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Lung stem cell behavior in development and regeneration

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ABSTRACT

New data have accumulated on the identification, behavior and cellular properties of embryonic and tissue-specific stem cells over the last decade. The modes of stem cell division, asymmetric versus symmetric, are tightly regulated during development and regeneration. The proper choice of a stem cell to divide asymmetrically or symmetrically has great consequences for development and disease because inappropriate asymmetric division disrupts organ morphogenesis, whereas uncontrolled symmetric division induces tumorigenesis. Therefore, understanding the behavior of lung stem cells could identify innovative solutions for restoring normal morphogenesis and/or regeneration of different organs. In this concise review, we describe different types of lung stem/progenitor cells and mechanisms controlling their proliferation and differentiation as well as recent studies in our laboratory about the mode of division of lung epithelial stem cells. We also compare asymmetric cell division in the lung stem cells with other tissues in different organisms.

Keywords: Asymmetric stem cell division, Differentiation and characterization, Lung stem/progenitor cells, Regeneration of lung stem cells, Types of lung stem cells

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INTRODUCTION

Stem cells are multipotent source of multiple cell lineages. They are undifferentiated cells, which have the potential to self-renew and develop into various cell types that carry out different functions. These cells are an essential driving force for fast-growing fields of both regenerative medicine and functional tissue engineering. Stem cells are critical players during development, and both tissue repair and regeneration after injury as well as healthy homeostatic cell turnover because of their ability to self-renew and pluripotency.

The lung has a much slower turnover that has hampered the identification and characterization of putative lung progenitor cells, unlike other epithelial

tissues that undergo rapid regeneration such as the gastrointestinal tract and skin. Thus, little is known about the existence of specific self-renewing cells in the lung, in contrast to other organs. In addition, we do not fully understand whether a single lung stem cell suffices to generate the more than 40 distinct cell types that are required for mature lung function. Several other factors have also hampered progress in the field of lung stem cell research, including the lack of both stem cell markers and clonality assays to identify and isolate them.

Recent reports on adult murine airway have shown that, in addition to both airway smooth muscle stem cells and endothelial stem cells in the pulmonary vasculature, there are at least five putative epithelial stem/progenitor cell niches. In addition, circulating stem cells, which may take up residence in the lung, are another source of stem cells in the lung [1, 2].

In this concise review, we discuss recent advances and research progress about lung stem cell development and behavior during lung morphogenesis, repair/regeneration.

TYPES OF LUNG STEM AND PROGENITOR CELLS

1 Prenatal Endogenous Embryonic Epithelial Progenitors and Postnatal Alveolar Epithelial Progenitor Cells

1.1 Localization and Characterization

Several reports have noted that distal tips of the branching tubules contain undifferentiated epithelial multipotent progenitor cells, which express several characteristic genetic markers at least at the pseudoglandular stage of lung development. Presumed endogenous epithelial stem/progenitor cells in adult lung reside in the basal layer of upper airways within the alveolar epithelium, the bronchoalveolar junction, and within or near the pulmonary neuroendocrine cell rests, as shown in several studies [3–11]. It has been shown that cell cycle kinetics of distal epithelial cells differs from that of other epithelial cells and most of these cells incorporate bromodeoxyuridine (BrdU), the analog of thymidine during one-hour pulse [12]. Furthermore, Rawlins et al. have shown that the distal lung epithelium has multipotent progenitors, which contribute descendents to bronchi as well as alveoli during lung development [13]. Several lines of evidence have shown that descendants of distal lung epithelial progenitors are left behind in the stalks during epithelial branching, while proliferative stem cells stay in the epithelial budding tips. For instance, unique high expression patterns of the transcription factors: SRY-box containing gene 9 (Sox 9), inhibitor of differentiation 2 (Id2), v-myc myelomatosis viral related oncogene (N-myc), and ets variant gene 5 (Etv5/ERM) have been observed in the distal lung epithelial cells. Furthermore, distal lung epithelial cells have been regulated by several key signaling pathways, such as that of fibroblast growth factor (FGF), sonic hedgehog (SHH), bone morphogenetic protein (BMP), and Wnt protein [14–16]. Similarly, these signaling mechanisms are reiterated in other organs such as pancreas [17, 18].

