Malcolm Albergo-Radisch

HON 498

Contract Deliverable

5/1/26

This project, focused on the spatial dynamics of lung regeneration, is a research initiative conducted under the supervision of Dr. Jorge A. Piedrahita. During the semester, I maintained a laboratory schedule of Mondays (8:15 AM – 1:15 PM), Wednesdays (8:15 AM – 1:15 PM), and Fridays (1:00 PM – 2:30 PM), totaling around 11.5 hours per week, with Tuesdays reserved for makeup hours as needed.

LGR5-expressing mesenchymal cells have been identified as regulators of epithelial fate through Wnt and SHH signaling. Making the LGR5+ cells relevant to study around regenerative medicine and developmental biology. While their role in general tissue homeostasis is emerging, their specific spatial distribution across the proximal-to-distal pulmonary axis in the developing lung remains poorly defined. Understanding how these cell populations fluctuate between newborn (0-3 weeks) and juvenile (4–8 weeks) porcine models is critical for identifying regional regenerative capacities. This project utilizes a gene-edited LGR5-H2BGFP porcine model to map these cells and establish a framework for site-specific therapeutic strategies in pulmonary medicine.

My primary laboratory responsibilities involved analyzing lung tissue sections. Immunohistochemistry (IHC) was utilized to stain EPCAM (epithelial marker), ASMA (smooth muscle marker), and DAPI (nuclear marker) to resolve the spatial architecture of the peribronchial niche. Using confocal microscopy, high-resolution images were captured to track the H2B-GFP signal, allowing for the precise localization of LGR5+ fibroblasts relative to airway

structures. Through this work, I gained significant expertise in multi-channel fluorescent imaging and the handling of transgenic porcine tissues.

A major component of my work this semester was learning computational tools. I used QuPath to perform digital pathology analysis, specifically defining the peribronchial interstitial space and automating the detection of LGR5+ nuclei. I developed and documented a formal protocol for these techniques, including the use of H&E Trichrome staining to define fibrosis levels. To support the broader lab environment, I assisted in training others (both members of Piedrahita Lab and of other labs in the building) on these software tools.

Beyond image acquisition, I was responsible for the statistical rigor of the project. I generated relevant graphs and comparative statistics to quantify cell densities across varying airway dimensions and ages. My analysis aimed to validate the hypothesis of a regional gradient, specifically, that distal airways maintain a higher ratio of LGR5+ to LGR5- cells, suggesting a higher distal regenerative signaling capacity. As well as the temporal aspect of different ages having varied levels of LGR5+ cell density. I organized these findings into professional presentation materials, which were reviewed by the PI during regular one-on-one meetings and lab presentations. And some of these statistics made it into a grant proposal.

The conclusions drawn are that there is consistently high, ~80%, density of LGR5+ cells in the distal airways while there is a big drop off of LGR5 signal in the proximal airways between newborn and juvenile pigs. This is relevant because it suggests a reason as to why lungs get worse at repairing themselves with age and also presents the distal region as a good target of regenerative potential regardless of age.

This work establishes the foundation for my continued research in the summer, where I will expand the study to include fetal and aged/boar porcine lungs to complete a full lifespan atlas of LGR5 distribution in the airway. These efforts are directed toward a final manuscript

submission detailing the porcine lung's stromal development. Thank you for your interest in my work, please check out my poster for more information and images (I made the lab logo this semester also, top left on the poster).

-Malcolm Albergo-Radisch