A "GLI-tch" in Alveolar Myofibroblast Differentiation

Alveolar septation is dependent upon the precise production and remodeling of lung extracellular matrix performed by alveolar interstitial myofibroblasts. Although it is well established that alveolar interstitial myofibroblast dysfunction is a prominent feature of idiopathic pulmonary fibrosis, emphysema, and bronchopulmonary dysplasia, the mechanisms by which myofibroblasts primarily direct alveolar septation remain poorly understood. Efforts to define alveolar myofibroblast function have been hindered by a lack of cell-specific markers that can differentiate alveolar myofibroblasts from other interstitial fibroblasts, and identification continues to rely heavily on spatial location (at elongating septal tips) and α-smooth muscle actin (α-SMA) expression. Furthermore, a lack of unique lineage markers has made it difficult to elucidate the hierarchy of intermediates ranging from embryonic progenitors to mature myofibroblasts. Although the presence of functional alveolar myofibroblasts is required for alveolar septation, much of what is known about the role of alveolar myofibroblasts in normal development and disease continues to be descriptive.

In this issue of the *Journal*, Kugler and colleagues (pp. 280–293) identify the Sonic Hedgehog (Shh) signaling pathway as a key component of alveolar development that regulates alveolar myofibroblast proliferation, maturation, and function (1). Through postnatal lineage-tracing experiments, they demonstrate that cells expressing *Gli1* in response to epithelium-derived Shh are highly proliferative, increase in number during early alveolarization, localize to growing septal tips, and transiently express α-SMA (1), confirming a previous report that *Gli1* expression identifies a population of fibroblasts that contribute to the pool of postnatal myofibroblasts (2). Through carefully timed pharmacological inhibition of canonical Shh signaling during postnatal saccular and alveolar lung development, they convincingly show that inhibition of Shh signaling during early postnatal saccular development (Postnatal Day 1 [P1]–P3) essentially eliminates *Gli1* expression and α-SMA production by alveolar myofibroblasts (1). However, whereas very early inhibition (P1) results in alveolar simplification due to impaired septation, slightly later inhibition (P3) results in increased alveolar size due to increased lung compliance (1). Although they do not explore the mechanisms linking Shh signaling to septation, the authors observe a striking downregulation of the proteoglycan versican during early alveolar septation. More importantly, saccular Shh inhibition is associated with a selective, significant reduction in $Gli1^+$ fibroblast proliferation (1). Notably, inhibited proliferation of alveolar interstitial fibroblasts was previously associated with failure of alveolar septation (3, 4). Finally, lineage-tracing experiments reveal that abnormal maturation and differentiation, rather than increased cell death or a decreased number of $Gli1^+$-lineage cells, are associated with abnormalities in alveolarization (1).

It is well established that in the developing fetal lung, the interaction between the epithelium and its surrounding mesenchyme is the major driving force that induces proximal-distal patterning. In contrast to a well-developed understanding of lung epithelial lineages, we are just beginning to understand the lineage relationships among some of the fibroblast subpopulations. Although differences in the inherent potential of proximal versus distal lung mesenchyme to induce proximal or distal lung epithelial differentiation, respectively, were identified decades ago (5, 6), further discrimination and functional analysis of postnatal fibroblast subpopulations have only recently become the focus of a growing number of laboratories. The field has been challenged by a lack of fibroblast-specific markers and genetic tools to identify specific contributions of interstitial fibroblast subpopulations to lung development and repair. Although the use of transcription factors and key players in major signaling pathways as lineage tags has proven very useful in the lung epithelium, the use of similar lineage tracers in fibroblasts is complicated due to (*1*) recurrent reactivation of transcription programs during lung development, and (*2*) the use of similar gene signaling pathways by multiple fibroblast subpopulations. Recent and future data on single-cell sequencing comparing normal and diseased lungs of humans, as well as preclinical disease models, may enable the development of novel fibroblast-specific markers (7).

