Osteoprogenitor YAP and TAZ Promote Bone Fracture Repair

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Introduction

Bone fracture healing requires progenitor cell mobilization, differentiation, and matrix deposition, but the molecular mechanisms remain incompletely understood. Recently, we found that yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) promote bone matrix development through regulation of collagen expression and organization[1]; however, the roles of YAP and TAZ in osteogenesis remain controversial. Separately, we have found in endothelial cells that YAP and TAZ regulate feedback control of cytoskeletal dynamics to control acto-myosin equilibrium and enable motility[2]. In osteoprogenitor cells, cytoskeletal remodeling regulates activation of the osteogenic transcriptional program[3], potentially through YAP/TAZ. Here, we tested the hypothesis that osteoprogenitor YAP and TAZ promote bone fracture healing and regulate cytoskeletal reorganization to control osteogenic differentiation.

Methods

We generated two mouse models: 1) constitutive allele dosage-dependent YAP/TAZ conditional knockout mice and 2) adult-induced double homozygous conditional knockout mice (YAPfl/fl;TAZfl/fl;TetOff-Osx-Cre, hereafter cDKOi), by ablating YAP and/or TAZ by Cre-recombination under control of the Osterix1 promoter. All animal experiments were approved by the IACUC. In inducible knockouts, Cre expression was repressed by doxycycline (dox) administration from conception to 14 weeks of age, while constitutive knockouts were bred and raised without exposure to dox. Unilateral femoral fractures were created at 16 weeks of age. Bone marrow stromal cells (MSCs) were isolated from both WT and Osterix conditional YAP/TAZ- deficient mice. MSCs were cultured in osteogenic media prior to either RNA isolation or staining for filamentous actin. Comparisons were made using Student's t-tests. Non-parametric Mann-Whitney tests were used if necessary. A p-value less than 0.05 was considered significant.

Results

In vivo, both WT and cDKOi mice exhibited callus formation in response to bone fracture (Fig.1 A). Compared to WT littermates, cDKOi mice had significantly lower bone volumes, at both one and two weeks post-fracture ($p < 0.05$), but had equivalent total callus volumes (Fig.1 B-C, $N = 6 - 12$). In vitro, cDKOi MSCs had increased filamentous actin intensity and increased stress fiber formation after 7 days in osteogenic media, which persisted to 14 days (Fig.2 A-B). Expression of YAP, TAZ, Osterix, and collagen1a1 (Col1a1) were not different prior to Osterix induction (day 0; Fig. 3A); however, Osterix-mediated Cre-recombination reduced

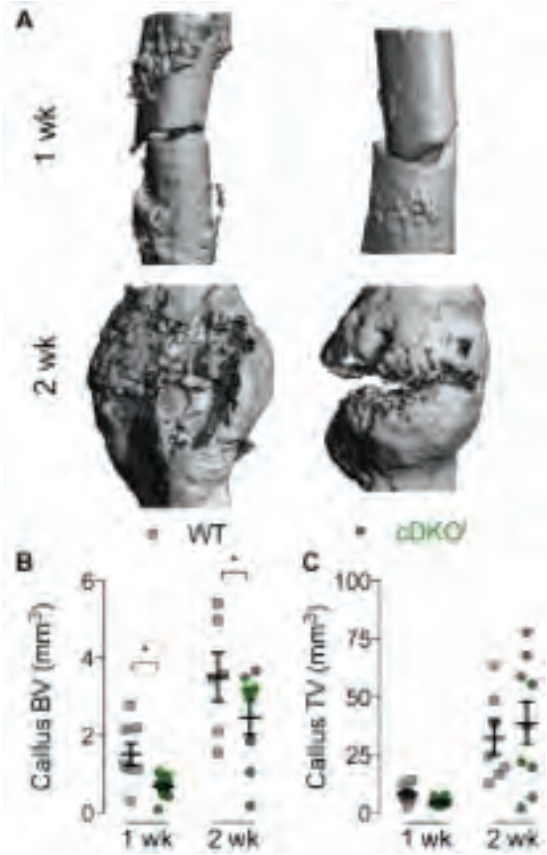

Figure 1. Inducible dual homozygous YAP/TAZ deletion impaired *in vivo* fracture healing. **(A)** representative 3D CT reconstructions. Quantification of calus **(B)** and **(C)** total bone volume.

