CHAPTER 2

PRENATAL LUNG DEVELOPMENT

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Introduction

Lung development requires integration of multiple regulatory factors that mediate patterns of cell proliferation, differentiation, migration, and death. These developmental programs, which include transcription factors and signaling molecules, are likely re-enlisted during recovery of the lung following injury. Understanding these processes could provide important insight into controlling cell differentiation and regeneration for therapeutic purposes.

This chapter describes the morphological features that characterize the defined stages of lung development and also addresses epithelial cell differentiation and vasculogenesis of the airways. In the latter part of the chapter, an attempt will be made to highlight specific cellular and molecular events during development that may influence lung maintenance in adult disease states.

Stages of Lung Development

During gestation, the fetal lung undergoes significant morphological changes to provide at birth an organ capable of maintaining respiration and gas exchange. Although lung development is continuous during embryogenesis, five developmental stages have been delineated, based on anatomic and histologic characteristics.1 (Table 2–1). The early embryonic and pseudoglandular stages elaborate the conducting airways; the latter canalicular, saccular, and alveolar stages are characterized by reduction of mesenchyme and vascularization to form a thin air-blood barrier. Birth does not signal the end of lung development. There is a continuing complex process of lung growth after birth, permitting changing relationships of airway size, alveolar size, and surface area. At birth, the newborn infant, with approximately 50 million alveoli, has the potential to add another 250 million alveoli and increase surface area from approximately 3 to 70 m².2 There are more than 40 different cell types in the lung, with different functions.2 How they establish their final appropriate physical and numerical relationships with each other is still unknown.

Embryonic Stage (3 to 7 Weeks)

The human fetal lung originates as a ventral diverticulum that arises from the laryngotracheal groove of the foregut endoderm.2 The laryngotracheal groove separates dorsoventrally from the primitive esophagus to form the tracheal rudiment and, at the same time, gives rise laterally to the two primary bronchial buds (Figure 2–1A). The lung bud grows into adjacent splanchnic mesoderm where it is induced to branch repeatedly, giving rise to the future respiratory tree. This primitive lung bud is lined by endodermally derived epithelium, which differentiates into specialized cells that line both the conducting and respiratory airways.3 Mesenchymal cells condensed around the primitive airways give rise to blood vessels, smooth muscle, cartilage, and other connective tissues of the lung.4

Pseudoglandular Stage (7 to 16 Weeks)

During the pseudoglandular stage, the stage of branching morphogenesis, there is rapid proliferation of the primi-

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**TABLE 2–1. Stages of Lung Development**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Developmental Age</th>
<th>Major Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic</td>
<td>3–7 wk</td>
<td>Lung budding from the foregut endoderm, with formation of trachea and mainstem bronchi</td>
</tr>
<tr>
<td>Pseudoglandular</td>
<td>7–16 wk</td>
<td>Airway division completed, with formation of 25,000 terminal bronchioles; cartilage, smooth muscle derived from mesenchyme</td>
</tr>
<tr>
<td>Canalicular</td>
<td>16–24 wk</td>
<td>Capillarization, with acinar formation; type I and II epithelial cells first differentiate</td>
</tr>
<tr>
<td>Saccular</td>
<td>24–36 wk</td>
<td>Progressive thinning of epithelial cells; terminal saccular formation; surfactant production</td>
</tr>
<tr>
<td>Alveolar (postnatal)</td>
<td>36 wk through infancy</td>
<td>Appearance of true alveoli; alveolar septation and expansion of air spaces</td>
</tr>
<tr>
<td></td>
<td>40 wk through infancy</td>
<td></td>
</tr>
</tbody>
</table>

wk = weeks.
tive airways so that all airway divisions are more or less completed by 16 weeks. This translates into 12 to 17 branches in the upper lobes, 18 to 23 branches in the middle lobes, and 14 to 23 branches in the lower lobes. The branching pattern does not change after this stage and is similar to that of the adult lung. The most peripheral structures, the terminal bronchioles, will further differentiate to form the future respiratory bronchioles and alveolar ducts. The name pseudoglandular is derived from the histologic appearance of the lung, which on cross section consists of hollow tubular-like structures (glands) surrounded by clusters of mesenchymal cells (see Figure 2–1B). The columnar epithelial cells that line the tubules contain cytoplasmic glycogen; a few become ciliated as early as 8 weeks while others begin to differentiate into goblet cells. During this period, cartilage begins to form around the larger airways and smooth muscle forms around airways and major blood vessels.