1.2 Repair and Regeneration of Alveolar Epithelial Cells

Several studies have proposed that decline in regeneration and repair of tissues during ageing could be a result of endogenous stem cell failure. The "ready-reserve" for replacement of injured alveolar surface has been proposed to contain large number of alveolar epithelial cells. For instance, acute oxygen damage of the alveolar epithelial cells leads to up-regulation of telomerase expression, which is a stem/progenitor cell marker [19]. It has been proposed that either alveolar epithelial cells contain a comparatively large subpopulation of stem cells, or most of alveolar epithelial cells have the capability to be reactivated into a stem cell-like state following tissue injury [19]. Furthermore, bronchoalveolar stem cells (BASCs), which carry stem cell properties, have the capability to resist naphthaline induced lung injury [9]. These BASC cells express epithelial cell markers, such as alveolar surfactant protein-C (SP-C) and airway Clara Cell 10 kD Protein (CC10), as well as co-express stem cell antigen-1 (Sca-1), and identified within or near bronchiolar-alveolar junctions. BASCs can undergo proliferation and differentiation into either alveolar cells or Clara cells. In vitro clonal assay of BASCs reveals self-renewal, and differentiation as well as multipotency. Additionally, several other studies have demonstrated that the variant Clara cells are endogenous lung stem cells, which occasionally proliferate in steady-state conditions and may be responsible for repopulation of the distal airway epithelium as a response to tissue damage [20]. These variant Clara cells are identified by its capability to express Clara cell secretory protein. However, they differ from the more abundant Clara cells because they can survive against naphthalene-induced injury. Moreover, the kinetics of [3H] thymidine-labeled Clara cell in growing lung revealed that these cells are capable of self-renewal and act as progenitors for ciliated cells during the early postnatal period [21, 22]. Perl et al. recently supported this finding by lineage labeling data [23]. However, further evidences are still needed. In addition, it has been elucidated that alveolar epithelial type II cells proliferate and result in type I cells following injury of adult alveoli. This is supposed to occur during postnatal growth [24]. The existence of these various types of endogenous putative alveolar stem cell populations probably leads to novel targets for rational regenerative lung therapies.

1.3 Molecular Mechanisms That Control Alveolar Epithelial Cells During Development, Repair and Regeneration

Some transcription factors have crucial roles in development, repair and regeneration of epithelial progenitor cell [25]. In our laboratory, we found that eyes absent homolog 1 (EYA1) and sine oculis homeobox 1 (SIX1) are important transcription factors for the lung epithelial stem/progenitor cells maintenance. Mice deficient in EYA1 or SIX1 mice do not have the epithelial progenitor cell markers and highly express differentiation markers in their lungs. Additionally, their lungs are markedly hypoplastic with decreased epithelial branching capacity and intensive mesenchymal cellularity [26, 27]. Furthermore, E74-like transcription factor-3 (ELF3) has a pivotal role in control of lung cell self-renewal and asymmetric cell division during recovery of the injured bronchiolar airway epithelium in response to Clara cell-specific injury [28]. Moreover, a recent study has suggested that thyroid transcription factor-1 (TTF-1) has an important role in modulating, and probably initiating, the early phase of compensatory lung growth [29] (Figure 1 and 2).

Several other studies have drawn attention on the influential role of growth factors, including FGF family members, in development of lung epithelial cells and protection of alveolar cells against lung damage [30]. Interestingly, FGF7 has been evaluated as a curative agent for animal models of alveolar injury [31, 32]. Therefore, it has been hypothesized that preserving progenitor cell function using small molecules like inosine could be an effective therapeutic option in clinical trials. Buckley et al. has supported this hypothesis by showing that FGF7 and inosine treatment can alleviate DNA damage in alveolar epithelial cells as well as enhance mitochondrial conservation and the alveolar epithelial cell migration and repair in an in vitro scratch assay [33]. In addition, inosine can potentially reduce the damage occurred due to oxygen injury by glutathione repletion, mitochondrial preservation and decreased apoptosis as well as increased vascular endothelial growth factor (VEGF) expression [34]. Additionally, FGF10 has been found to have an important function in antagonizing lung injury and fibrosis [35]. Other studies manifested that epithelial FGF9 mainly influences epithelial branching, while mesothelial FGF9 as well as mesenchymal Wnt2A are primarily responsible for maintain signaling of mesenchymal FGF-Wnt/beta-catenin [36].

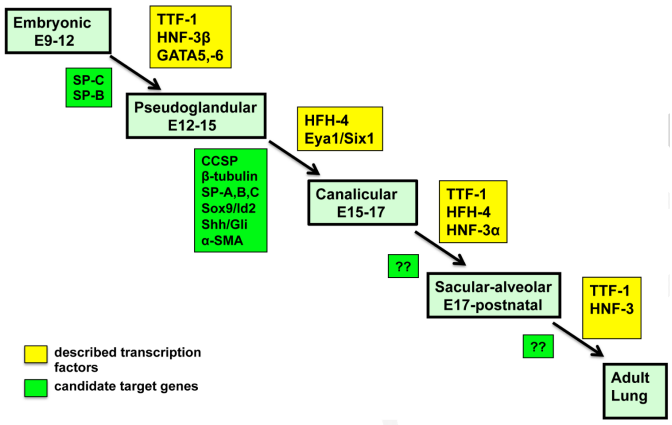

Figure 1: Model for some key transcription factors and their target genes that are involved at each developmental stages of the embryonic lung. Details are described in the text. Abbreviations: Clara cell secretory protein (CCSP), Thyroid TF1 (TTF-1/NKX-2), Hepatocyte nuclear factor3 (HNF3/ Foxd1=), Forkhead box J1 (HFH-4/FOXJ1).

