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Creating a molecular map of the pediatric lung

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CREATING A MOLECULAR MAP OF THE PEDIATRIC LUNG

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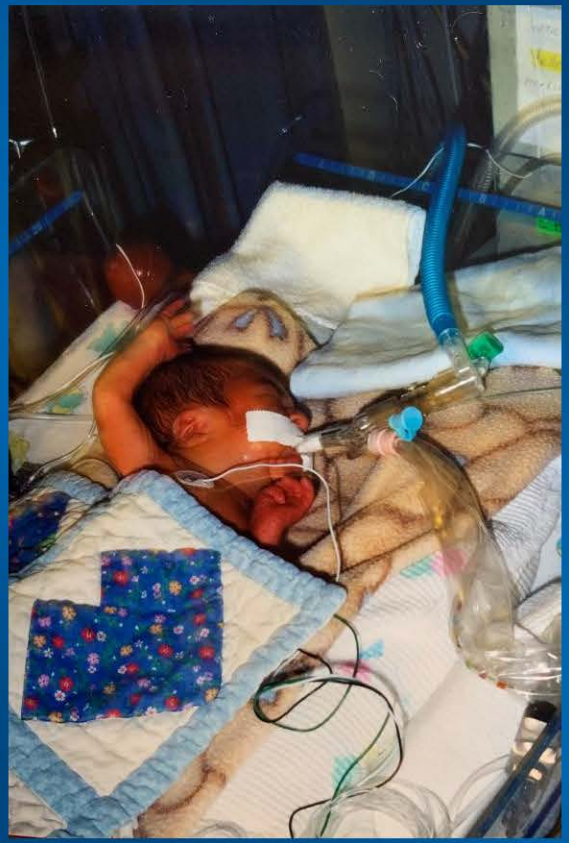
Background → Building the LungMAP → Using the LungMAP

BACKGROUND



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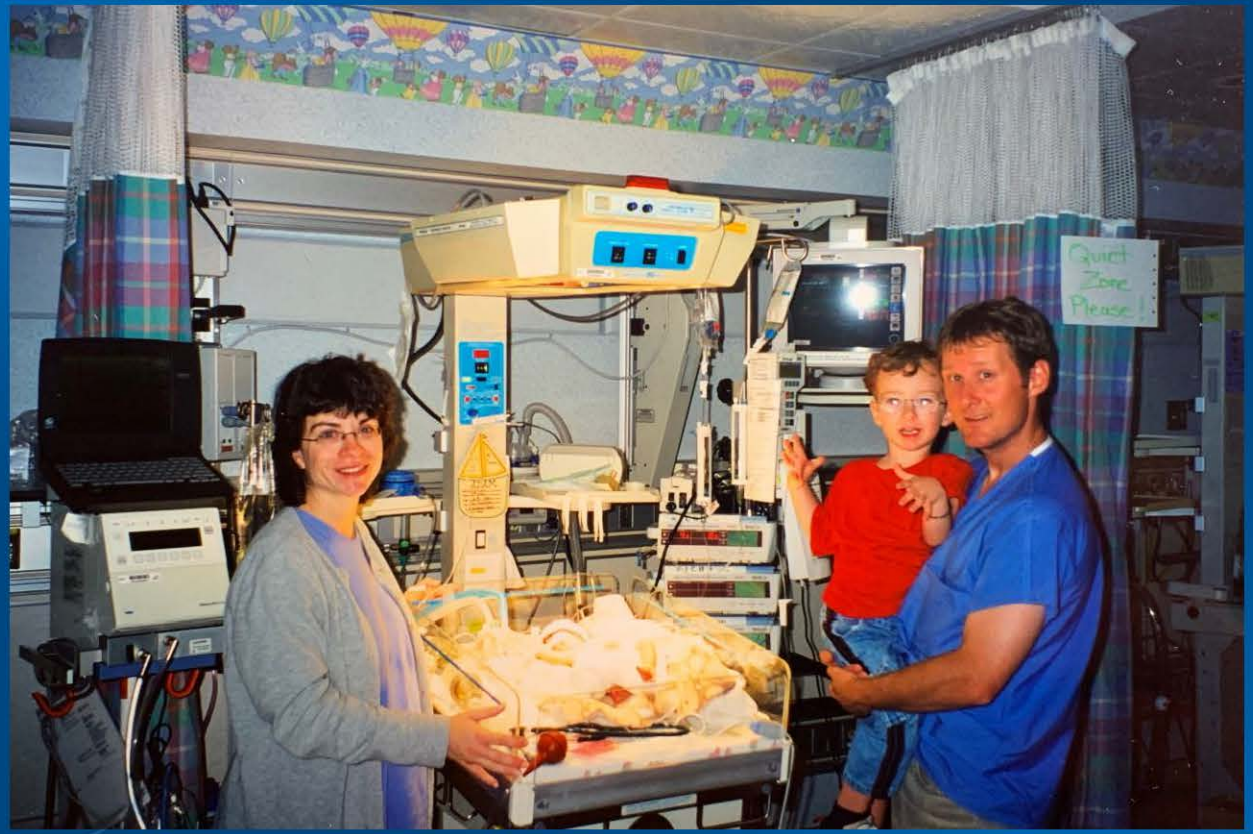

