PROSPECTS
Journal of Cellular Biochemistry 115:1469–1477 (2014)

Journal of Cellular Biochemistry

Tissue Crosstalk in Lung Development

Elizabeth A. Hines and Xin Sun*

Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

Lung development follows a stereotypic program orchestrated by key interactions among epithelial and mesenchymal tissues. Deviations from this developmental program can lead to pulmonary diseases including bronchopulmonary dysplasia and pulmonary hypertension. Significant efforts have been made to examine the cellular and molecular basis of the tissue interactions underlying these stereotypic developmental processes. Genetically engineered mouse models, lung organ culture, and advanced imaging techniques are a few of the tools that have expanded our understanding of the tissue interactions that drive lung development. Intimate crosstalk has been identified between the epithelium and mesenchyme, distinct mesenchymal tissues, and individual epithelial cells types. For interactions such as the epithelial–mesenchymal crosstalk regulating lung specification and branching morphogenesis, the key molecular players, FGF, BMP, WNT, and SHH, are well established. Additionally, VEGF regulation underlies the epithelial–endothelial crosstalk that coordinates airway branching with angiogenesis. Recent work also discovered a novel role for SHH in the epithelial-to-mesenchymal (EMT) transition of the mesothelium. In contrast, the molecular basis for the crosstalk between upper airway cartilage and smooth muscle is not yet known. In this review we examine current evidence of the tissue interactions and molecular crosstalk that underlie the stereotypic patterning of the developing lung and mediate injury repair. J. Cell. Biochem. 115: 1469–1477, 2014.

KEY WORDS: DEVELOPMENTAL SIGNALING; TRACHEA; AIRWAYS; EPITHELIUM; MESENCHYME

The lung is a complex organ comprised of cells derived from all three germ layers. Beginning in the earliest stage of lung specification and extending through adult lung injury response, these diverse cell types coordinate their spatial and temporal development. Multiple past studies demonstrated that lung epithelial branching and airway patterning follow a highly stereotypical program [Weaver et al., 1999; Mucenski et al., 2003; Shu et al., 2005; Chen et al., 2007; Metzger et al., 2008]. A continuous crosstalk between the distal epithelium and mesenchyme establishes this pattern [Bellusci et al., 1997; Park et al., 1998; Weaver et al., 2000]. Concomitant with these epithelial morphogenesis events, mesenchymal progenitors that will give rise to the support tissues of the lung, are being specified and allocated to the proper compartments through epithelial–mesenchymal and intra-mesenchymal tissue crosstalk. These support tissues include the upper airway cartilage, smooth muscle (SM), pulmonary vasculature, lymphatic vessels, and neurons. Following branching morphogenesis, the proximal and distal epithelial cells differentiate into specialized cell types; a process regulated by intra-epithelial signals and mesenchymal to epithelial crosstalk. Numerous congenital and acquired diseases originate from tissue imbalances that can arise during lung development. Therefore, further investigation into the developmental mechanisms that regulate pulmonary tissue patterning may lead to novel therapies for these conditions. This review highlights the importance of tissue crosstalk in the proper formation of the conducting airways. For additional information on topics not addressed here including alveologenesis, airway stem cells and transcriptional control of lung development, please see additional reviews [Maeda et al., 2007; Morrisey and Hogan, 2010; Rock and Hogan, 2011; Wansleeben et al., 2013; Herriges and Morrisey, 2014].

EARLY EPITHELIAL–MESENCHYMAL CROSSTALK DRIVES LUNG SPECIFICATION, BUDDING, AND BRANCHING

The initial pulmonary cell fate is induced in the endoderm-derived foregut epithelium by the surrounding mesoderm-derived mesenchyme. Ventral mesenchyme expressed signaling molecules FGF, BMP, and WNT, play key roles in specifying the pulmonary domain in the ventral foregut endoderm in part through transcriptional activation of *Nkx2-1* [Serls et al., 2005; Que et al., 2007; Goss

Grant sponsor: National Science Foundation Graduate Research Fellowship; Grant number: 2011101268; Grant sponsor: National Heart, Lung, and Blood Institute; Grant number: R01 HL113870.

*Correspondence to: Xin Sun, Laboratory of Genetics, University of Wisconsin, 425-G Henry Mall, Madison, WI 53706. E-mail: xsun@wisc.edu

Manuscript Received: 15 March 2014; Manuscript Accepted: 18 March 2014

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 19 March 2014

DOI 10.1002/jcb.24811 • © 2014 Wiley Periodicals, Inc.

et al., 2009; Harris-Johnson et al., 2009; Domyan et al., 2011]. *Nkx2-1* is the earliest marker of the pulmonary lineage and its expression is induced at approximately embryonic day 9.0 (E9.0). Genetic loss of *Nkx2-1* results in tracheo-esophageal fistula and severe lung branching defects, although interestingly, the lung primordium is correctly specified [Lazzaro et al., 1991; Kimura et al., 1996, 1999 Minoo et al., 1999; Yuan et al., 2000]. Ex vivo culture studies have demonstrated that high levels of FGF can induce *Nkx2-1* expression in endoderm explants, whereas lower FGF concentrations result in expression of *albumin* (*Alb*), a liver specific gene [Serls et al., 2005]. *Fgf1*, *Fgf2*, and *Fgf10* are expressed in the ventral mesoderm adjacent to the foregut epithelium during the time of pulmonary cell fate specification [Serls et al., 2005; Que et al., 2007]. These findings suggest FGF signals are important for the proper spatial specification of pulmonary fate along the anterior–posterior axis.

In contrast to FGF, BMP signals have been shown to be essential for pulmonary fate specification along the dorsal–ventral axis [Que et al., 2006; Domyan et al., 2011]. A crucial gradient of BMP signaling develops across the embryonic foregut through the expression of the BMP antagonist *Noggin* in the dorsal mesenchyme and *Bmp4* in the ventral mesenchyme [Weaver et al., 1999; Que et al., 2006]. Inactivation of *Noggin* causes a partially penetrant tracheo-esophageal fistula where the trachea is specified correctly, but the esophagus fails to separate [Que et al., 2006]. This defect can be rescued through the genetic loss of one copy of *Bmp4*, indicating that low levels of BMP signaling are necessary for esophageal fate to be specified in the dorsal foregut. Inactivation of BMP receptors, *Bmpr1a* and *Bmpr1b* (*Bmpr1a/b*), results in loss of *Nkx2-1* expression, expansion in the esophageal *Sox2* expression domain, and subsequent tracheo-esophageal fistula [Domyan et al., 2011]. Interestingly, genetic inactivation of *Sox2* in *Bmpr1a/b* mutants restores *Nkx2-1* expression in the ventral foregut, suggesting that BMP signaling indirectly promotes *Nkx2-1* expression through its repression of *Sox2* [Domyan et al., 2011]. Therefore, proper mesenchymal BMP levels across the dorsal–ventral axis of the embryo are essential for proper induction of pulmonary fate in the foregut epithelium.