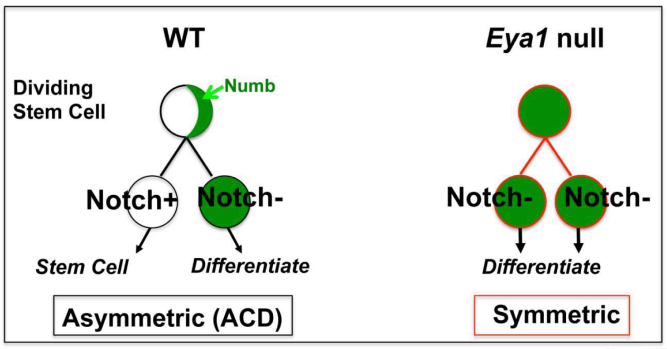

Figure 2: A schematic model of Numb segregation and inheritance in mitotic distal lung epithelial stem cells. The difference in Numb (green) expression levels between two daughter cells mediates asymmetric cell division (ACD, as shown in wild type [WT] lungs), whereas Numb inheritance by both daughters will allow them to execute the stem cell self-renewal program by inhibiting Notch signaling activity and thus allowing symmetric cell division (as shown in Eya1-/- mutant lungs).

2 Bronchial and Tracheal Epithelial Stem and Progenitor Cells

2.1 Localization and Characterization

Recently, studies on lung injury and repair models have identified multiple candidate endogenous stem/ progenitor cells in the epithelium of trachea and bronchi. For example, Hong et al. has shown that subsets of keratin-14 (K-14) -positive basal cells in the trachea can restore a differentiated epithelium following injury and these cells are different from basal cells in the bronchi [37]. Furthermore, these K-14$^+$ cells have the capability to act as progenitors, however ciliated cells cannot, as shown by some lineage-tracing studies in the adult mouse lung and trachea [37, 38]. Additionally, it has been shown by Rawlins et al. that secretoglobin 1A1 (SCGB1A1) -expressing Clara cells are capable of self-renewal and differentiation as a result of tracheal injury, however it is probably not the main mechanism of tracheal regeneration [39].

2.2 Repair and Regeneration of Bronchial and Tracheal Epithelial Stem/Progenitor Cells

It has been shown in an important study from Hogan laboratory that specific epithelial stem/progenitor cells populations have a pivotal role in maintaining lung alveoli [39]. Rawlins et al. has exploited restricted expression of SCGB1A1, a marker of Clara cells by producing a "knockin" transgenic mouse with a tamoxifen (TM) inducible Cre-recombinase (Scgb1a1-CreER™) resulting in lineage labeled Clara cells of the airway. Using different doses and schedule of TM, these Clara cells have been found to proliferate and differentiate into ciliated cells. Therefore, these Clara cells contribute to reconstitution of the epithelium during tracheal repair. While, BASCs, which co-express SCGB1A1 and surfactant protein C (Sp-C) have no clear role in maintenance or regeneration of postnatal lung cells as previously proposed [39]. However, Hong et al. has found that keratin-14 (K-14)-positive basal cells are alternative progenitor cells, which contribute to self-renewal and proliferation of injured bronchial epithelium after depletion of Clara cells [37]. Using lineage tracing method, Hogan et al. has drawn attention on the behavior of a population of basal cells as stem cells in the trachea. Considering that a population of based cells expressing cytokeratin 5 (Krt5) is present in the tracheal pseudostratified epithelium of mouse and human, Krt5-CreER^{T2} transgenic mouse line was used for lineage tracing. This study suggested that BCs of the murine trachea act as progenitor cells, in postnatal growth and during the steady state, as well as in recovery of tracheal epithelial following sulfur dioxide-induced injury [40]. Clonality assay study from Rock laboratory demonstrated the importance of based cells in lung as they are capable of self-renewal and differentiation into mucus and ciliated cells in the lack of stromal or columnar epithelial cells in mouse and human airways. Recently, grainyhead-like 2 (GRHL2) transcription factor was shown to play important roles in the development of bronchial epithelial cells, both as undifferentiated progenitor cells and as organized mucociliary epithelium [41].

Several studies also demonstrated that a diverse population of stem cells in lung expresses both airway and mesenchymal origin molecular markers. These cells are characterized as a Hoechst dye efflux side population (SP) cells [5]. Additionally, Hackett et al. showed the significant proliferative capacity of the CD45$^-$ side population cells, which are present in tracheobronchial epithelium of human. They also suggest that dysregulated pluripotent cells may have a principal role in the pathogenesis of Asthma [42]. Another study demonstrated that some of both lung CD45$^+$ and CD45$^-$ cells have endothelial progenitor cell characteristics following hyperoxic exposure during lung development [43].

