# The Role of Nuclear Lamins in Regulating Mouse Incisor Regeneration

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## ABSTRACT

The mouse incisor grows continually due to stem cells in the epithelial and mesenchymal tissue of the incisor. The epithelial stem cells differentiate into transit-amplifying cells (TACs), which become pre-ameloblasts and enamel-secreting ameloblasts. These populations exhibit distinct nuclear rigidity levels and cell behavior. This study investigates the role of nuclear lamins (A/C, B1, B2) in nuclear shape and cellular function. Epithelial knockout of all three isoforms resulted in pre-ameloblasts with shortened nuclei and altered nuclear spatial organization. ScRNA-seq of epithelial cells from control and knockout mice yielded 11 cell clusters. Gene-set enrichment of the clusters revealed upregulation of genes related to nuclear and cellular integrity. Cell trajectory analysis showed forking of the triple-knockout differentiation pathway at a relatively TAC-like cluster compared to the same control cluster. These findings help explain the triple-knockout phenotype and associate lamin depletion with cellular plasticity, supporting a role for nuclear rigidity in regulating cell differentiation.

## BACKGROUND

The incisor exhibits compensatory regeneration through adulthood to compensate for ongoing abrasion at the tip of the incisor. Regeneration of enamel and dentin occurs due to dental epithelial stem cells (DESCs) and mesenchymal stem cells respectively. DESCs in the labial cervical loop differentiate into TACs, which become pre-ameloblasts and later ameloblasts that secrete enamel. Mesenchymal stem cells in the dental pulp differentiate into pre-odontoblasts and odontoblasts, which secrete dentin.

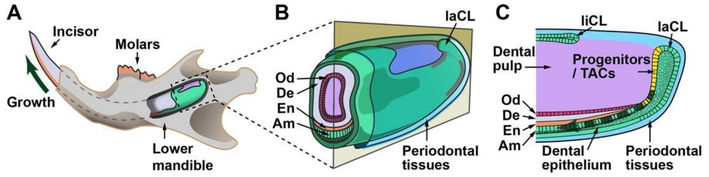


**Fig 1. Position and structures of incisor in mouse mandible.[3]**

The cell types in the pathway from DESCs to ameloblasts exhibit distinct nuclear rigidity levels as well as differentiation states. We showed that knockout of all three lamin isoforms (A/C, B1, B2) in the K14$^{CreER}$, R26$^{mTmG}$ mouse model ("mutant") resulted in pre-ameloblasts with shortened nuclei and altered organization of the nucleus. E-cadherin expression patterns are also altered.

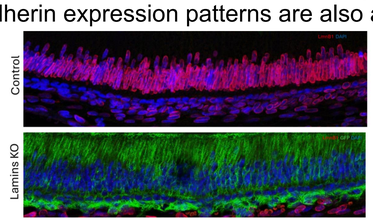


**Fig 2. Pre-ameloblasts in the laCL under control and mutant conditions. DAPI-stained nuclei in mutant are shortened and disorganized with a pseudostratified appearance.**

## RESEARCH QUESTION

**How do nuclear lamins (A/C, B1, B2) regulate cell behavior and cellular plasticity?**

## APPROACH

- Single-cell RNA sequencing to sequence epithelial and mesenchymal cells from the control and mutant lower incisor
- Clustering using CCA and cell type annotation
- Differential expression and gene set enrichment analysis
- Cell trajectory analysis using PAGA_TREE model
- Gene regulatory network analysis using SCENIC
- Cell-to-cell communication using CellChat

