Indeterminate Differentiation of Peripheral Respiratory Epithelial Cells in Idiopathic Pulmonary Fibrosis (IPF). Yan Xu[1], Anusha Sridharan[1], Anne-Karina T. Perl[1], Liya Huo[1], Yina Du[1], Takako Mizuno[2], Barry R. Stripp[2], Jeffrey A. Whitsett[1] on behalf of LungMap and Lung Repair and Regeneration Consortium sponsored by NHLBI. [1]Division of Neonatology, Perinatal and Pulmonary Biology, Perinatal Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]Cedars Sinai Medical Center, Los Angeles, CA.

Rationale: Idiopathic pulmonary fibrosis (IPF) is a common pulmonary disorder causing respiratory failure caused by tissue remodeling and fibrosis. While genetic causes and environmental exposures have been identified in rare subsets of patients, IPF remains an idiopathic life-threatening lung disease, and a common cause for lung transplantation. This study was undertaken to identify cellular and gene expression patterns underlying the pathogenesis of IPF. *Methods:* To identify structural and genetic abnormalities underlying IPF, we used immunofluorescence, cell sorting, and NexGen RNA-sequence (Seq) analysis to identify the cellular and genetic networks controlling epithelial cell differentiation in IPF. Confocal imaging was used to identify epithelial cell types in peripheral IPF lesions (n=12) that were compared with relatively normal human lung tissue obtained for transplantation (n=4). RNA-Seq was obtained from CD326/HTII-280 sorted "epithelial" cells from both normal and IPF tissue (n=3 per group) and single cell RNA-Seq was assessed. *Results:* RNAs from sorted HTII-280 cells expressed high levels of type II alveolar cell markers, including *ABCA3*, *SFTPC*, *SFTPB*, and *LPCAT1* from both normal and IPF lung tissue; however, RNAs from IPF samples included expression of genes characteristic of "proximal" airway epithelial cells, e.g., KRT14, MUC5B, PAX9, and TP63. "Pathways" altered in IPF included signaling via TGF-ß, ß-catenin, TP53, TP63, and ETS family transcription factor signaling. Biological processes enriched in the IPF tissue included "cell migration, adhesion, motility, extracellular matrix, and epithelial development." Surprisingly, downregulated processes including "protein modification, redox, and response to unfolded proteins." Confocal microscopy identified disturbed epithelial cell differentiation and tissue architecture in IPF, epithelial cells expressing RNAs indicating incomplete, aberrant or indeterminate differentiation. *Conclusions:* NexGen RNA sequencing of fractionated human AT2 cells provides novel insights into molecular pathway, genes and processes involved in the pathogenesis of IPF. Present analyses provide deep RNA-Seq analysis of normal human type II epithelial cells, identify IPF disease-related changes in type II epithelial cell fates, and provide new insights into pathways associated with IPF disease progression that has potential for the development of new biomarkers. **Supported by: HL122642 and HL110967**