Giving New Identities to Alveolar Epithelial Type I Cells

Two types of epithelial cells line the alveolar wall of the distal lung. Alveolar type (AT) 2 are large, cuboidal cells that produce and recycle surfactant phospholipids and proteins, express innate immune molecules, function as adult stem cells, and can contribute to lung disease by undergoing oncogenic transformation and perhaps epithelial–mesenchymal transition. In contrast, AT1 cells are thin squamous cells that exchange gases with the adjacent capillary network, maintain ion and fluid balance at the air–liquid interface, and may be a source of proinflammatory mediators. Despite this, we still know much less about AT1 cells than AT2 cells, because they have historically been difficult to isolate and culture. The recent identification of several novel AT1-specific genes, described by Marconett and colleagues in this issue of the *Journal* (pp. 310–321), may be the critical breakthrough needed to finally understand how AT1 cells are produced during lung development, are regenerated after lung injury, and function in alveolar homeostasis (1).

AT2 cells are perhaps best known for their expression of surfactant protein C (SFTPC). Because SFTPC is expressed solely in the lung by AT2 cells, it was used to target AT2-specific expression of numerous genes, including Cre recombinase required for differentiation and lineage tracing experiments. The ability to overexpress and delete genes or fluorescent reporters provided a powerful way to understand the biology of these cells. Hence, there has been a keen interest in applying the same approach to AT1 cells. Several AT1-specific genes have been identified by characterization of genes found in the lung, subtractive hybridization of isolated rat AT1 cells and AT2 cells, and gene expression profiling of *in vitro*-differentiated rat AT2 cells (2, 3). As Marconett and colleagues point out, aquaporin 5, advanced glycosylation end-product-specific receptor (AGER, previously “RAGE”), podoplanin, caveolin 1, and homeobox-only protein homeobox are all expressed by AT1 cells. However, these genes are not expressed in the lung solely by AT1 cells, which limits their usefulness as cell-specific markers. An example of this problem is AGER. Although highly expressed by AT1 cells (2), it can also be found in pulmonary capillary endothelial cells (4). AGER mRNA, but not protein, can be detected in AT2 cells (5).

To identify new AT1-specific genes, Marconett and colleagues used a *tour de force* approach of comparing the transcriptional profile of purified rat AT2 cells, purified rat AT1 cells, *in vitro*-differentiated AT1-like cells derived from AT2 cells, and multiple rat tissues. They identified 54 candidate genes, which were then honed to 18 candidates after cross-referencing with genes, the expression of which increased as cultured human AT2 cells differentiated into AT1-like cells. After validation by quantitative

RT-PCR, Western blot analysis, and immunohistochemistry, glycosyltransferases, Rab-like GTPase activators and Myotubularins (GRAM) domain 2 (*Gramd2*), semaphorin 3B, semaphorin 3E, and the sodium channel epithelial subunit 1 gamma (*Scnn1g*), were considered the best candidates for AT1-specific expression. Further characterization in human, rat, and mouse revealed that these genes were expressed by AT1 cells, but not AT2 cells, lung endothelium, or bronchial epithelium. Of course, this conclusion is based entirely upon the sensitivity of the assay used to detect mRNA or protein expression, and one must remember that other lung cells could express these genes during development or injury/repair.

Nonetheless, this is an important finding, because it provides an opportunity to create new tools needed to move the field forward. A logical first approach will be to target the tamoxifen-inducible CreERT2 gene into the mouse *Gramd2* or *Scnn1g* locus. Since AT1 cells and AT2 cells share expression of many genes, the outcome of deleting a specific gene in AT1 cells using *Gramd2*-CreERT2 or *Scnn1g*-CreERT2 mice could be compared against deleting the same gene in AT2 cells using the *Sftpc*-CreERT2 mouse (6). This would help distinguish how these two epithelial cells respond to specific growth factors and proinflammatory molecules present at the air–liquid interface. Such knowledge is desperately needed to create effective cell-based therapies for treating distal lung disease.

The same CreERT2 mouse could be used to study how AT1 cells are generated during development or regenerated after injury. AT1 cells were historically considered terminally differentiated, incapable of self-renewal or differentiation, and produced from adult AT2 cells (*see* Reference 7 for review). However, growing evidence questions these conclusions. AT1-like cells derived from cultured AT2 cells can undergo reversible transdifferentiation (8), and isolated rat AT1 cells can proliferate and produce some AT2-specific genes (9). Recent studies suggest that AT1 cells may not be derived developmentally from AT2 cells (10, 11). Furthermore, AT1 cells may be derived from Scgb1a1⁺ (12), Krt5⁺ (13), and even a lineage-undefined progenitor cell (14). Hence, having CreERT2 lines that conditionally target fluorescent reporters or toxins to AT1 cells should provide the rigor required to understand how AT1 cells are generated during development and regenerated after injury.