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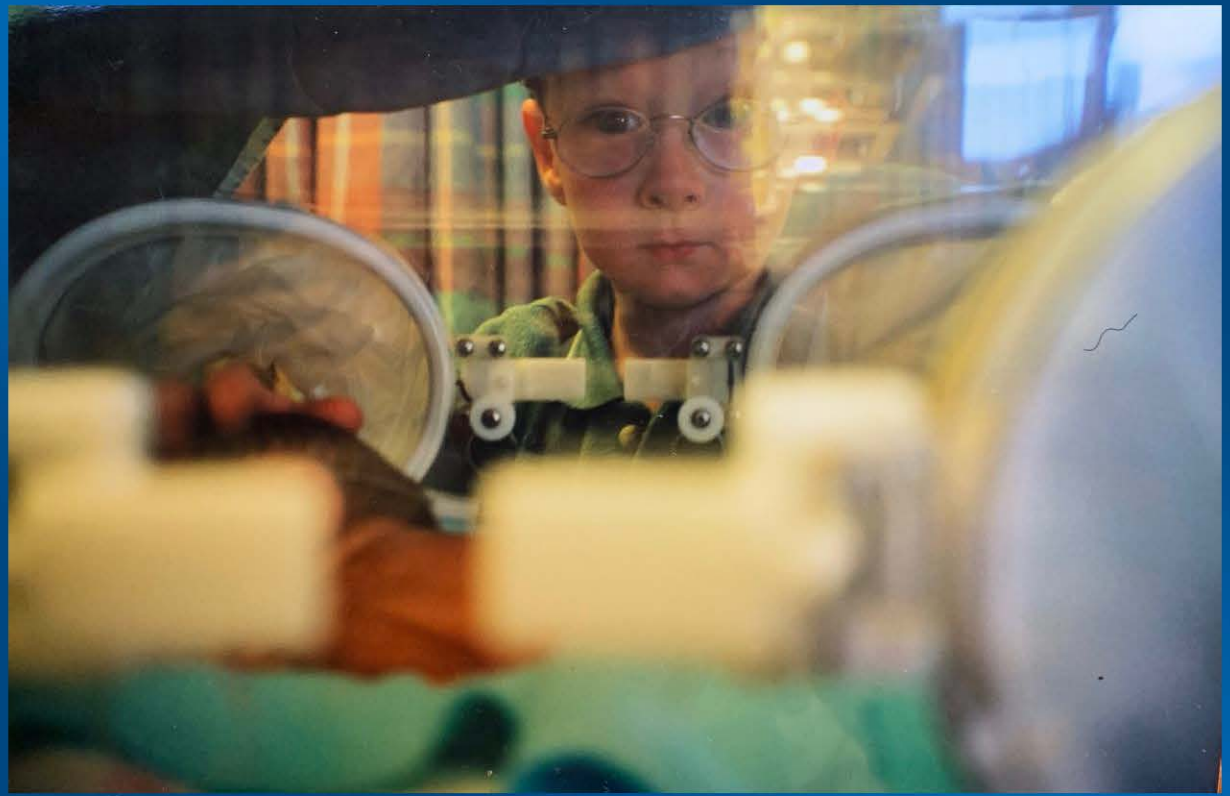