Canalicular Stage (16 to 24 Weeks)
The canalicular stage is so named because the potential air spaces are being “canalized” and approximated by a network of capillaries (see Figure 2–1C). The pulmonary acinar units, which eventually contain alveolar ducts, alveolar sacs, and alveoli, develop during this period. “Acinus” is the term applied to the gas exchange unit associated with a single terminal bronchiole. It follows that primitive lung lobules will have formed by the beginning of the canalicular stage. Each lobe contains three to five terminal bronchi and, by the end of 27 weeks, approximately 25,000 terminal bronchioli. A gradual decrease in mesenchymal tissue results in close apposition of the pulmonary vasculature to the epithelium. By 20 to 22 weeks’ gestation, type I and type II alveolar cells can be differentiated from the cuboidal epithelial cells in the most peripheral parts of the lung. Lamellar bodies associated with surfactant synthesis begin to appear in the cytoplasm of type II cells. Type I alveolar lining cells, which differentiate from type II cells, begin their flattening process and attenuate to provide an air-blood interface. The conducting airways have fully developed smooth muscle, and lymphatic structures now begin to appear. The developing pulmonary arteries and veins follow the development of the branching airways but lag behind it somewhat. By the end of the canalicular period, the potential air-blood barrier is thin enough to

![Embryonic Stage](image1) ![Pseudoglandular Stage](image2)
FIGURE 2-1. Schematic and light-microscopic appearance of human fetal lung at different stages of development.
support gas exchange. The bronchial artery system may be as critical for lung development as the pulmonary artery although the role of the bronchial artery in pulmonary differentiation and growth is currently unknown. It has been suggested that the most peripheral parts of the developing lung are supplied only by the pulmonary arterial vasculature.

Saccular Stage (28 to 35 Weeks)
The term “saccule” derives from the saclike appearance of the most peripheral air spaces, which represent the future alveolar ducts and alveoli (see Figure 2–1D). According to Boyden, each acinus supplied by a terminal bronchiole has three to four respiratory bronchioles that end in a transitional duct from which the saccules arise. The major changes that occur during the saccular stage are further compression of the intervening interstitium, thinning of the epithelium, and the beginning of alveolar septation, with the formation of small mesenchymal ridges. There is lengthening and widening of saccules distal to the terminal bronchioles and the addition of the last generations of future alveolar spaces. Continual differentiation of type I and II alveolar cells occurs during this period, so that the alveolar epithelial cells become the most abundant epithelial cells in the lung. The flattened type I alveolar cells make up the majority of these cells. Type II cells, ultrastructurally distinguished by their production of lamellar bodies, expand in size and number, with increased storage of surfactant lipids and less cytoplasmic glycogen.

Alveolar Stage (36 Weeks through Infancy)
Several million alveoli form before birth although this final stage of lung development primarily occurs during postnatal life. The beginning of this stage is not sharply defined; some alveolar formation probably begins a few weeks earlier. Alveolar formation is closely linked to the deposition of elastin in the saccular lung. Terminal saccules become invaginated by protrusions from the wall of epithelial cells and contain a double-walled capillary system (see Figure 2–1E). These protrusions elongate and thin, forming primitive alveoli that at first resemble shallow cups and then become deeper as development continues.

Postnatal Lung Growth
During the postnatal phase, lung growth is geometric, and there is no increase in airway number. There is proportionately less growth in the conducting airways in comparison with alveolar-capillary tissue. Estimates of the number of alveoli at birth vary widely, but an average of 50 million is generally accepted. These alveoli provide a gas-exchanging surface of approximately 3 to 4 m². Alveoli greatly increase in number after birth, to reach the adult range of 300 million by 2 years of age and the surface area of 75 to 100 m² by adulthood. There is substantial remodeling of the parenchyma after birth, with morphologic changes in the septa. Alveolarization occurs through the formation of numerous short, blunt tissue crests or ridges, and their protrusion into alveolar sacs increases the internal surface of the lung. The development of the alveolar crest is closely linked with elastin deposition and the local proliferation of interstitial and epithelial cells.