Let us not forget that AT1 cells express these genes for a reason. GRAMD2 is especially worth studying, because its function is presently not known. It contains a GRAM domain found in glycosyltransferases, myotubularins, and other membrane-associated proteins (15). The GRAM domain is similar in structure to pleckstrin homology domains used for protein- and lipid-binding intracellular signaling. Because AT1 cells cover 95% of the alveolar surface, GRAMD2 may serve as an important sensor of the

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alveolar environment. It will be of great interest to also understand how its expression is regulated and restricted to AT1 cells.

Likewise, further study of SCNN1G or one of the other AT1-specific genes should aid in our understanding of AT1 cells.

In summary, the Marconett and colleagues article is important, because it has given new identities to AT1 cells that should help us understand what defines an AT1 cell, how AT1 cells are generated during development, and how they are regenerated after injury. It is also timely, because the findings should help the LungMAP Consortium (<http://www.lungmap.net>) as it seeks to build a molecular and cellular atlas of early lung development. ■

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References

1. Marconett CN, Zhou B, Sunohara M, Pouldar TM, Wang H, Liu Y, Rieger ME, Tran E, Flodby P, Siegmund KD, *et al.* Cross-species transcriptome profiling identifies new alveolar epithelial type I cell-specific genes. *Am J Respir Cell Mol Biol* 2017;56:310–321.
2. Dahlin K, Mager EM, Allen L, Tighe Z, Goodglick L, Wadehra M, Dobbs L. Identification of genes differentially expressed in rat alveolar type I cells. *Am J Respir Cell Mol Biol* 2004;31:309–316.
3. Qiao R, Zhou B, Liebler JM, Li X, Crandall ED, Borok Z. Identification of three genes of known function expressed by alveolar epithelial type I cells. *Am J Respir Cell Mol Biol* 2003;29:98–105.
4. Katsuoaka F, Kawakami Y, Arai T, Imuta H, Fujiwara M, Kanma H, Yamashita K. Type II alveolar epithelial cells in lung express receptor for advanced glycation end products (RAGE) gene. *Biochem Biophys Res Commun* 1997;238:512–516.
5. Mangalmurti NS, Friedman JL, Wang LC, Stolz D, Muthukumar G, Siegel DL, Schmidt AM, Lee JS, Albelda SM. The receptor for advanced glycation end products mediates lung endothelial activation by RBCs. *Am J Physiol Lung Cell Mol Physiol* 2013;304:L250–L263.
6. Rock JR, Barkauskas CE, Counce MJ, Xue Y, Harris JR, Liang J, Noble PW, Hogan BL. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proc Natl Acad Sci USA* 2011;108:E1475–E1483.
7. Hogan BL, Barkauskas CE, Chapman HA, Epstein JA, Jain R, Hsia CC, Niklason L, Calle E, Le A, Randell SH, *et al.* Repair and regeneration of the respiratory system: complexity, plasticity, and mechanisms of lung stem cell function. *Cell Stem Cell* 2014;15:123–138.
8. Danto SI, Shannon JM, Borok Z, Zabski SM, Crandall ED. Reversible transdifferentiation of alveolar epithelial cells. *Am J Respir Cell Mol Biol* 1995;12:497–502.
9. Gonzalez RF, Allen L, Dobbs LG. Rat alveolar type I cells proliferate, express OCT-4, and exhibit phenotypic plasticity in vitro. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L1045–L1055.
10. Desai TJ, Brownfield DG, Krasnow MA. Alveolar progenitor and stem cells in lung development, renewal and cancer. *Nature* 2014;507:190–194.
11. Yee M, Gelein R, Mariani TJ, Lawrence BP, O'Reilly MA. The oxygen environment at birth specifies the population of alveolar epithelial stem cells in the adult lung. *Stem Cells* 2016;34:1396–1406.
12. Zheng D, Limmon GV, Yin L, Leung NH, Yu H, Chow VT, Chen J. Regeneration of alveolar type I and II cells from Scgb1a1-expressing cells following severe pulmonary damage induced by bleomycin and influenza. *PLoS One* 2012;7:e48451.
13. Zuo W, Zhang T, Wu DZ, Guan SP, Liew AA, Yamamoto Y, Wang X, Lim SJ, Vincent M, Lessard M, *et al.* p63(+)Krt5(+) distal airway stem cells are essential for lung regeneration. *Nature* 2015;517:616–620.
14. Vaughan AE, Brumwell AN, Xi Y, Gotts JE, Brownfield DG, Treutlein B, Tan K, Tan V, Liu FC, Looney MR, *et al.* Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. *Nature* 2015;517:621–625.
15. Doerks T, Strauss M, Brendel M, Bork P. GRAM, a novel domain in glucosyltransferases, myotubularins and other putative membrane-associated proteins. *Trends Biochem Sci* 2000;25:483–485.