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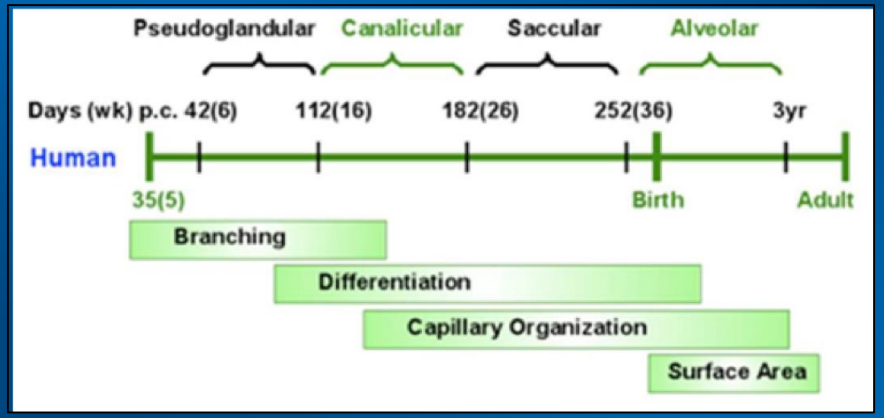


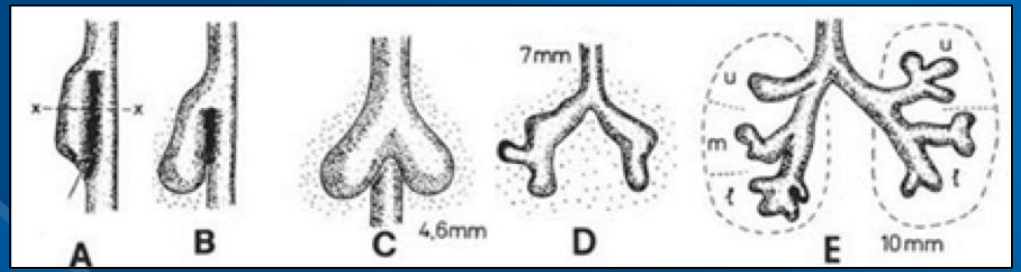


Figure 1. Stages of human lung development. Timeline through fetal development and birth of lung development. Images A-E demonstrate lung branching morphogenesis. (Bhattacharya, S. & Mariani, T. *Pediatr. Res.,* 2013).

BACKGROUND

- **Upon leaving the womb, the transition from fetus to newborn is one of the most complex adaptations that occurs during human life (Hillman, N., et al. *Clin. Perinatol.* (2012).**
- Involves transition from fluid environment to air environment where gas exchange must occur.
- Involves clearance of fetal lung fluid, surfactant secretion, and onset of regular air breathing.

BACKGROUND

- **Development of the lung proceeds through unique phases and involves gene regulation and dynamic cross talk between pulmonary cell types that uniquely contribute to the development of the lung (Whitsett, JA.** *Physiol. Rev.* (2019).
  - Broadly classified into epithelial, endothelial, mixed immune, and mesenchymal cells with numerous subtypes within each category.