In addition to BMP and FGF, canonical WNTs comprise a third group of signaling molecules necessary for lung specification. *Wnt2* and *Wnt2b*, are expressed in the ventral mesenchyme surrounding the foregut and genetic inactivation of *Wnt2/2b* leads to an absence of foregut *Nkx2-1* expression and complete lung agenesis [Goss et al., 2009]. Similarly, conditional inactivation of *β-catenin* in the ventral foregut results in loss of *Nkx2-1* expression and lung agenesis [Goss et al., 2009; Harris-Johnson et al., 2009]. Together these results indicate that WNT2/2B have additional mediators beyond *Nkx2-1*, since the WNT/β-catenin mutants exhibit lung agenesis, whereas lung specification still occurs in *Nkx2-1* null embryos.

Following pulmonary fate specification, mesenchymally expressed FGF10 signals to the epithelium through FGFR2 isoform B to initiate and promote lung bud outgrowth [Bellusci et al., 1997; Abler et al., 2009]. FGF10 is necessary for bud outgrowth since *Fgf10* null embryos exhibit normal tracheal specification, however the lung buds fail to emerge [Min et al., 1998; Sekine et al., 1999; Ohuchi et al., 2000]. Additionally, ex vivo lung culture studies have demonstrated that FGF10 is sufficient to produce bud outgrowth [Weaver et al., 2000]. FGF signaling activates epithelial expression of *Spry2*, a gene encoding an intracellular negative regulator of receptor tyrosine kinase signaling [Minowada et al., 1999; Mailleux et al., 2001; Tefft et al., 2002; Tang et al., 2011]. In culture, a reduction in *Spry2* expression results in greater epithelial branching, whereas an increase in *Spry2* expression inhibits epithelial branching and proliferation [Tefft et al., 1999; Mailleux et al., 2001]. Therefore, the FGF-induced increase in SPRY2 at prior bud tips prevents further outgrowth of the same cells by reducing epithelial competence to respond to FGF signals [Mailleux et al., 2001].

In addition to its role promoting bud outgrowth, FGF10 signaling to the epithelium upregulates expression of genes that will in turn negatively regulate bud outgrowth and *Fgf10* expression. FGF10 induces expression of *Bmp4* at the epithelial bud tips [Weaver et al., 2000]. In culture, exogenous BMP4 inhibits epithelial lung bud growth toward an FGF10 soaked bead, indicating that BMP4 acts downstream of FGF10 to negatively regulate budding [Weaver et al., 2000]. This mechanism allows for branching after budding, since the cells that previously experienced high FGF10 are inhibited from growing, whereas the epithelium flanking this BMP4 high site is able to receive FGF10 and bud out, creating a bifurcation and subsequent branches. SHH is also expressed by the epithelial bud tips and numerous in vitro and in vivo studies have demonstrated that SHH negatively regulates mesenchymal FGF10 expression [Pepicelli et al., 1998; Lebeche et al., 1999; Chuang et al., 2003]. The continual crosstalk between the epithelium and mesenchyme in the developing lung results in the formation of a highly branched stereotypic structure, with initial buds emerging at E10.0 and completion of the branching program in late gestation [Metzger et al., 2008].

ENDOTHELIAL–EPITHELIAL CROSSTALK ESTABLISHES THE PROPER BRANCHING PATTERN OF THE EPITHELIUM AND VASCULATURE

Concurrent with the epithelial branching program, the pulmonary vasculature is established within the lung mesenchyme. Various studies employing distinct methodologies have suggested that both angiogenesis and vasculogenesis play a role in forming the primitive vascular plexus; however, the extent that each contributes remains contested. Pulmonary angiogenesis initiates when new vessels sprout from the aortic sac (subsequently the pulmonary artery). Located anterior to the developing lung, this sprouting begins at E10.0 and parallels the outgrowth of the buds [Schachtner et al., 2000]. Vasculogenesis occurs in the distal mesenchyme as a result of angioblast differentiation. Angioblast-like structures are located in the mesenchyme surrounding the developing foregut prior to lung bud outgrowth and past studies have differentiated lung mesenchyme-derived cell lines into endothelium [deMello et al., 1997; Akeson et al., 2000]. Lineage labeling studies at E10.5 using *Kdr* (also known as *Flk1* and *Vegfr2*) promoter driven *LacZ* (*Kdr-LacZ*) expression identified thin, lumen-less vessels in the distal mesenchyme that form via vasculogenesis as well as the proximal lumen-containing pulmonary artery that originate via angiogenesis

[Schachtner et al., 2000]. Both types of vessels appear to be continuous with each other at E10.5, however the distal vessels do not develop a definitive lumen until after E11.5. Circulating hematopoietic cells are not present in the distal vascular plexus until E12.5. More recent lineage studies using *Tek-LacZ* (also know as *Tie2-LacZ*) suggest that distal vessels are always continuous with the proximal vessels, implying that the pulmonary vasculature forms through angiogenesis of a vascular plexus which surrounds the foregut prior to bud outgrowth [Parera et al., 2005]. For more information regarding the formation of the early pulmonary vasculature, please see additional recent reviews [Park et al., 2013; Peng and Morrisey, 2013].

The nascent pulmonary vascular network maintains a close spatial juxtaposition with the lung epithelium as they both undergo elaborate morphogenesis. These temporal and morphological parallels suggest an intimate crosstalk occurs between these structures during development. A variety of studies have provided evidence to support this notion. Ex vivo experiments demonstrated that E13.0 mesenchyme recombined with E13.0 epithelium develops a proper vascular plexus, whereas the mesenchyme cultured alone contains few disorganized endothelial cells [Gebb and Shannon, 2000]. This result suggests that the epithelium provides cues to promote angiogenesis of existing vessels. Recently this epithelial requirement was demonstrated in vivo using a lung agenesis model of a conditional *β-catenin* knockout [Peng et al., 2013]. In these mutant embryos, the pulmonary vasculature fails to progress from a primitive plexus to a branched network in the absence of lung buds, indicating the essential role of the lung epithelium in promoting vascular growth and branching.

Vascular endothelial growth factor (VEGF) has been identified as the key signal responsible for epithelial to endothelial communication and it is sufficient to promote angiogenesis in ex vivo lung culture [Healy et al., 2000]. From E12.5 until E14.5, *Vegfa* is expressed by both the epithelium and mesenchyme, however, after E14.5 its expression becomes restricted to the epithelium [Gerber et al., 1999; Gebb and Shannon, 2000; Ng et al., 2001]. Epithelial expression of FGF9 and SHH has been shown to promote the mesenchymal expression of *Vegfa* [White et al., 2007]. FGF9 and SHH are therefore necessary to establish the proper vascular density in the developing lung. An additional link between epithelial branching and angiogenesis was made when it was shown that mesenchymal FGF10, acting through SPRY2, activates mTOR1 and consequently HIF-α, upregulating VEGF expression at distal epithelial tips [Scott et al., 2010]. In addition to VEGF, angiopoietin-1(ANG-1) is another angiogenic cytokine produced by the airway epithelium that binds TEK receptors on endothelial cells to regulate their growth [Grzenda et al., 2013]. ANG-1 along with FGF2, can increase endothelial branching in *Shh* deficient lungs, which exhibit significantly reduced epithelial branch number, providing further evidence that epithelial signals coordinate endothelial growth and branching [van Tuyl et al., 2007].

