20.440 Analysis of Biological Networks
Tyler Dao, Nicholas Hutchins
March 24, 2021

Characterizing Cellular Identities and Gene Expression Profiles in Axolotl Regeneration

The axolotl is unique amongst vertebrates in their ability to efficiently regenerate. Their cells maintain an embryonic-like cell characteristic which allows for fine tuned regeneration of multi-tissue structures across their lifespan. Previous studies have described the regenerative response as a migration of specialized wound epidermis (WE) to the injury site under which progenitor cells aggregate and form a blastema. The blastema is composed of lineage-restricted and multipotent progenitors that can differentiate to reform the structure of a regenerated limb or organ[1,2]. However, while histological analysis and transcriptomic studies have identified cellular migration and key genes in regeneration, key insights on the cellular population at the blastema and their interactions remain poorly understood[3].

Here, we propose to characterize the population heterogeneity of axolotl cells across the span of regeneration. We hypothesize that novel cell populations derived from the blastema express species specific genes and autonomous patterning and regeneration, offering one explanation for why regeneration is not conserved. To identify key cells in regeneration, we propose to profile the populations at the site of injury in regenerating limbs as compared to healthy tissue using single cell RNA sequencing, focusing on rare subpopulations that were previously not identified. Our aims for this project are as follows:

Aim 1. *Identify rare populations of cells at different time points during regeneration.* We anticipate observing new populations at each stage as the progenitor cells in the blastema differentiate. While the dataset has been annotated as described by canonical cell markers, we hypothesize that rare, novel cellular populations may be overlooked due to population heterogeneity. Therefore, we will explore models of representational learning to learn latent space representation of each cell type in hopes of finding new populations. The MARS autoencoder is a promising approach as it has been previously shown to effectively learn cellular representations for classification, particularly of transitioning cells in a longitudinal study. A potential pitfall of this approach is that representational learning may not identify new cell states; in this case, we will perform clustering to identify new subpopulations.

Aim 2. *Dissect the genetic drivers of cell state transition at different time points during regeneration.* We aim to characterize novel cellular populations present at each state of regeneration at a transcriptomic level. We will perform differential gene expression and a pseudotime analysis, paying attention to canonically described tissue-formation genes and tumor suppressor genes which drive and control proliferation. This is of clinical significance as regenerative processes have been implicated in the uncontrolled growth of cancer and targets of cancer therapeutics[4]. With key genes identified, we will then aim to characterize genetic networks by performing gene set enrichment analysis (GSEA) to determine the difference in corresponding gene sets across cellular states and Gene Ontology (GO) enrichment analysis to understand the functional profile of enriched sets to better parse the underlying biology[5].

For an initial analysis, we took the single-cell RNA-seq data set of the uninjured axolotl arm and identified clusters of genes that are involved in tissue formation across the clusters (TNMD which encourages chondrocyte growth, SPARC which calcifies bone, ASPN which inhibits chondrogenesis, HMGN2 - chromatin regulation). A further analysis comparing how cell populations change post amputation of the arm and which genes are regulated at each point would allow us to understand what genes regulate this process.

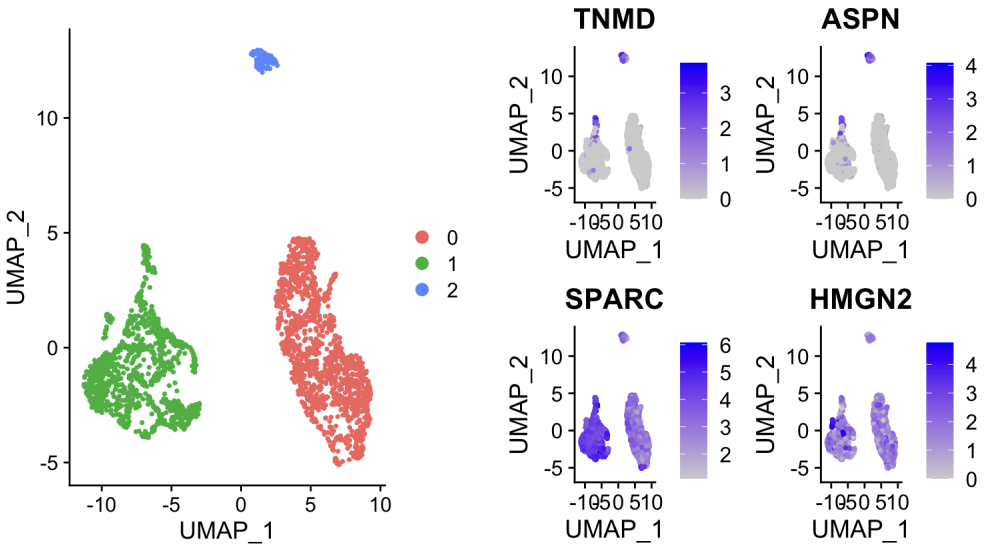

Figure 1. Clusters of cells in a UMAP projection of the uninjured axolotl arm. The expression of genes known to regulate tissue formation and were differentially expressed (TNMD, SPARC, ASPN, HMGN2) are overlaid on the clusters.

The data for this project is all published and publicly available and available in cell-by-gene matrices that can be read into standard scRNA-seq analysis libraries such as Seurat. A difficulty of this project may be trying to integrate data from multiple time points as well as inferring meaningful pseudotime trajectories for cells and identifying rare subpopulations. However, meaningful information can still be drawn from static pictures of each time point and a new single-cell tool MARS can be used to identify rare cell identities[6]. We hope to both identify rare subpopulations of cells and the tissue regulation genes that are present in those clusters to help to create a more comprehensive picture of axolotl regeneration.

By profiling novel cellular populations across the span of regeneration and identifying differentially expressed genes, our analysis will further our understanding of autonomous patterning. This will enable comparative studies between the human and axolotl genome to identify species specific genes that drive regeneration which could be translated to human health. Understanding the genomic drivers and regulators of controlled cell growth could translate into cancer therapeutics, identifying targets to modulate malignant neoplasia. Future work could further translate axolotl regeneration for *in vitro* growth of mammalian and vertebrate tissue and therapeutics for limb and tissue loss.

References

1. Leigh ND, Dunlap GS, Johnson K, et al. Transcriptomic landscape of the blastema niche in regenerating adult axolotl limbs at single-cell resolution. *Nat Commun*. 2018;9(1):5153.
2. Gerber T, Murawala P, Knapp D, et al. Single-cell analysis uncovers convergence of cell identities during axolotl limb regeneration. *Science*. 2018;362(6413).
3. Qin T, Fan CM, Wang TZ, et al. Single-cell RNA-seq reveals novel mitochondria-related musculoskeletal cell populations during adult axolotl limb regeneration process. *Cell Death Differ*. 2021;28(3):1110-1125.
4. Oviedo NJ, Beane WS. Regeneration: The origin of cancer or a possible cure?. *Semin Cell Dev Biol*. 2009;20(5):557-564. doi:10.1016/j.semcdb.2009.04.005
5. Sibai M, Parlayan C, Tuğlu P, Öztürk G, Demircan T. Integrative Analysis of Axolotl Gene Expression Data from Regenerative and Wound Healing Limb Tissues. *Sci Rep*. 2019;9(1):20280. Published 2019 Dec 30. doi:10.1038/s41598-019-56829-6
6. Brbić, M., Zitnik, et al. MARS: discovering novel cell types across heterogeneous single-cell experiments. *Nature methods*. 2020, *17*(12), 1200–1206.