Currently, the most inclusive lineage marker of lung fibroblasts is T-box transcription factor 4 (*Tbx4*). *Tbx4* is expressed in a population of mesenchymal cells before branching morphogenesis that give rise to the entire postnatal lung stroma (8). Peribronchiolar and perivascular smooth muscle precursors are defined by expression of WNT2/GLI1/ISL1 (9). As supported by the present work (1) and previous reports (2), *Gli1*-expressing mesenchymal cells are progenitors of peribronchiolar smooth muscle cells and alveolar myofibroblasts, but not lipofibroblasts. In contrast, FGF10-expressing mesenchymal cells of the developing distal lung can give rise to peribronchiolar smooth muscle and alveolar lipofibroblasts, but not alveolar myofibroblasts (10). The alveolar interstitial myo- and lipofibroblasts are also characterized by expression of platelet-derived growth factor receptor α (PDGFRα) (11–16). Pharmacological or genetic inactivation of either PDGF-A or PDGFRα signaling results in loss of alveolar myofibroblasts and alveolar simplification. Additionally, lung fibroblasts isolated from preterm infants with bronchopulmonary dysplasia, a chronic lung disease characterized by inhibited alveolarization (4, 17, 18), express reduced levels of PDGFRα (19). In previous work we demonstrated that during both postnatal alveolarization and realveolarization after partial pneumonectomy, the pool of $PDGFR\alpha^+$ fibroblasts contains a mixture of lipofibroblasts ($LipidTox^+$), myofibroblasts ($\alpha\text{-}SMA^+$), and matrix fibroblasts, and these interstitial fibroblast subpopulations undergo dynamic alterations in their relative proportions and gene-expression programs (12–14, 20). Given that the absence of $PDGFR\alpha^+$ fibroblasts results in severe deficits in alveolar septation, the fact that at P6 (the period of rapid alveolarization) Kugler and colleagues identified PDGFRα expression in 50% of all $Gli1^+/\alpha\text{-}SMA^+$ myofibroblasts and in 10% of all $Gli1^+$ fibroblasts

is reassuring (1). The knowledge that abrogation of PDGFRα expression has a more severe septation defect than what is presently shown by inhibition of canonical Shh signaling would suggest that although the pools of myofibroblasts that rely on PDGFRα$^+$ or Shh signaling overlap, the roles played by the two signaling pathways are distinct. Future endeavors to understand how the Shh and PDGFRα signaling pathways interact to guide interstitial fibroblast development are warranted.

Together with previously published lineage-relationship data, Kugler and colleagues' report shows that the lineage decisions of fibroblast populations are more complex than was initially perceived. Hence, it is clear that a combinatorial use of multiple lineage tags will be necessary to further dissect fibroblast subpopulations. A better understanding of the mechanisms that direct the fate and function of interstitial fibroblast subpopulations that are critical for alveolar formation, homeostasis, and regeneration will aid in efforts to overcome the current limitations of pulmonary regeneration. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Shawn K. Ahlfeld, M.D.
Anne Karina Perl, Ph.D.
Divisions of Neonatology and Pulmonary Biology
Cincinnati Children's Hospital Medical Center
Cincinnati, Ohio

