Spatial Transcriptomics Reveals the Crosstalk between ADGRG6 and SOX9 in Maintaining Chondrocyte Fate and Survival in Postnatal Growth Plates

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INTRODUCTION: The growth plate is essential for maintaining skeletal growth, however, the mechanisms that control postnatal growth plate homeostasis are poorly understood. Here we show that ADGRG6, a cartilage-enriched G protein-coupled receptor (GPCR), is dispensable for embryonic limb development but plays a crucial role in regulating postnatal growth plate homeostasis. Using a novel 10xGenomics spatial transcriptomics workflow that applies to mineralized knee joint tissues in combination with the single-cell RNA sequencing (scRNA-seq) technique, we uncovered that ADGRG6 regulates postnatal chondrocyte homeostasis by maintaining chondrocyte fate and survival through crosstalk with SOX9 and Indian Hedgehog (IHH) signaling.

METHODS: Mice lacking *Adgrg6* in cartilage were generated by crossing mice carrying the *Adgrg6* floxed allele to *Col2Cre* and *AcanCre^{ERT2}* strains. P20 knee joints of *Col2Cre; Adgrg6$^{f/f}$* mutant mice and Cre (-) littermate controls were harvested and processed with the spatial transcriptomics FFPE workflow according to 10xGenomics. P20 tibia growth plates of control and mutant mice were also harvested for scRNA-seq analysis. Histology, immunohistochemistry (IHC), and immunofluorescence (IF) analyses were used to assay known biomarkers of cartilage homeostasis. Western blot, RNA-seq, and qPCR analyses were used to measure protein and transcript expression. This study is approved by IACUC at USC and UT-Austin.

RESULTS: ADGRG6 is a cartilage-enriched G protein-coupled receptor (GPCR) that is highly expressed in the resting and proliferative chondrocytes of the growth plate (Fig. 1J). Loss of *Adgrg6* in osteochondral progenitor cells or postnatal chondrocytes leads to reduced cellularity of the resting and proliferative growth plate, due to increased apoptosis coupled with decreased cell proliferation. Histological analysis of knee joints also revealed a disorganized extracellular matrix and disturbed terminal hypertrophic differentiation (Fig. 1I, I'). To further understand the molecular regulators of these defects, we established a novel protocol that applies to formalin-fixed, paraffin-embedded (FFPE) tissue sections of mineralized knee joint tissues for spatial transcriptomics workflow (Fig. 2). Using this innovative technique in combination with scRNA-seq, we demonstrate that *Adgrg6* ablation results in a precocious chondrogenic-to-osteogenic conversion of the growth plate cells associated with reduced SOX9 expression (Fig. 3). Additionally, clustering and pathway analyses of growth plate cell populations revealed the formation of precocious osteogenic cell types in the *Adgrg6* mutant growth plate associated with increased IHH and POSTN/integrin signaling, coupled with ectopic upregulation of catabolic enzymes. We further uncovered that loss of *Adgrg6* results in reduced expression of PTHRP in the resting zone of the growth plate, suggesting that ADGRG6 regulates growth plate homeostasis by maintaining the PTHRP (+) progenitor cells within the resting zone.

DISCUSSION: This study utilizes cutting-edge techniques, including the FFPE workflow of spatial transcriptomics in combination with scRNA-seq, to investigate the functional role of ADGRG6 in postnatal cartilage homeostasis. We demonstrate that ADGRG6 regulates some crucial factors, including SOX9, involved in maintaining chondrocyte fate, survival, and terminal differentiation. Our study also suggests that ADGRG6 maintains the progenitor cells within the resting zone of the growth plate to maintain postnatal growth plate homeostasis.

SIGNIFICANCE: This is one of the first studies that applied spatial transcriptomics to mineralized joint tissues. This study reveals that ADGRG6, a cartilage-enriched GPCR, maintains chondrocyte fate by preventing precocious chondrogenic-to-osteogenic conversion via regulating SOX9 expression, maintains appropriate hypertrophic differentiation by regulating IHH signaling, and keeps resting zone chondrocytes survive by maintaining progenitor cells.