Epithelial Cell Differentiation
Maturation of the epithelium during development starts in the proximal airways and progresses distally into the intrapulmonary airways. Epithelial cell lineages are arranged in a distinct proximal-distal spatial pattern in the airways, which become morphologically apparent.
during the pseudoglandular stage. At least 11 different epithelial cell types have been described in the conducting and respiratory portions of the lung. The basal, secretory, and ciliated cells are the major cell types constituting the pseudostratified portion of the proximal tracheobronchial epithelium while type I and type II alveolar cells make up the distal respiratory epithelium. The lineage relationships between the different cell types have not been delineated, and the existence and identity of progenitor cells, which may play a role in lung injury and repair, are currently under study. There is some evidence from cell kinetic studies to suggest that basal cells, Clara cells, and type II alveolar cells are the primary progenitor cells for the pulmonary epithelium. Following acute lung injury, Clara cells and type II cells regain the capacity to proliferate and repopulate the damaged bronchiolar and alveolar epithelium, respectively. Lineage studies have not addressed the relationship of pulmonary neuroendocrine cells to the putative stem cells (type II cells and Clara cells) although neuroendocrine cells differentiate morphologically before any other epithelial cell type. Bombesin, a peptide secreted by pulmonary neuroendocrine cells, stimulates branching morphogenesis and lung maturation, in culture and in vivo.

The onset of cellular differentiation is signaled by the expression of differentiated gene products. Lung-specific gene products include the surfactant proteins (surfactant proteins A, B, C, and D [SP-A, SP-B, SP-C, and SP-D]) and Clara cell secretory protein (CCSP). The surfactant-associated proteins are abundant phospholipid-associated proteins that are expressed primarily in alveolar type II epithelial cells, although SP-A and SP-B are also detected in subsets of nonciliated epithelial (Clara) cells of the conducting airways and tracheobronchial glands. Expression of these genes is extinguished when type II cells undergo terminal differentiation to type I cells that constitute most of the gas exchange surface of the alveolus. For the most part, CCSP is a marker for the proximal Clara cells of the bronchiolar epithelium (Figure 2–3). Recent transgenic studies support a potential role for CCSP in the control of inflammatory responses; CCSP-deficient mice have an increased sensitivity to hyperoxia-induced lung injury characterized by lung edema and induction of pro-inflammatory cytokine messenger ribonucleic acids (mRNAs). Hepatocyte nuclear factor/korkhead homologue 4 (HFH-4), a winged helix transcription factor, has been implicated in ciliated cell development (see Figure 2–3), with mutational abnormalities in the mouse similar to those seen in Kartagener’s syndrome.

Early on in embryonic development, the undifferentiated epithelium coexpresses several lineage markers, including SP-A, CCSP, and calcitonin gene-related peptide, a marker of neuroendocrine cells. It is during the pseudoglandular stage that pulmonary epithelial cell lineages become restricted to proximal and distal regions of the airways. Of interest, after bleomycin-induced injury in the adult lung, there is again coexpression of lineage-specific markers, suggesting that a progenitor-type cell may be re-enlisted during epithelial repair. Tissue recombination experiments have demonstrated that a proximal versus a distal epithelial cell phenotype is dictated by soluble factors from the adjacent mesenchyme. Within a restricted time window of development, distal lung mesenchyme can reprogram rat tracheal epithelium to express type II cell differentiation, and conversely, tracheal mesenchyme can induce distal lung epithelium to express tracheal cytodifferentiation.

Analysis of transcriptional elements in the surfactant protein genes and CCSP has provided an understanding of the shared mechanisms of gene regulation and expression in respiratory epithelial cells. A homeodomain protein, Nkx2.1 (also known as thyroid transcription factor-1 [TTF-1]), and thyroid enhancer binding protein [T/ebp] plays an essential role in several phases of lung development, including epithelial cell lineage determination. Nkx2.1 is the earliest marker of the developing respiratory epithelium, with onset of expression at the time of lung bud formation from the foregut endoderm. Distribution of Nkx2.1 expression in the fetal lung includes respiratory epithelial cells of the trachea, bronchi, and developing respiratory tubes although expression is most robust in distal alveolar cells (see Figure 2–3). In vitro, Nkx2.1 binds and activates SP-A, SP-B, SP-C, and CCSP promoter elements as well as Nkx2.1 itself. Mice with a null mutation of Nkx2.1 have tracheosophageal fistulas and fail to form bronchiolar and alveolar structures distal to the lobular bronchi. Pulmonary-specific gene expression including SP-B, SP-C, and CCSP is extinguished within transgenic lungs, which do, however, contain ciliated and mucus-secreting cells. Thus, Nkx2.1 is felt to function as a “master gene” that induces and maintains lung morphogenesis as well as differentiation of certain epithelial cell lineages.

