Chapter 1
The Regulation of Branching Morphogenesis in the Developing Lung

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Overview

Lung branching morphogenesis generates a tree-like structure and allows efficient airflow to millions of gas exchange units, the alveoli. The initiation, continuation, and termination of lung branching morphogenesis require precise control of the specification, maintenance, and depletion of lung epithelial progenitors. Here I will focus on these progenitors and describe the branch morphology across species and in comparison with other branching organs; the underlying cell biology including polarity, movement, and matrix interaction; and an initial gene regulatory network that can be expanded using genetic and genomic approaches. I will also describe when and how branching ends and its implications in premature birth and developmental plasticity.

Relationship Between Lung Epithelial Progenitors and Branching Morphogenesis

The air lumen from the proximal airway to the peripheral gas exchange compartment is bordered by a continuous monolayer of epithelial cells embedded in mesenchymal tissues. The developmental process forming the respiratory tree, termed branching morphogenesis, is not simply the sculpting of a homogeneous epithelial sheet, but rather concerted morphogenesis and differentiation of specialized epithelial progenitors. These progenitors are commonly defined by expression

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of transcription factors *Sox9* and *Id2* [1, 2], and the expression of the former has been traced throughout development [3, 4]. The *Sox9* progenitors are specified in the embryonic foregut, build the entire respiratory tree including both the airway and gas exchange compartments, and are depleted after birth (Fig. 1.1). The temporal and spatial relationships between epithelial progenitors and branching morphogenesis are described below.

Temporal: The respiratory primordium forms in the ventral portion of the anterior foregut around embryonic day (E) 9 in mouse and gestation week 5 in human [1, 5]. A combination of *Wnt*, *Bmp,* and *Fgf* signalings is required to activate the respiratory lineage factor *Nkx2.1* [6–10]. The *Nkx2.1* respiratory primordium gives rise to both the trachea and the lung. A subset of *Nkx2.1* cells express *Sox9*, constituting the lung epithelial buds that will subsequently undergo branching morphogenesis (Fig. 1.1) [3].

After specification, *Sox9* progenitors undergo branching morphogenesis forming new branches at an exponential rate. The basic unit of a branch consists of a branch tip and a branch stalk. *Sox9* progenitors are always associated with branch tips throughout the branching process (Fig. 1.1) and thus must be precisely regulated in at least three aspects. First, the progenitors need to self-renew at a rate that matches the exponentially growing respiratory tree. Second, a controlled fraction of their progeny needs to exit the progenitor pool, forming branch stalks and differentiating into airway and alveolar cells [3, 4]. Third, depending on whether branch tips are split in a symmetric or asymmetric manner to form bifurcation or lateral branches, respectively, the progenitors need to be partitioned between new branch tips accordingly (Fig. 1.2).

Branching morphogenesis does not continue forever, and *Sox9* progenitors are depleted after birth (Fig. 1.1). A time course analysis following branch tips along the lobe edge shows that the branch tip-stalk structure disappears after birth as the stalks widen, presumably as a result of differentiation into alveolar ducts and air filling. Meanwhile, *Sox9* progenitors persist as clusters at branch tips as late as

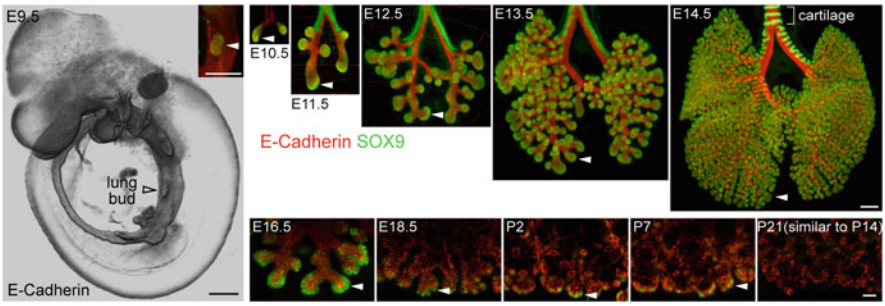

Fig. 1.1 A complete developmental history of SOX9 lung epithelial progenitors. Optical projection tomography (OPT, *top row*, scale: 250 um) and confocal images (*bottom row*, scale: 20 um) of immunostained whole embryo (E9.5), lungs (E10.5–E14.5) and lobe edges (E16.5–P21). SOX9 progenitors are indicated by *arrowheads*. *Open arrowhead*: lung bud forming on the foregut. Adapted from [3]