Recent studies have also identified an epithelial dependence on the vasculature for proper airway branching. Treatment of cultured lungs with antisense oligonucleotides against HIF-α or VEGF, inhibits angiogenesis and subsequently stalls epithelial branching [van Tuyl et al., 2005]. Additionally, exogenous VEGF increases epithelial branching and FLK-1 positive cells when added in culture, whereas treatment with antisense oligonucleotides for FLK-1 decreases epithelial *Bmp4* expression and branching [Del Moral et al., 2006]. In vivo overexpression of a decoy VEGF receptor (sVEGFR1) suppresses VEGF signaling, reduces the vascular plexus, and decreases epithelial branching [Lazarus et al., 2011]. Interestingly, switching off sVEGFR1 expression at E12.5 can restore epithelial branching and vascular development. Secreted endothelium-derived signals mediate this effect on epithelial branching because endothelial cell-conditioned media can partially rescue the epithelial branching defects in sVEGFR1-cultured lungs. sVEGFR1 lungs exhibit an upregulation of FGF10 in the mesenchyme at E12.5, which is preceded and accompanied by an increase in epithelial *Spry2* expression. As *Spry* genes are negative regulators of FGF signaling, this result suggests that endothelium-derived signals make the epithelium less competent to receive FGF signaling, thereby regulating branching [Lazarus et al., 2011]. An additional example of epithelial dependence on the vasculature for proper development is observed at a later stage of lung maturation in platelet endothelial cell adhesion molecule 1 (*Pecam1*)-deficient mice. *Pecam1* null mice exhibit a primary endothelial cell migration defect and a subsequent decrease in alveolarization [DeLisser et al., 2006]. These results demonstrate that the growth and patterning of the epithelium and endothelium are coordinated through a crosstalk of molecular signals.

INTRA-MESENCHYMAL CROSSTALK ESTABLISHES EXTRAPULMONARY AIRWAY STRUCTURES

The trachea and main bronchi are surrounded by a variety of mesenchymal structures including the cartilage, smooth muscle (SM), vasculature, lymphatic vessels, and neurons. These mesenchymal tissues of the extrapulmonary airways are specified and spatial patterned during the same time that the intrapulmonary airways undergo branching morphogenesis. Cartilage and airway SM develop in spatial and temporal juxtaposition to establish upper airways that possess a balance of rigidity and elasticity [Hines et al., 2013]. The tracheal vasculature and lymphatics develop in a stereotypical spatial arrangement with regard to the cartilage rings [Ni et al., 2010]. The cell bodies of neural crest-derived intrinsic neurons line up along the midline of the SM domain [Langsdorf et al., 2011]. Emerging evidence suggests that crosstalk between extrapulmonary mesenchymal tissues exists and that it is important for their establishment and patterning.

The lung mesenchyme originates from cardiac mesoderm surrounding the anterior foregut. A recent study demonstrated that WNT2+/GLI1+/ISL1+ cardiac-pulmonary progenitor cells (CPPs) contribute to airway SM, vascular SM, and proximal endothelium of the lung [Peng et al., 2013]. While the cartilage progenitors were not specifically addressed in this study, it is likely that they originate from the broader ISL1+ cell population surrounding the anterior foregut. A subset of these mesenchymal cells surrounding the developing trachea and bronchi begin to express *Sox9*, a transcription factor required for cartilage development. Shortly after lung budding at E10.5, these cartilage precursors

become localized to the ventral trachea and lateral main bronchi. By E11.5, these cartilage precursors differentiate into cartilage cells and express differentiated marker *Col2a* [Hines et al., 2013]. In parallel, by E10.5, airway SM differentiates from a SRF+/MYOCARDIN+ precursor population. The earliest cells to express differentiated SM marker ACTA2 (also termed smooth muscle actin) are found at the carina (intersection of the trachea and main bronchi) [Hines et al., 2013]. SM differentiation then proceeds anteriorly up the dorsal side of the trachea and posteriorly along the medial main bronchi, developing in juxtaposition with the ventral/lateral cartilage cells. Beginning around E13.0, the sheet of COL2A+ cartilage cells morphs into segmented C-shaped rings by a mechanism that is currently unknown. In mice, airway cartilage is only present in the non-branching regions of the lung and distal to the main bronchi, SM surrounds the airway circumference. In humans, cartilage extends into the branching regions of the lung and the C-shaped rings transition to flattened plates in the more distal airways. These plates rest atop a layer of airway SM that encircles the intrapulmonary airways [Fraser, 1999].

Recent work by our laboratory demonstrated that spatial and temporal juxtaposition of cartilage and SM is necessary for the proper development of these tissues [Hines et al., 2013]. Utilizing conditional mutants for upstream transcription factors for cartilage (*Sox9*) and SM (*Srf*) specification, we genetically prevented each tissue's formation during development and assessed its effect on the remaining tissue type. Airway SM loss increases the cartilage cell number in both the trachea and the bronchi. We observed similar compensatory changes in our cartilage mutants; SM cells are increased in both the trachea and main bronchi. Interestingly, in both mutants, the cell number increase corresponds with an increase in airway coverage in the bronchi, but not in the trachea. These experiments presented the first evidence for cartilage and SM crosstalk in upper airway development. The molecular nature of the communication remains unknown, however future studies comparing gene expression profiles of developing cartilage and SM may identify the possible pathways.

In the trachea, the vascular and lymphatic systems develop in a stereotypic pattern through a process of growth and remodeling [Ni et al., 2010]. By E16.5, a primitive vascular plexus has formed in the trachea, with arterioles, venules, and lymphatic vessels preferentially located in the inter-cartilage space, and the capillaries extended over the cartilage rings. Beginning around P1, the capillaries regress from the cartilage region, creating a striated vascular pattern in the trachea. This pattern is transient; at around P4, the capillaries regrow over the cartilage rings and form the mature vascular network that persists through adulthood. This vascular regrowth process is dependent on VEGF signaling because treatment with a VEGFR2 antagonist prevents capillary regrowth [Ni et al., 2010]. It is possible that the cartilage rings are responsible for this capillary regression and regrowth since cartilage has been shown to express HIF-α and VEGF during limb growth plate development [Schipani et al., 2001], however, this crosstalk mechanism has not been elucidated in the trachea. Conversely, it is also possible that alterations in epithelial expression of angiogenic factors are responsible for this remodeling. Recent evidence has demonstrated that lymphatic vessels respond to inflammatory cues, suggesting a potential role for vessel remodeling in disease remediation. For example, overexpression of cytokine IL-1B induces abnormal lymphatic vessels sprouting over the cartilage rings in a similar pattern to the vascular capillaries, an abnormality since lymphatic vessels are normally limited to the inter-cartilage regions [Baluk et al., 2013]. The exact mechanism that patterns the vascular and lymphatic systems with regard to tracheal cartilage remains to be elucidated.

Two types of neurons innervate the developing lung, extrinsic, and intrinsic neurons [reviewed in Aven and Ai, 2013]. Extrinsic neurons' cell bodies are located in ganglia outside of the lung whereas neural crest-derived intrinsic neurons' cell bodies are situated in the lung [Langsdorf et al., 2011]. A recent study found that the cell bodies of intrinsic neurons become localized to the dorsal trachea, adjacent to the airway SM, beginning by E13.5 and persisting to adulthood. These neurons innervate the trachealis muscle and are likely involved in its contraction. Additionally intrinsic neurons extend to the ventral inter-cartilage regions. The GDNF family member *Nrtn* is expressed diffusely around the trachea including the SM region, and NRTN receptors GFRα1/2 are expressed by neurons and airway SM. In culture, NRTN can promote intrinsic neurogenesis [Langsdorf et al., 2011]. These findings support the existence of a crosstalk mechanism between the airway SM and intrinsic neurons to establish the proper upper airway innervation.