Cellular and Molecular Mechanisms of Lung Development

During the past few years, major progress has been made in identifying key determinants of branching morphogenesis and cellular differentiation within the lung. New insights have been derived through the characterization of signaling molecules that regulate gene expression and that are functionally conserved through evolution in invertebrate species such as *Drosophila* and *Caenorhabditis elegans*, which are functionally conserved through evolution. Null muta-
tions in mouse models have identified several nuclear transcription proteins, which determine respiratory-cell fate and pattern formation via the activation and repression of downstream target genes. The recurrent theme emerging from these studies is that lung development extends in a coordinated manner from successive epithelial-mesenchymal interactions. Growth factor signaling and induction of responsive transcription factors mediate this interplay between developing epithelial and mesenchymal structures. The following section will detail specific cellular and molecular events during lung morphogenesis, which may be recalled during tissue injury and regeneration.

Early Lung Differentiation

The lung shares a common embryological origin of other foregut derivatives including the liver, pancreas, gastrointestinal tract, and thyroid (Figure 2–4). While many studies have focused on events critical to organ morphogenesis and terminal cell differentiation, few have revealed how initial cell type choices are made. In Drosophila, the initial specification of tissues within the gut endoderm is caused by signals from overlying mesoderm. Studies within the liver have demonstrated that changes in gene expression and tissue morphology define discrete phases in organ development. Hepatic gene expression and differentiation begin in the foregut endoderm immediately after the endodermal epithelium interacts with the cardiac mesoderm, in the 8.5-day-old embryo. Recent studies have demonstrated that fibroblast growth factors (FGFs) ema

FIGURE 2-3. Comparison of N-myc, thyroid transcription factor-1 (TTF-1 or Nkx2.1), hepatocyte nuclear factor/forkhead homologue 4 (HFH-4), and Clara cell secretory protein (CCSP) messenger ribonucleic acid (mRNA) expression in human fetal xenograft lung, with the appearance of the alveolar stage of lung development, shown on dark-field photomicrographs of serial sections. Expression of N-myc is not detectable in the xenograft lung at this time point. The signal for TTF-1 is restricted to distal alveolar spaces whereas CCSP and HFH-4 are localized to the proximal tracheal and bronchiolar epithelium. (T = trachea; b = bronchi; a = alveoli.)
specification are currently unknown, preliminary studies suggest that dosage-dependent growth factor signaling from the cardiac mesoderm might play a role (G.H. Deutsch, unpublished observations) (see Figure 2–4).

The early pattern of expression of the homeobox gene Nkx2.1 is consistent with its role in the morphogenesis of the lung. Targeted disruption of the gene has demonstrated that while Nkx2.1 is not required for the initial specification of the lung primordia, Nkx2.1 is essential for further pulmonary development and cell differentiation. Study of the cis-acting regions in the Nkx2.1 promoter reveals that Nkx2.1 has multiple binding sites for both ubiquitous and specific transcription factors, including those of the hepatocyte nuclear factor (HNF) and GATA zinc finger families, which are required for the development of the foregut endoderm. The HNF-3β null mutation results in an early embryonic lethal phenotype, in which the primitive foregut endoderm fails to close into a tube; consequently, there is no development of foregut derivatives such as the lung.

Pattern Formation of the Lung

In the fully differentiated lung, epithelial cells from the proximal tracheal, and bronchial tubules greatly differ from the distal alveolar tubules in morphology and gene expression. The general principle of pattern formation states that cells are instructed to assume a specific function according to their position along a concentration gradient of a signal. Cells located closer to the source of signal recognize a higher concentration and assume a different phenotype from those cells located at a distance, which recognize low concentrations of the same signal. Recognition of their position is followed by the activation or repression of target genes, which ultimately determine the regional differences in cell identity along the lung axis. We have previously discussed how the presence of proximal versus distal mesenchyme can dictate cell differentiation in adjacent epithelial cells. Early studies have also shown that the quantity of mesenchyme is influential in inducing a specific epithelial cell fate. A small amount of recombined mesenchyme is able to direct cell differentiation toward a bronchiolar phenotype; however, an increased amount of the same mesenchyme will induce the epithelial cells to differentiate toward an alveolar phenotype. This study implies that variable concentrations of the same signals or morphogens expressed by the mesenchyme can induce different cell fates in identical epithelial cells.