## Clustering reveals absence of a transition population in mutant epithelium

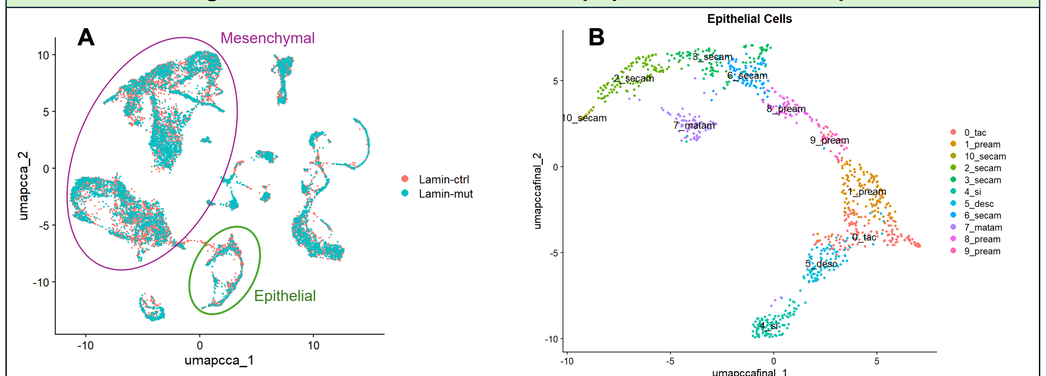


**Fig 3. (A) CCA cluster plot of control and mutant datasets. Manual annotation of epithelial and mesenchymal cell clusters. Additional control cluster "bridge" between epithelial and mesenchymal clusters, visually indicating epithelial-mesenchymal plasticity. Top 50 genes in expression profile of "bridge" cluster compared to other epithelial clusters includes epithelial-to-mesenchymal transition (EMT) and mesenchymal markers with p values << 0.001, such as Twist1, Snai1, Foxf1, Foxf2, Osr2, and Tbx3. Lamin knockdown has been shown to impact EMT, so it is possible the transition population is EMT.[7] (B) CCA cluster plot of epithelial cells. 11 clusters shared in both control and mutant. Continuous progression of clusters from SI to ameloblasts supports current understanding of differentiation pathway in labial cervical loop and importance of differentiation pathway in defining cell types.**

## Cellular plasticity indicators in mutant differentiation pathway

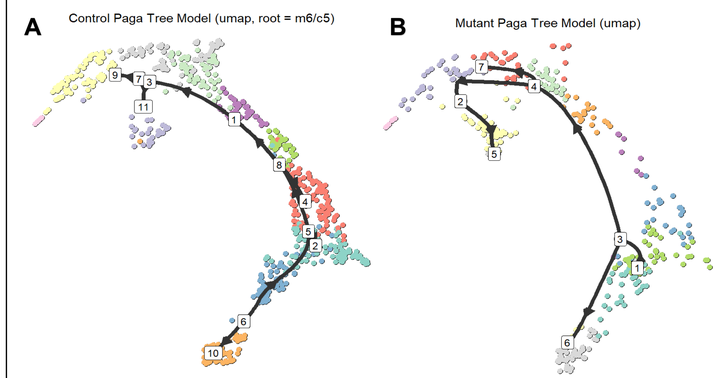


**Fig 4. Cell trajectory analysis of control (A) and mutant (B) epithelial cells. Numbers indicate milestone IDs. Control trajectory follows expected SI ← DESC → TAC → pre-ameloblast → secretory ameloblast → mature ameloblast with slight branching at mID 3. Mutant trajectory exhibits similar overall direction with additional branching at mID 4 (cluster 6). Mutant cluster 6 expresses some TAC-like genes with more significance and fold-change than control cluster 6, including Foxi3. Uhrf1 (related to stem cell fate) is also expressed more highly in mutant cluster 6.**

## Diverse Gene Regulatory Networks Altered in Mutant Epithelium

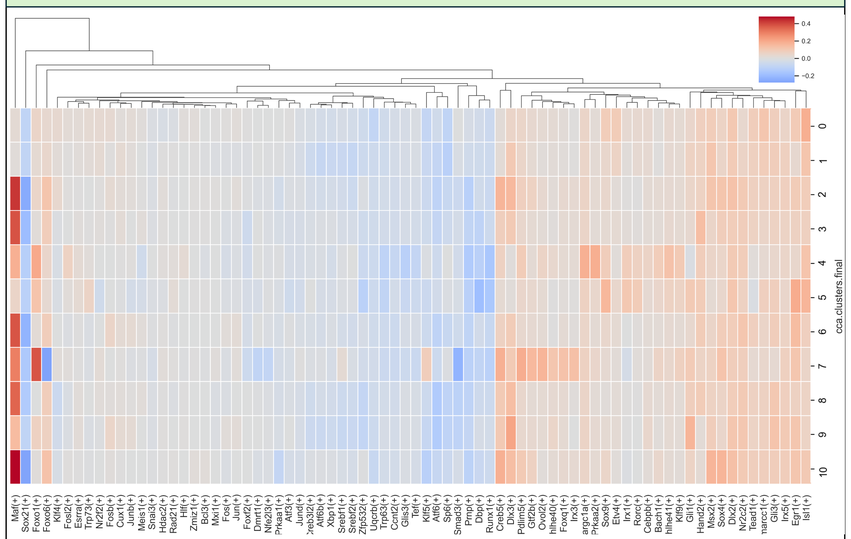


**Fig 5. Up (red) and down (blue) regulation of gene regulatory networks in mutant epithelial cells. Altered GRNs include Maf, which are expressed in developing ameloblasts and have diverse functions in cell development and differentiation[5], and Sox21, which is believed to regulate dental epithelial differentiation by inhibiting EMT through Anapc10.[6]**

