Spatial Transcriptomics and Lineage Analysis for Craniofacial Mesenchymal Fate Determination

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INTRODUCTION

The differentiation of post-migratory cranial neural crest cells (CNCCs) into spatially and genetically distinct mesenchymal subpopulations is critical for craniofacial development. In this study, using palate development as a model, we aim to establish a real-time, high-resolution spatial transcriptomic and cell-type atlas to reveal the diversification of CNCC-derived mesenchyme during craniofacial development.

MATERIALS AND METHODS

Single-cell RNA sequencing was conducted on mouse palatal shelves at E12.5, E13.5, E14.5, E15.5, and E18.5. SeqFISH spatial genomics was performed on cryosections from E12.5, E13.5, and E15.5 using a custom gene panel. For cell lineage tracing, all pregnant female mice received tamoxifen at a dosage of 1.0 mg/10 g or 1.5 mg/10 g body weight.

RESULTS

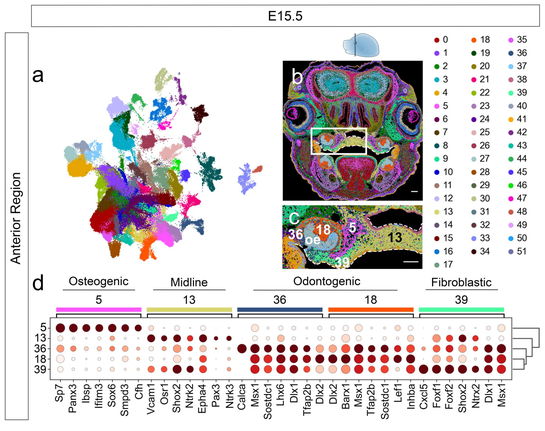

Fig. 1. Spatial mapping of mesenchymal subpopulations in the E15.5 hard palate region using seqFISH.

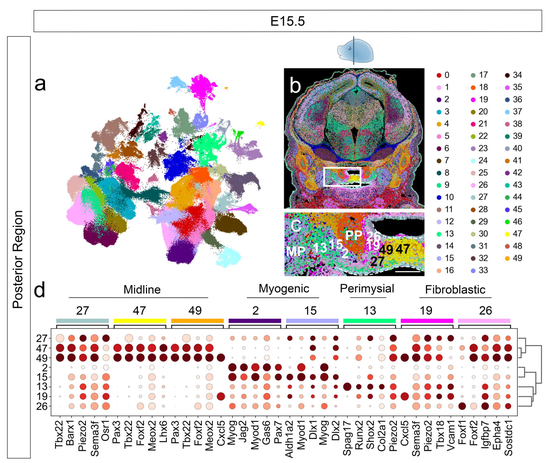

Fig. 2. Spatial mapping of mesenchymal subpopulations in the E15.5 soft palate region using seqFISH.

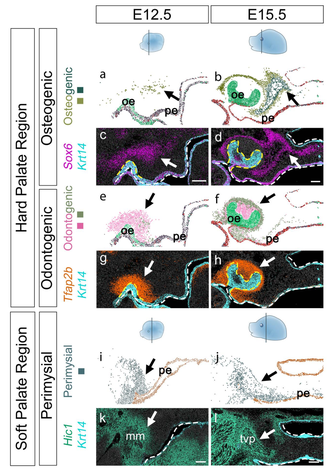

Fig. 6. SeqFISH analysis reveals expression patterns of early mesenchymal lineage markers at E12.5.

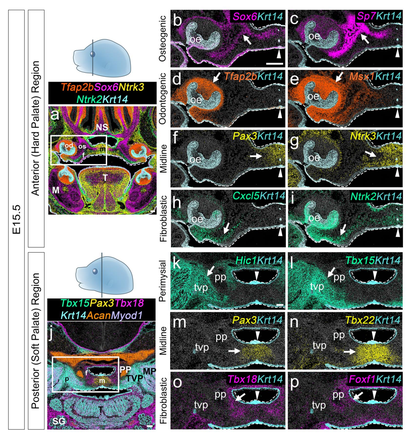

Fig. 3. Spatial mapping of markers for mesenchymal subpopulations in the E15.5 palatal region using seqFISH.

