Identifying the heterogeneity of pulmonary mesenchymal cell lineages via single cell RNA-seq analysis

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Rationale: While lineage relationships among epithelial cells are becoming increasingly understood in the developing mouse lung, the identities and lineage relationships among mesenchymal cell subsets remain poorly defined. Hierarchical relationships, dynamic expression patterns and associated functions of the diverse cell types in fetal lung mesenchyme remain largely unknown. The present study sought to develop and utilize systems biology approaches to identify ontogenetic changes in mesenchymal cell types during fetal lung maturation.

Methods: RNA-seq analysis of single cells isolated from mouse lung at E16.5 identified a diversity of mesenchymal cell types including endothelial, pericyte, bone marrow derived, and five distinct fibroblast subtypes we termed (Proliferative Mesenchymal Progenitors-“PMP”, Myofibroblasts-“MyoF”, Matrix Fibroblasts-“MFB”, Intermediate Fibroblasts 1&2 – “IF1&2”) and their associated signature genes. Using the expression patterns of mesenchymal cell-specific signatures as input data, we employed two independent analytic approaches 1) Bayesian analysis of lung development time series using RNA expression data from E15.5 to birth, and 2) a novel unbiased cell-ordering algorithm based on cellular network “entropy” to reveal the relative lineage relationships among lung mesenchymal cell types. We used the term “entropy” to define a cell state function; high entropy is associated with unstable transitional stages and low entropy with more differentiated cells. The “entropy” calculation was based on the degree of uncertainty in protein-protein interactions associated with cell-selective gene signatures.

Results: Through integrative analysis of mesenchymal cell-specific RNA expression signatures, we placed individual cells into sequential order based on the proliferative property and the predicted “pseudotime”. The five fibroblast subtypes were ordered: “PMP” serves as a common progenitor cell. From this common root, two main developmental branches were identified: PMP to MyoF, and PMP to MFB. Temporal patterns and key regulators of each cell type were identified for both branches. Data provide a working model defining the lineages of major pulmonary mesenchymal cells during the saccular phase of lung morphogenesis and provide expression signatures to benchmark with fibroblast cell types associated with lung repair and pathology (Supported by R01HL105433, U01HL110964-Lungmap and U01HL110964-LRRC).