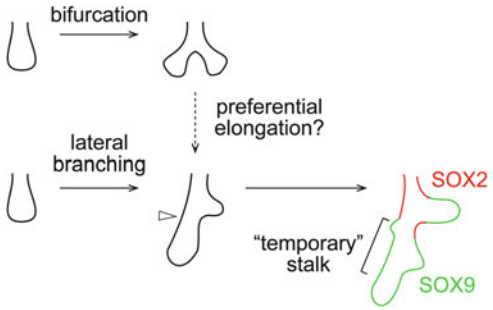

Fig. 1.2 Two modes of new branch formation. New branch tips form via bifurcation or lateral branching. One branch that is preferentially elongated after the symmetric bifurcation may appear as the main branch in the asymmetric lateral branching, suggesting that the two branching modes may be more similar than their final appearance. The main branch contains a "temporary" branch stalk that does not express SOX2 and can initiate new branches (*open arrowhead*)

postnatal day (P) 7 and potentially could continue to divide into smaller clusters, reminiscent of branch tip-splitting during branching morphogenesis. Given their location in the most distal portion of the respiratory tree and beyond alveolar ducts, these *Sox9* progenitor clusters presumably differentiate into primary saccules that will be further subdivided to form mature alveoli. Therefore, cessation of branching morphogenesis parallels depletion of *Sox9* progenitors.

Spatial: For bifurcation where branch tips form by splitting existing tips, it is intuitive that *Sox9* progenitors are located in branch tips, while their differentiating progeny, commonly marked by *Sox2* expression, are located in branch stalks. However, for lateral branching where branch tips form on existing stalks, I would like to introduce the concept of "temporary" branch stalks, in which a morphological branch stalk is molecularly a branch tip. Specifically, although "temporary" branch stalks are surrounded by airway smooth muscle [11], they are *Sox2* negative as supported by the absence of labeled distal epithelial cells in $Sox2^{CreER}$ lineage tracing experiments [4]. In other words, before *Sox9* progenitors become *Sox2*-positive "permanent" branch stalks, they remain capable of branching regardless of the tip-stalk morphology (Fig. 1.2). Therefore spatially, *Sox9* progenitors are located in the branching region of the developing respiratory tree.

The close temporal–spatial correlation between branching morphogenesis and *Sox9* epithelial progenitors suggests that regulation of branching morphogenesis is fundamentally regulation of *Sox9* progenitors by intrinsic (epithelial) and extrinsic (mesenchymal) signalings.

Morphological Features of Branching Morphogenesis

The building blocks, branch tips and stalks, form in two modes: bifurcation and lateral branching. Deployment of these branching modes must be regulated in a deterministic manner to form a respiratory tree of a highly reproducible shape and

lobation pattern that is specific for each species. Meanwhile, space-filling must also play a role to ensure maximal space utilization without branch collision. This section will focus on the mouse lung whose branching has been studied in depth [12]; and this will be compared with lungs from other species and other branched organs.

Lobation: On a global level, branching morphogenesis is constrained by the size, shape, and arrangement of lobes termed lobation. The mouse lung consists of a single left lobe and four right lobes: cranial, middle, accessory, and caudal lobes. This asymmetry is regulated as part of the bilateral asymmetry of the body plan as situs inversus mutants, such as the *Dnahc11* mutant [12], have left-right inverted lungs as well as other internal organs. Closer examination of the branching structure suggests that the mouse lung might be more symmetric than its appearance with the exception of the accessory lobe (Fig. 1.3). Specifically, the caudal lobe can be considered the equivalent of the caudal portion of the left lobe (starting from L.L3) as both consist of a central medially curved lobar bronchus and multiple parallel lateral branches. The axis of the middle lobe parallels the lateral branches of the caudal lobe and thus may be the L.L2 equivalent, while the anterior portion of the cranial lobe is reminiscent of the L.L1.A1 branch. Thus, the main difference between left and right lobes is that individual right lobes are encased and thus separated by a single-layered mesothelium. Future studies of the mesothelium may shed light on mechanisms of normal lobation and abnormal lobe fusion.

