Sime PJ, Bringas P Jr., et al. Spatial-specific TGF-beta1 adenoviral expression determines morphogenetic phenotypes in embryonic mouse lung. Eur J Cell Biol 1999; 78:715–725.
109. Zeng X, Gray M, Stahlman MT, et al. TGF-beta1 perturbs vascular development and inhibits epithelial differentiation in fetal lung in vivo. Dev Dyn 2001; 221:289–301.
110. Zhou L, Dey CR, Wert SE, et al. Arrested lung morphogenesis in transgenic mice bearing an SP-C-TGF-beta 1 chimeric gene. Dev Biol 1996; 175:227–238.
111. Lecart C, Cayabyab R, Buckley S, et al. Bioactive transforming growth factor-beta in the lungs of extremely low birthweight neonates predicts the need for home oxygen supplementation. Biol Neonate 2000; 77:217–223.
112. Toti P, Buonocore G, Tanganelli P, et al. Bronchopulmonary dysplasia of the premature baby: an immunohistochemical study. Pediatr Pulmonol 1997; 24:22–28.
113. Sime PJ, Xing Z, Graham FL, et al. Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. J Clin Invest 1997; 100:768–776.
114. Zhao J, Shi W, Wang YL, et al. Smad3 deficiency attenuates bleomycin-induced pulmonary fibrosis in mice. Am J Physiol Lung Cell Mol Physiol 2002; 282:L585–L593.
115. Gauldie J, Galt T, Warburton D. Transfer of active TGFβ1 gene to newborn rat lung induces changes consistent with Bronchopulmonary dysplasia. Am J Pathol 2003; 163:2575–2584.
116. KomatsuY, Shibuya H, Takeda N, et al. Targeted disruption of the Tab1 gene causes embryonic lethality and defects in cardiovascular and lung morphogenesis. Mech Dev 2002; 119:239–249.
117. Li DY, Sorensen LK, Brooke BS, et al. Defective angiogenesis in mice lacking endoglin. Science 1999; 284:1534–1537.
118. Sterner-Kock A, Thorey IS, Koli K, et al. Disruption of the gene encoding the latent transforming growth factor-beta binding protein 4 (LTBP-4) causes abnormal lung development, cardiomyopathy, and colorectal cancer. Genes Dev 2002; 16:2264–2273.
119. Chen H, Sun J, Buckley S, et al. Abnormal mouse lung alveolarization caused by Smad3 deficiency is a development antecedent of centrilobular emphysema. Am J Physiol Lung Cell Mol Physiol 2005; 288:L683–L691.
120. Shi W, Zhao J, Anderson KD, et al. Gremlin negatively modulates BMP-4 induction of embryonic mouse lung branching morphogenesis. Am J Physiol Lung Cell Mol Physiol 2001; 280:L1030–L1039.
121. Bragg AD, Moses HL, Serra R. Signaling to the epithelium is not sufficient to mediate all of the effects of transforming growth factor beta and bone morphogenetic protein 4 on murine embryonic lung development. Mech Dev 2001; 109:13–26.
122. Bellusci S, Henderson R, Winnier G, et al. Evidence from normal expression and targeted misexpression that bone morphogenetic protein (Bmp-4) plays a role in mouse embryonic lung morphogenesis. Development 1996; 122:1693–1702.
123. Eblaghie MC, Reedy M, Oliver T, et al. Evidence that autocrine signaling through Bmpr1a regulates the proliferation, survival and morphogenetic behavior of distal lung epithelial cells. Dev Biol 2006; 291:67–82.