References

1. Kugler MC, Loomis CA, Zhao Z, Cushman JC, Liu L, Munger JS. Sonic Hedgehog signaling regulates myofibroblast function during alveolar septum formation in murine postnatal lung. *Am J Respir Cell Mol Biol* 2017;57:280–293.
2. Li C, Li M, Li S, Xing Y, Yang CY, Li A, Borok Z, De Langhe S, Minoo P. Progenitors of secondary crest myofibroblasts are developmentally committed in early lung mesoderm. *Stem Cells* 2015;33:999–1012.
3. Ahlfeld SK, Wang J, Gao Y, Snider P, Conway SJ. Initial suppression of transforming growth factor-β signaling and loss of TGFBI causes early alveolar structural defects resulting in bronchopulmonary dysplasia. *Am J Pathol* 2016;186:777–793.
4. Lau M, Masood A, Yi M, Belcastro R, Li J, Tanswell AK. Long-term failure of alveologenesis after an early short-term exposure to a PDGF-receptor antagonist. *Am J Physiol Lung Cell Mol Physiol* 2011;300: L534–L547.
5. Shannon JM. Induction of alveolar type II cell differentiation in fetal tracheal epithelium by grafted distal lung mesenchyme. *Dev Biol* 1994; 166:600–614.
6. Shannon JM, Nielsen LD, Gebb SA, Randell SH. Mesenchyme specifies epithelial differentiation in reciprocal recombinants of embryonic lung and trachea. *Dev Dyn* 1998;212:482–494.
7. Guo M, Wang H, Potter SS, Whitsett JA, Xu Y. SINCERA: a pipeline for single-cell RNA-seq profiling analysis. *PLoS Comput Biol* 2015;11: e1004575.
8. Zhang W, Menke DB, Jiang M, Chen H, Warburton D, Turcatel G, Lu CH, Xu W, Luo Y, Shi W. Spatial-temporal targeting of lung-specific mesenchyme by a Tbx4 enhancer. *BMC Biol* 2013;11:111.
9. Peng T, Morrisey EE. Development of the pulmonary vasculature: current understanding and concepts for the future. *Pulm Circ* 2013;3:176–178.
10. El Agha E, Herold S, Al Alam D, Quantius J, MacKenzie B, Carraro G, Moiseenko A, Chao CM, Minoo P, Seeger W, *et al*. Fgf10-positive cells represent a progenitor cell population during lung development and postnatally. *Development* 2014;141:296–306.
11. Barron L, Gharib SA, Duffield JS. Lung pericytes and resident fibroblasts: busy multitaskers. *Am J Pathol* 2016;186:2519–2531.
12. Endale M, Ahlfeld S, Bao E, Chen X, Green J, Bess Z, Weirauch MT, Xu Y, Perl AK. Temporal, spatial, and phenotypical changes of PDGFRα expressing fibroblasts during late lung development. *Dev Biol* 2017;425:161–175.
13. Green J, Endale M, Auer H, Perl AK. Diversity of interstitial lung fibroblasts is regulated by platelet-derived growth factor receptor α kinase activity. *Am J Respir Cell Mol Biol* 2016;54:532–545.
14. Chen L, Acciani T, Le Cras T, Lutzko C, Perl AK. Dynamic regulation of platelet-derived growth factor receptor α expression in alveolar fibroblasts during realveolarization. *Am J Respir Cell Mol Biol* 2012;47:517–527.
15. McGowan SE, McCoy DM. Regulation of fibroblast lipid storage and myofibroblast phenotypes during alveolar septation in mice. *Am J Physiol Lung Cell Mol Physiol* 2014;307:L618–L631.
16. Kimani PW, Holmes AJ, Grossmann RE, McGowan SE. PDGF-Rα gene expression predicts proliferation, but PDGF-A suppresses transdifferentiation of neonatal mouse lung myofibroblasts. *Respir Res* 2009;10:119.
17. Boström H, Willetts K, Pekny M, Levéen P, Lindahl P, Hedstrand H, Pekna M, Hellström M, Gebre-Medhin S, Schalling M, *et al*. PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. *Cell* 1996;85:863–873.
18. Lindahl P, Karlsson L, Hellström M, Gebre-Medhin S, Willetts K, Heath JK, Betsholtz C. Alveogenesis failure in PDGF-A-deficient mice is coupled to lack of distal spreading of alveolar smooth muscle cell progenitors during lung development. *Development* 1997;124:3943–3953.
19. Popova AP, Bentley JK, Cui TX, Richardson MN, Linn MJ, Lei J, Chen Q, Goldsmith AM, Pryhuber GS, Hershenson MB. Reduced platelet-derived growth factor receptor expression is a primary feature of human bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol* 2014;307:L231–L239.
20. Perl AK, Gale E. FGF signaling is required for myofibroblast differentiation during alveolar regeneration. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L299–L308.