

**Spatial Multiomics Reveal Pax9-dependent Wnt Signaling Fine Tunes Palatal Osteogenesis**

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Multiple genetic and environmental etiologies contribute to the pathogenesis of cleft palate, which constitutes the most common among the inherited disorders of the craniofacial complex. Insights into the molecular mechanisms regulating osteogenic differentiation and patterning in the palate during embryogenesis are limited and needed for the development of innovative diagnostics and cures. Our recent work has defined the transcriptomic basis of murine secondary palate development using bulk, single-cell, and whole-transcriptome spatial RNA-sequencing technologies. This multimodal approach enabled the identification of several novel enriched genes at specific developmental time points. We then differentially analyzed and expanded this baselined palate development roadmap using high-throughput spatial RNA localization and single-cell multiomic technologies in comparison to a mouse genetic model organism engineered to lack the Pax9 transcription factor, critical for orchestrating Wnt/β-catenin signaling in the secondary palate, without which results a consistent phenotype of cleft secondary palate. While prior research had identified upregulation of Wnt pathway modulators Dkk1 and Dkk2 in Pax9-/- palate mesenchyme, limitations of spatial resolution and technology restricted a more robust analysis. Here, we provide evidence of a distinct relationship between Pax9+ and osteogenic populations in the developing palate. Loss of

Pax9 results in spatially restricted osteogenic domains bounded by Dkk2, which normally interfaces with Pax9 in the mesenchyme. Taken together, Pax9-dependent Wnt signaling modulators influence osteogenic programming during palate formation, potentially contributing to the observed cleft palate phenotype. These modulators may prove to be viable therapeutic targets to augment palatal bone during surgical or minimally invasive interventions for cleft palate.