EPITHELIAL–MESENCHYMAL CROSSTALK REGULATES CELL DIFFERENTIATION AND AIRWAY PATTERNING

Early tissue recombination studies demonstrated that the mesenchyme is responsible for specifying the proximal versus distal cell fate in the adjacent epithelium [Shannon et al., 1998]. Recombination culture of tracheal epithelium with distal lung mesenchyme, and distal lung epithelium with tracheal mesenchyme, demonstrated that the mesenchyme is instructive and imposes its regional identity on the epithelium. This didactic role of the mesenchyme becomes prominent in the injured adult lung. Following SO_2 airway injury, epithelial cells in the sub-mucosal glands located in the inter-cartilage regions of the upper trachea and near the edge of cartilage rings in the lower trachea, proliferate to repopulate the denuded airway. When labeled with BrdU, these cells retain the marker months after, suggesting that tracheal cartilage may serve as a niche for progenitor cells [Borthwick et al., 2001]. Interestingly, these inter-cartilage regions also contain a high density of endothelial vasculature and lymphatic vessels, raising the possibility that these mesenchymal structures may also provide a niche environment.

Recent genetic studies have identified a novel role for tracheal cartilage in epithelial cell differentiation during embryonic development [Hines et al., 2013; Turcatel et al., 2013]. At prenatal stages within the wild type trachea, basal cells are present at greater density in the ventral epithelium adjacent to the cartilage domain compared with the dorsal epithelium adjacent to the SM. In mutants devoid of tracheal cartilage, overall basal cell density is decreased to a density comparable to the wild type dorsal (SM) side of the trachea. Interestingly, a precocious differentiation of club cells (formerly Clara cells) accompanies this decrease in basal cells. These in vivo

data demonstrate that mesenchymal structures can impact the development of the airway epithelium and they suggest a mesenchyme to epithelial communication may be at work.

Recent results suggest that FGF10 is a strong candidate for the mesenchyme to epithelium signal that regulates basal cell density. At E14.5 when basal cells are beginning to differentiate, *Fgf10* is expressed in the inter-cartilage mesenchymal cells [Sala et al., 2011]. Additionally, while cartilage is still present in the *Fgf10* mutant tracheas, they do contain significantly fewer basal cells than control tracheas, suggesting FGF10 is required for establishing or maintaining proper basal cell density [Volckaert et al., 2013].

SM surrounding the intra-pulmonary airways is innervated and thus the neuronal axons must continue to grow and establish new connections as the epithelium branches and the SM expands to surround the lengthening airways. The intra-pulmonary airway SM cells are innervated by extrinsic neurons, since these neuronal projections persist in *Ret* mutants that lack intrinsic neurons [Langsdorf et al., 2011]. The growth of these extrinsic neurons is regulated by brain derived neurotrophic factor (BDNF), since *Bdnf* mutant lungs lack neuronal projections to the airway SM [Radzikinas et al., 2011]. *Bdnf* is expressed in the mesenchyme surrounding the epithelium beginning around E11.5 and continuing through E15.5, the same temporal and spatial window as airway SM is being established. BDNF was found to be regulated post-transcriptionally by miR-206, which is itself transcriptionally regulated by epithelial-derived SHH [Radzikinas et al., 2011]. These results demonstrate that an elegant signal relay from epithelium to airway SM to extrinsic neurons is responsible for proper innervation of the developing lung.

INTRA–EPITHELIAL COMMUNICATION AMONGST DIFFERENTIATING CELLS DIRECTS AIRWAY PATTERNING

Differentiated airway epithelium is composed of a variety of cell types including club cells, ciliated cells, pulmonary neuro-endocrine cells (PNECs), and goblet cells. Notch signaling, which acts in a cell–cell contact dependent manner, plays crucial roles in coordinating the differentiation and spatial placement of these cells types. The *Notch1-3* receptors along with their ligands, *Jagged1 Jagged2*, *Dll1*, and *Dlk1*, are expressed in the lung epithelium and mesenchyme at various levels during development [Post et al., 2000; Taichman et al., 2002; Kong et al., 2004]. A series of studies utilizing both gain-of-function and loss-of-function approaches have demonstrated that an epithelial–epithelial communication is essential for the proper differentiation and distribution of each cell type [Ito et al., 2000; Guseh et al., 2009; Tsao et al., 2009, 2011; Morimoto et al., 2010, 2012; Zhang et al., 2013]. Epithelial loss of Notch signaling components, *Pofut1*and *Rbpjk*, prevents club cell differentiation, increases ciliated cell and PNEC differentiation prenatally, and expands goblet cell differentiation postnatally [Tsao et al., 2009, 2011; Morimoto et al., 2010]. Similar results are observed in *Jagged1* loss-of-function mutants, and when Notch signaling is inactivated in culture by pharmacological γ-secretase inhibitor [Tsao et al., 2009; Zhang et al., 2013]. Consistent with these findings, null mutants for *Hes1*, the downstream target of Notch signaling, have more PNECs than controls [Ito et al., 2000]. In contrast to these loss-of-function results, mutants with gain-of-function Notch signaling (*Sftpc-cre* driven expression of the Notch intra-cellular domain [ICD]) exhibit decreased ciliated cells and increased mucous producing cells [Guseh et al., 2009]. Transcriptionally, Notch has been shown to promote club cell marker *Scgb1a1* expression and inhibit postnatal transcription of goblet cell marker *Muc5ac* through HES5 [Tsao et al., 2011; Guha et al., 2012], providing a mechanism by which the above described phenotypes manifest. Three Notch receptors are expressed in the lung, and until recently the individual role for each receptor was not understood. A recent study utilizing conditional inactivation of *Notch1* and *Notch2* along with a null allele for *Notch3*, assorted these roles [Morimoto et al., 2012]. *Notch2* was found to be expressed by club cells and be the primary regulator of the club/ciliated cell fate decision in lung development. Additionally, each Notch receptor contributes to the patterning and regulation of NEB size and number because the subsequent loss of *Notch* alleles reduces NEB number, increases PNEC number within each NEB, and increases *Cgrp* expression stepwise. *Notch2* and *Notch3* are expressed in the SSEA1+ variant club cells adjacent to NEBs and epithelial overexpression of *Notch2* ICD promoted SSEA1+ cell differentiation without producing more SCGB1A1+ (also known as CC10+) club cells [Morimoto et al., 2012]. Formation of *Scgb3a2* and *Upk3a* expressing cell clusters in the lower airways is dependent on *Asc1* expression, NEB formation, and consequentially Notch [Guha et al., 2012]. In vitro, Notch signaling together with NKX2-1, promotes transcription of variant club cell genes *Scgb3a2*, *Upk3a*, and *Scgb1a1* [Guha et al., 2012]. The cumulative results from a variety of mutants propounds that Notch signaling is an essential component of the intra-epithelial crosstalk regulating epithelial cell differentiation.

