Dysbiosis alters transcription programs within the lung mesenchyme after *Streptococcus pneumoniae* infection

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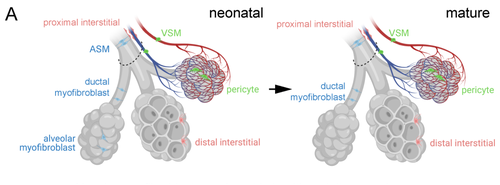
Introduction

- 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.
- Perinatal antibiotics (ABX) exposure is an early life stressor.
- 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. While this concept termed '***gut-lung axis'*** is exemplified by several observations linking ABX-exposure and dysbiosis with RTI, its mechanistic basis remains unclear.
- It is unknown how dysbiosis disrupts transcription programs in lung mesenchymal cells and intercellular communication.
- Here, we use a three-axis classification to study the role of dysbiosis during RTI within the mouse lung mesenchyme.

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



B

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

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. (Narvaez del Pilar, O. et al. 2022)

Vascular smooth muscle and pericyte envelop the lung vasculature

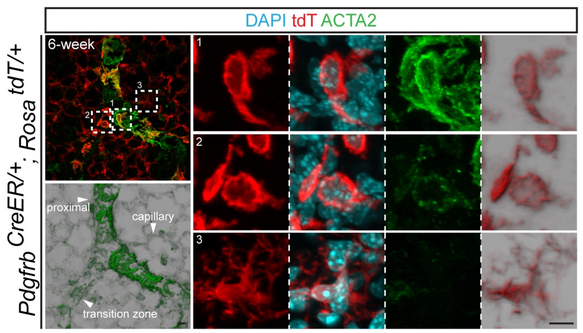


Figure 2. Vascular axis. Vascular smooth muscle cells envelop blood vessels and transition to pericytes that surround capillaries. Scale 10 um.

Ductal and alveolar myofibroblasts support the neonatal lung epithelium

Epithelial axis. The epithelial tree is comprised of the airway smooth muscle, ductal myofibroblast and alveolar myofibroblast.

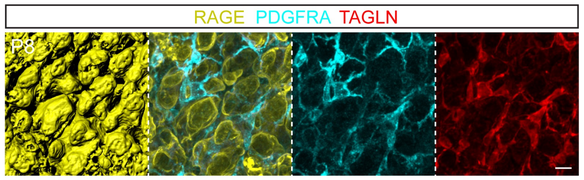


Figure 3. The alveolar myofibroblasts (PDGFRA) are wedged between alveolar septa and are known to drive alveolar septation.

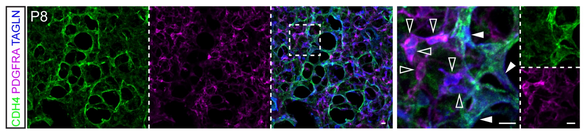


Figure 4. The ductal myofibroblasts (CDH4/HHIP) envelop alveolar ducts and their function is poorly understood.

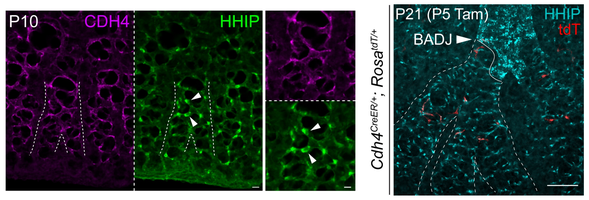


Figure 5. The ductal myofibroblasts persist into adulthood.

