Investigating the effects of microtubules on gene expression in the mouse dental epithelium using scRNA-seq

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Abstract

Understanding the stem cell's behavior within the niche in response to the environmental cues is pivotal in discerning the mechanisms regulating regeneration. The mouse incisor as the stem cell model provides valuable information on the cells' behavior and organization. The epithelial and mesenchymal stem cells within the dental pulp that arise at the proximal end of the incisor enable a sustained cell cycling of cells that allows for continuous regeneration of the incisor. These cell populations are governed by molecular signaling and mechanical forces that influence their proliferative and differentiation potentials. Mechanical forces affecting the organization are dependent on the cell shape and packing. Microtubules are a crucial cytoskeletal component that contributes to cell shape, among other functions. The severing of microtubules through spastin overexpression perturbed cell shape, affecting cellular organization. To identify the effects of severed microtubules, we combined Seurat, CellChat, and SCENIC workflows to analyze the transcriptional landscape of the affected pathways. This analysis elucidated the transcriptional regulatory networks and modulation of cell population functions in the epithelial tissue layer as a consequence of microtubule perturbation. In studying the signaling interaction we showcased by perturbing cell shape and packing, triggers a cascade of transcriptional changes across the epithelial cell populations, ultimately leading to a widespread failure in cell differentiation, proliferation, and metabolic function, thereby compromising the regenerative capacity of the mouse incisor. We elucidate the mechanistic consequences of microtubule disruption on oral epithelial regeneration, highlighting its influence on cellular behavior.

Spastin Overexpression – Ameloblast Populations

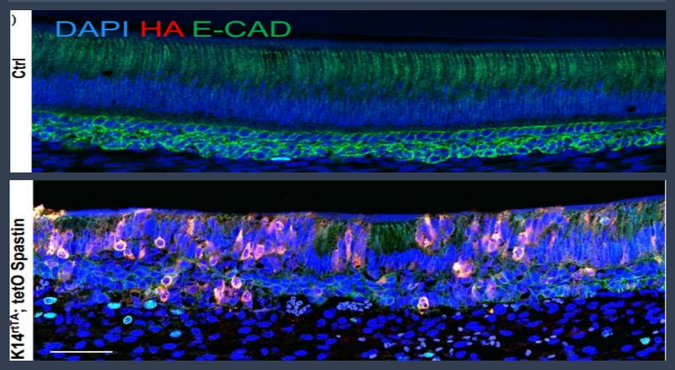

Figure 1 Doxycycline fed mice induced genetic overexpression of the Microtubule severing protein, Spastin. Validated with the expression of Hemagglutinin (HA) . Revealed loss of adherence protein – E-Cadherin in the ameloblasts.

Methods

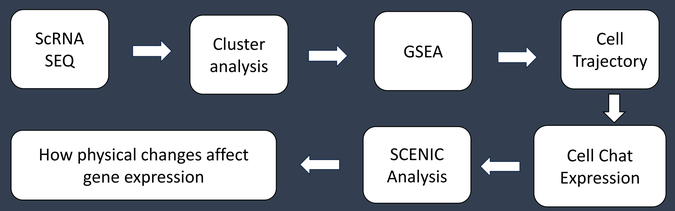

Figure 2 For gene expression studies, we used Seurat to examine epithelial clusters, fgsea R package with gene sets from MsigDB, Dynverse to examine cell lineages, CellChat to examine cell-cell communication, and SCENIC to examine transcriptional regulatory networks.

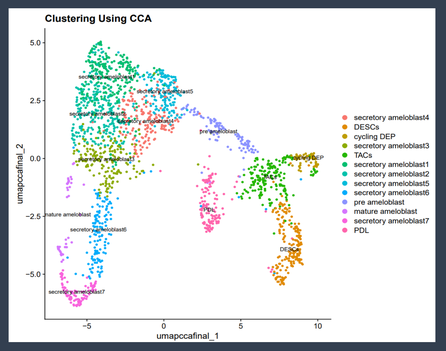

Figure 3 Enamel regeneration in the mouse incisor begins with the cells in the labial cervical loop. DESCs replace mature enamel-secreting epithelial cells known as ameloblasts. As DESCs differentiate and move distally they become TACs. These cells continue to progress transitioning into pre-ameloblasts, proliferate into secretory ameloblasts, and finally mature ameloblasts.