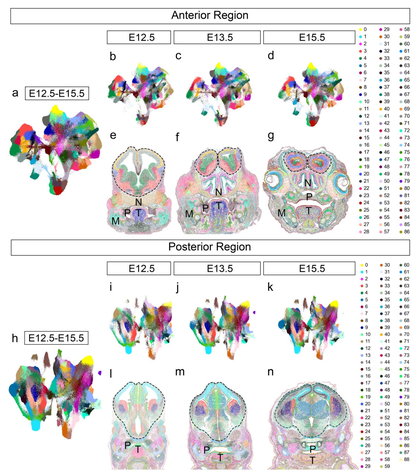

Fig. 4. Cell type mapping of anterior and posterior craniofacial tissues through integrative analysis of E12.5-E15.5 (E12.5, E13.5, and E15.5) spatial genomics data.

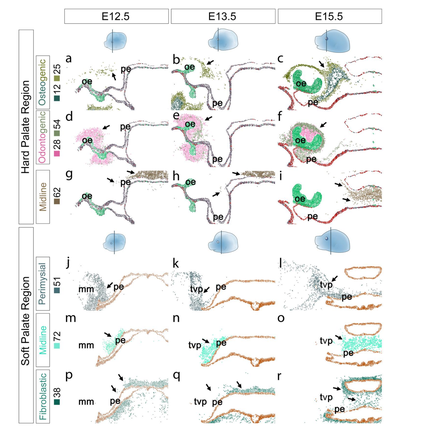

Fig. 5. Mapping of mesenchymal cell distribution during palate development using integrated analysis of E12.5, E13.5, and E15.5 spatial genomics data.

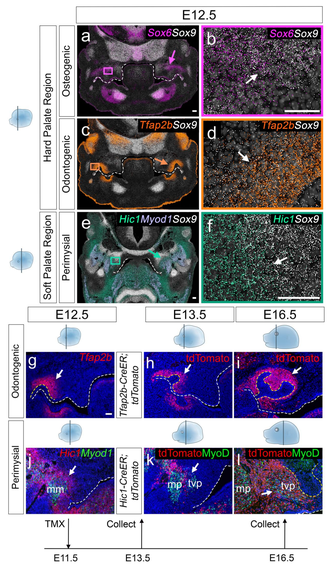

Fig. 7. seqFISH maps *Sox9*+ subsets at E12.5, with *Tfap2b* marking odontogenic and *Hic1* labeling perimysial cells, confirmed by lineage tracing.

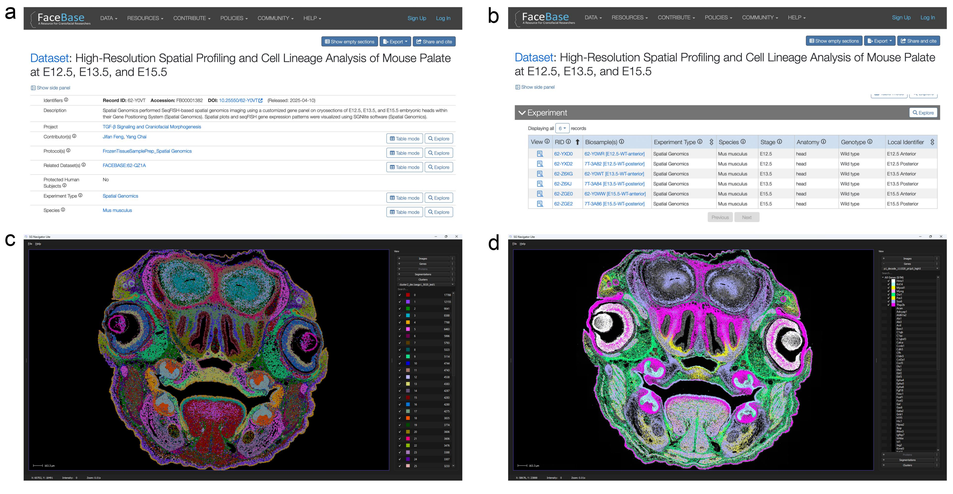

Fig. 8. The raw and processed sequencing-FISH data for embryonic heads at E12.5, E13.5, and E15.5 are available in the FaceBase database.

CONCLUSION

Our findings reveal the dynamic cellular positioning and gene expression patterns during the specification of CNCC-derived mesenchymal subpopulations throughout palatogenesis.

ACKNOWLEDGMENTS

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