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When Development of the Alveolar Gas Exchange Unit Fails
Universal Single-Cell Lessons from Rare Monogenic Disorders

Alveolar capillary dysplasia with misalignment of the pulmonary veins (ACDMPV) is a lethal lung developmental disorder in which most affected infants die within the first few hours or days of life (1). Histologically, ACDMPV is characterized by thickened alveolar septae with enlarged pulmonary capillaries that are reduced in number and do not directly contact the alveolar epithelium. Often, pulmonary veins are mispositioned in proximity to the bronchovascular bundle (1, 2). ACDMPV is caused, in most cases, by *de novo* mutations in the *FOXF1* gene locus, and *Foxf1* haploinsufficient mice display most features of human ACDMPV (3, 4). However, how exactly the loss or decreased expression of *FOXF1* affects the cells of the developing human lung was largely unknown.

In this issue of the *Journal*, Guo and colleagues (pp. 709–725) analyzed lung tissues from infants who died of ACDMPV within 2 to 5 weeks of birth (severe phenotypes) and from subjects who survived to lung transplant at 3.5 years and 9 months of age (less severe phenotypes) (5). Using single-nucleus RNA sequencing and single-nucleus chromatin accessibility analysis, the researchers identified 35 distinct cell types in ACDMPV, including major epithelial, endothelial, mesenchymal, and immune cell types. Single-cell RNA sequencing is a powerful technology that allowed generation of cell atlases of various lung diseases (6) and led to the identification of two previously unknown microvascular endothelial cell types in mice and humans, termed "aerocytes" and "general alveolar capillaries cells" (or "gCap") (7–9). Of note, *FOXF1* is most strongly expressed in aerocytes and general capillaries and to a much lesser degree in other endothelial subpopulations or fibroblasts (9). In ACDMPV, Guo and colleagues observed a severity-associated loss of aerocytes and reduced expression of the *FOXF1* gene in gCap, indicating a disruption in the formation of the pulmonary microvasculature in a dose-dependent manner. In contrast, ACDMPV was characterized by an expansion of *COL15A1*-positive systemic venous endothelial cells, previously described in the subpleural und peribronchial spaces in the healthy lung and in the fibrotic areas of lungs with idiopathic pulmonary fibrosis (9, 10). Whether the expansion of *COL15A1*-positive endothelial cells reflects a compensatory systemic circulation caused by the lack of pulmonary capillary perfusion, as the authors suggest, or a change in endothelial phenotype in an altered extracellular matrix in the thickened alveolar septa of ACDMPV remains to be shown in the future.

In the mesenchyme, pericyte frequencies were significantly reduced in ACDMPV lungs, whereas one fibroblast population was increased (labeled alveolar fibroblast 1 = "AF1"), the other ("AF2") being decreased. Here, we suggest an alternative interpretation.

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In accordance with previous publications, AF1 had been identified as alveolar fibroblasts with *TCF21* as an important transcription factor (11, 12). However, the marker gene *MFAP5* of the "alveolar" fibroblast AF2 suggests an alternative identity, namely that of adventitial fibroblasts (11, 12). Although *FOXF1* is expressed at extremely low levels in both fibroblast populations, at least in adults, *FOXF1* is more highly expressed in adventitial fibroblasts than in alveolar fibroblasts, potentially suggestive of a *FOXF1* transcription factor insufficiency as well (9). Alternatively, the relative increase of true alveolar fibroblasts could imply a missing aerocyte- or AT1-derived proliferation-inhibitory factor.

Although *FOXF1* was not expressed in the epithelium, differentiation of alveolar epithelial cells was dramatically impaired in ACDMPV lungs as well, with a loss of mature AT1 cells and an increase in AT1/AT2 transitional cells. Cell–cell communication analysis offered a potential clue to this observation: In the healthy lung, and as previously reported, the AT1 to capillary signaling dominates the alveolar intercellular communication, especially through the *VEGFA–KDR* axis (7, 13). In ACDMPV lungs, signaling from both capillary populations was largely disrupted, with *COL15A1*-positive cells becoming the new signaling hub of the endothelium. Together with the extremely close physical proximity of aerocytes and AT1 cells, this finding highlights the absolute interdependency of the two cell types in forming the alveolar gas exchange unit. Just as important as what has changed is what has not changed: AT2 cells were profiled at similar frequencies, suggesting that their development is not dependent on major cues from the microvasculature.

This study highlights the critical role of *FOXF1* in the development of the lung in general, of the pulmonary microvasculature specifically, and of the alveolar epithelium consecutively. It identifies disrupted signaling patterns among alveolar cell types in ACDMPV, affecting normal cell interactions and pathways involved in alveolar development. It also represents a new and exciting phase in the research of rare human monogenic disorders. Not so long ago, investigators were excited to discover mutations that explained a rare congenital syndrome, allowing early detection and better diagnoses. Then, through the careful use of genetically modified cell and animal models, they were able to infer the role of the protein encoded by the mutated gene, and even, in some limited cases, develop therapies. In this study, the authors use the powerful technology of single-cell profiling to elucidate the impact of a single mutation, limited mostly to endothelial cells, on multiple cells and their interactions in the affected organ, moving us forward toward elucidating the design principles of the developing alveolus. Thus, the direct impact of the atlas of cellular abnormalities in ACDMPV is that it serves as a basis for further research to identify prenatal biomarkers that will allow prenatal gene therapy to detect and cure this terrible disease even *in utero*. But the ACDMPV atlas is also important because it provides insights into how *FOXF1*, in endothelial cells, regulates the alveolar cellular unit, a topic of relevance to far more common conditions in which the alveolar gas exchange unit fails, such as chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis, and where improving endothelial cell function may enhance repair. Indeed, it was recently suggested that *FOXF1* in lung endothelial cells may regulate fibrosis (14). Thus, beyond its relevance to ACDMPV, this study provides a blueprint for using rare monogenetic diseases to identify the general design principles of the human lung that will potentially be useful to develop better therapies also for other diseases in which the lung fails. An impressive feat indeed. ■

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