Loss of Dental Epithelial Populations in Response to Microtubule Severance

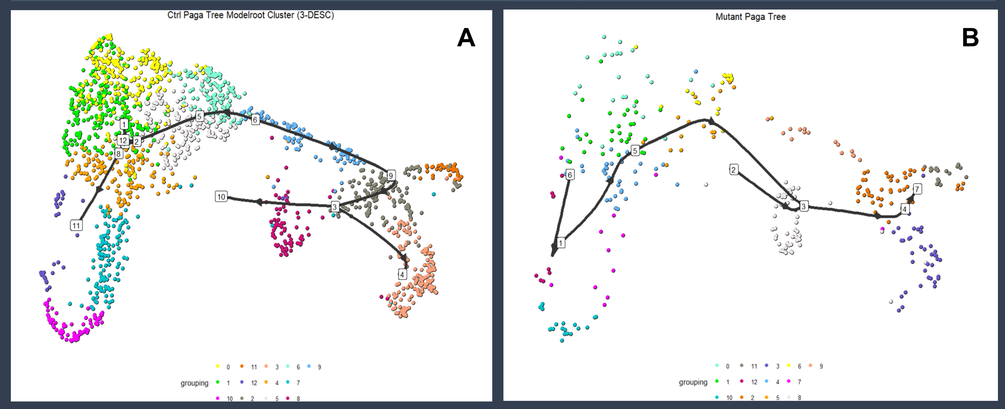

Figure 4 The oral epithelium is progressively differentiated into mature populations along the loop (A). Epithelial cells are initially more proliferative, but shift towards other fates. Spastin overexpression (B) reveal loss of secretory and mature cell populations due to microtubule severance, stem cell populations remain relatively intact.

Transcriptomic Response to Microtubule Depletion in Dental Epithelium

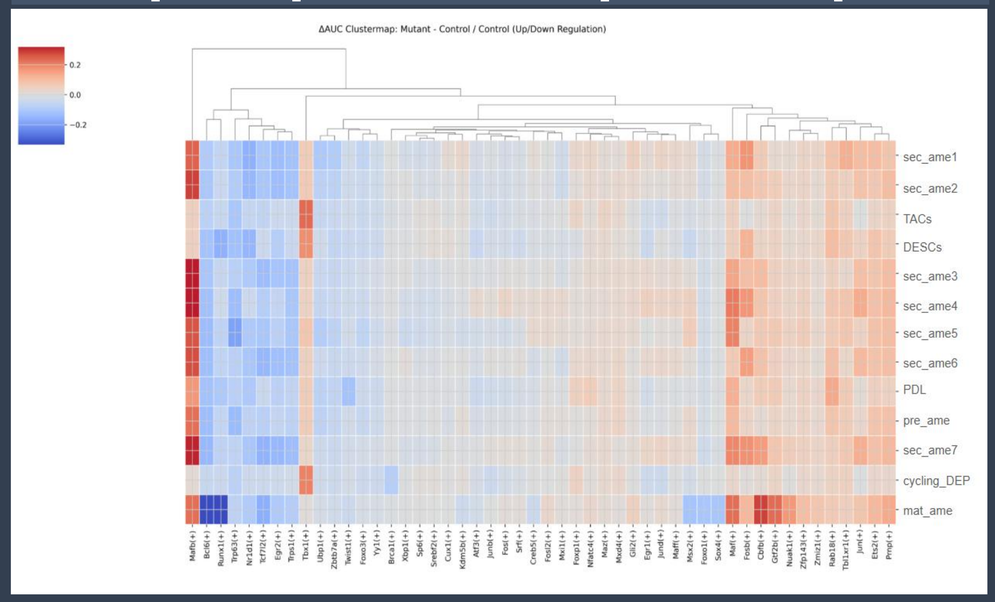

Figure 5 Tbx1 is progressively upregulated in the mutant TACs, DESCs, and cycling DEP in reaction to other pathways involved in cell fate, differentiation, and proliferation.

