Tropomyosin 3.1 Stabilizes F-actin To Prevent Chondrocyte Degeneration and Death

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INTRODUCTION: Osteoarthritis (OA) is an irreversible, debilitating disease caused in part by aberrant mechanical overload onto cartilage. We suspect that amplified mechanical overloading on superficial zone chondrocytes (SZCs) has major implications in the etiology of post-traumatic osteoarthritis (PTOA) via activation of chondrocyte degeneration and cell death pathways[1]. Recently, we showed that destabilization of the medial meniscus leads to decreased cortically organized filamentous (F-)actin[2]. We speculate that this decrease in cortical F-actin is pivotal. In a separate study, we have shown that disruption of F-actin in SZC reduces lubricin (proteoglycan-4; Prg4) expression[3], which is critical for proper surface lubrication of the cartilage. Additionally, reduced F-actin promotes cellular apoptosis in neuroepithelial cells[4,5], leading to the speculation that reduced F-actin may contribute to the characteristic apoptosis seen in PTOA. Therefore, maintaining proper F-actin organization may be critical for chondrocyte/cartilage health. In this study, we sought to determine the actin binding proteins vital for stabilizing chondrocyte F-actin. Tropomyosins (Tpms) are master regulators of actin organization that bind and stabilize F-actin. There are over ~40 non-redundant Tpm isoforms, each with the ability to differentially stabilize F-actin. *We hypothesize that specific Tpm isoforms exist in chondrocytes to promote F-actin stability and that they are chondroprotective to prevent chondrocyte degeneration and death.*

METHODS: All procedures involving mice were conducted following approved animal protocols from the University of Delaware Institutional Animal Care and Use Committee (IACUC). To identify the expression of F-actin stabilization Tpms in chondrocytes, we performed semi-quantitative PCR followed by Sanger Sequencing. We next examine the expression of the specific isoform Tpm3.1 in mouse, bovine, and human chondrocytes. Using confocal microscopy, we examined the localization of TPM3.1 in chondrocytes, both in native mouse hip tissue sections and *in-vitro* in isolated primary bovine cells. We determined the effect of TPM3.1 inhibition on cell degeneration and death markers via qRT-PCR and imaging. Using a whole mount confocal imaging protocol we performed super high-to-super resolution imaging of mouse hip cartilage, and examined the effect of Tpm3.1 knockout on F-actin. We also visualized F-actin and TPM3.1 organization by immunostaining cryosections of Tpm3.1 wild type and knockout mice. Finally, we determined the effect of chondrocyte overloading on F-actin stability and Caspase-3 expression by exposing primary bovine chondrocytes on silicone substrates to 30% uniaxial stretch.

RESULTS: We determined that Tpm3.1 and 4.2 are expressed in mouse chondrocytes. We also found that Tpm3.1 is expressed in bovine and human chondrocytes. Confocal microscopy of primary chondrocytes, revealed that TPM3.1 is associated in microdomains along cortical F-actin in primary chondrocytes. In comparison to wild-type controls, F-actin is reduced in Tpm3.1 knockout mice (Fig. 1). Similarly, inhibition of TPM3.1 in primary bovine chondrocytes reduces F-actin. Both knockout of Tpm3.1 in mouse hip cartilage or inhibition in bovine cells represses Prg4 expression (Fig. 2). Furthermore, in bovine cells, TPM3.1 inhibition substantially reduces F-actin in a subpopulation of cells which correlates with elevated expression for apoptosis marker Caspase-3 (Fig. 3). Finally, we determine that mechanical overloading of chondrocytes led to a subpopulation of reduced F-actin, mimicking the effects of TPM3.1 inhibition.

DISCUSSION: Our findings show that mechanical overloading reduces F-actin in chondrocytes. We determined that the specific isoform Tpm3.1 contributes to the stabilization of F-actin in chondrocytes. Our results lead to the suggestion that the dysregulation of Tpm3.1 in PTOA may contribute to disease progression by causing PRG4 loss and eventually cellular apoptosis. Further studies aim to investigate if overexpression of Tpm3.1 will prevent F-actin depolymerization and offer chondroprotection.

SIGNIFICANCE/CLINICAL RELEVANCE: Elucidating the contribution of F-actin to PTOA progression and the mechanisms leading to F-actin destabilization may provide for new therapeutic insights to halt/reverse disease progression.

REFERENCES: [1]Novakofski, K. D. *et al.* (2015); [2]Chan, B. *et al.* (2023); [3]Delve, E. *et al.* (2020); [4]Desouza-Armstrong, M., Gunning, P. W. & Stehn, J. R. (2017); [5]Currier, M. A. *et al.* (2017).

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IMAGES:

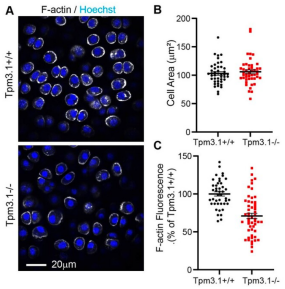

Fig. 1. F-actin is reduced in Tpm3.1-/- as compared to Tpm3.1 +/+ mouse hip cartilage. (A) Whole mount confocal microscopy of SZC. Quantification of (B) SZC area and (C) F-actin fluorescence.

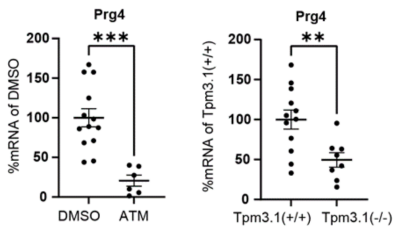

Fig. 2. Inhibition (ATM) or knockout of Tpm3.1 represses Prg4 mRNA levels in bovine chondrocytes and mouse hip cartilage chondrocytes, respectively.

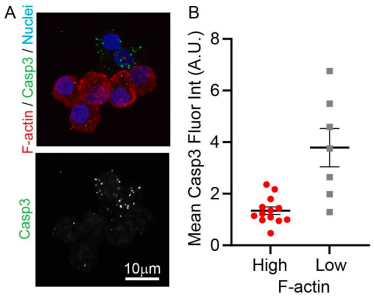

Fig. 3. Tpm3.1 inhibition leads to a subpopulation of cells with substantially low F-actin. Cells with low F-actin are positive for cleaved Caspase-3.