Alveolar Epithelium: Beyond the Barrier

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Abstract

I am deeply honored to have been awarded an *American Thoracic Society Recognition Award for Scientific Accomplishment* for 2014. Over the last 20 years, it has become clear that the alveolar epithelium, my area of research focus, is not simply a gas exchange surface and barrier to leakage of fluid and protein into the alveoli, but is an active participant in the pathogenesis of a number of lung diseases, including pulmonary fibrosis. Recognition by this Award stimulates a review of the awardee's contributions to the field, as summarized in this perspective.

Why Alveolar Epithelium?

My entry into the study of alveolar epithelial cell (AEC) biology was in many ways serendipitous when I joined the laboratory of Dr. Edward Crandall as a junior faculty member. A specific research focus was less important to me at the time than my desire that the area be relevant to human disease. The laboratory's focus on AEC biology and regulation of alveolar epithelial barrier properties appealed to me because of its direct relevance to my clinical interests in acute lung injury and the acute respiratory distress syndrome. As the field evolved over the years, it has become clear that the alveolar epithelium is far more than "just a barrier," and is increasingly appreciated to play an active and important role in the pathogenesis of human disease (1, 2).

Regulation of Alveolar Epithelial Barrier Properties

My early work focused on characterization of ion transport molecules expressed by AECs, and elucidation of their transcriptional regulation to understand mechanisms regulating alveolar fluid clearance and alveolar barrier properties (3–6). The complexity of the distal lung necessitated the use of simplified models to characterize these properties. Many of our studies were conducted using a model of AECs in primary culture on semipermeable supports, which recapitulates many of the properties of alveolar epithelium *in vivo* (7, 8), and in which alveolar type II (AT2) cells in primary culture undergo transdifferentiation to a type I (AT1) cell–like phenotype (9). Using this model, we established serum-free conditions for AEC culture (8) and identified factors (e.g., epidermal growth factor and keratinocyte growth factor [KGF]) that up-regulate transport properties through effects on both Na pumps and channels (3–5, 10), and members of the claudin family of tight junction proteins, suggesting that these factors might be useful for enhancing fluid clearance after lung injury.

Type I–Like AEC Monolayers versus Type I Cells

Our *in vitro* model of AT2 cells in primary culture used for studies of AEC physiology initially encountered skepticism, because it was thought to represent dedifferentiation of AT2 cells rather than differentiation to another interrelated phenotype. With the advent of new AT1 cell phenotypic markers, it became clear that AT2 cells in primary culture acquire many characteristics of AT1 cells over time (9, 11, 12), recapitulating the process *in vivo* where AT2 cells serve as progenitors of AT1 cells (13). This led to the concept that AT2 cells in primary culture undergo transdifferentiation to an "AT1 cell–like" phenotype. This concept was somewhat controversial, and I recall having to insert a caveat into grant proposals indicating that "while AT1-like cells acquire many but not all the properties of AT1 cells, they nevertheless constitute a useful model of alveolar epithelium, studies of which provide significant mechanistic insights into AEC function and biology." This

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begged the question of how closely AT1-like cells resembled AT1 cells *in vivo*, and led us to develop methods for isolation of AT1 cells from rat lung (14). Although our studies of AT1-like cells had suggested a role for AT1 cells in ion transport in distal lung, a direct contribution of AT1 cells to alveolar homeostasis had not yet been demonstrated. To address this question, we and others developed techniques for isolation of highly purified (~95% purity) populations of AT1 cells from rat lung (14, 15). We demonstrated that freshly isolated AT1 cells indeed express subunits of Na pumps and Na channels, confirming observations in AT1-like cells and implicating AT1 cells in ion transport and alveolar fluid homeostasis. Successful isolation of these highly purified populations of AT1 cells formed the basis for subsequent phenotypic characterization of these important lung cells using one of the early microarray platforms (which could analyze expression of only 1,000 genes!) and further studies of the contributions of AT1 cells to alveolar and lung biology (16). Many of our initial observations in AT1-like cells were subsequently corroborated in isolated AT1 cells or *in vivo*, emphasizing the utility of this now generally accepted model for functional studies of AT1 cells.