EPITHELIUM TO MESOTHELIUM COMMUNICATION INDUCES EMT

In addition to the epithelium and mesenchyme, a third layer of tissue, the mesothelium, serves as the external wrapping of the lung lobes. This cell population undergoes an epithelial-to-mesenchymal (EMT)-like transition to contribute to various mesenchymal structures in the lung [Que et al., 2008; Dixit et al., 2013]. Mesothelium-derived cells however, do not comprise a significant portion of the total lung mesenchyme. For example, at E14.5, only around 5% of lung mesenchyme is mesothelium-derived [Dixit et al., 2013]. Expression of *Wilm's tumor-1* (*Wt1*) marks this mesothelial cell population during early lung development and analysis of WT1 expression reveals that the lung mesothelium differentiates between E9.5 and E10.5 in an anterior to posterior manner [Que et al., 2008]. *Wt1* expression peaks at E11.5 and then is progressively down-regulated until birth, with few WT1+ cells present in adult lungs [Que et al., 2008; Dixit et al., 2013]. *Wt1* expression is also down-regulated in mesothelial cells undergoing EMT. Lineage tracing of mesothelial cells using an inducible *Wt1-cre* demonstrated that these cells move interiorly between E10.5 and E17.0 where they contribute to airway SM, vascular SM, and alveolar

fibroblasts [Dixit et al., 2013]. This time period coincides with when the epithelium expresses *Shh* and recently HH signaling was found to be necessary for mesothelial EMT [Dixit et al., 2013]. HH signaling antagonist cyclopamine inhibited EMT of mesothelial cells in ex vivo culture of *Wt1-creERT2*; *Rosa-tmRed* lineage-labeled lungs. Additionally, conditional inactivation of downstream HH signaling component *Smo* prevented mesothelium EMT. Since HH signaling becomes up-regulated following adult lung bleomycin injury, it would be interesting to examine if this increase in signaling corresponds with a reactivation of mesothelial EMT [Liu et al., 2013]. These results provide the first evidence for epithelial SHH as the regulator of mesothelial EMT.

TISSUE CROSSTALK FOLLOWING AIRWAY DAMAGE

Following injury, developmental programs are often reactivated to repair tissue damage and return the organ to homeostasis. This paradigm holds true in the lung and a variety of signaling pathways including FGF, Notch, and WNT are up-regulated following airway injury. These signals are the basis for communication and crosstalk between distinct cell types, either within the epithelium or between epithelial and mesenchymal cells. Additionally, resident or infiltrating immune cells can signal to the lung epithelium to disrupt airway development and repair.

An epithelial–SM crosstalk mechanism was recently demonstrated to regulate intra-pulmonary airway re-epithelialization after naphthalene injury [Volckaert et al., 2011]. *Wnt7b* expression was re-activated in the remaining epithelial cells 3 days post-injury. This *Wnt7b* expression corresponded to an increase in TOPGAL (WNT signaling readout) expression in the airway SM and variant (naphthalene-resistant) club cells. A WNT ligand-dependent upregulation of *Fgf10* expression was observed in airway SM cells 3 days post-injury. Overexpression of secreted FGFR2 (sFGFR2) caused a significant decrease in both FGF10 signaling and in the ability of the airways to re-epithelialize. Additionally, FGF10 signaling was found to up-regulate Notch signaling in variant club cells 3 days post-injury. Interestingly, a recent study demonstrated that Notch1 is required for proper club cell regeneration post-naphthalene injury [Xing et al., 2012]. This WNT-FGF10-Notch signaling relay from the epithelium to the mesenchyme and back to the epithelium illustrates a tissue crosstalk mechanism important for airway re-epithelialization post-injury.

Tracheal epithelial repair following SO_2 injury requires Notch signaling for basal cell differentiation and re-epithelialization [Rock et al., 2011]. Tracheal basal cells express Notch pathway components *Notch1*, *Jag1*, and *Dll* in homeostasis. However, Notch signaling and consequentially downstream target gene (*Heyl* and *Hey1*) expression becomes up-regulated in the basal cells that remain following SO_2 injury. Notch ICD overexpression in basal cells can promote their differentiation into secretory and goblet cells but not into ciliated cells. Additionally, inhibition of Notch signaling blocks the differentiation of basal cells into secretory cells and impedes the proper re-epithelialization of the damaged airway. Despite this, Notch is not required for basal cell maintenance in the tracheal epithelium. This demonstrates that basal cells communicate with each other via Notch signaling to coordinate repair the airway following injury.

Bronchopulmonary dysplasia (BPD) is a lifelong lung condition diagnosed in approximately 60% of infants born before 28 weeks of age [Stoll et al., 2010]. One of the causes of BPD is believed to be the exposure of the premature lung to high oxygen concentrations, which results in lung inflammation and disrupted development. Past studies have demonstrated that the inflammatory trigger, *Escherichia coli* lipopolysaccharide (LPS), can inhibit branching in lung culture studies in the absence of the circulatory system and immune cells [Prince et al., 2005]. A recent study has provided evidence that when activated, resident lung macrophages inhibit epithelial branching morphogenesis [Blackwell et al., 2011]. In E15 lung explant culture, LPS activates NF-κβ signaling and increases expression of *Il-1β* and *Tnf-α* in resident macrophages. Depletion of lung macrophages both in culture using clodronate and in vivo using macrophage specific *Ikkβ* deletion, provides a protective effect on airway branching following LPS exposure. Additionally, macrophage Ikkβ overexpression in vivo inhibits saccular airway branching and decreases epithelial expression of *Bmp4* and *Wnt7b*. In some lethal BPD cases, *Fgf10* expression is decreased [Benjamin et al., 2007]. When this is modeled in LPS-treated primary fetal mesenchymal cell culture, NF-κβ, IL-1β, and TNF-α can all reduce *Fgf10* expression [Benjamin et al., 2010; Carver et al., 2013]. Ex vivo lung cultures studies demonstrate that activation of Toll-like receptors 2 and 4 (TLR2/4) decreases *Fgf10* expression and disrupts sacculation [Benjamin et al., 2007]. Together these results demonstrate that immune cell signaling in the developing lung can influence epithelial development and future studies elucidating this mechanism may reveal new therapeutic targets for the treatment of BPD.

CONCLUSION

Here we examined the evidence for crosstalk and communication between tissues and cell types in the lung during development and injury. In some cases, such as in lung bud specification and branching, the molecular players in the tissue crosstalk have been identified. However, in others, such as upper airway cartilage and SM patterning, the molecular mediators are currently unknown. While most factors are secreted, including FGF, BMP, WNT, VEGF, GDNF, and BDNF, other transmembrane molecules such as Notch are also involved. In the future, it is likely that other modes of tissue crosstalk will be investigated. One possibility for this new crosstalk mechanism may be mechanical forces. Work in a tracheal occlusion model has demonstrated that greater luminal pressure during lung branching morphogenesis can increase the number of airways branches and upregulate *Fgf10*, *Spry2*, *Shh*, and *Vegf* expression [Unbekandt et al., 2008]. This pressure driven increase in branching is dependent on FGF10 signaling in the epithelium. Additionally, mechanical stretch can increase neurotransmitter release in a PNEC tumor derived cell line [Pan et al., 2006]. Future studies into the role of mechanical forces in the lung may identify the tissues that are exerting and receiving forces as well as the effect forces have on cell behavior and gene expression. Understanding the types of cellular

crosstalk and tissue communication that occur during normal development and injury repair will likely inform the design of novel treatments for acute and chronic lung diseases.

