# Multi-omics Analyses Reveal the Skeletal Progenitor Cells that Drive Long Bone Growth at Different Postnatal Stages in Mouse Growth Plates

Jingyu He[1], Courtney Leong[1], Fangzhou Bian[1], Holly Katherine Johnson[1], and Zhaoyang Liu[1]
[1]University of Southern California, Los Angeles, CA.
Email of Presenting Author: hejingyu@usc.edu **Disclosures:** The authors have nothing to disclose.

**INTRODUCTION:** The cartilage growth plate is a specialized structure that plays an essential role in the linear growth of the long bone. Growth plate defects can severely affect the height of the long bone and lead to short stature, affecting about 2.5% of the population. The quality of life (QoL) in individuals with short stature is significantly worse and causes a tremendous socioeconomic burden. Therefore, an in-depth understanding of the molecular mechanisms that regulate growth plate activities, especially the progenitor cell populations that affect adolescent growth spurt, warrants further investigation. In this study, we integrate single-cell RNA sequencing and spatial transcriptomics (10xGenomics Visium HD) in combination with mouse genetics and pharmacological approaches to uncover the complexity of the postnatal growth plate cell landscape and reveal that the G-protein coupled receptor (GPCR)-mediated Gs/cAMP signaling promotes long bone growth in distinct cell populations of the growth plate at different ages.

**METHODS**: Mice overexpressing a Gs-coupled DREADD (Designer Receptor Exclusively Activated by Designer Drugs, called GsD) in growth plates were generated by crossing *Rosa-GsD*$^{f/+}$ mice with the *Col2Cre*, *AcanCre*$^{ERT2}$, *PthrpCre*$^{ERT2}$, or *Axin2Cre*$^{ERT2}$ strains. scRNA-seq was performed on P9, P14, and P21 tibia epiphyseal growth plates of wild-type mice (n=3). Spatial transcriptomics was performed on P21 knee joints of wild-type and *Col2Cre; Rosa-GsD*$^{f/+}$ mice utilizing the 10xGenomics Visium HD platform (n=2). Histology, immunohistochemistry (IHC), immunofluorescence (IF), and fluorescent in situ hybridization (FISH) were used to assay known and newly identified markers in growth plates. BrdU/EdU incorporation was used to analyze proliferative cells. Both sexes are analyzed (n=3-5). This study is approved by IACUC at USC.

**RESULTS**: Our previous studies identified ADGRG6 as the top 1 enriched GPCR in non-hypertrophic chondrocytes, which is required to maintain the stem/progenitor cells in the resting zone of the growth plate and can signal through Gs/cAMP signaling to promote cell proliferation in mature chondrocytes (1). Here, we demonstrate that stimulating Gs/cAMP signaling in osteochondral progenitor cell lineages of 3-week-old mice (*Col2Cre; Rosa-GsD*$^{f/+}$ mice, induced with the synthetic drug CNO) leads to robust cell proliferation in the entire growth plate, but also results in bone fibrosis mimicking fibrous dysplasia (Fig. 1A). On the other hand, Gs/cAMP activation in mature chondrocytes (*AcanCre*$^{ERT2}$*; Rosa-GsD*$^{f/+}$ mice induced at 8 weeks) leads to region-specific cell proliferation mainly at the center of the growth plate (Fig. 1B). These results suggest a complex cell landscape of the postnatal growth plates and indicate that the edges of the growth plate may house a group of *Col2a1* (+) *Acan* (weak) progenitor populations that controls long bone growth during later stages of adolescence. To uncover the progenitor cell landscape in postnatal growth plates, we performed scRNA-seq on P9, P14, and P21 tibia epiphyseal growth plates of wild-type mice and spatial transcriptomics on P21 wild-type and *Col2Cre; Rosa-GsD*$^{f/+}$ mice (Fig. 2A). Integration of multi-omics analyses allows us, for the first time, to precisely map the cell populations of the postnatal growth plates at a sub-cellular resolution, and to identify Gs/cAMP signaling targets in various cell populations. We were able to map 20 clusters identified by scRNA-seq, including 9 clusters of chondrocytes and bone lineages within the body of the growth plate, and 11 clusters of perichondrium/periosteum/synovium around the edges of the growth plate (Fig. 2D, E). We first confirmed our mapping results with some well-established progenitor cell clusters, including the resting zone cells (*Pthlh/Pthrp*+ and *Adgrg6* +), osteoprogenitors at the periosteum (*Ctsk*+ and *Postn*+), and the fibroadipogenic cells (*Clec3b*+) at the synovial insertion around the perichondrium. We also identified several new clusters of progenitor-like cells, including an *Apoe*+ and *Adgrg6*+ cluster at the middle layer of the perichondrium (Fig. 2A, C, cluster 1), and an *Axin2*+ and *Adgrg6*+ cluster above the groove of Ranvier (Fig. 2A, C, E, cluster 2). Pseudotemporal cell trajectory analyses projected that cluster 1 may serve as the branch point of three different bone-forming trajectories: to form cortical bone via the periosteum, to form trabecular bone via the resting zone to the center of the growth plate, and to form trabecular bone via cluster 2 to the edges of the growth plate (Fig. 2B). To better characterize this *Axin2* (+) cluster 2 cells, we generated *Axin2Cre*$^{ERT2}$; *Td-Tomato*$^{f/+}$ reporter mice and induce recombination at 2 months. We demonstrated that these *Axin2* (+) cells are localized at the edges of the growth plate and can contribute to the formation of the lateral growth plate at 7 days post-labeling (Fig. 3). To compare the role between the center and the lateral of the growth plate (targeted by *PthprCre*$^{ERT2}$ and *Axin2Cre*$^{ERT2}$, respectively), we generated *PthrPCre*$^{ERT2}$*; Rosa-GsD*$^{f/+}$ mice and *Axin2Cre*$^{ERT2}$*; Rosa-GsD*$^{f/+}$ mice and induce Gs/cAMP activation at various postnatal stages. Our preliminary data suggest that the *PthrPCre*$^{ERT2}$ targeted cells are more responsive at early adolescence, while the *Axin2Cre*$^{ERT2}$ targeted cells are responsive until late adolescence (8-10 weeks). Some Gs/cAMP targets in cluster 1 and cluster 2 identified via spatial transcriptomics (Fig. 2D) were validated with IF/IHC/FISH.

