Identification of synchondrosis-specific genes contributing to cranial base cartilage formation

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Objectives: The cranial base drives craniofacial growth and determines overall craniofacial skeletal patterns. Deficiencies in cranial base growth cause midfacial hypoplasia and skeletal Class III malocclusions. The cranial base is formed by endochondral ossification mediated by unique bidirectional growth plates termed the synchondroses, which are structurally similar to the long bone growth plates with distinct layers of resting, proliferating and hypertrophic chondrocytes. The objective of this study is to identify the genes that are uniquely expressed by chondrocytes of the cranial base synchondrosis.

Experimental Methods: We analyzed single-cell RNA-sequencing (scRNA-seq) datasets of cells of the cranial base synchondrosis and the long bone growth plate in mice, at three distinct stages of postnatal 0 (P0), P6 and P12, which represent before and after the formation of the epiphyseal stem cell niche. Chondrocytes expressing *fibroblast growth factor receptor 3 (Fgfr3)-GFP* were isolated from the spheno-occipital synchondrosis (SOS) and femur growth plate by fluorescence activated cell sorting (FACS) and subjected to the 10X Genomic Chromium platform. R packages Seurat and LIGER were employed to computationally analyze scRNA-seq data. Subsequently, immunohistochemistry was performed to determine the *in situ* expression pattern of the genes that were specifically expressed in the cranial base.

Results:. Genes encoding several key transcription factors, such as *Pax1, Pax9*, and *Six2*, were highly expressed in the SOS. Immunohistochemistry demonstrated that Pax1 was expressed by resting and proliferating chondrocytes in the SOS, but not by those of the long bone growth plate.

Conclusion: The unique characteristics of cranial base chondrocytes may be determined by a set of genes that are specifically expressed by these cells, such as those identified by this study. Future studies are warranted to interrogate the *in vivo* function of the cranial base synchondrosis-specific “metagenes” identified in the current study using mouse genetics approaches.

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