

**Spatial Transcriptomics and Lineage Analysis for Craniofacial Mesenchymal Fate Determination**

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The differentiation of post-migratory cranial neural crest cells (CNCCs) into distinct mesenchymal lineages is essential for proper craniofacial development. Here, we combined single-cell RNA sequencing (scRNA-seq) with a seqFISH-based spatial genomics approach to generate high-resolution, spatially resolved gene expression profiles of the developing palate and other craniofacial regions in mouse embryos. We systematically defined mesenchymal cell types by linking their transcriptomic profiles to spatial identities. Integrative analysis of spatial transcriptomic data from E12.5 to E15.5 further revealed that mesenchymal lineage specification occurs at or prior to the onset of palatogenesis. The raw and processed scRNA-seq data from embryonic palatal tissue (E12.5, E13.5, E14.5, E15.5, and E18.5), along with the corresponding seqFISH data from anterior and posterior embryonic head regions (E12.5, E13.5, and E15.5), have all been deposited in FaceBase under DOIs 10.25550/62-QZ1A and 10.25550/62-Y0VT, respectively.