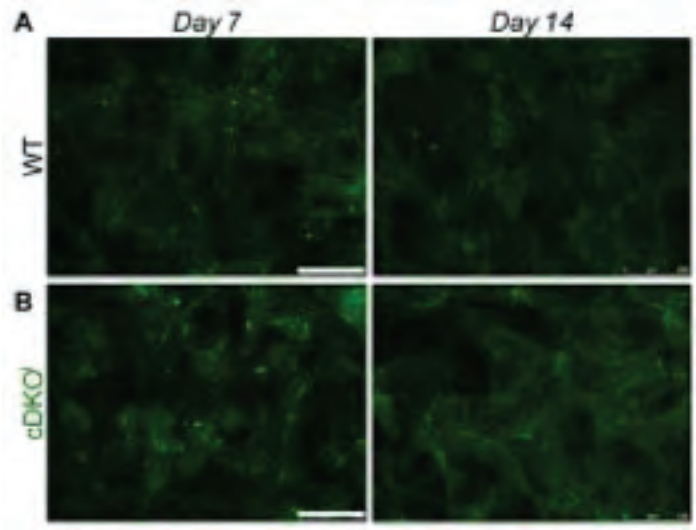

Figure 2. Increased stress fiber formation *in vitro* following Osteriz-induced YAP/TAZ deletion. **(A)** WT and **(B)** cDKO MSCs in osteogenic induction media for 7 and 14 days. Scale bar = 250μM.

TAZ mRNA expression levels after seven days in osteogenic media while differences in YAP expression did not reach significance (Fig. 3B). At day 7, Osterix-conditional YAP/TAZ deletion reduced Col1a1, osteocalcin (Ocn), alkaline phosphatase (Alp), and bone sialoprotein (Bsp) expression, while Runx2 and Osterix mRNA levels were not significantly altered (Fig.3 B-C).

Discussion

YAP/TAZ deletion from osteoprogenitor cells reduced fracture callus ossification, but did not impair cartilaginous anlage formation, consistent with our prior observations in developmental endochondral ossification[1]. In osteoprogenitor cells, YAP/TAZ deletion upon Osterix induction increased actin stress fiber formation, suggesting conservation of the cytoskeletal feedback mechanism observed in endothelial cells[2]. In addition to stress fiber persistence,YAP/TAZ deletion reduced osteogenic gene expression. Together, these data implicate YAP/TAZ in cytoskeleton-dependent control of osteogenesis during endochondral fracture repair.

Significance

A mechanistic understanding of how these proteins combinatorically regulate osteogenesis could guide future therapeutic strategies for bone regeneration.

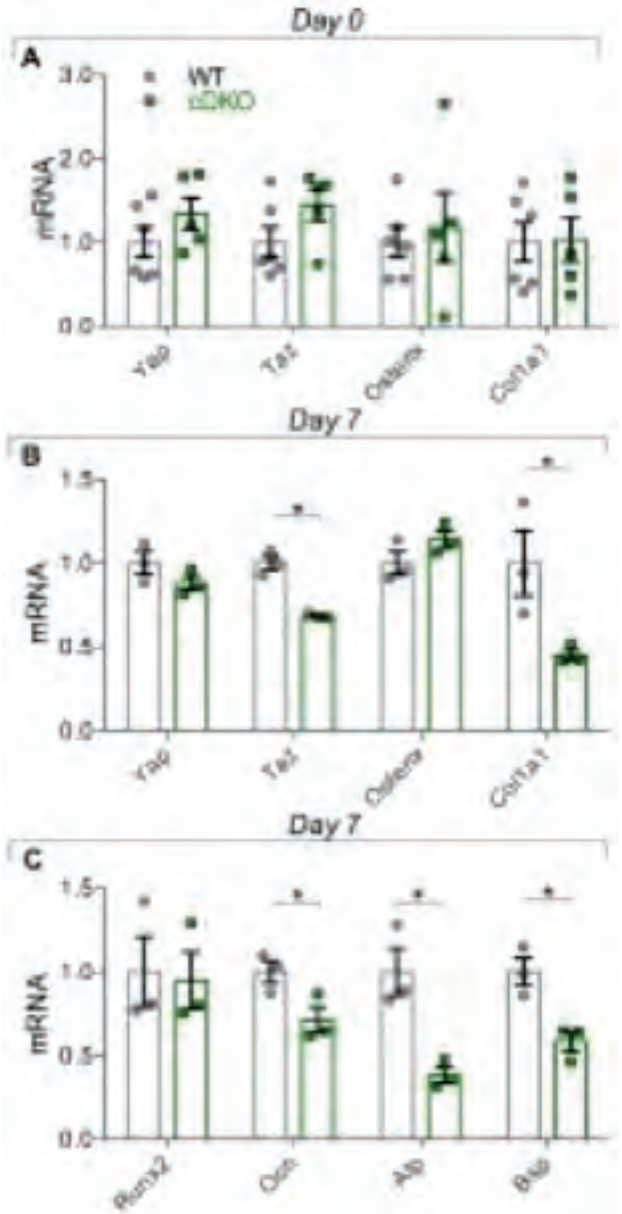

Figure 3. Decreased osteogenic gene expression following Osterix-mediated YAP/TAZ ablation *in vitro.* **(A)** mRNA expression levels of YAP, TAZ, Osterix, and collagen 1a1 (Col1a1) prior to Osteoinduction (Day 0), **(B)** mRNA expression levels of YAP, TAZ, Osterix and col1a1 following Osterix-induction (Day 7) **(C)** mRNA expression levels of Runx2. Ossification (Ocn), alkaline phosphatase (Alp) and bone sialoprotein (Bsp) at day 7.

References

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