Defining the identities and the origins of two distinct types of cementoblasts

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Objectives: The tooth root is covered by thin acellular cementum (AC) and thick cellular cementum (CC), and the AC is important for periodontal tissue regeneration. However, fundamental characteristics of two different types of cementoblasts remain unknown. Previously, we found that *Pthrp* is a specific marker for cementoblasts. The aim of this study was to characterize AC and CC cementoblasts and to determine their origin.

Experimental Methods: We used *Pthrp-mCherry* reporter mice to label cementoblasts and isolated fluorescently labeled cells at 6, 15 and 25 days and 8 weeks postnatally. The FACS sorted cells were subjected to a single cell analysis platform (10X Genomics) and the datasets were integrated using LIGER. To identify the cellular origin of cementoblasts, we used the tamoxifen-inducible Wnt inhibitory factor 1 (*Wif1*)-creER knock-in mouse model to perform *in vivo* cell lineage tracing of *Wif1*$^+$ cementoblast precursor cells. Tamoxifen was injected at 6, 15, 25 days and 8 weeks postnatally and analyzed at 9 weeks.

Results: *PTHrP-mCherry*$^+$ dental follicle (DF) cells differentiate into *Osteocalcin* (*Ocn)*$^+$ cementoblasts on the AC and CC. The UMAP showed two distinct clusters that were determined to be AC cementoblasts and CC cementoblasts, and *Wnt5a* was specifically upregulated in the cluster of AC cementoblast cluster. The RNA velocity revealed a putative point of cell origin where *Wif1* was highly expressed. *Wif1-creER*-marked *tdTomato*$^+$ cells were localized to the peri-epithelial apical mesenchyme at postnatal day 6, and we found that these *tdTomato*$^+$ cells give rise to cementoblasts on both AC and CC. In contrast, the contribution of *Wif1*$^+$ cells to cementoblasts on AC progressively decreased when these cells were pulsed at later stages.

Conclusion: Our results demonstrate that cementoblasts on the AC represent a unique matrix-producing cell type that can be generated only during the initial stage of tooth root formation.

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