**DISCUSSION**: This study utilizes multi-omics techniques in combination with mouse genetics to reveal that (1) postnatal growth plate houses distinct progenitor cell populations that contribute to long bone growth, and (2) Gs/cAMP activation can stimulate distinct progenitor cell populations at different ages to promote bone elongation, potentially through activating IGF signaling and suppressing BMP signaling.

**SIGNIFICANCE**: To our knowledge, this is one of the first studies that precisely and unbiasedly maps growth plate cell populations at a sub-cellular resolution. Our findings that GPCR-mediated signaling promotes long bone growth in distinct cell populations at different ages may shed light on the development of therapeutic approaches to correct long bone growth in adolescent patients of different ages and prevent precocious growth plate closure.

**REFERENCE:** (1) Bian F et.al., J Bone Miner Res. 2024. PMID: 39236220. **ACKNOWLEDGEMENTS**: R00AR077090 and R01AR083966 to Z.L.

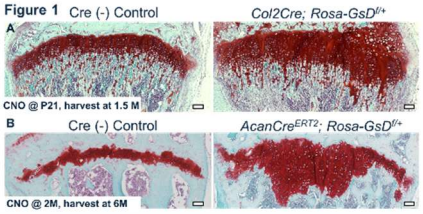


**Fig.1 (A, B)** Histological analysis of growth plate in *Col2Cre; Rosa-GsD*$^{f/+}$ (**A**) and *AcanCre*$^{ERT2}$*; Rosa-GsD*$^{f/+}$ (**B**) mice. (n=5)

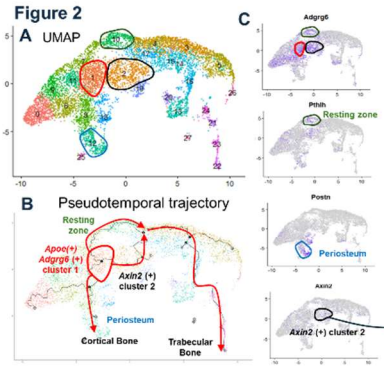


**Fig. 2 (A, B)** UMAP and pseudotemporal trajectory of scRNA-seq of P9, P14, and P21 growth plate cells. **(C)** Feature plots of typical progenitor markers and the *Axin2* (+) cluster 2. **(D, E)** Spatial feature plot of P21 growth plates and the mapping of scRNA-seq clusters.

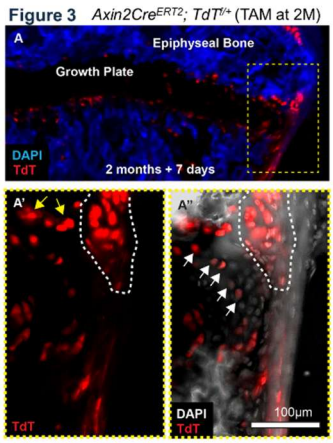


**Fig. 3 (A)** The *Axin2* (+) cells located at the perichondral region can migrate to the lateral growth plates (yellow arrows in **A'**) and contribute to cell proliferation (white arrows in **A''**).