Homeobox (Hox) genes are transcription factors, which are known to regulate body axis patterning and specification of regional identity during embryonic development, although the mechanisms by which they do so are largely unknown. The Hox genes likely regulate downstream factors that influence cell proliferation, migration, and cell death. The pattern of Hox gene expression at the beginning of lung development is consistent with its role in elaborating the proximal-distal orientation of the lung and in branching morphogenesis. In the mouse, Hoxb-3 and Hoxb-4 are expressed in the mesenchyme of the entire developing lung (proximal and distal) while Hoxb-2 and Hoxb-5 expression are restricted to the mesenchyme of distal lung buds. The levels of Hox-gene expression decline with advancing gestational age. Hoxb genes may be involved in patterning the developing foregut by specifying regional differences in lung mesenchyme. Hoxb-3 transactivates the rat Nkx2.1 gene promoter, which infers that Hoxb-3 could be involved in lung-specific gene regulation through Nkx2.1 as an intermediate. In cultured mouse embryonic lung, retinoic acid induces Hoxa-5, Hoxb-5 and Hoxb-6 gene expression, while Hoxb-5 is negatively regulated by epidermal growth factor (EGF) and transforming growth factor-β (TGF-β). Expression of Hox genes in the adult lung may be relevant to neoplasia as well as to pathologic disease states such as pulmonary hypertension and emphysema. Retinoids have also been shown to
influence the proximal-distal pattern of lung development. Retinoic acid, in dose-dependent concentrations, has been demonstrated to favor the growth of proximal airways and gene expression at the expense of distal structures. It is probable that Hox genes mediate the retinoic acid–induced alteration in lung patterning.

Bone morphogenetic proteins (Bmps), members of the TGF-β–related family of signaling molecules, are also implicated in the control of the proximal-distal patterning of the lung and in branching morphogenesis. Bone morphogenetic protein 4 (Bmp-4) is restricted to the tips of distal buds and to the adjacent mesenchyme, locally inhibiting endoderm proliferation and forcing the outgrowth of lateral branches (Figure 2–5). Inhibition of Bmp signaling in transgenic animals results in complete proximalization of the respiratory epithelium, including ciliated cells in the most distal portions of transgenic lungs. It is hypothesized that Bmps provide a concentration gradient to control proximal versus distal differentiation of the lung endoderm. Endodermal cells located at the periphery of the lung, which are exposed to high levels of Bmp-4, maintain or assume a distal identity while cells below a certain threshold of the Bmp-4 signal initiate a proximal differentiation program.

**FIGURE 2–5.** Epithelial-mesenchymal interactions in the developing lung during branching morphogenesis. Factors are represented only at sites where the expression is most abundant. Fibroblast growth factor (FGF) signals in the mesenchyme act as a chemotactic focus for the epithelium during lung budding. As the bud extends toward the FGF signals in the mesenchyme, endoderm cells that escape a high bone morphogenetic protein 4 (Bmp-4) signal assume a proximal cellular identity; those that retain a high Bmp-4 signal take on a distal identity. A high concentration of Bmp-4 signal also serves to locally inhibit endoderm proliferation, thereby inducing the lateral outgrowth of new airway branches. Sonic hedgehog (Shh) at the distal tips functions to downregulate FGF-10 expression in the mesenchyme, which limits local budding. Transforming growth factor-β (TGF-β) signaling also prevents local budding, by decreasing endodermal proliferation and by stimulating synthesis of matrix components at branch points (FGFR = fibroblast growth factor receptor; Hoxb = homeobox b.).