The shape of individual lobes is apparently established early in development (Fig. 1.1), raising the interesting question of how branching morphogenesis at

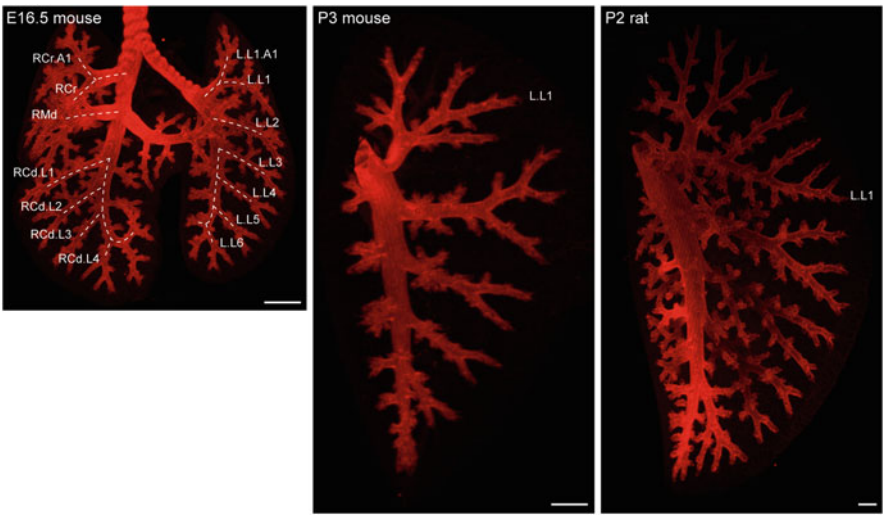

Fig. 1.3 Mouse and rat airways. OPT images of mouse and rat lung or left lobes immunostained for an airway marker SOX2. The mouse lung and left lobe are shown at the same magnification and the rat left lobe is half of that magnification. Scale: 500 um. The branch lineage is labeled to show symmetry between the left and right lobes

different locations advances the lobe surface in a concerted fashion to preserve lobe shape over time. Alternatively, lobe geometry is regulated by other means and feeds into the branching pattern control. Intriguingly, the rat lung is remarkably similar in lobe shape and branching pattern to the mouse lung, but is not simply a scaled-up version as it has more airway branch generations (Fig. 1.3).

Branching subroutines: Careful comparison of hundreds of developmental intermediates has revealed that branching in the mouse lung can be accounted for by three branching subroutines: domain branching, planar, and orthogonal bifurcation [12]. Furthermore, these three subroutines are deployed in three sequences, where orthogonal bifurcation represents a "dead-end" subroutine that will only form rosette-like branches and no longer switch to alternative subroutines [12]. It is interesting to note that, compared to orthogonal bifurcation, domain branching and planar bifurcation, by definition, form longer branch stalks that extend to the lobe edge and can initiate lateral branches. Thus, as discussed below, branch length (or elongation) may be an additional parameter other than branch angle that distinguishes the subroutines.

Dichotomous and monopodial branching: Branching in the mouse lung is often monopodial featuring minor daughter branches on the side of a major parent branch, while branching in the human lung is often dichotomous featuring symmetric daughter branches of comparable size [13]. Although this difference might reflect differential usage of lateral branching versus bifurcation across species, an additional contributing factor may be a difference in branch elongation after, but not during, branch formation. Specifically, differential elongation of one daughter branch after dichotomous branching can form an asymmetric structure reminiscent of the one formed via monopodial branching (Fig. 1.2). Consistent with this possible dichotomous-branching origin of monopodial branches, the major parent branch in monopodial branching is often curved (Fig. 1.3). Furthermore, no gene has been identified whose expression distinguishes dichotomous (bifurcation) and monopodial (lateral) branching, and computer simulation suggests a deterministic role of branch elongation in generating different branching modes [14]. The difference in branch elongation may be ultimately linked to lobe shape, which differs significantly between mouse and human.

