Lung mesenchymal cells undergo transcriptional reprogramming after dysbiosis and *Streptococcus pneumoniae* infection

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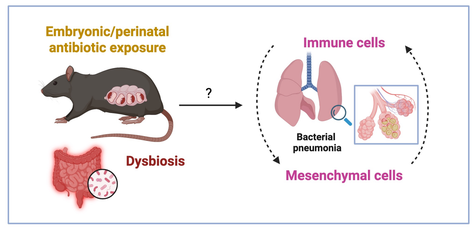
Introduction

- Eradicating pulmonary pathogens while maintaining gas exchange is a critical ongoing challenge for the infant after birth. Unfortunately, these priorities often go awry since lower respiratory tract infections (RTI) remain a leading cause of morbidity and mortality in infants and children.
- While current practices reduce infant mortality, antibiotic use during the critical postnatal assembly phase of the gut microbiome has negative consequences related to loss of microbial diversity (dysbiosis).
- Intestinal dysbiosis profoundly affects the lung immune homeostasis.
- We use the three-axis classification to approach the study of the lung mesenchyme in the setting of dysbiosis and RTI.
- The transcriptional programs among lung mesenchymal cells in the setting of dysbiosis and bacterial infection is currently unknown.
- A unifying framework explaining how early-life dysbiosis remodels lung mesenchymal cell transcriptional program and rewires mesenchymal-immune crosstalk remains poorly understood.



Three-axis classification as a strategical approach to study mouse lung mesenchyme

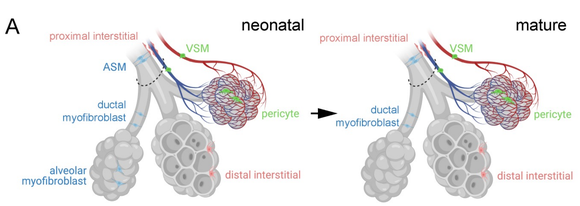


Figure 1. **Three-axis classification system as a strategy to analyze cellular constituents of the lung mesenchyme.** (A) Diagram shows the general anatomic location of individual mesenchymal cell types based on the structures they support: vascular tree (**vascular smooth muscle [VSM], pericyte**), epithelial tree (**airway smooth muscle [ASM], ductal and alveolar myofibroblast**), and interstitium (**proximal interstitial [PIC], distal interstitial [DIC]**). (B) Table summarizes markers for mesenchymal cell types (next column). (Narvaez del Pilar, O. et al. 2022)

Molecular markers per axis

B

Three-axis classification	cell type	markers
vascular	vascular smooth muscle	Acta2, Tagln, Pdgfrb, Notch3
vascular	pericyte	Pdgfrb, Notch3
epithelial	airway smooth muscle	Actc1, Acta2, Tagln, Lgr6
epithelial	ductal myofibroblast	Hhip, Cdh4, Lgr6, Acta2, Tagln
epithelial	alveolar myofibroblast	Pdgfra^{high}, Acta2, Tagln
interstitial	proximal interstitial	Meox2, Pdgfra^{low}, Pdgfrb, Il33, Dner (neonatal), Pi16 (mature)
interstitial	distal interstitial	Meox2, Pdgfra^{low}

The lung vasculature is enveloped by the vascular smooth muscle and pericyte

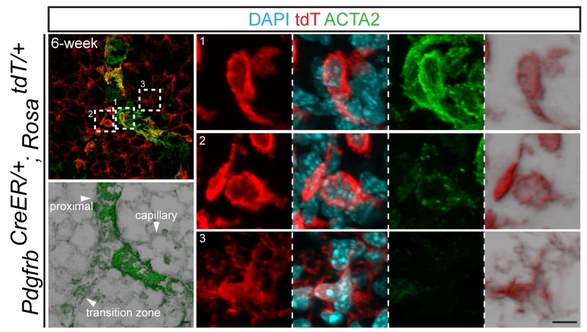


Figure 2. Vascular axis. Vascular smooth muscle cells envelop blood vessels and transition to pericytes that surround capillaries. Scale 10 um.

The lung epithelium is supported by the ductal and alveolar myofibroblast

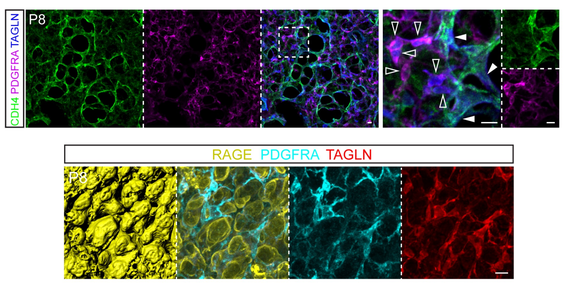


Figure 3. The ductal myofibroblasts (CDH4/HHIP) envelop alveolar ducts, whereas the alveolar myofibroblasts (PDGFRA) are wedged between alveolar septa.

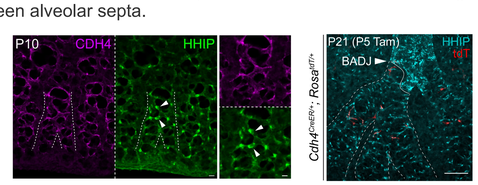


Figure 4. The ductal myofibroblasts persist into adulthood.