MEOX2-expressing mesenchymal cells occupy the lung interstitium

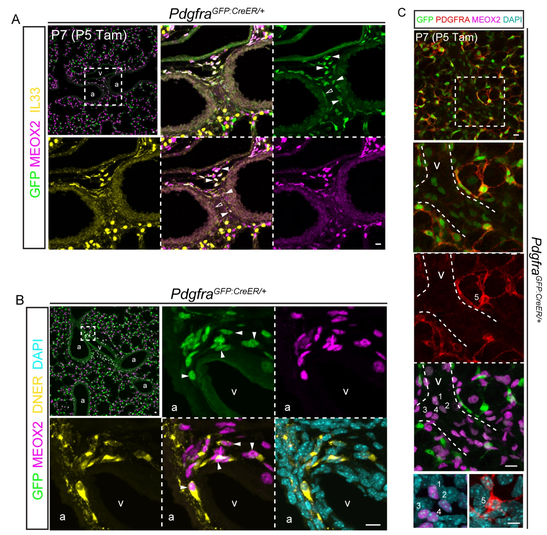


Figure 6. Interstitial Axis. Proximal and interstitial mesenchymal cells are distinct from PDFRA-expressing alveolar myofibroblasts.

ScRNA-seq reveals transcriptional differences after perinatal antibiotic exposure and bacterial infection

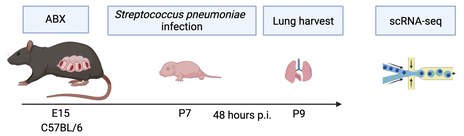


Figure 7. Experimental design. To pinpoint whether dysbiosis alters the transcriptional landscape after a respiratory infection within the lung mesenchyme, we infected mouse lungs with *Streptococcus pneumonaie*, a common bacterial pathogen that affects neonates.

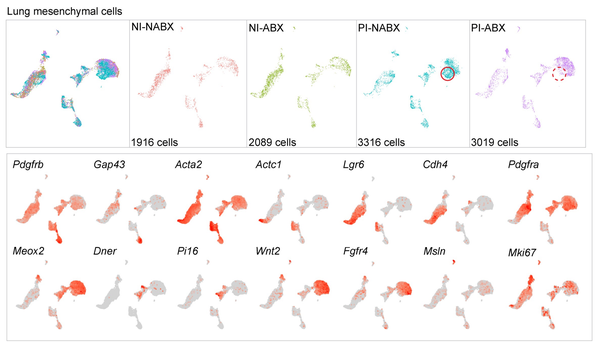


Figure 8. scRNA-seq analysis reveals a transcriptionally different subpopulation of distal interstitial cells after dysbiosis and *Streptococcus pneumoniae* infection.

Dysbiosis alters mesenchymal-mediated inflammatory response

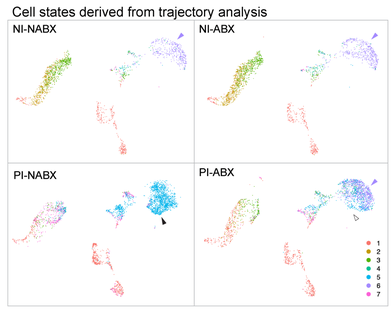


Figure 9. Trajectory analysis of the lung mesenchyme reveals cell states after dysbiosis and bacterial infection.

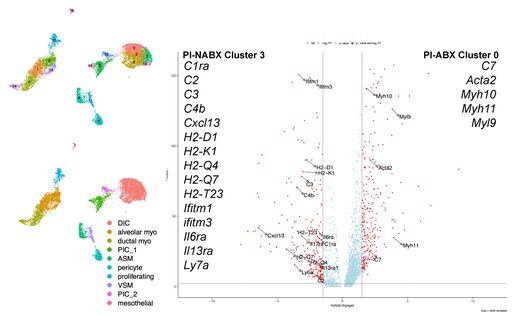
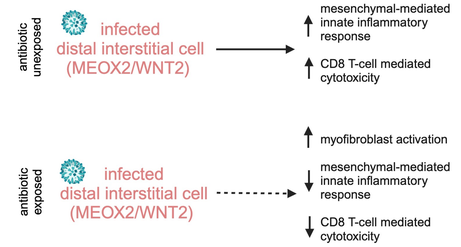


Figure 10. Dysbiosis downregulates innate inflammatory response and upregulates expression of smooth muscle genes in the distal interstitial cells.

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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