ACKNOWLEDGEMENTS: This study is supported by: NIH-NIAMS K99AR077090 to Z.L., R00AR075899 to C.L.W., and R01AR072009 to R.S.G. The Start-up fund from USC to Z.L.

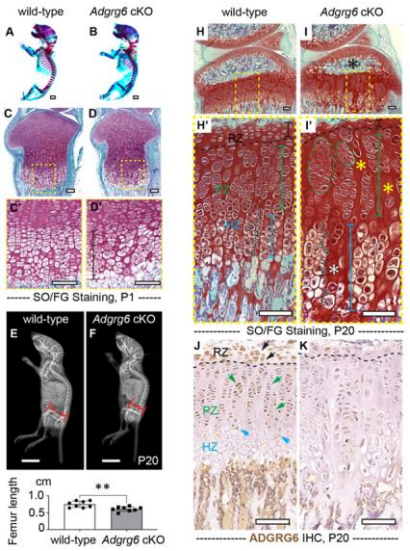

Fig. 1 (A-D') Ablation of *Adgrg6* with *Col2Cre* (*Adgrg6* cKO) does not affect embryonic limb development at P1. **(E-F)** X-ray analysis shows reduced femur length of the *Adgrg6* cKO mice at P20. **(H-I')** Histology analysis shows growth plate defects of P20 *Adgrg6* cKO mice. **(J, K)** IHC analysis shows reduced ADGRG6 expression in mutant growth plates at P20. (n=8)

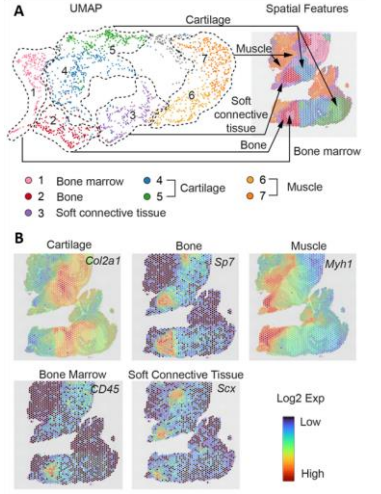

Fig. 2 (A) UMAP and spatial feature plots of knee joint cell populations identified by spatial FFPE workflow. **(B)** Spatial expression plots of representative markers.

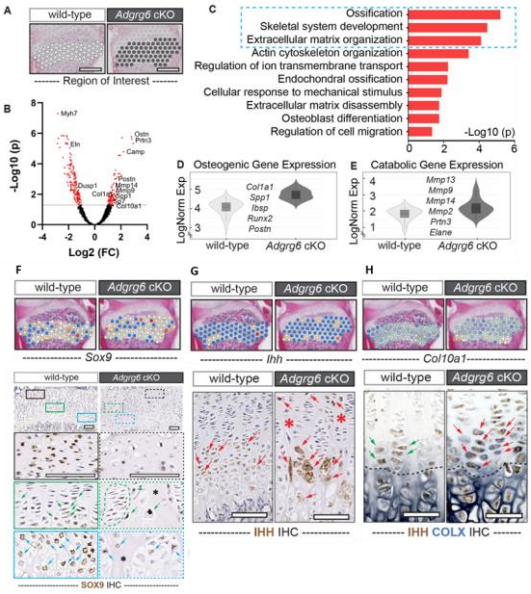

Fig. 3 (A) Spatial spots that are mapped to the control (wild-type) and *Col2Cre; Adgrg6$^{f/f}$* mutant (*Adgrg6* cKO) growth plates were selected for spatial analysis. **(B)** 240 differentially expressed genes were identified between control and mutant growth plates. **(C)** GO enrichment analysis of the differentially expressed genes revealed altered biological processes. **(D, E)** Violin plots of differentially expressed osteogenic and catabolic genes that are precociously upregulated in the mutant growth plate. **(F-H)** Spatial plot and IHC analyses revealed reduced expression of SOX9 (F) and increased expression of IHH (G) and COLX (H) in the mutant growth plates. (n=3)