Tip morphology in comparison with other branched organs: Compared to a bead-on-a-string design, branching morphogenesis packs a large number of terminal units without incurring a tortuous transport route. Thus, it is used in multiple organs, including the kidney, mammary, and salivary glands. However, the branch tip morphology among organs differs substantially. While branch tips in the lung are bulbous with obvious luminal space, the ureteric tree frequently contains trifurcating branch tips [15]; the salivary gland tips split via clefting due to replacement of cell–cell junctions with cell–matrix interactions [16]; and mammary gland branching requires epithelial-to-mesenchymal transition and migration of a bi-layered epithelium [17]. Although the Drosophila trachea system mediates respiratory function and requires the same fibroblast growth factor (*Fgf*) signaling as the lung, its branching appears more similar to vertebrate angiogenesis, where selected tip cells lead the rest toward a morphogen source [18]. It is worth noting

that in the developing lung, the bulbous branch tip may appear on sections as a large diameter tube, but should not be mistaken as proximal airways, which do not have as large a lumen as those in the adult lung.

Cell Biology of Branching Morphogenesis

The cellular behaviors underlying lung branching morphogenesis are not well understood, in part because existing explant culture systems [19] are limited in recapitulating in vivo development. In particular, cultured lungs are constrained in size by the supporting filters, and the resulting limited branch outgrowth may disrupt the epithelium–mesenchyme cross talk that is essential for normal branching. Furthermore, cultured lungs flatten due to gravity; this disrupts branch shape, morphogen distribution, and potentially intraluminal fluid pressure, a mechanical stimulus possibly involved in branching [20]. In addition, given the exponential nature of branch tip number increase, a small difference in the initial tip number due to variation in developmental timing, coupled with ambiguity in branch tip counting due to altered morphology, may be misinterpreted as treatment effects. Nevertheless, genetic and culture experiments have implicated cell polarity, movement, and matrix interaction in lung branching.

Cell polarity: The developing lung epithelium is a monolayer polarized both along the apical–basal axis and within the monolayer plane. Epithelial-specific deletion of *Integrin beta-1* (*Itgb1*), a major mediator of cell–matrix interaction, completely abrogates branching [21]. The mutant epithelium frequently contains abnormal apical–basally oriented cell division and becomes multilayered with distinct gene expression among layers, reminiscent of a stratified epithelium such as the epidermis [22]. Overactivation of *Kras*, a small GTPase mediating receptor tyrosine kinase signaling, or loss of *Spry1* and *Spry2*, phosphatases limiting receptor tyrosine kinase signaling, shortens and widens branch tips in association with altered proportions of longitudinal versus circumferential cell divisions [23]. Although computer modeling suggests that abnormal cell division orientation largely explains the observed branch morphology phenotype, a similar mechanism has been proposed for formation of cystic renal tubes but may not be causal to their phenotype in some cases [24].

Cell movement: Although constrained by tight and adherent junctions, the lung epithelium is fluid with frequent interkinetic nuclear migration (INM) along the apical–basal axis and neighbor exchange via intercalation. Best known for its role in neurogenesis, INM positions the nucleus at the basal or apical side during the S or M phase of the cell cycle, respectively, and potentially exposes cells to location-specific signals [25, 26]. Asynchrony in INM among cells makes a monolayered epithelium appear pseudostratified and, depending on cell density, generates bottle-shaped cells with apical and basal thin extensions of varying length [27].

Real-time imaging of cultured lungs has confirmed the association between cell division orientation and branch growth in the longitudinal versus circumferential

directions [28]. In addition, no migratory cellular extensions are observed, and instead epithelial cells move via neighbor exchange, a process reminiscent to intercalation during germ band elongation [29]. Unlike intercalation, the net apical surface area of lung epithelial cells can decrease locally via actomyosin contraction, leading to epithelium deformation and branch initiation, in culture and in a *Wnt* signaling mutant [27, 30]. Future genetic mosaic analyses are necessary to understand whether and how morphogenetic signals, including *Wnt* and *Fgf,* regulate cell shape in a cell-autonomous manner. Intriguingly, in the kidney, neighbor exchange occurs even during mitosis via a mitosis-associated cell dispersal process where apically dividing daughter cells move apart and insert back to the epithelium at distant sites [31]. If similar cell behavior also occurs in the lung, it would be interesting to determine how it might be integrated with oriented cell division to regulate branch morphology.

Extracellular matrix (*ECM*): The lung epithelium is surrounded by a basement membrane that contains fibronectin, collagen, nidogen, and laminin and is thicker around branch stalks than tips [32], suggesting a potential role of ECM in branching morphogenesis. Although such a role is demonstrated in cultured lungs [16, 33], genetic mutants either die before lung formation or exhibit little branching phenotype, presumably due to essential roles of ECM in cell survival or functional redundancy among related genes [34]. Interestingly, several ECM mutants, including those affecting ECM turnover, have abnormal perinatal lung maturation, highlighting the importance of ECM in supporting the air-filled alveolar region [34–37].