**Regulation of Branching Morphogenesis**

Elegant recombination experiments have demonstrated that mesenchyme is necessary for initial lung budding and branching and that the mesenchyme surrounding the trachea and mainstem bronchi has a different regional identity than the mesenchyme surrounding the distal lung bud. There is abundant data to suggest that soluble growth factors are likely mediators of this interaction. Candidate growth factors whose signaling peptides and cognate receptors are expressed in the early embryonic mouse lung include FGFs, EGFs, TGFs, hepatocyte growth factor (HGF), and platelet-derived growth factor (PDGF). Their influences on lung development have been demonstrated by gain vs. loss of function experiments in embryonic mouse lung organ cultures and in transgenic mice. In general, growth factor receptors with tyrosine kinase intracellular signaling domains (EGF, FGF, and PDGF receptors [EGFR, FGFR, and PDGF-R]) stimulate lung morphogenesis, whereas those with serine/threonine kinase intracellular signaling domains (such as the TGF-β family) are inhibitory.

Branching of the respiratory tracheae in *Drosophila* is considered a conserved genetic paradigm for the morphogenesis of the mammalian lung. Primary branching in *Drosophila* is spatially regulated by the *branchless* gene, of which FGF-10 is the mammalian homologue. The *branchless* gene activates the *breathless* gene in tracheal cells, to induce their migration and thus primary branching. *Breathless* is homologous to the FGFR family. This FGF homologue-signaling pathway appears to be evolutionarily conserved in mammals. Fibroblast growth factor-1 (FGF-1), FGF-7, and FGF-10 are synthesized by the pulmonary mesenchyme, and they bind to and activate FGFRs on endodermal cells of the developing lung buds. Fibroblast growth factor-10 may play a chemotactic role similar to that of *branchless* in inducing epithelial branching and cell migration. Transcripts of FGF-10 are present at discrete sites in the mesenchyme and have been demonstrated to govern the directional outgrowth of lung buds (see Figure 2–5). At early stages, FGFR-2, the receptor for FGF-10, is expressed at high levels along the entire proximal-distal axis of the respiratory-tract epithelium. Disruption of FGF signaling by the generation of FGF-10–deficient mice, or expression of a dominant negative form of its receptor (FGFR-2), interferes with branching morphogenesis and peripheral cell differentiation; transgenic lungs are characterized by only a trachea or a trachea and two primary bronchi. Thus, it appears that the elaboration of branched airways involves the directional movement of epithelial cell precursors toward a localized source of an FGF ligand, either by cell migration or by outgrowth of epithelial buds.
Despite high-sequence homology and use of the same receptor, FGF-7 and FGF-10 exert distinct effects on the developing lung. Rather than being chemotactic, FGF-7 influences airway branching by promoting epithelial cell proliferation and expansion.118,119,120 Misexpression of FGF-7 in mice during the pseudoglandular stage disrupts branching morphogenesis and epithelial cell differentiation.118 Transgenic lungs have large cystic spaces that resemble human congenital cystic adenomatoid malformations. Fibroblast growth factor-7 as a differentiation factor for the developing lung has been demonstrated in lung organotypic cultures. In the absence of mesenchyme or serum, exogenous FGF-7 can induce a type II cell–like phenotype.117,119 Despite evidence that FGF-7 plays a role in lung development, mice homozygous for a null mutation in the FGF-7 gene exhibit no apparent pulmonary abnormalities.120 This suggests that other members of the FGF family (perhaps FGF-10) can serve as functionally redundant molecules.

Many studies have shown that certain signaling and growth factors are negative regulators of lung epithelial proliferation, which may play a role in counteracting the bud-promoting effects of FGFs. Fibroblast growth factor-10 is downregulated in lungs of transgenic mice overexpressing sonic hedgehog (Shh),85 which is a secreted signaling protein that induces gene expression and proliferation of adjacent mesenchymal target cells in the lung.93,121 Low levels of Shh are expressed throughout the primary respiratory epithelium, with high levels seen at the distal tips85,122 (see Figure 2–5). Treatment of embryonic lung mesenchymal cells with recombinant Shh markedly inhibits FGF-10 mRNA expression.123 In the absence of Shh, FGF-10 expression is no longer restricted to focal areas but becomes widespread throughout the distal mesenchyme.124 This finding raises the possibility that one of the roles for Shh signaling in branching is to spatially restrict FGF-10 levels in the mesenchyme surrounding the bud tips.124 Sonic hedgehog–null mutant mice have tracheoerosophageal fistulas and bilateral rudimentary sacs, due to failure of branching and growth after formation of the primary lung buds.121,124 The lung mesenchyme shows enhanced cell death and decreased cell proliferation.121 Conversely, overexpression of Shh leads to hypercellularity of the lung, with an absence of normal alveolar septa because of excessive mesenchyme between the alveolar spaces.125 Sonic hedgehog expression in the endoderm may also be essential for the proliferation and differentiation of the mesoderm, which in turn is required for proper differentiation of the primitive pulmonary epithelium.121 Of interest, treatment of cultured lungs with retinoic acid increases the level of Shh expression while decreasing the level of FGF-10 expression and the degree of airway branching.126,127 These data support the concept that distinct signaling and transcriptional pathways are interrelated during lung development.

