Thrombospondin-2 serves a time-dependent role in mediating nociceptive behavior, structural remodeling, and the synovial transcriptome in post-traumatic osteoarthritis

Lindsey Lammlin[1], Isabelle Smith[1], Adrienne Giannone[1], Scarlet Howser[1], Michael Newton[1], Paul Lais[1], Sofia Gonzales-Nolde[1], Andrea Alford[1], Kurt Hankenson[1], Tristan Maerz[1]
[1]University of Michigan, Ann Arbor, MI
zlammlin@umich.edu

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INTRODUCTION: Thrombospondin-2 (TSP2, *Thbs2*) is a matricellular protein with roles in anti-angiogenic signaling, collagen fibrillogenesis, fibroblast activation, bone formation, and wound healing – processes relevant to post-traumatic osteoarthritis (PTOA), but the role of TSP2 in PTOA pathogenesis is unknown. Our goal was to employ a novel inducible, global TSP2 knockout mouse model to establish the impact of TSP2 ablation on PTOA progression.

METHODS: With IACUC approval, Cre-mediated recombination was induced in novel *Thbs2*$^{fl/fl}$-RosaCre^{ERT2} mice. Cre^{ERT2}(-) floxed mice were used as WT controls; Cre^{ERT2}(+) floxed mice are designated as the knockout (KO). All mice received tamoxifen at -4, -2, 0, and +2 days post-ACLR. Whole knee joints were harvested 7d post-ACLR for recombination assessment using Western blot. WT and KO mice were randomized to non-invasive anterior cruciate ligament rupture (ACLR) to induce post-traumatic osteoarthritis (PTOA) or sham procedure (anesthesia and analgesia only)[1]. The injured group was further randomized to 7d or 28d timepoints. Shams were collected 28d post-ACLR. Mice underwent a suite of PTOA phenotyping assessments including knee hyperalgesia testing, micro-computed tomography (μCT), and histological assessment of PTOA and synovitis severity via Safranin-O/Fast Green staining. Synovia were collected from separate cohorts of WT and KO sham, 7d ACLR, and 28d ACLR mice for single cell RNA sequencing (scRNAseq) of whole synovium. For each biological replicate, one male and one female sample of the same group/genotype were pooled (n=2 replicates per group/genotype were sequenced, 20k cells input, 25k reads/cell). Quality control removed debris and low-quality cells. Dimensionality reduction and clustering identified major cell types, and cluster-by-cluster differential gene expression and pathway analysis (PantherDB, GO) compared WT vs KO synovia at each timepoint.

RESULTS: We measured 90% reduction in TSP2 protein in whole joints (Fig. 1A). At 7d and 28d post-ACLR, KO mice had worse knee hyperalgesia than WT, as per ACLR/contralateral withdrawal threshold ratio (Fig. 1B). Female KOs had greater subchondral bone thickness, epiphyseal BV/TV, epiphyseal mineral density, and osteophyte mineral density than female WT mice at 28d, but no genotype-based difference was observed in any μCT outcomes in males (Fig. 1C-G). 7d ACLR male KOs had lower histologic PTOA scores compared to group- and sex-matched WTs (Fig. 1H), driven largely by less severe structural damage (Fig. 1J yellow arrows), but no difference was observed at 28d between genotypes. Additionally, 7d ACLR male KOs had marginally lower synovitis scores and qualitatively greater maintenance of areolar adipose tissue compared to 7d ACLR male WTs (Fig. 1I, 1K blue arrows). Single-cell RNAseq yielded ~74k total synovial cells across WT and KO (Fig. 1L). Compared to KO, WT fibroblasts and endothelial cells exhibited enhanced gene expression of pro-inflammatory, pro-fibrotic, and neurotrophic pathways including "collagen fibril organization" (marked by ↑*Col1a1*, ↑*Anxa2*), "cellular response to TGF-β signaling", "angiogenesis" (↑*Anxa2*, ↑*Ccn3*), "chondrocyte development" and "bone development" at 7d (Fig. 1M-N). Several pathways were enhanced at 7d in WT fibroblasts ("regulation of cell migration", "BMP signaling", "regulation of Wnt signaling"), or both WT fibroblasts and WT endothelial cells, such as "neurogenesis" and "axon guidance" (↑*Nfib*, ↑*Sema3a*), which were then downregulated at 28d post-ACLR compared to KO. These scRNAseq results suggest a blunted pro-inflammatory, pro-fibrotic, and pro-neuroangiogenic response in KO at 7d compared to WT, which appears to reverse at 28d.

