Hypertrophic chondrocyte-autonomous roles of YAP/TAZ signaling in embryonic bone development

Christopher J. Panebianco[1,2], Joel D. Boerckel[1,2]
[1]Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA
[2]Center for Engineering Mechanobiology (CEMB), University of Pennsylvania, Philadelphia, PA

INTRODUCTION: The appendicular skeleton forms through endochondral ossification, in which chondrocytes proliferate, undergo hypertrophy, and then are remodeled at the chondro-osseous junction to form bone.[1] Previously, we found that deleting the transcriptional regulators Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motifs (TAZ) from Osterix (Osx)-expressing cells (YAP/TAZ cKO^{Osx}) caused bone fragility, perinatal lethality, growth plate hypertrophic zone (HZ) elongation, and a unique conical chondro-osseous junction.[2] Since Osx is expressed in both hypertrophic chondrocytes (HCs) and osteoblast-lineage cells, it remains unclear whether the growth plate phenotype was caused by HC-autonomous defects or by disrupted chondro-osseous junction remodeling. To address this, we established and characterized a collagen 10a1 (Col10)-specific YAP/TAZ conditional knockout (YAP/TAZ cKO^{Col10}).

METHODS: Mice featuring allele dosage-dependent deletion of YAP and TAZ in Col10-expressing cells (*i.e.*, HCs) were generated by crossing mice harboring loxP-flanked exon 3 alleles in both YAP and TAZ with Col10a1-Cre mice, which have tissue-specific activity in hypertrophic cartilage.[3] At postnatal day (P) 0, YAP/TAZ cKO^{Col10} mice and Cre-negative wildtype (WT$^{fl/fl}$) littermates were harvested and imaged using microcomputed tomography (μCT) with X-ray intensity of 145 μA, energy of 55 kVp, integration time of 400 ms, and resolution of 10.4 μm. Harvested P0 mice were also stained with Alizarin red and Alcian blue for whole mount imaging. To explore the embryonic basis of potential phenotypes in YAP/TAZ cKO^{Col10} mice, embryonic day (E) 17.5 mouse hindlimbs were harvested for paraffin-embedding and staining with Safranin-O/fast green. E17.5 hindlimbs were also harvested for cryo-embedding and immunostaining with YAP and TAZ to determine the efficiency and specificity of YAP/TAZ deletion in HCs. Student's t-tests were used to find significant differences between groups ($\alpha = 0.05$).

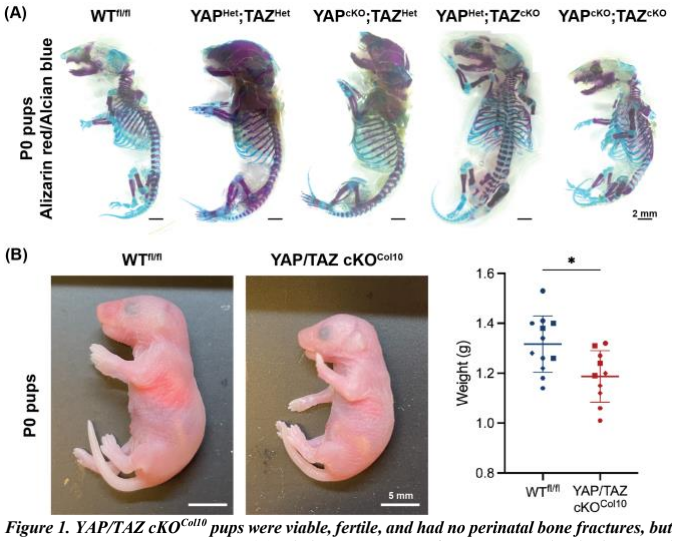

Figure 1. YAP/TAZ cKO^{Col10} pups were viable, fertile, and had no perinatal bone fractures, but were smaller than WT littermates. (A) *Whole mount images of P0 pups stained with Alizarin red and Alcian blue. WT = wildtype, Het = heterozygous, cKO = conditional knockout. Bar = 2mm.* ***(B)*** *P0 pups with mass. Bar = 5 mm. * = p<0.05.*

RESULTS: In contrast to YAP/TAZ cKO^{Osx} mice, YAP/TAZ cKO^{Col10} mice were viable, fertile, and had no perinatal bone fractures (**Fig 1A**). Whole mount imaging of P0 mice featuring allele dosage-dependent deletion of YAP/TAZ in Col10a1-expressing cells showed that heterozygous deletion of YAP and/or TAZ had little effect on skeletal formation. Thus, future analyses focused on homozygous YAP/TAZ cKO^{Col10} mice and WT$^{fl/fl}$ littermates. Homozygous Col10a1-condition YAP/TAZ deletion reduced pup size and body mass at birth (**Fig 1B**), likely driven by reduced skeletal structure and bone volume (**Fig 2**). Next, we analyzed E17.5 embryos to better understand the embryonic basis of this phenotype. To determine the efficiency and specificity of YAP/TAZ deletion in HCs, we used immunostaining to quantify the numbers of YAP- and TAZ-expressing cells in the HZ and proliferative zone (PZ) of the E17.5 distal femur. YAP/TAZ cKO^{Col10} mice exhibited significant reductions in the percentage of YAP^{+} and TAZ^{+} cells (60.0% and 57.7% reduction, respectively), but no significant change in the PZ. Through morphometric analysis of the developing proximal tibia, we found that YAP/TAZ cKO^{Col10} mice had a normal flat chondro-osseous junction, but an elongated HZ and a shortened proliferation zone (PZ). YAP/TAZ deletion also decreased the distance between the tip of the proximal tibia and the start of the HZ (**Fig 3**).