## Mutant compensation for lamin deletion

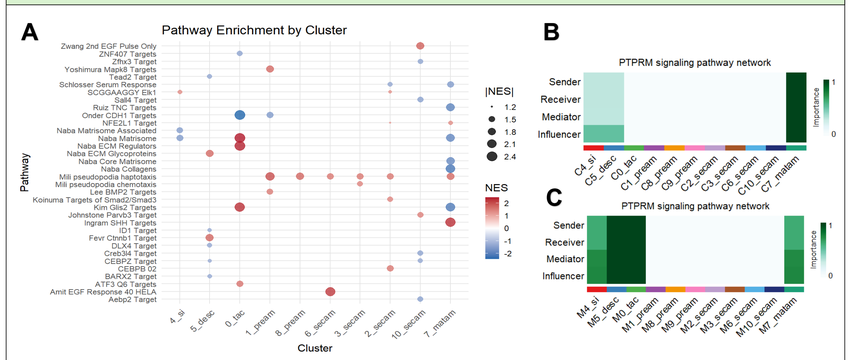


**Fig 6. (A) Selected gene set enrichment analysis results of mutant vs control epithelial clusters. Red = upregulated in mutant. Upregulated pathways include cellular integrity pathways (Naba Matrisome, ECM Regulators), pointing to possible compensation for loss of lamins and explaining relatively mild phenotype associated with lamin knockdown. Upregulation of cell adhesion pathways (ex. ECM Glycoproteins) may compensate for dysregulation of E-cadherin in mutant. PTPRM signaling pathway between control (B) and mutant clusters (C). PTPRM (involved in homophilic adhesion) upregulated in mutant DESCs and TACs, possibly compensating for dysregulation of E-cadherin.**

## Summary of Outgoing Cell Signaling Pathways in Epithelium

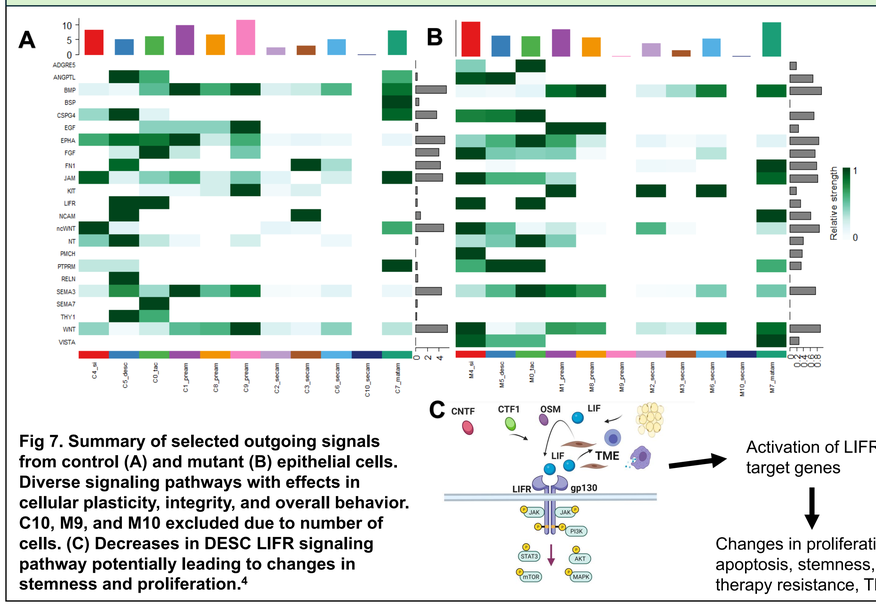


**Fig 7. Summary of selected outgoing signals from control (A) and mutant (B) epithelial cells. Diverse signaling pathways with effects in cellular plasticity, integrity, and overall behavior. C10, M9, and M10 excluded due to number of cells. (C) Decreases in DESC LIFR signaling pathway potentially leading to changes in stemness and proliferation.[4]**

## CONCLUSIONS

- Mutant cells may partially compensate for loss of lamins through upregulation of cellular integrity and cell adhesion pathways and signaling networks, reducing the severity of the observed phenotype
- Indications of changes in cellular plasticity and differentiation between control and mutant, including "bridge" cluster, expression of Foxi3, Maf and Sox21 regulatory networks, and additional branching of differentiation pathway
- Findings support possibility of a link between nuclear rigidity and cell differentiation
- Upregulated genes and pathways provide a guide for future experiments to explore and confirm results

## REFERENCES

[3] Hu et al, *Genesis* (2013)
[4] Viswanadhapalli et al, *Genes and Diseases* (2022)
[5] Pemberton et al, *Developmental Dynamics* (2015)
[6] Saito et al, *iScience* (2020)
[7] Jia et al, *Journal of Experimental Medicine* (2019)

## CONTACTS

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