Plasticity of AEC Phenotype

Using this *in vitro* model of AEC transdifferentiation, we made the important observation that the phenotype of AECs in primary culture could be significantly modulated by exogenous soluble factors or interactions with substratum (17–20). It had been believed that, over time, AT2 cells in primary culture invariably default to an AT1 cell phenotype. Whether or not this transition could be modulated was unknown. Using a number of AT1 cell phenotypic markers, we demonstrated that AECs that had already acquired AT1 cell characteristics in culture could be induced to revert to an AT2 cell phenotype through exposure to KGF or changes in cell shape, indicating that AEC phenotype is actively regulated. Effects of KGF were subsequently shown to be mediated by c-Jun N-terminal kinase signaling (21). The demonstration of reversible AEC phenotypic transitions challenges the widely held belief that AT1 cells are terminally differentiated. Although still to be demonstrated by lineage tagging *in vivo* using mice that we have developed with AT1 cell–specific reporter expression (22), the notion that AT1 cells may not be terminally differentiated is supported by observations of others that isolated AT1 cells can proliferate in culture (23, 24). It also suggests greater plasticity of AEC phenotype than previously appreciated, a concept that is becoming more widely accepted with recent demonstrations of stem cell plasticity/reprogramming.

To elucidate mechanisms regulating AEC phenotype and further characterize interrelationships between AT2 and AT1 cells, we characterized the promoter of the water channel, aquaporin-5, which, within distal lung epithelium, is selectively expressed in AT1 cells (25, 26). We used *Aqp5* as a prototype AT1 cell gene with which to elucidate molecular mechanisms that regulate phenotype transitions between AT2 and AT1 cells by transcriptional activators (e.g., GATA-binding factor 6 (27) and repressors (e.g., Forkhead box p2 and A1 Foxp2 and FoxA1 (28, 29) that are also implicated in lung development through their interactions with other lung-enriched transcription factors (TFs). Extending our studies of single genes and TFs, we recently undertook a genome-wide analysis of transcriptomic and epigenetic changes accompanying AEC differentiation *in vitro*. Integration of transcriptional changes with surrounding chromatin modifications enabled characterization of molecular signaling events that were activated or repressed during adult AEC differentiation and identification of putative novel regulators (e.g., hepatocyte nuclear factor 4a) and signaling pathways (e.g., retinoid X receptor pathway) involved in this process (30). The demonstration that AEC phenotype is actively regulated and elucidation of underlying regulatory pathways are of particular relevance when considering strategies to modulate AEC differentiation to enhance alveolar epithelial repair.

Central Role of Alveolar Epithelium in Pulmonary Fibrosis: Apoptosis, Epithelial–Mesenchymal Transition, and Epithelial–Fibroblast Cross-Talk

Our observations of AEC plasticity raised the question of whether AECs could also give rise to other (nonepithelial) cell types. We showed that AECs exposed to transforming growth factor (TGF)-β undergo epithelial–mesenchymal transition (EMT) *in vitro*, as evidenced by loss of epithelial and acquisition of mesenchymal markers (31, 32). We further demonstrated colocalization of epithelial and mesenchymal markers in up to 80% of hyperplastic AECs in lung biopsies of patients with idiopathic pulmonary fibrosis (IPF), suggesting that EMT may contribute to the pathogenesis of fibrosis *in vivo*. Subsequent demonstration that endoplasmic reticulum (ER) stress (either chemically induced or in response to accumulation of misfolded mutant surfactant protein) induces EMT in AECs in a dose-dependent manner (with lower levels of ER stress inducing EMT and higher levels inducing apoptosis) suggests that expression of mesenchymal markers may represent an adaptive response of AECs to injury, which, when overwhelmed, leads to cell death (33, 34). Lineage tracing studies have since raised questions about the precise role of EMT in fibrogenesis (because the number of EMT-derived fibroblasts has been variable among different studies, and sometimes low to absent) (35–38). Nevertheless, a number of studies in addition to our own have shown that AECs in IPF are abnormal and express mesenchymal markers (37, 39, 40), leading to a major paradigm shift that suggests a central role of alveolar epithelium in IPF pathogenesis (2, 41). The demonstration that deletion of the TGF-β type II receptor specifically in lung epithelium protects mice from bleomycin-induced pulmonary fibrosis (42, 43) further supports a central role for alveolar epithelium in fibrogenesis, even if indirectly, as a result of aberrant epithelial–mesenchymal interactions (44). Evidence of AEC abnormalities (including morphologic changes, apoptosis [45], EMT, up-regulation of TGF-β [46] and other cytokines [47], and evidence of ER stress [33, 48]) in IPF lung, and of fibrosis in association with genetic abnormalities (e.g., surfactant and telomerase mutations) that affect the alveolar epithelium, strongly support the evolving notion that epithelial abnormalities as a result of injury (as yet of unknown cause) and aberrant repair contribute to IPF pathogenesis (41). Demonstration that the injured alveolar epithelium is abnormal or "reprogrammed" in IPF has identified new areas of