Borthwick et al. has noted that cells from gland ducts are the main source of regenerated airway tracheal epithelium following induced injury [44]. Another study conducted by Lu et al. has shown that the submucosal gland ducts found in the proximal airway probably contain putative stem cells [1]. Recently, a study has shown the role of airway submucosal gland (SMG) duct cells in repair of the SMG tubules as well as surface epithelium (SE) following sever hypoxic-ischemic injury. Using in vivo and in vitro stem cell model systems and lineage tracing, Hegab et al. showed that the cells of SMG duct have the capability to proliferate and differentiate into SMGs and SMG duct cells. They can also form the SE in the area adjacent to the sub-mucosal duct. They deduced that SMG duct cells are repairing stem cells for airway epithelium, which may play pivotal role in the treatment of lung diseases as figuring out the repairing cell populations leads to discovering new therapeutic targets and novel cell-based therapies for airway diseases [45].

3 Endogenous Mesenchymal Stem and Progenitor Cells

Little is known about mesenchymal stem/progenitor cells compare by epithelial stem/progenitor cells. Nonetheless, signals from lung mesenchymal cells were found to have a main role in branching morphogenesis and differentiation of lung epithelium. For example, beta-catenin signaling is essential for mesenchymal FGF signaling, which in turn has a critical role in control of mesenchymal proliferation [36]. Moreover, FGF10 signaling is activated and controlled by mesothelial-derived FGF9 from the peripheral mesenchyme to epithelium. This occurs by assembly of a signaling complex comprising FGFR2b, SHP2, Grb2, Sos, and Ras in the epithelium as well as Sprouty2, which is an inducible negative modulator of this signaling pathway [46–49].

Recently, glycogen synthase kinase-3beta/beta-catenin signaling has been considered as an important regulator of the differentiation of lung mesenchymal stromal cells of neonatal murine lung into myofibroblasts [50]. Moreover, SIX1/EYA1 signaling can control SHH signaling, which negatively regulates FGF10, at normal level needed for appropriate lung growth. Thus, SHH expression increases above normal levels in the absence of either SIX1 or EYA1, as well as its ectopic expression inhibits FGF10 signaling pathway, resulting in sever abnormalities of the lung mesenchyme and progenitors of epithelial branching. Moreover, Lüdtke et al. and his collaborators has shown that absence of T-box transcription factor 2 (Tbx2) transcription factor in murine lung leads to highly hypoplastic mesenchymal cells with severely decreased proliferation but prematurely induced mesenchymal differentiation into fibrocytes [51]. Hence, different types of endogenous mesenchymal progenitors such as smooth muscle progenitors and vascular progenitors are discussed below.

3.1 Smooth Muscle Progenitor and Stem Cells

In peripheral airway smooth muscles, FGF10–expressing peripheral mesenchymal cells have been proposed to serve as progenitor cells for peripheral airway smooth muscles during early development of lung. Several studies showed that the airway smooth muscle progenitors evolved as FGF10-expressing cells, which distribute with the elongation of peripheral airway during the development of the airway like wearing a leg sock [30, 52–54]. These studies likewise have shown that SHH and BMP4 signals expressed in the distals of airways control the trans-differentiation of airways smooth muscle progenitors and make them express alpha-smooth muscle actin fibers. Additionally, a population of progenitors in airway smooth muscle has been found to originate from the proximal mesenchyme [55], which are activated by Wnt signaling because Wnt2 signaling regulates expression of myocardin/myocardin-related transcription factor-B (MRTF-B) as well as FGF10 in the lung [56].

3.2 Vascular progenitor and Stem Cells

Some studies emphasize the importance of vascular endothelial growth factor (VEGF), erythropoietin, and nitric oxide in on the mobilization and homing of lung endothelial progenitor cells (EPCs) during developmental changes such as bronchopulmonary dysplasia (BPD) [57]. Balasubramaniam et al. has found that decreasing the surface area of gas exchange in alveolar and vascular compartments has been occurred as a response to oxygen toxicity. These developmental disturbance have been associated with decreased expression of endothelial nitric oxide synthase, VEGF, and erythropoietin receptor as well as reduced number of EPCs in both blood and bone marrow [58].

Two studies have demonstrated the differentiation of hemangioblasts to form a stereotypic capillary network that circles the bronchial, and segmental, as well as lobar branches of the airway by the activation of epithelial-derived VEGF [30, 49]. Appropriate organization of this vascular plexus may play an important function for correcting airway branching as well as tissue perfusion. Therefore, mesothelial mesenchymal epithelia endothelial crosstalk matches epithelial and vascular progenitor function and may play a critical role in lung repair and regeneration [25].

Que et al. has found a population of progenitors in the mesothelium overlying the lung, which gives rise to pulmonary vascular smooth muscle cells during embryonic growth [59]. However, endogenous circulating bone marrow progenitors or vascular wall progenitor cells may give rise to vascular endothelial progenitor cells. Moreover, using a transgenic mouse line carrying a BMP-responsive eGFP reporter allele showed that canonical BMP pathway is active mainly within the airway smooth muscle layer and the vascular network during the pseudoglandular stage of lung development [60]. However, further studies are still needed to find more lung progenitors in the other locations in pulmonary vasculature.