The lung interstitium is occupied by MEOX2-expressing mesenchymal cells

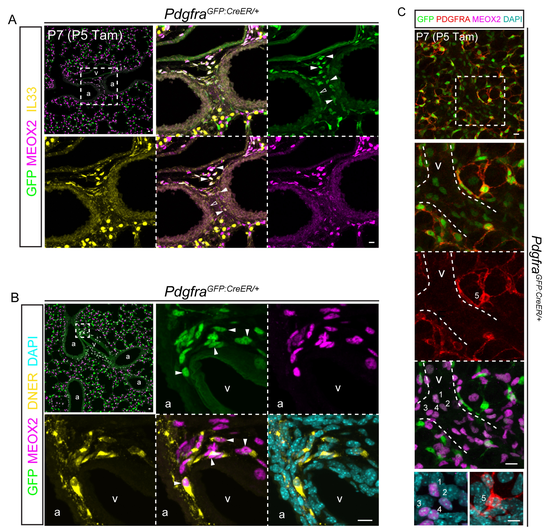


Figure 5. Proximal and distal interstitial mesenchymal cells are distinct from PDFRA-expressing alveolar myofibroblasts.

Application of scRNA-seq to investigate transcriptional programs after dysbiosis and infection

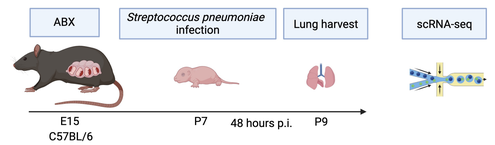


Figure 6. Experimental design. To pinpoint whether dysbiosis alters the transcriptional landscape after a respiratory infection within the lung mesenchyme, we infected mouse lungs with *Streptococcus pneumonaie*, a common bacterial pathogen that affects neonates.

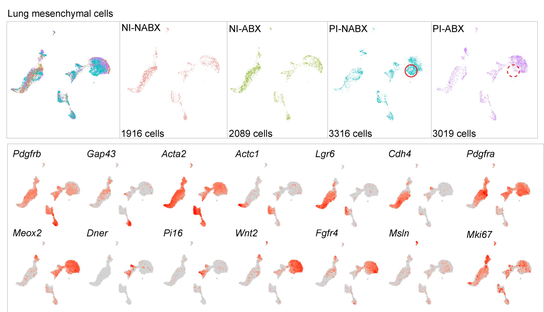


Figure 7. scRNA-seq analysis reveals a transcriptionally different subpopulation of distal interstitial cells after dysbiosis and *Streptococcus pneumoniae* infection.

Infected DICs in the setting of dysbiosis display a reduced inflammatory response and heightened expression of smooth muscle genes

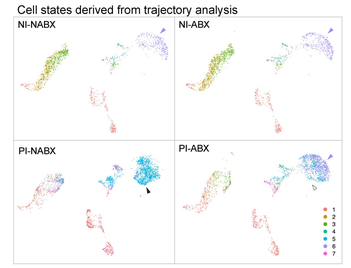


Figure 8. Trajectory analysis of the lung mesenchyme reveals cell states after dysbiosis and bacterial infection.

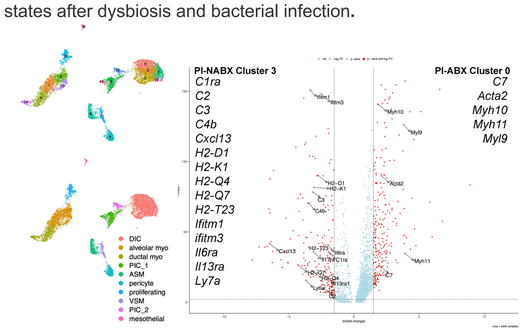
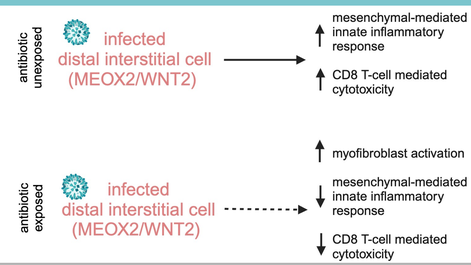


Figure 9. Dysbiosis alters transcriptional immune mediated response in the distal interstitial cells and promotes expression of smooth muscle cell genes.

Research Model



Bibliography, Funding & Acknowledgements

Narváez del Pilar, O., Gacha-Garay, M., & Chen, J. 2022. Three-axis classification of mouse lung mesenchymal cells reveals two populations of myofibroblasts. *Development.* Mar 15;149(6). doi: 10.1242/dev.200081. **Funded by F31D and U54 MD/PhD MDACC and UPR partnership program during PhD training in Dr. Jichao Chen's lab.**

Stevens, J. *et al. The Balance between Protective and Pathogenic Immune Responses to Pneumonia in the Neonatal Lung Is Enforced by Gut Microbiota. Sci. Transl. Med* vol. 14 https://www.science.org (2022).

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