Cell – Cell Interactions Summary

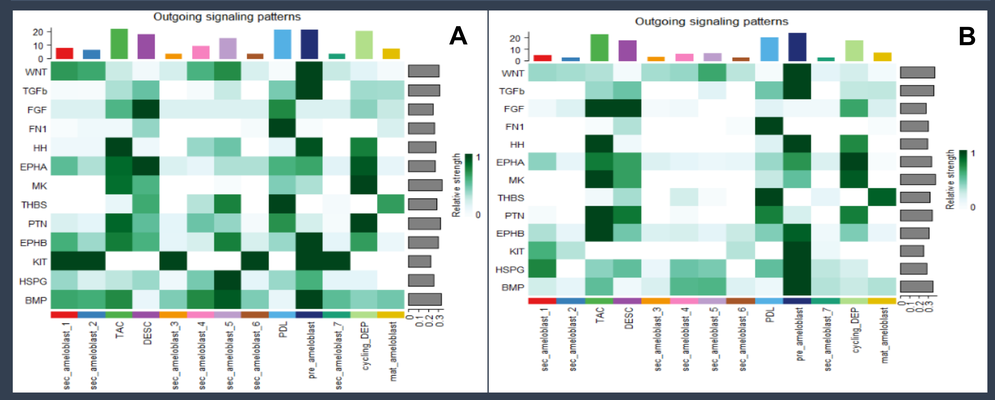

Figure 6 Cell Chat for systematic analysis of cell-cell communication reveals distinct downregulation of out signaling in secretory ameloblast populations (B), particularly in the HSPG pathway. In response, pre ameloblasts upregulate HSPG, potentially due to loss of cell adhesion in the ameloblasts.

HH Pathway Compensation by Pre Ameloblasts

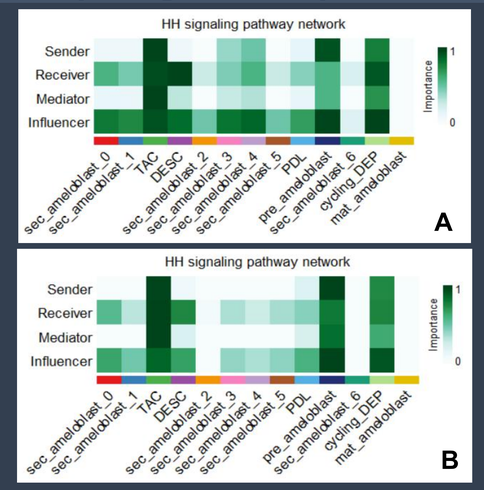

Figure 6 Reduction in Sonic Hedgehog Pathway genes that influence cell growth in ameloblasts, influence the upregulation HH in the TACs and pre ameloblasts.

GSEA – Pathway Enrichment

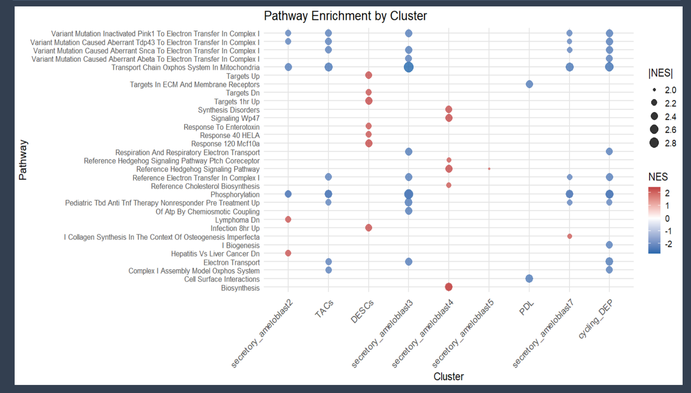

Figure 7 Dot plot showing Gene Set Enrichment Analysis (GSEA) results for selected biological pathways across identified cell clusters. Showcases, continuous downregulation of Hedgehog Pathway related genes. Likely, an effect, is the increase of mitochondrial activity to meet heightened energy demands for cellular reorganization or repair of the cilia.

Conclusions

- Loss of microtubules and their transcriptional effects were prominent in the pre ameloblast and ameloblast populations
- Loss of expression in the DESC cascade into changes of expression in TACs and cycling DEP
- Downregulation of transcription factors such as Sox4, Runx1, Trp63, Msx2 suggest disturbance in **epithelial stem cell maintenance and differentiation**
- **Future studies can investigate the relationship between cell arrangement and cell shape**

References

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