investigation aimed at understanding how the abnormal epithelium contributes to fibrosis and elucidating genetic and other mechanisms underlying epithelial injury.

Translational Aspects

Aberrant wngless-related MMTV integration site/β-catenin signaling in alveolar epithelium has been implicated in the pathogenesis of IPF as a result of epithelial hyperplasia, altered AEC differentiation, and altered epithelial–mesenchymal cross-talk (49, 50). Interactions of β-catenin with the homologous transcriptional coactivators, cAMP response element–binding protein–binding protein (CBP) and p300, have been shown to differentially regulate subsets of target genes, leading to different functional outcomes (51). We have shown that interactions between β-catenin and TGF-β pathways are implicated in EMT, and are dependent on specific interactions of β-catenin with the transcriptional coactivator, CBP (52). Importantly, treatment with ICG-001, a selective small-molecule inhibitor of β-catenin–CBP interactions, both prevents and reverses bleomycin-induced fibrosis while preserving epithelial integrity, suggesting a potential novel therapeutic approach to pulmonary fibrosis (53). We also recently demonstrated that the peroxisome proliferator–activated receptor γ agonist troglitazone ameliorates TGF-β–induced EMT in a peroxisome proliferator–activated receptor γ–independent manner in AECs, likely through inhibition of β-catenin–dependent signaling downstream of TGF-β. Understanding the contribution(s) of alveolar epithelium to fibrogenesis may offer unique opportunities for development of new therapeutic interventions in IPF.

Beyond the Barrier

Research in AEC biology, as with other fields, has, to a large extent, been driven by technological advances and feasibility. In my case, what began as physiologic measurements in simplified cell culture models (3, 4, 54), and subsequently highly purified populations of isolated AECs (14), evolved to elucidation of the molecular bases of AEC function and phenotype. From initial analyses of expression of selected genes and TFs of interest at baseline and after injury (5, 6, 25), and subsequent generation of mice with the capacity for modulation of genes of interest in AT1 cells *in vivo* (22), our studies have culminated recently in genome-wide analyses leading to insights into transcriptomic and epigenetic changes that accompany AEC differentiation (30). We have come full circle, with the field now being poised for the study of cellular heterogeneity with advances in technology for single-cell RNA-Seq and generation of differentiated AT2 cells through direct cellular reprogramming.

Going Forward

When starting my research career, I remember being worried that I would run out of questions to ask. After more than 20 years, I know now that the opposite is true: the questions are infinite, and each result leads to more questions. It has been exciting to be working in such a rapidly advancing field, where the importance of alveolar epithelium in both acute and chronic lung disease has become much better appreciated. Rather than a passive bystander, the epithelium is actively involved in human disease pathogenesis. Building on our initial *in vitro* studies, we plan to further investigate differential contributions of AT2 and AT1 cells to alveolar function, fibrogenesis, and repair *in vivo* and, applying genome-wide approaches to our *in vitro* model and purified AEC populations, elucidate signaling pathways that are aberrantly activated in AECs in the fibrotic lung. We hope to apply knowledge gained from elucidation of pathways regulating normal and aberrant AEC differentiation to enhance epithelial repair mechanisms, with the ultimate goal of ameliorating fibrotic lung disease. ■

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

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