ACKNOWLEDGEMENTS

We thank members of the laboratory of X.S. for helpful discussions and suggestions, particularly John Herriges, Jamie Verheyden, and David McCulley. This work was supported by National Science Foundation Graduate Research Fellowship 2011101268 (to E.A.H.) and National Heart, Lung, and Blood Institute R01 HL113870 (to X.S.).

REFERENCES

Abler LL, Mansour SL, Sun X. 2009. Conditional gene inactivation reveals roles for Fgf10 and Fgfr2 in establishing a normal pattern of epithelial branching in the mouse lung. Dev Dyn 238:1999–2013.

Akeson AL, Wetzel B, Thompson FY, Brooks SK, Paradis H, Gendron RL, Greenberg JM. 2000. Embryonic vasculogenesis by endothelial precursor cells derived from lung mesenchyme. Dev Dyn 217:11–23.

Aven L, Ai X. 2013. Mechanisms of respiratory innervation during embryonic development. Organogenesis 9:194–198.

Baluk P, Hogmalm A, Bry M, Alitalo K, Bry K, McDonald DM. 2013. Transgenic overexpression of interleukin-1beta induces persistent lymphangiogenesis but not angiogenesis in mouse airways. Am J Pathol 182:1434–1447.

Bellusci S, Grindley J, Emoto H, Itoh N, Hogan BL. 1997. Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung. Development 124:4867–4878.

Benjamin JT, Carver BJ, Plosa EJ, Yamamoto Y, Miller JD, Liu JH, van der Meer R, Blackwell TS, Prince LS. 2010. NF-kappaB activation limits airway branching through inhibition of Sp1-mediated fibroblast growth factor-10 expression. J Immunol 185:4896–4903.

Benjamin JT, Smith RJ, Halloran BA, Day TJ, Kelly DR, Prince LS. 2007. FGF-10 is decreased in bronchopulmonary dysplasia and suppressed by Toll-like receptor activation. Am J Physiol Lung Cell Mol Physiol 292:L550–L558.

Blackwell TS, Hipps AN, Yamamoto Y, Han W, Barham WJ, Ostrowski MC, Yull FE, Prince LS. 2011. NF-kappaB signaling in fetal lung macrophages disrupts airway morphogenesis. J Immunol 187:2740–2747.

Borthwick DW, Shahbazian M, Krantz QT, Dorin JR, Randell SH. 2001. Evidence for stem-cell niches in the tracheal epithelium. Am J Respir Cell Mol Biol 24:662–670.

Carver BJ, Plosa EJ, Stinnett AM, Blackwell TS, Prince LS. 2013. Interactions between NF-kappaB and SP3 connect inflammatory signaling with reduced FGF-10 expression. J Biol Chem 288:15318–15325.

Chen F, Desai TJ, Qian J, Niederreither K, Lu J, Cardoso WV. 2007. Inhibition of Tgf beta signaling by endogenous retinoic acid is essential for primary lung bud induction. Development 134:2969–2979.

Chuang PT, Kawcak T, McMahon AP. 2003. Feedback control of mammalian Hedgehog signaling by the Hedgehog-binding protein, Hip1, modulates Fgf signaling during branching morphogenesis of the lung. Genes Dev 17:342–347.

Del Moral PM, Sala FG, Tefft D, Shi W, Keshet E, Bellusci S, Warburton D. 2006. VEGF-A signaling through Flk-1 is a critical facilitator of early embryonic lung epithelial to endothelial crosstalk and branching morphogenesis. Dev Biol 290:177–188.

DeLisser HM, Helmke BP, Cao G, Egan PM, Taichman D, Fehrenbach M, Zaman A, Cui Z, Mohan GS, Baldwin HS, Davies PF, Savani RC. 2006. Loss of PECAM-1 function impairs alveolarization. J Biol Chem 281:8724–8731.

deMello DE, Sawyer D, Galvin N, Reid LM. 1997. Early fetal development of lung vasculature. Am J Respir Cell Mol Biol 16:568–581.

Dixit R, Ai X, Fine A. 2013. Derivation of lung mesenchymal lineages from the fetal mesothelium requires hedgehog signaling for mesothelial cell entry. Development 140:4398–4406.

Domyan ET, Ferretti E, Throckmorton K, Mishina Y, Nicolis SK, Sun X. 2011. Signaling through BMP receptors promotes respiratory identity in the foregut via repression of Sox2. Development 138:971–981.

Fraser RS. 1999. Fraser and Paré's diagnosis of diseases of the chest. Philadelphia: W.B. Saunders .p. 4 v. (xiii, 3190, 61 p.) .

Gebb SA, Shannon JM. 2000. Tissue interactions mediate early events in pulmonary vasculogenesis. Dev Dyn 217:159–169.

Gerber HP, Hillan KJ, Ryan AM, Kowalski J, Keller GA, Rangell L, Wright BD, Radtke F, Aguet M, Ferrara N. 1999. VEGF is required for growth and survival in neonatal mice. Development 126:1149–1159.

Goss AM, Tian Y, Tsukiyama T, Cohen ED, Zhou D, Lu MM, Yamaguchi TP, Morrisey EE. 2009. Wnt2/2b and beta-catenin signaling are necessary and sufficient to specify lung progenitors in the foregut. Dev Cell 17:290–298.

Grzenda A, Shannon J, Fisher J, Arkovitz MS. 2013. Timing and expression of the angiopoietin-1-Tie-2 pathway in murine lung development and congenital diaphragmatic hernia. Dis Model Mech 6:106–114.

Guha A, Vasconcelos M, Cai Y, Yoneda M, Hinds A, Qian J, Li G, Dickel L, Johnson JE, Kimura S, Guo J, McMahon J, McMahon AP, Cardoso WV. 2012. Neuroepithelial body microenvironment is a niche for a distinct subset of Clara-like precursors in the developing airways. Proc Natl Acad Sci USA 109:12592–12597.

Guseh JS, Bores SA, Stanger BZ, Zhou Q, Anderson WJ, Melton DA, Rajagopal J. 2009. Notch signaling promotes airway mucous metaplasia and inhibits alveolar development. Development 136:1751–1759.

Harris-Johnson KS, Domyan ET, Vezina CM, Sun X. 2009. Beta-Catenin promotes respiratory progenitor identity in mouse foregut. Proc Natl Acad Sci USA 106:16287–16292.

Healy AM, Morgenthau L, Zhu X, Farber HW, Cardoso WV. 2000. VEGF is deposited in the subepithelial matrix at the leading edge of branching airways and stimulates neovascularization in the murine embryonic lung. Dev Dyn 219:341–352.

Herriges M, Morrisey EE. 2014. Lung development: Orchestrating the generation and regeneration of a complex organ. Development 141:502–513.

Hines EA, Jones MK, Verheyden JM, Harvey JF, Sun X. 2013. Establishment of smooth muscle and cartilage juxtaposition in the developing mouse upper airways. Proc Natl Acad Sci USA 110:19444–19449.

Ito T, Udaka N, Yazawa T, Okudela K, Hayashi H, Sudo T, Guillemot F, Kageyama R, Kitamura H. 2000. Basic helix-loop-helix transcription factors regulate the neuroendocrine differentiation of fetal mouse pulmonary epithelium. Development 127:3913–3921.