MODE OF CELL DIVISION AND ITS REGULATORY MECHANISMS IN MAMMALIAN LUNG EPITHELIAL STEM CELLS

Proper regulation of epithelium stem cells is crucial for development of mammalian lung [25, 61]. Congenital defects of lung stem cells lead to fatal abnormalities such as bronchopulmonary dysplasia (BPD) or lung hypoplasia via affecting vital processes such as the capacity of gas diffusion [25, 61–63]. To maintain tissue homeostasis of different organs including lung, stem cell self-renewal and differentiation have to be balanced. Tissue hyperplasia and/or tumorigenesis may result from excess of stem cell self-renewal. However, excessive differentiation may result in tissue degeneration and/or aging. Understanding the mechanism of proper balance between lung stem cells proliferation and differentiation probably leads to figuring out new solutions to repair of the gas diffusion surface and restore lung morphogenesis. During lung development, asymmetric cell division is definitely essential for proper balance between proliferation and differentiation, and correct temporal and spatial specification of epithelial cell lineages [64, 65].

Homogenous growth of stem cells in lung occurs due to different fates of cell division (symmetric and asymmetric) that they undergo [1, 66]. Few feasible ways to differentiate between symmetric and asymmetric division are available; such as observing the spindle orientation or noticing differences in inheritance cytoplasmic or membrane-bound proteins like cell fate determinant Numb and atypical Protein kinase C zeta (aPKCζ) [67–71]. Either extrinsic or intrinsic fat determinants result in asymmetrically division of the stem cells. Microenvironment is an example of extrinsic fat determinants, while each daughter cell is placed in different microenvironment so undergoes different fate. On the other hand, cytoplasmic cell fate determinant is an example of intrinsic fate determinants. Asymmetric cell division is controlled by preferential segregation of intrinsic cell fate determinants such as numb into one of the daughter cells in *Drosophila* and mammalian epithelial cells [72, 73]. Asymmetrical localization of cell fate determinants in the dividing cells as well as definition of the axis of polarity determine the apical-basal orientation and allow a rapid switch from proliferation to differentiation [72]. Numb is expressed uniformly in the cell during interphase. However, it is localized asymmetrically in the cytoplasm during cell division, and then segregated to one of the two daughter cells. The cell with high numb levels can suppress notch activity for differentiation, while the cell receiving low numb levels preserves high notch signaling and has stem

cell fate [74–77]. In the embryonic lung, numb plays a key role in determining the asymmetric or symmetric cell division by distributed asymmetrically at the apical side of epithelial stem cells [70, 71]. Additionally, there is a positive correlation between perpendicular asymmetric cell division and segregation of numb to one daughter cell, which suggest that distal lung epithelial stem cells undergo asymmetric cell division [71]. Moreover, numb (-/-) murine lung epithelial cells 15 (MLE15) highly express Sox9/Id2, stem cell markers [71].

Cell polarity means asymmetric distribution of the cellular components within a single cell. Cell polarity and orientation of mitotic spindle have pivotal roles in the self-renewal and differentiation of epithelial cells and can affect normal physiological processes, such as epithelial differentiation, and tissue branching. Studies in our laboratory demonstrated that the embryonic lung distal epithelial stem/progenitor cells have polarized and highly mitotic characteristics [26, 70, 71]. These cells also divide in a perpendicular manner. Moreover, cell polarity helps in organizing and integrating complex molecular signaling. Therefore, these cells can decide their fate, proliferation, orientation and differentiation. For example, recent findings in our laboratory have demonstrated that lung stem cells polarization plays an important role in their perpendicular cell division [78]. Disturbing this polarization leads to imbalance between self-renewal and differentiation in lung stem cell culture [70]. In the epithelial cells of different mammals, there is a correlation between perpendicular cell division and asymmetric cell division [79]. Our laboratory confirmed this finding by highlighting the asymmetric localization of proteins controlling spindle orientation such as mouse Inscuteable (mInsc), G-protein signaling modulator 2 (GPSM2), and nuclear mitotic apparatus (NuMA) polarity proteins in mitotic distal epithelial stem cells within embryonic lung [70].