Genetic Control of Branching Morphogenesis

Defects in lung branching morphogenesis often lead to respiratory failure and death at birth, a characteristic phenotype that has allowed an effectively forward genetic screen and identification of a number of branching control genes and signalings [1, 38]. Future studies need to assemble the existing parts list into a gene regulatory network, especially with regard to their role in *Sox9* epithelial progenitors as they give rise to the entire epithelial tree. This section will summarize the major branching signalings focusing on the epithelial progenitors; highlight importance of nomenclature in phenotypic characterization; and describe available genetic and genomic tools to integrate existing knowledge toward a system-level understanding. Due to the aforementioned limitations of lung explant culture, only genetic mutants are included although caution is also warranted with the interpretation of these models because of the potential nonspecific toxicity of genetic tools [39–41].

Multiple signalings: The primary branching signal utilizes the *Fgf10-Fgfr2* pathway. *Fgf10* expression is dynamic and localized in the mesenchyme just ahead of growing branch tips [42], and both *Fgf10* and *Fgfr2* mutants completely lack branching [43, 44]. Epithelial-specific deletion of *Fgfr2* leads to a lung with only left and right main bronchi that are devoid of *Sox9* progenitors, while epithelial

overactivation of *Kras*, a mediator of receptor tyrosine kinase including *Fgf* signalings, leads to expansion and persistence of *Sox9* progenitors [45]. Such gain-of-function and loss-of-function data support a dominant role of the *Fgf* signaling in progenitor maintenance.

The *Wnt* signaling in the developing lung is complex as multiple *Wnt* genes are present and canonical *Wnt* activity, depending on the reporter, is detected in either the epithelial or mesenchymal compartment [46]. *Wnt2/2b*-dependent canonical *Wnt* signaling controls respiratory fate specification in the foregut [6, 7]. The *Wnt7b* mutant has a significantly smaller lung with fewer albeit normal looking branches [47]. The *Wnt5a* mutant also has a smaller lung and a higher density of epithelial cells at late stages, although it is unknown if the total number of branches is increased [48]. Epithelial deletion of *Ctnnb1*, the mediator of canonical *Wnt* signaling, decreases the number of *Sox9* progenitors whose domain becomes disproportional to that of their *Sox2* expressing progeny [4, 49, 50].

Similar complexity exists for the *Bmp* and *Tgf* signalings. Although *Bmp4* is specifically expressed in the *Sox9* progenitors and antagonizes *Fgf10* in culture, its in vivo role is still debatable [4, 51, 52]. Similarly, although deletion of *Alk3* (*Bmpr1a*), a *Bmp* receptor, using *Sftpc* genetic drivers disrupts branching [52, 53], Shh^{Cre}-mediated deletion of *Alk3* has no detectable phenotype (unpublished observation). Only deletion of both *Bmp* receptors, *Alk3* and *Alk6*, disrupts respiratory fate specification in the foregut [8]. Such redundancy among homologous genes may also apply to the *Bmp* ligands. Epithelial deletion of *Alk5*, a *Tgf* receptor, leads to a smaller lung with fewer and dilated branches [54].

Therefore, existing evidence indicates that the epithelial progenitors receive inputs from *Fgf*, *Wnt,* and *Tgf* signalings. Although not discussed here, the mesenchyme is also precisely regulated by multiple signalings including *Fgf9*, *Wnt,* and *Shh*. Disruption of these will also in turn affect epithelial branching [55–57].

Phenotype nomenclature: Gross histological examination prompted by the characteristic neonatal lethality phenotype often leads to a description of the lung phenotype as hypoplastic or hypercellular, which likely corresponds to defective branching or alveolar differentiation, respectively. Further analyses of such mutants need to define phenotypes using an increasing number of molecular markers available for each process.