Kimura S, Hara Y, Pineau T, Fernandez-Salguero P, Fox CH, Ward JM, Gonzalez FJ. 1996. 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 10:60–69.

Kimura S, Ward JM, Minoo P. 1999. Thyroid-specific enhancer-binding protein/thyroid transcription factor 1 is not required for the initial specification of the thyroid and lung primordia. Biochimie 81:321–327.

Kong Y, Glickman J, Subramaniam M, Shahsafaei A, Allamneni KP, Aster JC, Sklar J, Sunday ME. 2004. Functional diversity of notch family genes in fetal lung development. Am J Physiol Lung Cell Mol Physiol 286:L1075–L1083.

Langsdorf A, Radzikinas K, Kroten A, Jain S, Ai X. 2011. Neural crest cell origin and signals for intrinsic neurogenesis in the mammalian respiratory tract. Am J Respir Cell Mol Biol 44:293–301.

Lazzaro D, Price M, de Felice M, Di Lauro R. 1991. The transcription factor TTF-1 is expressed at the onset of thyroid and lung morphogenesis and in restricted regions of the foetal brain. Development 113:1093–1104.

Lazarus A, Del-Moral PM, Ilovich O, Mishani E, Warburton D, Keshet E. 2011. A perfusion-independent role of blood vessels in determining branching stereotypy of lung airways. Development 138:2359–2368.

Lebeche D, Malpel S, Cardoso WV. 1999. Fibroblast growth factor interactions in the developing lung. Mech Dev 86:125–136.

Liu L, Kugler MC, Loomis CA, Samdani R, Zhao Z, Chen GJ, Brandt JP, Brownell I, Joyner AL, Rom WN, Munger JS. 2013. Hedgehog signaling in neonatal and adult lung. Am J Respir Cell Mol Biol 48:703–710.

Maeda Y, Dave V, Whitsett JA. 2007. Transcriptional control of lung morphogenesis. Physiol Rev 87:219–244.

Mailleux AA, Tefft D, Ndiaye D, Itoh N, Thiery JP, Warburton D, Bellusci S. 2001. Evidence that SPROUTY2 functions as an inhibitor of mouse embryonic lung growth and morphogenesis. Mech Dev 102:81–94.

Metzger RJ, Klein OD, Martin GR, Krasnow MA. 2008. The branching programme of mouse lung development. Nature 453:745–750.

Min H, Danilenko DM, Scully SA, Bolon B, Ring BD, Tarpley JE, DeRose M, Simonet WS. 1998. Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless. Genes Dev 12:3156–3161.

Minoo P, Su G, Drum H, Bringas P, Kimura S. 1999. Defects in tracheoesophageal and lung morphogenesis in Nkx2.1(−/−) mouse embryos. Dev Biol 209:60–71.

Minowada G, Jarvis LA, Chi CL, Neubuser A, Sun X, Hacohen N, Krasnow MA, Martin GR. 1999. Vertebrate Sprouty genes are induced by FGF signaling and can cause chondrodysplasia when overexpressed. Development 126:4465–4475.

Morimoto M, Liu Z, Cheng HT, Winters N, Bader D, Kopan R. 2010. Canonical Notch signaling in the developing lung is required for determination of arterial smooth muscle cells and selection of Clara versus ciliated cell fate. J Cell Sci 123:213–224.

Morimoto M, Nishinakamura R, Saga Y, Kopan R. 2012. Different assemblies of Notch receptors coordinate the distribution of the major bronchial Clara, ciliated and neuroendocrine cells. Development 139:4365–4373.

Morrisey EE, Hogan BL. 2010. Preparing for the first breath: Genetic and cellular mechanisms in lung development. Dev Cell 18:8–23.

Mucenski ML, Wert SE, Nation JM, Loudy DE, Huelsken J, Birchmeier W, Morrisey EE, Whitsett JA. 2003. Beta-Catenin is required for specification of proximal/distal cell fate during lung morphogenesis. J Biol Chem 278:40231–40238.

Ng YS, Rohan R, Sunday ME, Demello DE, D'Amore PA. 2001. Differential expression of VEGF isoforms in mouse during development and in the adult. Dev Dyn 220:112–121.

Ni A, Lashnits E, Yao LC, Baluk P, McDonald DM. 2010. Rapid remodeling of airway vascular architecture at birth. Dev Dyn 239:2354–2366.

Ohuchi H, Hori Y, Yamasaki M, Harada H, Sekine K, Kato S, Itoh N. 2000. FGF10 acts as a major ligand for FGF receptor 2 IIIb in mouse multi-organ development. Biochem Biophys Res Commun 277:643–649.

Pan J, Copland I, Post M, Yeger H, Cutz E. 2006. Mechanical stretch-induced serotonin release from pulmonary neuroendocrine cells: Implications for lung development. Am J Physiol Lung Cell Mol Physiol 290:L185–L193.

Parera MC, van Dooren M, van Kempen M, de Krijger R, Grosveld F, Tibboel D, Rottier R. 2005. Distal angiogenesis: A new concept for lung vascular morphogenesis. Am J Physiol Lung Cell Mol Physiol 288:L141–L149.

Park C, Kim TM, Malik AB. 2013. Transcriptional regulation of endothelial cell and vascular development. Circ Res 112:1380–1400

Park WY, Miranda B, Lebeche D, Hashimoto G, Cardoso WV. 1998. FGF-10 is a chemotactic factor for distal epithelial buds during lung development. Dev Biol 201:125–134.

Peng T, Morrisey EE. 2013. Development of the pulmonary vasculature: Current understanding and concepts for the future. Pulm Circ 3:176–178.

Peng T, Tian Y, Boogerd CJ, Lu MM, Kadzik RS, Stewart KM, Evans SM, Morrisey EE. 2013. Coordination of heart and lung co-development by a multipotent cardiopulmonary progenitor. Nature 500:589–592.

Pepicelli CV, Lewis PM, McMahon AP. 1998. Sonic hedgehog regulates branching morphogenesis in the mammalian lung. Curr Biol 8:1083–1086.

Post LC, Ternet M, Hogan BL. 2000. Notch/Delta expression in the developing mouse lung. Mech Dev 98:95–98.

Prince LS, Dieperink HI, Okoh VO, Fierro-Perez GA, Lallone RL. 2005. Toll-like receptor signaling inhibits structural development of the distal fetal mouse lung. Dev Dyn 233:553–561.

Que J, Choi M, Ziel JW, Klingensmith J, Hogan BL. 2006. Morphogenesis of the trachea and esophagus: Current players and new roles for noggin and Bmps. Differentiation 74:422–437.

Que J, Okubo T, Goldenring JR, Nam KT, Kurotani R, Morrisey EE, Taranova O, Pevny LH, Hogan BL. 2007. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. Development 134:2521–2531.

Que J, Wilm B, Hasegawa H, Wang F, Bader D, Hogan BL. 2008. Mesothelium contributes to vascular smooth muscle and mesenchyme during lung development. Proc Natl Acad Sci USA 105:16626–16630.

Radzikinas K, Aven L, Jiang Z, Tran T, Paez-Cortez J, Boppidi K, Lu J, Fine A, Ai X. 2011. A Shh/miR-206/BDNF cascade coordinates innervation and formation of airway smooth muscle. J Neurosci 31:15407–15415.