The TGF-βs represent a family of peptides involved in cell growth and apoptosis control, connective-tissue formation, and embryonic development.128 Transforming growth factor-β1, TGF-β2, and TGF-β3 are indirect mitogens for certain mesenchymal cells and are marked stimulators of extracellular matrix deposition, including collagen, fibronectin, and proteoglycans.128 The TGF-βs are also potent inhibitors of epithelial cell proliferation. The TGF-β1 protein is localized at the interface between epithelial and mesenchymal cells, particularly around the bronchiolar ducts and airway branch points.90,129 Over expression of members of the TGF-β family, both in organ culture and in transgenic animals, has a negative regulatory effect on branching morphogenesis.106,108 Abrogation of TGF-β type II receptor signaling (either with antisense oligodeoxynucleotides or with blocking antibodies), stimulates lung morphogenesis twofold to threefold, and increases expression of the distal NKx2.1 and SPC genes.108 Transforming growth factor-β1 has been shown to downregulate the expression of N-myc and branching morphogenesis in murine organ lung cultures.106 The N-myc gene may play a role in lung development by maintaining cells in an undifferentiated state; its expression is most abundant in undifferentiated progenitor cells and primitive tumors.130,131 Targeted disruption of the N-myc gene results in embryonic lethality during the pseudoglandular stage of development, with a clear defect in branching morphogenesis; homozygous lungs consist of only a trachea and mainstem bronchi.132–135

Factors Related to Alveolarization

During postnatal life, FGFR-3 and FGFR-4, receptors that bind several of the FGFs, cooperate to regulate alveolarization; a double mutation of these genes results in a postnatal lethal pulmonary phenotype in which there is failure of alveolar septation, resulting in an emphysematous appearance.136 Lungs of transgenic mice demonstrate an aberration of elastin synthesis. Mutants lacking the growth factor PDGF-A also exhibit a defect in pulmonary alveogenesis, but this phenotype is apparently caused by a loss of alveolar smooth-muscle myofibroblasts and parenchymal elastic fibers.137 The defect in alveolization in neonatal EGF receptor–deficient mice is also associated with impaired branching morphogenesis.105 Inactivation of the EGF receptor affects type-II pneumocyte maturity, with decreased expression of the distal SPC marker. In contrast, exogenous EGF accelerates alveolar type-II cell differentiation in fetal lungs.138

Pulmonary Vasculogenesis

Like lung morphogenesis, pulmonary vascular development is a highly regulated process involving not only the formation and differentiation of new vessels but also the
inhibition of further vascular proliferation. Direct epithelial-mesenchymal interactions may be essential for vessel formation, with the participation of growth factors and matrix proteins. Pulmonary endothelial cell differentiation is still poorly understood, but studies suggest that receptors for the vascular endothelial growth factor (VEGF), located within the mesenchyme, facilitate early vessel formation within the embryo. Growth factors such as FGF-2 and VEGF are known stimulants of endothelial cell migration, proliferation, and transdifferentiation, leading to the appearance of vascular structures. Misexpression of VEGF in transgenic mice under the control of the SP-C promoter/enhancer induces gross abnormalities in lung morphogenesis, characterized by an increase in vascularity and concomitant decrease in distal acinar tubules and mesenchyme. Inhibition of the VEGF receptor KDR (VEGFR-2) also influences lung morphology by reducing vascular density and alveolarization in the newborn rat.

Endothelial monocyte-activating polypeptide II (EMAPII), localized primarily to the mesenchyme, has been demonstrated to have a negative effect on neovascularization in the developing lung. Its location correlates with the same area of expression as other regulators of vessel formation, including the VEGF receptors 1 and 2. Its continual expression within large vessels in adulthood leads one to theorize that EMAPII might function postnatally to stabilize or maintain existing vascular structures.

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