Another term commonly used is proximal–distal patterning, which requires clarification and discussion. First, patterning is classically defined as a onetime cell fate choice in a homogeneous field of naïve cells in response to a morphogen gradient, such as the anterior–posterior patterning of the Drosophila embryo [58]. Although the lung epithelium has differential gene expression proximal–distally (e.g., distal *Sox9* and proximal *Sox2*), the proximal region is not the equivalent of the distal region, but instead is made of the continuous influx of the progeny of the distally located *Sox9* progenitors. Therefore, proximal–distal patterning, or "proportion" to be exact in the context of branching, reflects cumulative, constant cell fate choices of *Sox9* progenitors between self-renewal and differentiation. Such a concept has been explored in recent studies [4, 59].

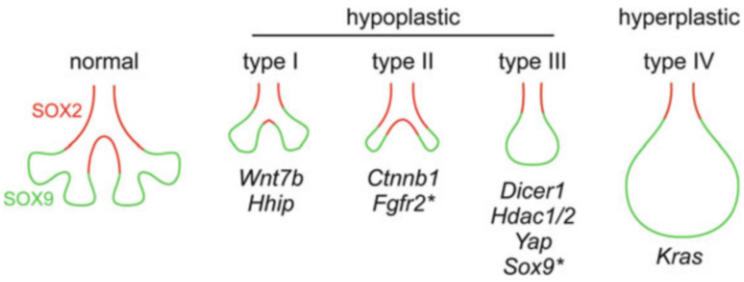

Fig. 1.4 Proximal–distal proportion in mutants with different branch morphology. Type I mutants [47, 57] have fewer normal-looking branches with a normal proximal–distal proportion. Type II mutants [4, 45, 49, 50] have fewer branching containing a smaller progenitor region. *Asterisk*: the *Fgfr2* mutant has no SOX9 progenitors. Type III mutants [45, 59, 60, 70] have fewer branches that are counterintuitively dilated as in the hyperplastic type IV mutant. Compared with the normal lung, type III mutants have a larger distal region with respect to individual branches, but a smaller distal region with respect to the whole lung. *Asterisk*: the *Sox9* mutant has a distal region that does not express SOX9. Type IV mutants [23, 45] have a larger lung with fewer and dilated branches

Second, definition of proximal–distal patterning may be confounded by branch morphology and depends on whether individual branches or the whole lung is compared between the control and mutant. As illustrated in Fig. 1.4, a simple slow branching mutant (type I) will have fewer branches with a normal proximal–distal proportion. Type II mutants can have a disproportionally small distal region as the result of decreased progenitor self-renewal with normal differentiation. Type III mutants are characterized by a smaller lung with fewer branches that are counterintuitively dilated. Due to this abnormal branch morphology, individual branches appear to have an expanded distal region while the total distal region in the whole lung is smaller. The apparent distal bias may be actually accompanied by decreased progenitor self-renewal as supported by the epithelial apoptosis phenotype in the *Dicer* mutant and the branch dilation phenotype from epithelial cell ablation in the *Sftpc-rtTA; tetO-DTA* mutant [4, 60]. Therefore, type II and type III mutants may be similar in having decreased progenitor self-renewal and total distal region in the whole lung, while their apparent difference in proximal–distal patterning with respect to individual branches may be confounded by branch dilation. Finally, Type IV mutants can have a disproportionally large distal region as the result of increased progenitor self-renewal with normal or decreased differentiation.

Last, proximal–distal patterning is often inferred from sections stained for distal and proximal markers, such as *Sox9* and *Sox2,* respectively. Caution should be taken in interpreting section-based results because sections near the lobe surface are expected to have more distal regions than those near the center. Furthermore, the proportion of distal versus proximal regions on sections reflects the average proximal–distal patterning in the whole lung instead of individual branches, an important difference for the type III mutants.

Therefore, while proximal–distal patterning is a useful phenotype descriptor, its interpretation may vary depending on mutant types (Fig. 1.4) and thus requires clarification, such as whether in the context of individual branches or the whole lung.

Gene regulatory network in epithelial progenitors: Despite the importance of tissue cross talk, branching morphogenesis ultimately is about self-renewal of *Sox9* progenitors to sustain branch formation and their differentiation into proximal airway progeny. Genetic and genomic tools are available to integrate known controlling signals into a gene regulatory network in the progenitors.