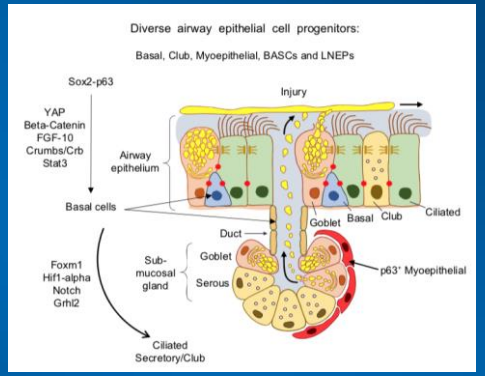
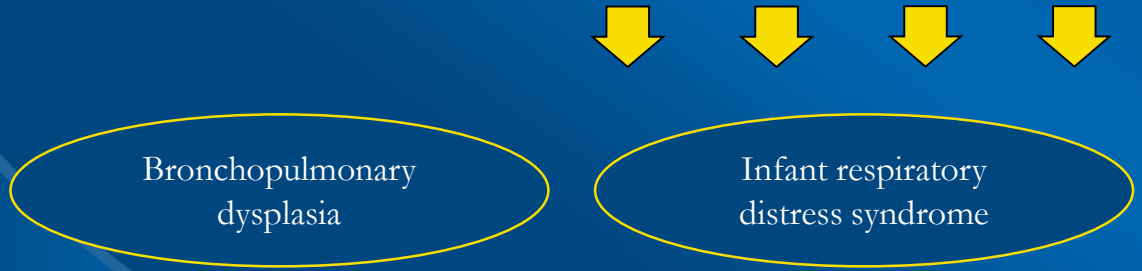


Figure 2. Diversity of lung progenitor cells repairing airway epithelium. Diverse progenitor cells repair the conducting airway epithelium. The conducting air- way surface and submucosal glands are shown (Whitsett, JA. *Physiol. Rev.* (2019).

BACKGROUND

- **Little is known of human lung development at this critical period when the diverse lung cells go through terminal differentiation and maturation and when the gas exchange units (alveoli) form.**

- Incomplete lung maturation and morphogenesis can lead to lifelong pulmonary dysfunction



Highest morbidity and mortality associated disease category for infants (Bhattacharya, S. & Mariani, T. *Pediatr. Res.,* 2013).

BACKGROUND

- Knowledge about the molecular interactions among diverse lung cells, the genes that regulate their functions and behavior, and the molecular and physical interactions among the cells is needed.
  - May support novel approaches to advancing treatments for lung injury repair and regeneration.

- Molecular profiles of the diverse cell types in the lung.
- Knowledge of the dynamics of three-dimensional (3D) cellular structure of the airways and alveoli.
- Integrative open- access database.

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BUILDING THE LUNGMAP

- The NHLBI organized the LungMAP initiative to build a molecular atlas of late-stage lung development to serve as a platform for discovery research to better understand critical events, including alveologenesis.
  - Funding was provided beginning in 2016.

GOALS

Build murine lung cell atlas from embryonic day 16.5 to PND 28

Human lung cell atlas profiling normal development

Integrated, publicly accessible database for researchers

BUILDING THE LUNGMAP

- **The LungMAP consortium consists of a set of research centers across the United States.**
  - Each center focuses on specific portion of LungMAP project.

- Images (immunofluorescence, in-situ, histology, **micro-CT**, Vibra-SSIM)
- Transcriptomics
- Lipidomics/proteomics
- Metabolomics
- Epigenetic

