Identification of Six2 as a cranial base synchondrosis-specific marker

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Objectives: The cranial base is crucial for craniofacial skeletal growth- containing the spheno-occipital synchondrosis (SOS) and the intersphenoid synchondrosis (ISS), sites of endochondral growth. Deficiencies in these structures lead to complications, including midfacial hypoplasia and skeletal Class III occlusions. The cranial base synchondroses are structurally similar to the epiphyseal growth plates of long bones, comprised of resting, proliferating, and hypertrophic chondrocytes. While the mechanism of cartilage formation in the growth plate is well studied, that of the cranial base synchondroses remains unclear. This project aims to identify the specific genes regulating SOS and ISS cartilage formation.

Methods: We analyzed single-cell RNA-sequencing (scRNA-seq) datasets of chondrocytes expressing fibroblast growth factor receptor 3 (FGFR3)-GFP in the cranial base synchondrosis and long bone of mice at three stages: postnatal 0 (P0), P6, and P12. Double transgenic mice carrying *Six2-creER* and *R26R-tdTomato* reporter alleles received one dose of tamoxifen (0.25mg) at P0 or P5 to trace the cell fate of Six2-positive cells in the cranial base synchondrosis.

Results: ScRNA-seq analysis revealed that *Six2* was highly expressed in the cranial base synchondroses at the three stages. Analysis of *Six2-creER*; *R26R-tdTomato* mice revealed that approximately 10-20% of resting and proliferating chondrocytes in the cranial base (ISS and SOS) expressed *Six2*, whereas no *Six2* expression was observed in the long bone. Six2 had greater expression in the ISS than in the SOS. Cell fate analysis revealed that a subset of $Six2^+$ cells formed columnar chondrocytes at P21 in the synchondrosis, suggesting that $Six2^+$ chondrocytes have stem cell ability.

Conclusion: Six2 is specifically expressed in the cranial base synchondrosis, particularly among resting and proliferating chondrocytes of the ISS. We aim to further investigate whether Six2 specifically regulates neural crest-derived chondrocytes in the ISS. We are working on generating Six2 knockout mice to define Six2's role in synchondrosis development.

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