The relationship between genes in a network is classically studied using epistasis analysis, where the mutant phenotype of genes functioning downstream is expected to be dominant over, or epistatic to, that of upstream genes [61]. Epistasis analysis is essential in identifying causal instead of associative changes in gene expression, especially in systems with robust feedback regulation such as branching morphogenesis. Several genetic tools are available to specifically target the lung epithelium including epithelial progenitors. Shh^{Cre} is highly efficient due to its early activity in the foregut epithelium, but may be limited in studying branching morphogenesis if progenitor specification is blocked as in the *Ctnnb1* mutant [6, 7]. $Sox9^{CreER}$ and $Id2^{CreER}$ allow specific targeting of the progenitors at desired time points and efficiencies, and generation of mosaic mutants to study cell-autonomous effects [2, 4, 45]. Additional targeting tools include *Sftpc-rtTA; tetO-Cre* and $Nkx2.1^{CreER}$ [45, 62]. A possible epistasis analysis to integrate two major signalings, *Fgf* and *Wnt*, is to examine the phenotype of $Kras^{LSL\text{-}G12D/+}$; $Ctnnb1^{CKO/-}$; $Sox9^{CreER/+}$ double mutants, where the single mutants have an opposite effect on progenitor self-renewal (Fig. 1.4) [4].

Targeted and genomic screens have generated an increasing list of progenitor markers besides *Sox9* and *Id2* [3, 45, 63, 64]. A genome-level molecular definition of progenitors will allow comprehensive mutant analyses and gene network construction. For example, progenitor marker analyses in the *Sox9* mutant identify *Sox9*-dependent (e.g., *Clu* and *Mia1*) and independent (e.g., *Bmp4*, *Spry2,* and *Id2*) genes that are placed downstream or parallel (potentially upstream) to *Sox9* [45]. Furthermore, a gene controlling markers that are a subset of those controlled by another gene is likely to function downstream of the other gene. Such marker analyses can be extended to the whole genome by transcriptome profiling of mutant lungs or purified progenitors.

Termination of Branching Morphogenesis

Early in development, *Sox9* progenitors expand at least several hundred folds from less than a few hundred at specification to clusters of tens at each of the thousands of branch tips at the peak of branching morphogenesis (Fig. 1.1). This expansion is due to progenitor self-renewal, outpacing their differentiation into airway progeny. However, late in development, progenitor self-renewal must slow down, leading to eventual progenitor depletion and differentiation into their alveolar progeny. It is unclear when this transition in the balance between self-renewal and differentiation occurs and whether it corresponds to the transition from airway to alveolar differentiation. The timing of progenitor depletion and alveolar differentiation must be

coupled with gestation periods to ensure a functional gas exchange region at birth. An intriguing candidate is glucocorticoid signaling as the maternal level of glucocorticoids peaks before birth and earlier administration of exogenous glucocorticoids activates alveolar differentiation prematurely. However, loss of the glucocorticoid receptor only causes a small delay in alveolar differentiation, suggesting the involvement of additional lung maturation factors [4, 65].

Additional insights may be obtained by examining lungs from species that do not have branching morphogenesis. Specifically, the frog lung consists of two main bronchi that do not branch but instead undergo alveolar differentiation after specification. Intriguingly, *Sox9*, although present in the frog genome, is not expressed at branch tips of the early frog lung, suggesting an antagonistic relationship between branching morphogenesis and alveolar differentiation [45, 66]. Consistent with this idea, loss of *Sox9* in the mouse lung impairs branching and allows premature expression of some alveolar markers, while overactivation of *Kras* leads to persistent *Sox9* progenitors and arrested alveolar differentiation [45]. Further cross species comparison using RNA sequencing may identify the molecular basis underlying lung evolution and specialization [67].

Given the exponential nature of branch number increase, the last few rounds of branching morphogenesis are expected to have a dominant effect on the final number of branches and consequently alveoli. This termination process may be sensitive to environmental perturbations such as oxygen and ventilation that are used to support premature birth and potentially contribute to bronchopulmonary dysplasia and chronic respiratory diseases. Even in clinically normal birth, suboptimal branching termination may lead to a reduced functional reserve or stressed stem cells that may manifest clinically upon injury or aging. This developmental plasticity matches epidemiological observations and has been explored experimentally in other organs [68, 69].

In summary, lung epithelial progenitors undergo branching morphogenesis to build both the airway and alveolar compartments. Cellular and genetic control of the progenitors via multiple signalings dictates branch number and morphology. Mechanistic understanding of progenitor specification, maintenance, and depletion has implications for clinical and subclinical respiratory diseases.

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