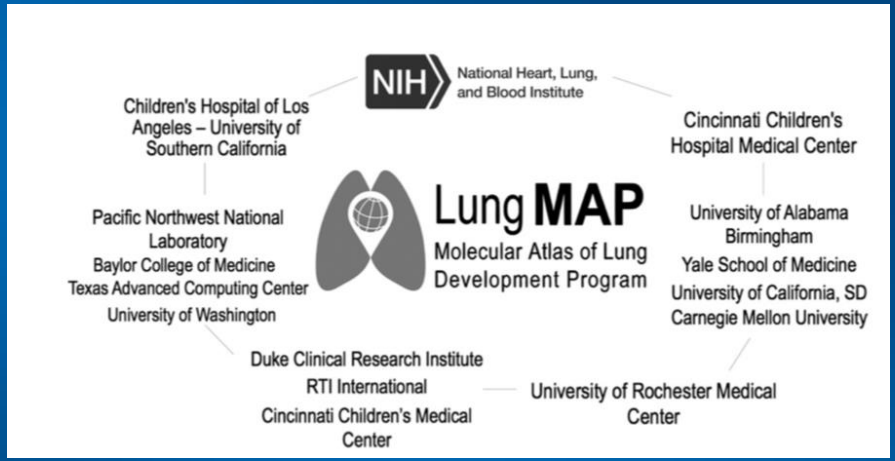

Figure 3. The LungMAP Consortium. Organizations funded by the National Heart, Lung, and Blood Institute for the Molecular Atlas of Lung Development Program. NIH, National Institutes of Health.

BUILDING THE LUNGMAP

- University of Rochester Medical Center – Rochester, New York.
- LungMAP Human Tissue Core: Biorepository for Investigation of Neonatal Diseases of Lung-Normal (BRINDL-NL)

- **Principal Investigator: Gloria Pryhuber, M.D.**

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BUILDING THE LUNGMAP

- **Procure, process, deposit and distribute normal late fetal, neonatal and early childhood human lung tissue and dissociated cells for LungMAP research working.**
- Transplantation quality tissues obtained through:
  - International Institute for the Advancement of Medicine
  - National Disease Research Interchange
  - United Network for Organ Sharing

What does this process look like for the HTC?

BUILDING THE LUNGMAP

- **At the HTC, donated lungs are:**
  - imaged by CT (computed tomography)
  - Reconstructed in 3D
  - Processed for histological analysis
  - Dissociated to provide a range of enriched cell populations including subsets of epithelial, endothelial, mesenchymal, lymphatic, immune and stem cells.

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USING THE LUNGMAP

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USING THE LUNGMAP

- **Whole transcriptome RNA and single-cell RNA sequencing methods can characterize pulmonary cell populations during development in addition to demonstrating dynamic gene expression patterns.**
- Many current human genomic studies of lung maturation suffer from limited sample size, limiting their applicability to longitudinal pediatric lung development.

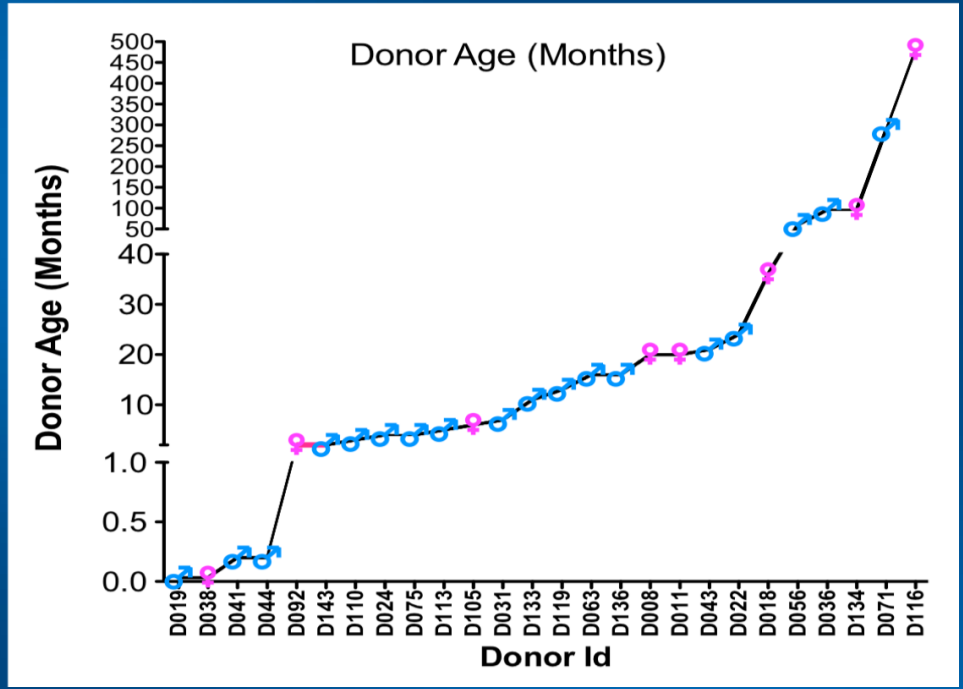
- **Time-series analysis of gene expression aims to bridge this gap.**
  - Short Time-series Expression Miner (STEM) (Ernst, J. & Bar-Joseph, Z. *BMC Bioinformatics*, 2006).
  - STEM utilizes unique methods to cluster, compare, and visualize short time-series gene expression data.

