Exploring the Effects of Lamins and Microtubules on Cellular Gene Expression in the Mouse Dental Incisor Epithelium Using scRNA-seq

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Abstract

The maintenance of many adult organs depends on the function of somatic stem cells, which undergo tightly regulated processes of self-renewal and differentiation in response to genetic and signaling cues. Generations of mice lacking lamins or microtubules in the incisor epithelium revealed that these structural proteins are critical for stem cell shapes and arrangements. This led us to perform single-cell RNA sequencing (scRNA-seq) to determine if mutant incisors also exhibit differentiation defects and fate changes. Clustering at a low resolution revealed 19-20 individual clusters, with 2-3 epithelial cell clusters in both the lamin and microtubule mutant mice. Subsetting out the epithelial cells and reclustering at a higher resolution, we further analyzed gene set enrichment with Metascape, cell lineages with Dynverse, cell-cell communication with CellChat, and transcriptional regulatory networks with SCENIC. These findings provided insights into how the absence of lamins and microtubules affects epithelial gene expression in the mouse incisor.

Background

The mouse mandible tissue samples we collected include various cell types, such as immune, mesenchymal, and glial cells, which are not pertinent to our analysis. Epithelial cells are isolated from this sample and classified based on their maturation stage at the time of data collection.

Enamel regeneration in the mouse incisor begins with cells in the labial cervical loop. Dental epithelial stem cells (DESCs) remain quiescent until new cells are needed to replace mature, enamel-secreting epithelial cells known as ameloblasts. As DESCs differentiate and move toward the distal end of the tooth, they first become transit-amplifying cells (TACs). These cells continue to proliferate and progress distally as they mature, transitioning into pre-ameloblasts and eventually into fully differentiated ameloblasts. Distinct gene expression patterns characterize each stage in the epithelial lineage. Accurately identifying the specific cell types from which the RNA is derived enhances the precision of comparisons between the control and mutant populations.

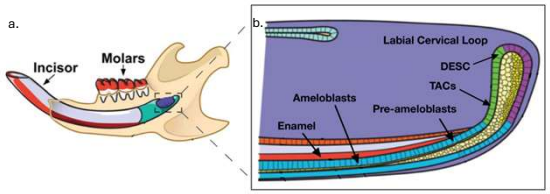

Figure 1. (a) Mouse Mandible and **(b)** Labial Cervical Loop Labeled[1]

Methods

- Quality Control
- UMAP Integration
- Dynverse: Cell Trajectory
- Dynverse: Gene Expression
- CellChat: Signaling Interactions

Results

Number of Microtubule scRNA-seq Samples:
QC: 18,739 → 14,298
Epithelial: 2,645

Number of Lamin scRNA-seq Samples:
QC: 15,703 → 14,617
Epithelial: 1,017

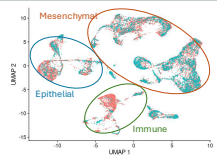

Figure 2a. Microtubule Integrated Clusters Labeled[2,3]

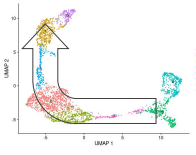

Figure 2b. Microtubule Epithelial Subcluster with Cell Trajectory

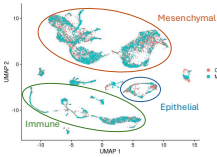

Figure 2c. Lamins Integrated Clusters Labeled[2,3]

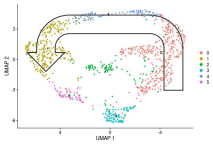

Figure 2d. Lamins Epithelial Subclusters with Cell Trajectory

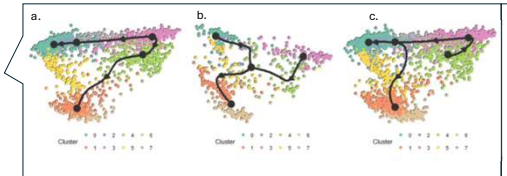

Figure 3. (a) Microtubule Control, **(b)** Mutant, and **(c)** Combined Cell Trajectories

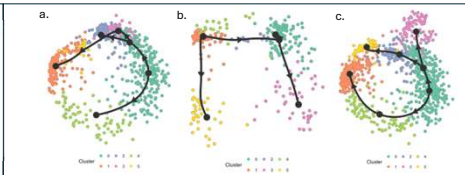

Figure 4. (a) Lamins Control, **(b)** Mutant, and **(c)** Combined Cell Trajectories

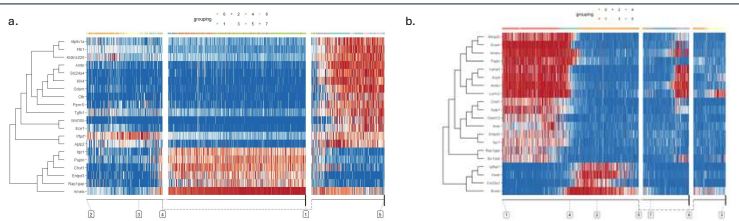

Figure 5a. Microtubule Gene Expression Patterns by Cluster & Trajectory

Figure 5b. Lamin Gene Expression Patterns by Cluster & Trajectory

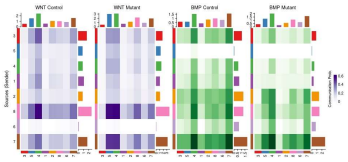

Figure 6. (a) Microtubule Control and **(b)** Mutant WNT Signaling. **(c)** Microtubule Control and **(d)** Mutant BMP Signaling.

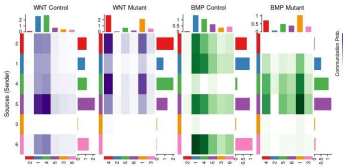

Figure 7. (a) Lamins Control and **(b)** Mutant WNT Signaling. **(c)** Lamins Control and **(d)** Mutant BMP Signaling.

Conclusions

- The two control populations (microtubules and lamins) exhibited unanticipated differences
- Cell trajectories consistently branched into two mature, unidentified ameloblast populations under both conditions
- Signaling interactions differed between control and mutant cells in immature populations under both conditions
- Various biological reasons may explain differences in control and mutant epithelial numbers
- Further analysis is necessary to decipher differences in gene expression under knockout conditions

References

1. Kuang-Hsien Hu, J., Mushegyan, V., & Klein, O. D. (2014). On the cutting edge of organ renewal: identification, regulation, and evolution of incisor stem cells. *genesis*, *52*(2), 79-92.
2. Krivanek, J., Soldatov, R. A., Kastriti, M. E., Chontorotzea, T., Herdina, A. N., Petersen, J., ... & Adameyko, I. (2020). Dental cell type atlas reveals stem and differentiated cell types in mouse and human teeth. *Nature communications*, *11*(1), 4816.
3. Sharir, A., Marangoni, P., Zilionis, R., Wan, M., Wald, T., Hu, J. K., ... & Klein, O. D. (2019). A large pool of actively cycling progenitors orchestrates self-renewal and injury repair of an ectodermal appendage. *Nature cell biology*, *21*(9), 1102-1112.