DISCUSSION: TSP2 ablation at the time of joint injury (bypassing deleterious impacts of TSP2 deletion on developmental and growth in constitutive knockouts) induces an early protective effect in the synovial transcriptome as shown by increased nociceptive, fibrotic, and tissue remodeling pathways in WTs compared to KOs at 7d post-ACLR. This is corroborated by histological PTOA and synovitis scoring. Paradoxically, KOs exhibited worse knee hyperalgesia throughout the study. A role for TSP2 in nociception is supported by the upregulation at 7d and downregulation at 28d post-ACLR of axon guidance-related pathways in WTs compared to KOs, suggesting a late-stage shift in pain-related signaling. Higher osteophyte volume and density in KOs supports sustained pain sensitization as osteophyte formation is known to correlate to pain clinically. Ongoing studies aim to augment (via AAV-TSP2) or ablate TSP2 at different disease timepoints to establish the kinetics of TSP2 involvement in PTOA pathogenesis.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings indicate that thrombospondin-2 mediates multiple aspects of PTOA pathogenesis, with clear time-dependent trends, and these results may establish translational approaches to leverage TSP2 signaling as a therapeutic target.

REFERENCES: [1]Rzeczycki+, *Osteoarthr. Cartil.*, 2021.

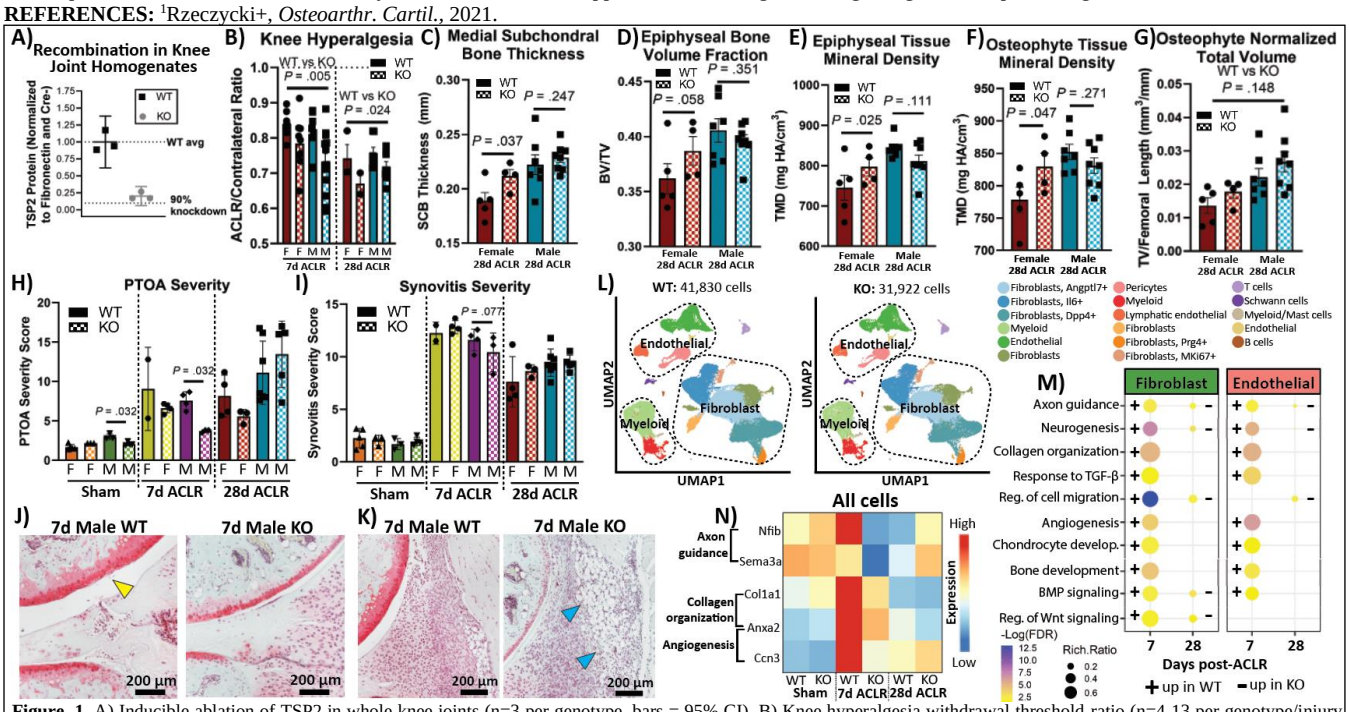

Figure. 1. A) Inducible ablation of TSP2 in whole knee joints (n=3 per genotype, bars = 95% CI). B) Knee hyperalgesia withdrawal threshold ratio (n=4-13 per genotype/injury condition/sex, bars = SEM). C-G) MicroCT bone morphometry, densitometry, and osteophyte analysis (n=4-8 per genotype/injury condition/sex, bars = SEM). H-I) Histological assessment of PTOA and synovitis severity (n=2-7 per genotype/injury condition/sex with 6-12 sections per limb, bars = SEM). J-K) Representative histology (yellow – structural damage, blue – areolar fat pad). L) Cell type clusters in WT and KO synovia. M) GO analysis of fibroblast and endothelial megaclusters. N) Select genes driving GO pathways.