In various organs, a characteristic apical-basal polarity has been shown by epithelial cells, such that the switch of epithelial cells from symmetric to asymmetric cell division occurs mostly with only subtle deviation of the spindle orientation resulting in an asymmetrical distribution of their adjacent adherent junctions and apical plasma membrane to the daughter cells [80, 81]. E-cadherin is a component of lateral epithelial cell plasma membrane and apicolateral junction complex [82]. The plasma membrane of mitotically dividing cells shows the 'cadherin hole' as a comparatively small, unstained segments in the cell surface by immunostaining of E-cadherin [71, 81], which is plasma membrane of lateral epithelial cell and apicolateral junctional complex component [82]. In the epithelium of various organs, the orientation of the cleavage plane relative to the cadherin hole can predict whether symmetric or asymmetric distribution of the plasma membrane to daughter cells occurs [81]. Recently, our laboratory analyzed cadherin hole of the lung epithelium. In embryonic lung, most distal epithelial stem cells divide asymmetrically [71]. The cleavage planes of these cells are supposed to circumvent the cadherin hole to the daughter cell. This is considered as another evidence for asymmetric cell division of embryonic distal lung epithelial cells. Moreover, our laboratory discovered that the EYA1 protein phosphatase control cell polarity and spindle orientation as well as distribution patterns of numb, a cell fate determinant. Therefore, EYA1 stimulate perpendicular division and asymmetric segregation of numb to one of the two daughter cells in distal epithelial cells of embryonic lung, apparently by regulating the phosphorylation level of aPKCζ [70]. Furthermore, in vivo or in vitro interfering with EYA1 function leads to impairment in both epithelial cell polarity mitotic spindle orientation [26]. Additionally, deficiency of EYA1 in lung hinders perpendicular division and leads to segregation of numb in both two daughter cells and inactivation of notch signaling in mitotic epithelial cells. However, activation of notch signaling genes could preserve EYA1 –deficient phenotype and leads to an increase in epithelial differentiation and a decrease in branching and deprivation of stem cells. Furthermore, we have demonstrated that EYA1 protein phosphatase regulates the balance between proliferation and differentiation of distal epithelial stem cells in embryonic lung regulating asymmetric cell division, which is essential for preserving tissue proliferation during maintenance and lung injury repair. This appropriate balance is absolutely needed for maintaining normal lung morphogenesis and repair. Therefore, deficiency of this balance probably leads to premature or injured lung. Proper outgrowth and branching of the epithelial cells is crucial for generating a large enough gas diffusion surface. Thus, developmental deformity in this smooth progression leads to improper differentiation and possible postnatal respiratory distress [25, 61].

In summary, several lines of evidence from our laboratory suggesting that asymmetric cell division are frequent in distal epithelial stem cells of embryonic lung. For example, the cleavage plane orientations are suggested to circumvent the cadherin hole, leading to asymmetric allocation of cadherin hole to the daughter embryonic distal lung epithelial cells [71]. Moreover, our finding that the majority of distal lung epithelial cells contain apically localized polarity proteins such as Par, LGN, NuMA and mInsc, and their mitotic spindle are aligned perpendicular to the basement membrane as well as asymmetric segregation of numb, are evidences for the asymmetric division of these cells [70]. Both phenomena of polarized localization of Par, LGN, NuMA, and mInsc proteins, and perpendicular alignment of mitotic spindles are rigorously coordinated with asymmetric cell division in mammalian and *Drosophila* epithelial cell types [73, 79, 83–85]. Additionally, we found that EYA1 protein phosphatase activity plays a crucial role in cell polarity, spindle orientation, and the asymmetric distribution

of numb in stem/progenitor cells of embryonic lung. Figuring out lung stem cells behavior may lead to novel treatment for abnormal lung morphogenesis. Knowing and understanding of the underlying mechanisms controlling asymmetric cell division can help finding out new targets for prevention and rescuing lethal lung diseases in infants and children, and for regeneration of injured lungs.

MOLECULAR MECHANISMS THAT CONTROL LUNG STEM/PROGENITOR CELL PROLIFERATION AND DIFFERENTITION

Several lines of evidence indicate that transcription factors and signaling molecules control the expression of genes that regulate progenitor cell self-renewal and proliferation during lung development. A subset of transcription factors plays critical role in lung development and differentiation. For example, TTF-1 plays a vital role in the development of distal lung progenitors and in marking the lineage commitment in the early embryonic lung [86]. Mice lacking TTF-1 was born dead with abnormal undifferentiated lung [87]. Another example is Sox9, the HMG transcription factor, which is highly expressed in the distal epithelial progenitors in embryonic lung; nonetheless, conditional deletion of Sox9 does not affect progenitor cell behavior in lung [23, 88, 89]. It has been suggested that other genes may act in concomitant with Sox9 to regulate progenitor cell proliferation. For instance, it has been observed that over-expression of the transcription factor; N-myc resulted in inhibition of differentiation, coupled with an increased expression of Sox9 in distal lung epithelial compartment [12]. Moreover, forkhead box (Fox) transcription factors are crucial for controlling lung progenitor cell self-renewing divisions and morphogenesis. Fox family members including FOXA1, FOXA2, FOXJ1, FOXF1, FOXP1, and FOXP2 may control gene expression and regulate cell differentiation in the lung. For instance, it has been found that conditional deletion of both FOXA1 and FOXA2 genes markedly decrease proliferation of lungs resulted in hypoplastic lungs [90]. Furthermore, conditional deletion of both foxp1 and FOXP2 also results in a similar lung phenotype to that found in case of FOXA1 and FOXA2 genes deletion. Thus, in foxp22/2; foxp11/2 double mutants, the lungs are small, with inhibited proliferation, but regular proximal–distal patterning [91].

