Induced Pluripotent Stem Cell-derived Alveolar Type II Heterogeneity: Revealed by SFTPC Expression

Human induced pluripotent stem cell (hiPSC) differentiation offers great opportunities to generate diverse, personalized, and disease-specific cells for future cell-based therapies and drug development. In addition, it also provides a powerful tool for understanding human organogenesis and disease pathogenesis. In studying the lung, numerous stepwise differentiation protocols have been reported to successfully convert hiPSCs to various types of airway epithelia and lamella body–containing alveolar epithelia. Alveoli are an important part of the respiratory system, playing a crucial role in oxygen and carbon dioxide exchange. Human and mouse alveolar epithelium consists of type I and type II cells. Alveolar epithelial type II cells (AEC2s) are the facultative progenitors of lung alveoli, as they maintain the alveolar epithelium through self-renewal and differentiation into alveolar epithelial type I cells for homeostatic maintenance and damage repair. In addition, the AEC2 is responsible for secreting surfactant to modulate alveolar surface tension. Thus, it is of great interest to generate a large quantity of functional and expandable AEC2s for the understanding of alveolar development, tissue regeneration, and lung disorders.

Previously, Dr. Kotton's group has reported generating AEC2-like cells from human iPSCs (thus named as iAEC2) through the use of knock-in SFTPC fluorochrome reporters (1). However, they noticed that distal lung epithelium generated from human SFTPC reporter iPSCs failed to manifest the full features of primary adult AEC2 (1). In this issue of the *Journal*, Sun and colleagues (pp. 442–460) revisited this reporter and found that the SFTPC reporter appears to only identify those iAEC2s expressing the highest SFTPC levels (2). This is likely due to the fact that SFTPC is not restricted to type II cells during lung development, as embryonic lung progenitors also express SFTPC. They hypothesized that ABCA3 (ATP binding cassette A3) would be a better candidate than SFTPC to designate mature alveolar type II cells. During human lung organogenesis, ABCA3 expression is detectable around week 28 of gestation, whereas SFTPC expression is detectable around week 15, showing that ABCA3 is a canonical biomarker for alveolar epithelial cells (3, 4). Importantly, ABCA3 plays a critical role in surfactant metabolism and lamellar body biogenesis, making it more relevant to alveolar physiological function and lung diseases. With this consideration, they generated an ABCA3 knock-in GFP fusion reporter (ABCA3:GFP) for tracking and isolating mature iAEC2 (2). Using the alveolar epithelial differentiation protocol they established (1), Sun and colleagues generated GFP cells that are positive for ABCA3 antibodies, suggesting stringency of the reporter. Careful examination further suggested that those GFP cells harbored lamellar bodies, organelles associated with both AEC2 maturation and type II function. Transcriptomic analysis also revealed that ABCA3:GFP iAEC2 express a characteristic type II alveolar epithelial signature.

However, their work went beyond just cell production. More intriguing information was disclosed when they took a close look at surfactant production of iAECs generated from SFTPC^{tdTomato} and ABCA3:GFP bifluorescent iPSC lines (2). They observed that two distinct populations appeared in the dish: ABCA3:GFP/SFTPC^{tdTomato} double positive cells and ABCA3:GFP single positive cells (SPs). Although both cell populations exhibit alveolar type II cell transcriptomic signatures, the double positive population exhibits greater type II function and mature gene expression, including those for surfactant protein secretion and phospholipid processing. On the other hand, the SPs were closer to immature alveolar precursors, as demonstrated by a more proliferative gene expression signature and increased expression of progenitor markers (2). Functional studies further confirmed with RNA sequencing data that SPs have increased clonogenicity with less surfactant secretion capability. Thus, they conclude that the iAEC2 population has cellular heterogeneity with an inverse relationship between proliferation genes and surfactant or maturation-related genes (2).

Alveolar epithelial type 2 cell heterogeneity has been documented in numerous studies. Within developing lungs of mice, SFTPC is expressed at various levels in SOX9 lung buds, lung bipotent progenitors, and type II cells. Increased expression of SFTPC is often associated with type II maturation during the course of development (5, 6). Recently, two studies have reported that a subpopulation of more proliferative, Wnt-responsive, Axin2 or TM4SF1-expressing, and SFTPC-low alveolar progenitor-like cells exist in both mouse and human adult lungs (7, 8). These cells remain relatively quiescent during steady-state but expand robustly after lung injury to repopulate the alveolar epithelium (7, 8). When Sun and colleagues performed scRNA-Seq analysis on their iAECs, they observed that the ABCA3^{+} SFTPC$^{low/negative}$ SP iAEC population is enriched for many Wnt signaling targets, such as AXIN2, LEF1, and NKD1, as well as TM4SF1. They thus suggested that the SP iAECs are likely equivalent to those Wnt responsive type II progenitors in the lung that were reported previously (7, 8).

Despite these intriguing results, one has to be cautious when extrapolating from *in vitro* studies to the *in vivo* context. Transcriptomic and functional heterogeneity is more pronounced in culture, whereas the cell lineage is more restricted in tissue. In particular, iPSC and their derivatives with loose chromatin show more plasticity than primary cells and are highly sensitive to perturbations induced by culture conditions. Indeed, the same lab has reported that some sorted NKX2.1^{+} cells (supposed to be lung lineages) changed into nonlung

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lineages during culture, illustrating the instability of iPSC-derived cells (1, 9). Consistently, Sun and colleagues also noticed that sorted double positive cell and SP iAEC populations are interchangeable, despite each retaining its original identity to some degree. Thus, we can't exclude the possibility that the heterogeneity in SFTPC expression and proliferative capability is just a reflection of culture-associated dynamic transition. Significantly, human and mouse alveoli during steady-state homeostasis are largely homogeneous and exhibit slow turnover. The heterogeneity only occurs in settings that require massive proliferation of AEC2s, such as during development, injury repair, and disease progression. Accordingly, when Sun and colleagues reanalyzed the reported scRNA-Seq data of healthy and diseased lungs, they found that AEC2s of healthy quiescent lungs are more or less homogeneous, whereas SFTPC expression heterogeneity become evident in injured or diseased lungs (2). Hence, even though the SP population is equivalent to the lung progenitors, they more resemble the progenitors that are in action. Furthermore, we need to differentiate between true heterogeneity and transitoriness. SFTPC expression can be transiently downregulated by infection, inflammation, and injury. For example, a dramatic loss or decrease in the expression of surfactant proteins SFTPC and SFTPB has been observed in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–infected alveolospheres (10). In this scenario, SFTPC expression is not a reliable marker to identify an AEC2 subpopulation, as its expression can be restored upon regeneration. Lastly, where is this SFTPC $^{low/negative}$ type II progenitor subpopulation located in the adult lung? In mouse lung, several lung progenitors for alveolar homeostasis and regeneration seem to have their unique niches. For example, Axin2^{+} type II progenitors often reside in juxtaposition to Wnt-expressing fibroblasts (7, 8), whereas CD44^{high} type II progenitor cells are preferentially located in the perivascular region (11) and bronchioalveolar stem cells inhabit the bronchioalveolar duct junction (12).

In summary, this work adds another valuable tool to the field of lung differentiation for the isolation and tracking of functionally mature type II cells (true iAEC2). In addition, identification of a subpopulation of type II cells differing in maturity and proliferative capacity extends our knowledge regarding the current concept of the complexities of the lung. Further work will be needed to define their *in vivo* equivalent in the lung, their native niches, and their distinctive biological functions. ■

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