Failure of Alveolar Type 2 Cell Maintenance Links Neonatal Distress with Adult Lung Disease

Infants born preterm often require ventilation and oxygen supplementation, which sometimes results in bronchopulmonary dysplasia (BPD). This disorder manifests mainly as airway inflammation and bronchiectasis, but follow-up studies in adults also commonly reveal reduced areas of attenuation on chest computed tomography scans suggestive of emphysema and even interstitial fibrosis (1). Although improved prenatal care has resulted in much milder BPD, patients today still exhibit an increased risk of later respiratory illness including chronic obstructive airway disease and viral infection (2), a finding mirrored in infants born in the lower-oxygen atmosphere of high altitudes (3). In this issue of the *Journal*, Yee and colleagues (pp. 453–464) provide a compelling explanation as to why this increased susceptibility to disease may exist, namely that these early injury conditions result in persistently diminished numbers of alveolar type 2 cells (AEC2s) in the mature lung (4).

AEC2s have long been recognized as a facultative progenitor population, capable of self-renewal and generation of new alveolar type 1 cells (AEC1s) after injury (5, 6). However, several studies in mice over the past few years have suggested that the regenerative capacity of these cells can be overwhelmed quickly by injury, a phenomenon especially apparent in severe influenza infection (H1N1, PR8), wherein there is a dramatic influx of alveolar epithelial cells expressing the basal cell marker Krt5 subsequent to widespread AEC2 ablation by the virus (7–11). This phenomenon has also been demonstrated to occur to a lesser degree after bleomycin injury (8, 12). Whether these basal-like cells develop after acute lung injury in humans has not yet been demonstrated definitively, but it is strongly suggested by the abundance of Krt5⁺ cells in so-called honeycombing cysts found in patients with interstitial lung disease (8, 13).

In their report, Yee and colleagues demonstrate that neonatal exposure to hypoxia and hyperoxia both resulted in ~50% reduction in the abundance of AEC2s in the adult lung. Although these lungs seemed otherwise grossly normal, subsequent infection with a mildly pathogenic H3N2 (HKx31) virus resulted in pulmonary fibrosis and an influx of Krt5⁺ epithelial cells, neither of which was observed after HKx31 infection in mice that had experienced normoxia only. They went on to demonstrate that AEC2 loss was the primary determinant of this remodeling/dysplastic response by genetic ablation of similar numbers of AEC2s by diphtheria toxin expression, which largely phenocopied the effect of neonatal hypoxia/hyperoxia.

In addition to an increased presence of Krt5⁺ cells, Yee and colleagues identified airway epithelial cells that appeared to transdifferentiate into AEC2s after infection, again only in combination with AEC2 ablation. Using Scgb1a1⁻, Sftpc⁻, and Krt5-CreERT2 mice, a lineage tracing methodology was used to characterize the source of these two newly apparent cell types. As expected, AEC2s and newly generated AEC1s could be traced by Sftpc, whereas the airway AEC2-like cells were largely traced by

Scgb1a1, suggesting transdifferentiation of club cells or Scgb1a1-expressing bronchoalveolar stem cells into AEC2s, as has been reported previously (14, 15).

Fate-mapping analysis of the injury-induced Krt5⁺ cells provided one of the most curious findings in this report. In contrast to a report by Zuo and colleagues (10), but in agreement with Vaughan and Ray and colleagues (8, 11), the vast majority of Krt5⁺ cells were untraced by any Cre driver, indicating their derivation from lineage-negative epithelial progenitors (LNEPs). However, additional Krt5-CreERT2-traced cells were identified in heavily injured regions and they expressed markers of AEC1s but never AEC2s. The authors interpreted this finding to be a preexisting Krt5⁺ progenitor cell, perhaps migrating from the trachea, which had the capacity to generate AEC1s apparently independent of an AEC2 or bipotent progenitor intermediate (6), and representing a separate population from the cellular source of the larger Krt5⁺ infiltration.

There are limitations to the lineage tracing analysis performed here. In every experiment, there was only a 7-day chase period between the induction of tracing (tamoxifen administration) and injury. This is potentially problematic in light of increased recognition that the long half-life of tamoxifen can result in spurious label induction several weeks after tamoxifen administration (8, 16). For instance, it is possible that the Krt5-traced AEC1-like cells are derived not from a separate Krt5⁺ progenitor, but rather from the “first responding” LNEPs (i.e., Krt5^{neg} progenitors that were activated by hypoxia/hyperoxia early in injury) up-regulating Krt5 and hence were more likely to become traced because of residual tamoxifen. The unlabeled “pods” could have been derived from the same LNEP pool that was activated somewhat later in injury and hence largely avoided Krt5-CreERT2 labeling.

Another curiosity is the fate of the AEC2s that arise after injury. In recent work by the same group, the authors observed a significant AEC2 expansion immediately after neonatal hypoxia/hyperoxia that not only does not persist, but actually declines, leading to an overall reduction in AEC2 numbers in the adult (17, 18). Furthermore, AEC2-like cells arise in the airways of mice exposed to hypoxia/hyperoxia at birth after viral infection as adults but do not appear to contribute to alveolar maintenance. Presumably the airway AEC2s represent an attempt by the lung to regenerate AEC2s from a non-AEC2 source, as has been demonstrated recently by several groups (5, 8, 14, 15, 19, 20). However, there are important unanswered questions. If AEC2s are uniformly stem/progenitor cells, then why do the expanding AEC2s after neonatal injury not continue self-renewal? What are the source(s) of non-AEC2s capable of becoming AEC2s and contributing to the alveolar pool? An additional caveat in attempting to answer such questions by lineage tracing is that the accuracy of tracing also depends on the specificity of the Cre driver. In the case of Scgb1a1, there is already evidence that this driver is active in more than one epithelial cell type, including mature AEC2s themselves (21),

limiting its usefulness in studies of cell fate. Although it is appealing to hypothesize that there are distinct subpopulations of AEC2s and LNEPs both very capable of AEC2 reconstitution and maintenance after major injury, it remains to be defined exactly when and to what extent these putative progenitors actually contribute to lung regeneration.

Caveats aside, this work represents an important step forward in understanding when and how nontraditional progenitor lineages become involved in lung repair, and what the functional consequences of these alternative lineages might mean for injury resolution, in particular the apparent increased likelihood of remodeling and fibrosis. Indeed, Yee and colleagues observe a close association between Krt5⁺ cell recruitment and fibrotic effectors such as collagen deposition and α -smooth muscle actin⁺ mesenchymal cells. Total AEC2 numbers seem to be a critical determinant, but how exactly this results in alternative progenitor recruitment is unclear. Although a 50% loss of AEC2s is obviously significant, this would not be expected to substantively change the overall epithelial surface lining because the vast majority of the epithelial barrier is made up of AEC1s. Furthermore, it has not been demonstrated that humans with childhood BPD actually possess fewer AEC2s, as seems to be true in the case of mice. Future work should focus on how exactly the lung senses the loss of AEC2s and the associated paracrine signals that activate and recruit these alternative progenitors, and whether these cells can be coaxed into contributing toward more physiologically normal alveolar epithelium, or conversely, whether AEC2 progenitor functionality can be better preserved in the face of severe lung injury. ■

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Andrew E. Vaughan, Ph.D.
Harold A. Chapman, M.D.

Department of Medicine and Cardiovascular Research Institute
University of California–San Francisco
San Francisco, California

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