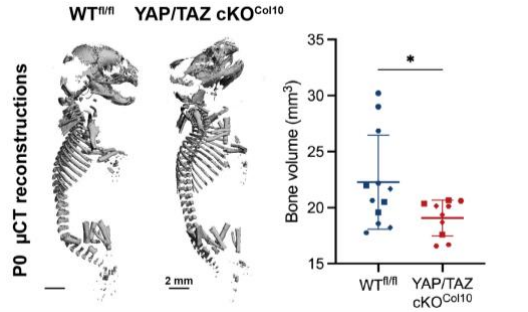

Figure 2. YAP/TAZ cKO^{Col10} pups had reduced bone volume compared to WT littermates. *P0 μCT reconstructions with bone volume quantification. Bar = 2 mm. * = p<0.05.*

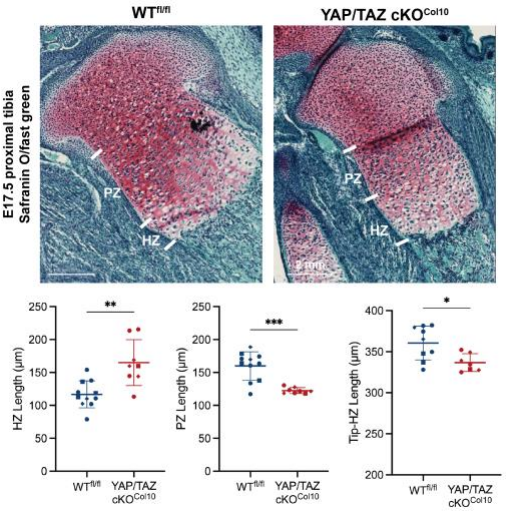

Figure 3. YAP/TAZ cKO^{Col10} embryos had elongated HZ, shorted PZ, and decreased Tip-HZ distance. *Safranin O/fast green stained E17.5 proximal tibia. HZ = hypertrophic zone, PZ = proliferative zone. Bar = 2 mm. * = p<0.05, ** = p<0.01, *** = p<0.001.*

DISCUSSION: Our results indicate an HC-autonomous contribution of YAP/TAZ signaling to endochondral ossification. Our previous work with YAP/TAZ cKO^{Osx} mice suggested that deleting YAP and TAZ from both HCs and osteoblast-lineage cells impaired growth plate remodeling through both HC-autonomous and non-autonomous mechanisms.[2] Here, we demonstrate that the loss of YAP/TAZ signaling from HC's elongated the HZ and shortened the PZ of the developing growth plate, while also decreasing the distance between the tip of the tibia to the start of the HZ. Since YAP and TAZ expression was effectively suppressed in HCs, but not proliferating chondrocytes, these data indicate that loss of YAP/TAZ signaling in maturing chondrocytes induced precocious hypertrophy. Furthermore, the elongated HZ, with flat chondro-osseous junction, suggests there are defects in HC-autonomous remodeling, rather than vascular/septoclastic resorption. These phenotypes differ from YAP/TAZ cKO^{Osx} mice, which had HZ elongation and a conical chondro-osseous junction. However, these phenotypes closely resemble those recently reported in collagen 2a1-specific YAP/TAZ conditional knockout (YAP/TAZ cKO^{Col2}), which showed that mice were smaller, displayed HZ elongation, and had a flat chondro-osseous junction without defects in PZ proliferation. Ongoing work will define the molecular mechanisms by which HC-autonomous YAP/TAZ signaling regulated hypertrophic initiation, maturation, and remodeling. Importantly, these data demonstrate that HC-autonomous YAP/TAZ signaling is necessary for proper endochondral ossification.

SIGNIFICANCE: This work is the first to demonstrate the HC-autonomous roles of YAP/TAZ signaling in embryonic bone development. Better understanding of how YAP and TAZ mediate endochondral ossification will help enhance treatment options for patients with developmental dysplasias and bone fractures.

REFERENCES: [1] Kozhemyakina+ *Development* 2015, **[2]** Collins+ *BioRxiv* 2023, **[3]** Gebhard+ *Matrix Biol* 2008, **[4]** Vanyai+ *Development* 2020

ACKNOWLEDGEMENTS: This work was supported by the CEMB, an NSF Science and Technology Center, under grant agreement CMMI: 15-48571.