Similarly, several reports have supported that some growth factors are essential for embryonic development. There are five key signaling molecules play a crucial role in embryonic development; FGF, hedgehog, notch, Wnt and transforming growth factor-b family (TGF-b). In various stem cell lines, these signaling pathways are essential at some levels and at some times to drive differentiation and lineage switching. Despite their important roles, little is known about the mechanisms of these signaling molecules in deriving lung cell differentiation. However, it has been revealed that canonical Wnt2/2b and beta-catenin signaling are essential for the specifying of lung endoderm progenitors in the growing foregut endoderm [92]. Using homologous recombination, null alleles of both Wnt2 and Wnt2b genes in murine embryos have been produced, and found to result in entire lung agenesis with complete absence of tracheal budding. Moreover, conditional inactivation and activation of beta-Catenin resulted in loss or gain of trachea/lung progenitor identity in murine foregut endoderm, respectively [92, 93].

Several studies have shown a fundamental functional role of FGF in specification of the lung lineages distal to the trachea [30, 94–96]. For instance, FGF10 is expressed by lung mesenchyme and plays a pivotal function in the amplification of pulmonary epithelial progenitors as well as organizes alveolar smooth muscle cell development and angiogenesis [30]. Additionally, FGF10 over-expression increases epithelial progenitor cell proliferation and leads also to hyperplasia of goblet cells [97]. Retinoic acid signaling was also found to have an important role in the proliferation of lung progenitors and for development of primary lung buds, by influencing FGF10 expression through TGF-beta signaling [98]. Another growth factor signaling which plays an essential role in epithelium proliferation and branching morphogenesis of embryonic lung is sonic hedgehog (SHH) signaling [99]. Martin et al. found that over-expression of Gli2, a mediator of SHH activity, is associated with increased cyclin expression as well as cell proliferation. These results suggest that Gli2 mediates SHH signaling influencing embryonic lung development through cyclin regulation [100]. Moreover, BMP signaling has been shown to be essential for proliferation of lung distal epithelial progenitors. Eblaghie et al. has provided evidence that autocrine signaling through BMP receptor 1 (BMPR1) controls cell division, survival and organ morphogenesis in murine distal lung epithelium. In this study, it has been found that deleting either lung epithelium BMPR1A or its ligand, BMP4, leads to smaller lungs, with lower levels of proliferation and reduced expression of both n-myc and FOXA2 [101]. Similarly, Wnt5a, which is a prominent Wnt ligand is also highly expressed in and around the distal epithelial tips. Conditional deletion of Wnt in mice leads to a significant increase cell proliferation, and an additional branch of the conducting airways [102]; nevertheless, further studies are needed to know whether this phenotype is related to defects in progenitor cells. The general conclusion is that these signaling mechanisms collaborate to regulate the proliferation of distal epithelial progenitor cells. However, little is known about the detailed interactions among them.

Recently, mesenchymal nuclear factor 1 B (NF1B) has been found to control epithelial cell self-renewal and differentiation during maturation of lung [103]. It has also been reported that HuR, an RNA-binding protein (RBP) is a crucial controller of mesenchymal responses during

branching of lung. Therefore, HuR binds and regulates mRNAs of Tbx4 and FGF10. Thus, its deletion diminished their post-transcriptional regulation, resulting in abolishing distal bronchial branches morphogenesis during early pseudoglandular stage [104]. Furthermore, canonical notch signaling is essential for both selection of Clara versus ciliated cell fate and identification of arterial smooth muscle cells in the developing lung [105].

Micro-RNAs (miRNAs), a class of small, non-coding RNAs have the capability to regulate gene expression and acting as a post-transcriptional modifier. The importance of miRNA in lung cell proliferation and differentiation has been shown in several studies. For example, over-expression of miR-17–92 in embryonic lung epithelium results in high level of proliferative, undifferentiated epithelial cells expressing Sox9, a progenitor cell marker [106]. This suggests that miR-17–92 stimulate proliferation of progenitors instead of differentiation. During pseudoglandular period, miR-17 as well as its paralogs, miR-20a, and miR-106b, are remarkably expressed, and have crucial function during embryonic lung growth [107]. In addition, concurrent in vitro down-regulation of these three miRNAs in isolated lung epithelium modified FGF10 induced budding morphogenesis. This effect was rescued by synthetic miR-17. Moreover, E-Cadherin levels were decreased, as well as its distribution was modified by down-regulation of these three miRNAs, however beta-catenin activity was increased, and expression of its downstream targets, including BMP4 as well as FGFr2b, were augmented [107]. This study also recognized both signal transducer and activator of transcription 3 (Stat3) and mitogen-activated protein kinase 14 (Mapk14) as crucial targets of miR-17, miR-20a, and miR-106b. Similarly, concurrent over-expression of Stat3 and Mapk14 alters E-Cadherin distribution as noted after co-down-regulation of miR-17, miR-20a, and miR-106b, indicating that mir-17 family of miRNA altered FGF10-FGFR2b downstream signaling by distinctively targeting Stat3 and Mapk14, thus control expression of E-Cadherin, which consecutively alters morphogenesis of epithelial bud in response to FGF10 signaling [107].

