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Dynamism of the Human Lung Proteome during Alveolarization
Moving beyond the Transcriptome

Human lung development extends throughout pregnancy and continues at least through the first decade of postnatal life (1). During alveolarization, the final stage of lung development, division of the primitive alveolar ducts into terminal saccules and marked expansion of the capillary bed significantly increases gas exchange surface area (2). However, completion of lung development after birth heightens the susceptibility of the lung to injuries that disrupt development. This is frequently observed in the context of premature birth, when impaired alveolarization leads to bronchopulmonary dysplasia, a chronic lung disease associated with significant morbidity and mortality. Thus, a comprehensive understanding of the molecular mechanisms that promote postnatal lung development might motivate the development of novel therapies to enhance lung growth and repair in infants and young children (3).

Over the past two decades, significant advances have been made relative to the molecular control of alveolarization. These include the identification of essential signaling pathways (4), transcription factors (5, 6), and key cell types that drive alveolarization (7, 8). Recently, the development and application of multiomic methodologies, including single-cell transcriptomics, have provided novel insight into the heterogeneity and maturation of the lung at single-cell resolution, providing comprehensive atlases of cell abundance and phenotype across development (9–11). However, many of these prior studies used experimental murine models, and there are key differences between human and murine lung development, including differences in timing, with alveolarization beginning before birth in humans but after birth in mice. Furthermore, the majority of these invaluable cell and expression maps were constructed using only gene expression data. Thus, there remains an absence of similar, comprehensive data sets defining how the lung proteome evolves during postnatal development, particularly in humans.

In this issue of the *Journal*, Clair and colleagues (pp. 208–218) performed deep proteomic profiling on postmortem human lung samples obtained from donors ranging from 1 day old to 8 years of age (12). The authors used two-dimensional liquid chromatography–mass spectrometry to identify almost 9,000 proteins, representing an almost fourfold increase in proteome coverage compared with a prior proteomic analysis of human lung development (13). Of the proteins identified, ∼50% changed across development, with the majority decreasing linearly over time, consistent with a gradual transition to quiescence as the lung reaches maturity. Further analyses of these dynamic proteins identified that proteins related to telomerase activity, the proteasome, RNA splicing, and post-translational modifications decreased with age, suggesting a gradual differentiation of lung cell progenitors and a significant contribution of post-transcriptional mechanisms to postnatal lung development. In contrast, proteins associated with immunity and inflammation, lipid metabolism, and extracellular matrix increased with age. Using principal component analysis, the authors identified four molecularly distinct substages that are present during alveolarization, consistent with the four substages previously identified in the murine lung using bulk RNA sequencing (14). The authors also demonstrate that the developmentally regulated proteome profiles they identified accurately predicted donor age in a separate validation cohort, indicating high consistency and reliability of these temporal changes in the lung proteome. Interestingly, when proteomic and transcriptomic analyses were performed on the same donor, the dynamic protein/transcript pairs only exhibit the same trend (e.g., linear decreasing or increasing, etc.) ∼50% of the time, highlighting the importance of validating potentially relevant transcriptomic changes with methodologies that assess protein expression rather than relying on gene expression alone.

Although this study represents a great resource for clinicians and scientists studying normal and aberrant lung development at the molecular and cellular level, there are some limitations, and a number of questions remain. The authors make the important point in the introduction that a key difference in human versus murine lung development is the onset of alveolarizations at 36 weeks in humans. However, in this study, the earliest time point included was 1 day after birth, precluding an elucidation of the molecular mechanisms driving early alveolarization in humans. Furthermore, it is important to note that the authors identified substages of alveolarization that were similar to those identified in the murine lung using transcriptomics, suggesting significant conservation of key regulatory mechanisms promoting alveolarization in both mice and humans.

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This result is particularly intriguing given that the authors also identified moderate discordance between the genes and proteins changing across development, suggesting the existence of greater concordance among the highly conserved pathways that drove the principal component and regression analyses to reveal similar substages of alveolarization.

In addition, the use of bulk proteomics did not permit the identification of cell-specific protein expression or important spatial information essential for a complete understanding of the complex biology underlying postnatal lung development. Recent single-cell transcriptomic studies on the mouse and human lung have identified marked changes in cell type abundance over time. Yet bulk proteomics does not permit the distinction between changes in protein expression per cell and aggregate changes in protein expression secondary to alterations in the abundance of that specific cell population. Significant advances in single-cell proteomic and spatial proteomic methodologies (15, 16) represent valuable future strategies to further enhance our understanding of molecular regulation of postnatal lung growth. Finally, although this study adds greatly to the field by contributing a broad resource of protein expression changes across time, sophisticated but user-friendly analytic algorithms that allow investigators to deftly interpret these complex, multidimensional data sets to better inform our understanding of the biology underlying development and disease remain elusive.

Despite these limitations and unanswered questions, this work by Clair and colleagues provides a comprehensive evaluation of protein expression of the human lung across postnatal development. These studies represent an invaluable data set likely to serve as a resource for the validation of murine and human transcriptomic data and a vital framework for the interpretation of future proteomic analyses performed on human tissue obtained from donors with aberrant lung development such as bronchopulmonary dysplasia. Although the immaturity of the lung at birth heightens the susceptibility to injury, this same quality appears to increase the capacity for regeneration, as developmental pathways are reinvoked to stimulate lung repair. Thus, deep discovery of the fundamental pathways directing human lung development as presented in this work has important clinical implications, providing knowledge that can be potentially leveraged into novel therapies to broadly treat lung diseases in infants and children. ■

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