USING THE LUNGMAP

Analyze whole-transcriptome RNA sequencing (RNAseq) data from mesenchymal cells collected from 24 pediatric donors at various ages using Short Time-series Expression Miner to detect novel gene expression patterns during development.

USING THE LUNGMAP

Figure 4. Subject Demographics. Ages and gender distribution of subjects enrolled in RNAseq study. X-axis represents donor ID arranged in ascending order of age and y-axis represents age. Two adult donors (D071 and D116) were excluded from the current analysis. Pink and blue symbols indicate female and male donors, respectively.



USING THE LUNGMAP

DATA ANALYSIS WITH STEM

RNAseq was performed using RNA obtained from lung mesenchymal cells, (n=24, (<1 d/o - 8 y/o, 17 m, 7 f) generating 24.3±5.5 million reads (depth of 10 million reads, 48.3±4.6% genome mapped). **Repeat time points were averaged and separated into a younger (n=9, <1 d/o – 1 y/o) and older (n=8, 1 y/o – 8 y/o) group**. Time-series analysis was performed with STEM, and 16 and 20 profiles were significant in the younger and older group, respectively, using Fisher's exact test ($p<0.05$).

FUNCTIONAL ENRICHMENT

7 profiles in the younger group and 8 profiles in the older group were selected for further functional analysis based on significance and directionality of gene expression changes. Lists of genes from indicated profiles were functionally enriched using ToppGene Functional Gene Enricher (Chen, J. et al. *BMC Bioinformatics*, 2007) for molecular functions, biological processes, cellular components, pathways, and ToppCell Atlas reference gene lists.

USING THE LUNGMAP

- **Gene expression associated with immune-like pathways increased in both groups. Immune like gene ontologies were also noted.**
  - Suggests dynamic immune response that may decrease after infancy and has considerable variability with individual donors. Contamination from immune cell type population is also possible.

- **Proliferative fibroblast and cell division associated gene expression decreased from birth to 1 year in the younger group.**
  - May be due to decrease in alveolarization of the lung after the rapid fetal growth and development of the lungs.

USING THE LUNGMAP

- **Cell signatures in the older group associated with the Wnt pathway decreased from 1 year until 2 years and then increased from 4 years to 8 years.**
- Suggests decrease in Wnt signaling pathways after infancy.

- **Matrix fibroblast associated gene expression increased and peaked at 2 years.**
- Detection of multiple mesenchymal like profiles validates cell enrichment.

- **Expression of genes associated with extracellular matrix components and production decreased in the younger group.**
  - These processes may decrease as development of the lung slows after rapid lung growth during growth of the fetus.

USING THE LUNGMAP

WHAT DOES THIS TELL US?

Unique heterogeneity of pulmonary mesenchymal cells throughout adolescence.

Gene expression associated with immune signatures during pediatric lung development was noted

USING THE LUNGMAP

HELPS FULFILL SECOND GOAL OF LUNGMAP

Build human lung cell atlas profiling normal development

Further validation and exploration using this technique may advance understanding of the diversity of pulmonary cell types and pathophysiology of pediatric lung disease.

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Human Tissue Core Lab

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