Rock JR, Gao X, Xue Y, Randell SH, Kong YY, Hogan BL. 2011. Notch-dependent differentiation of adult airway basal stem cells. Cell Stem Cell 8:639–648.

Rock JR, Hogan BL. 2011. Epithelial progenitor cells in lung development, maintenance, repair, and disease. Annu Rev Cell Dev Biol 27:493–512.

Sala FG, Del Moral PM, Tiozzo C, Alam DA, Warburton D, Grikscheit T, Veltmaat JM, Bellusci S. 2011. FGF10 controls the patterning of the tracheal cartilage rings via Shh. Development 138:273–282.

Schachtner SK, Wang Y, Scott Baldwin H. 2000. Qualitative and quantitative analysis of embryonic pulmonary vessel formation. Am J Respir Cell Mol Biol 22:157–165.

Schipani E, Ryan HE, Didrickson S, Kobayashi T, Knight M, Johnson RS. 2001. Hypoxia in cartilage: HIF-1alpha is essential for chondrocyte growth arrest and survival. Genes Dev 15:2865–2876.

Scott CL, Walker DJ, Cwiklinski E, Tait C, Tee AR, Land SC. 2010. Control of HIF-1{alpha} and vascular signaling in fetal lung involves cross talk between mTORC1 and the FGF-10/FGFR2b/Spry2 airway branching periodicity clock. Am J Physiol Lung Cell Mol Physiol 299:L455–L471.

Sekine K, Ohuchi H, Fujiwara M, Yamasaki M, Yoshizawa T, Sato T, Yagishita N, Matsui D, Koga Y, Itoh N, Kato S. 1999. Fgf10 is essential for limb and lung formation. Nat Genet 21:138–141.

Serls AE, Doherty S, Parvatiyar P, Wells JM, Deutsch GH. 2005. Different thresholds of fibroblast growth factors pattern the ventral foregut into liver and lung. Development 132:35–47.

Shannon JM, Nielsen LD, Gebb SA, Randell SH. 1998. Mesenchyme specifies epithelial differentiation in reciprocal recombinants of embryonic lung and trachea. Dev Dyn 212:482–494.

Shu W, Guttentag S, Wang Z, Andl T, Ballard P, Lu MM, Piccolo S, Birchmeier W, Whitsett JA, Millar SE, Morrisey EE. 2005. Wnt/beta-catenin signaling acts upstream of N-myc, BMP4, and FGF signaling to regulate proximal-distal patterning in the lung. Dev Biol 283:226–239.

Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, Hale EC, Newman NS, Schibler K, Carlo WA, Kennedy KA, Poindexter BB, Finer NN, Ehrenkranz RA, Duara S, Sanchez PJ, O'Shea TM, Goldberg RN, Van Meurs KP, Faix RG, Phelps DL, Frantz ID, III , Watterberg KL, Saha S, Das A, Higgins RD. 2010. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics 126:443–456.

Taichman DB, Loomes KM, Schachtner SK, Guttentag S, Vu C, Williams P, Oakey RJ, Baldwin HS. 2002. Notch1 and Jagged1 expression by the developing pulmonary vasculature. Dev Dyn 225:166–175.

Tang N, Marshall WF, McMahon M, Metzger RJ, Martin GR. 2011. Control of mitotic spindle angle by the RAS-regulated ERK1/2 pathway determines lung tube shape. Science 333:342–345.

Tefft D, Lee M, Smith S, Crowe DL, Bellusci S, Warburton D. 2002. MSprouty2 inhibits FGF10-activated MAP kinase by differentially binding to upstream target proteins. Am J Physiol Lung Cell Mol Physiol 283:L700–L706.

Tefft JD, Lee M, Smith S, Leinwand M, Zhao J, Bringas P, Jr., Crowe DL, Warburton D. 1999. Conserved function of mSpry-2, a murine homolog of Drosophila sprouty, which negatively modulates respiratory organogenesis. Curr Biol 9:219–222.

Tsao PN, Vasconcelos M, Izvolsky KI, Qian J, Lu J, Cardoso WV. 2009. Notch signaling controls the balance of ciliated and secretory cell fates in developing airways. Development 136:2297–2307.

Tsao PN, Wei SC, Wu MF, Huang MT, Lin HY, Lee MC, Lin KM, Wang IJ, Kaartinen V, Yang LT, Cardoso WV. 2011. Notch signaling prevents mucous metaplasia in mouse conducting airways during postnatal development. Development 138:3533–3543.

Turcatel G, Rubin N, Menke DB, Martin G, Shi W, Warburton D. 2013. Lung mesenchymal expression of Sox9 plays a critical role in tracheal development. BMC Biol 11:117.

Unbekandt M, del Moral PM, Sala FG, Bellusci S, Warburton D, Fleury V. 2008. Tracheal occlusion increases the rate of epithelial branching of embryonic mouse lung via the FGF10-FGFR2b-Sprouty2 pathway. Mech Dev 125:314–324.

van Tuyl M, Groenman F, Wang J, Kuliszewski M, Liu J, Tibboel D, Post M. 2007. Angiogenic factors stimulate tubular branching morphogenesis of sonic hedgehog-deficient lungs. Dev Biol 303:514–526.

van Tuyl M, Liu J, Wang J, Kuliszewski M, Tibboel D, Post M. 2005. Role of oxygen and vascular development in epithelial branching morphogenesis of the developing mouse lung. Am J Physiol Lung Cell Mol Physiol 288:L167–L178.

Volckaert T, Campbell A, Dill E, Li C, Minoo P, De Langhe S. 2013. Localized Fgf10 expression is not required for lung branching morphogenesis but prevents differentiation of epithelial progenitors. Development 140:3731–3742.

Volckaert T, Dill E, Campbell A, Tiozzo C, Majka S, Bellusci S, De Langhe SP. 2011. Parabronchial smooth muscle constitutes an airway epithelial stem cell niche in the mouse lung after injury. J Clin Invest 121:4409–4419.

Wansleeben C, Barkauskas CE, Rock JR, Hogan BL. 2013. Stem cells of the adult lung: Their development and role in homeostasis, regeneration, and disease. Wiley Interdiscip Rev Dev Biol 2:131–148.

Weaver M, Dunn NR, Hogan BL. 2000. Bmp4 and Fgf10 play opposing roles during lung bud morphogenesis. Development 127:2695–2704

Weaver M, Yingling JM, Dunn NR, Bellusci S, Hogan BL. 1999. Bmp signaling regulates proximal-distal differentiation of endoderm in mouse lung development. Development 126:4005–4015.

White AC, Lavine KJ, Ornitz DM. 2007. FGF9 and SHH regulate mesenchymal Vegfa expression and development of the pulmonary capillary network. Development 134:3743–3752.

Xing Y, Li A, Borok Z, Li C, Minoo P. 2012. NOTCH1 is required for regeneration of Clara cells during repair of airway injury. Stem Cells 30:946–955.

Yuan B, Li C, Kimura S, Engelhardt RT, Smith BR, Minoo P. 2000. Inhibition of distal lung morphogenesis in Nkx2. 1(−/−) embryos. Dev Dyn 217:180–190.

Zhang S, Loch AJ, Radtke F, Egan SE, Xu K. 2013. Jagged1 is the major regulator of Notch-dependent cell fate in proximal airways. Dev Dyn 242:678–686.