Current and Future Perspectives

In our laboratory, we have shown resemblance in the stem cell division mode between lung and other organs. For example, stem cells in lung distal epithelium prefer perpendicular polarized division rather than parallel symmetric division, similar to other different tissues [65, 70, 79]. Moreover, similar to satellite muscle cells and neural stem cells, inheritance of Numb and asymmetric segregation may be a prevalent mode of asymmetric cell division control in stem cells of lung [68]. Resemblance can also be observed in the apical localization of polarity proteins, Par, LGN, NuMA, and mInsc, which regulate orientation of spindle in mitotically dividing epithelial cells in mammals [79]. We showed that most distal lung epithelial stem cells have apically localized polarity proteins, with mitotic spindles perpendicularly aligned to the basement membrane. The balance between cell proliferation and differentiation is essential for normal regeneration of tissues of different organs, including lung. Thus, lack of this balance in lung results in diseases like bronchopulmonary dysplasia (BPD) and congenital lung hypoplasia, wherein a remarkable deficiency of progenitors probably occurs, are general characteristics of prematurity and/or lung injury in human. These abnormalities are common public health problems in human newborn infant and a significant cause of death during infancy. In normal lungs, the epithelial stem cells leave the progenitor pool to produce lineage-committed precursor cells, which in turn give rise to fully differentiated cell lineages. Rapid and controlled self-renewal of lung progenitors and differentiation of these progenitors produce sufficient large alveolar gas diffusion surface to maintain normal postnatal life. Defects in this balanced progression of lung development may result in abnormal differentiation and therefore postnatal respiratory distress [25, 61]. As given in our studies, there is similarity in asymmetric stem cell division between the lung and other organs and this may lead to find novel solutions for restoration of normal lung development and morphogenesis.

Recent studies suggested several mechanisms for maintaining the production of new stem cells as well as generation of differentiated cells by focusing on asymmetric cell division in different organs and species. For example, *Drosophila* has been used as an invertebrate model to discover the role of different extrinsic signals and intrinsic influences in control of stem cell division providing paradigms for how both these signals and influences work to direct asymmetric divisions. Recently, several studies show similar mechanisms in vitro models. Nonetheless, in vivo characterization of stem cells and elucidation of the mechanisms of regulating asymmetric cell division in mammals, including humans, needs further improvements in the research techniques like isolation and purification of stem cells, and real time imaging. Higher number of symmetric divisions may be needed temporarily to provide the tissue with enough number of stem cells during tissue regeneration and repair. Therefore, studying the mechanisms controlling asymmetric cell division is crucial for organ development as well as during tissue repair and regeneration. Moreover, it is important to understand that several factors can act to retard or even prohibit stem cell from switching from symmetric back to asymmetric cell division mode. For example, chronic inflammation or damage of a tissue might modify the ability of stem cells to respond properly to repair injured tissues. This tissue damage may also prevent stem cells to switch from symmetric to asymmetric cell division. Inappropriate regulation of tissue repair may ultimately lead to choosing stem cells that are rebellious to normal growth control signals, which is a hallmark of

cancer cells. Thus, elucidating signaling mechanisms that control asymmetric cell division in all types of stem cells is essential for developing approaches that exploit the ability of these cells to repair diseased and injured tissues. Further exploration of these mechanisms will direct us to new potent strategies to prohibit initiation of cancer in different cell types and may develop novel targets for anti-cancer therapies. In addition, understanding the molecular mechanisms and components controlling the mode of proliferation and differentiation of adult stem cells will provide new strategies for maintaining and expanding stem cells in culture meanwhile preserving their differentiation potential. Moreover, this will direct the differentiation of stem cells into various cell types, which can be utilized in regenerative medicine.

Current and future elucidation of mechanisms for asymmetric cell division and identification of novel components involved in the mechanisms regulating asymmetric cell division in lung epithelial stem cells, which is the main mode for controlling the balance between lung proliferation and differentiation, will aid to define new targets for the prevention and treatment of lethal lung abnormalities in infants and children and for lung repair after injury.

CONCLUSION

Elucidation of the molecular mechanisms, which maintain the balance between proliferation and differentiation of lung stem cells, has a fundamental role in developing techniques for tackling the ability of stem cell to repair lung injury.

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Conflict of Interest
Authors declare no conflict of interest.

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