

Stress Deprivation of Tenocytes Depolymerizes Filamentous Actin to Directly Regulate Gene Expression

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INTRODUCTION: Tendinosis is a painful degenerative condition caused, in part, by mechanical overload. We speculate that tendon mechanical overload counterintuitively causes the under-stimulation of tenocytes. Tenocyte under-stimulation alters tenocyte molecular expression levels by decreasing tenogenic mRNA levels while increasing both chondrogenic and protease mRNA levels. Concomitant with these gene expression changes are alterations in tenocyte filamentous (F-) actin, whereby cells depolymerize F-actin into monomeric, globular (G-) actin units. However, it is unknown whether F-actin depolymerization leads to altered gene expression and if so, what are the mechanisms by which F-actin depolymerization regulates genes? In other cell types, F-actin depolymerization regulates gene expression through myocardin-related transcription factor (MRTF). MRTF is a co-activator of serum response factor which binds to the promoter region of genes to enhance gene expression. In addition, MRTF has a high affinity for G-actin. During F-actin depolymerization, the increase in G-actin results in sequestration of MRTF in the cytoplasm of cells causing a downregulation of MRTF-regulated genes. The objectives of this study were to: (1) evaluate the effect of tendon overload and under-stimulation of tenocytes on actin polymerization status; and (2) test our hypothesis that F-actin depolymerization regulates gene expression through MRTF.

METHODS: *In vivo model:* We used an *in vivo* rat tendinosis model to evaluate the effects of mechanical overload on plantaris tendon through ablation of the synergistic Achilles tendon (SynAb)¹. To examine the effect of plantaris overload on micro-to-nano scale matrix and cell features, we performed serial block face scanning electron microscopy (SBF-SEM). To determine the effect of SynAb on F-actin polymerization status, paraformaldehyde fixed plantaris tendons were sectioned and stained for Phalloidin and DNase-I for visualization of F-actin and G-actin respectively. *Ex vivo tissue culture model:* We used an *ex vivo* model of stress deprivation² to examine effects on F-actin polymerization status. Tail, Achilles, and plantaris tendons from mice were isolated and maintained in floating (mechanically stress deprived) cultures for 1 day. We examined F-actin polymerization status in fixed tissues via sectioning and staining. *In vitro cell culture model:* Using *in vitro* cultures of isolated tail tenocytes, we examine the regulation of genes by actin polymerization status by either stimulating F-actin depolymerization (latrunculin A) or polymerization (TGFβ1). In another set of experiments, to investigate the specific regulation of genes by MRTF, we exposed tenocytes to MRTF inhibitor, CCG1423. We investigated MRTF localization via confocal imaging as well as downstream regulation of molecules via qRT-PCR (mRNA levels) and WES Capillary electrophoresis (protein levels). This study was approved by IACUC. Experiments were performed on at least 3 separate occasions using tendons from different animals. A t-test was used to determine differences between 2 groups of data, whereas an ANOVA followed by Dunnett's post-hoc was used to determine differences between 3 or more groups.

RESULTS: Our *in vivo* SynAb of the Achilles demonstrated an increase in distance between collagen fibrils and cells in plantaris tendons (Fig.1A). In addition, we observed a decrease in the proportion of F/G-actin (Fig.1B). Similarly, *ex vivo* stress deprivation of tendons decreases F/G-actin. Direct perturbation of F-actin by latrunculin treatment of *in vitro* cultured tenocytes reduced nuclear MRTF (Fig.2). This coincided with decreases in tenogenic (collagen-1, scleraxis, tenascin-C, and α-smooth muscle actin) mRNA levels and increases in chondrogenic (Sox9) and protease (Mmp-3 and Mmp-13) mRNA levels. As compared to latrunculin treatment, TGFβ1 treatment led to the reverse effects by increasing F/G-actin and nuclear MRTF. Furthermore, TGFβ1 treatment upregulates tenogenic mRNA levels, while downregulating chondrogenic and protease mRNA levels. Exposure of tenocytes to a MRTF inhibitor, CCG1423, prevents nuclear accumulation of MRTF. While CCG1423 led to significant decreases in tenogenic mRNA levels, there are no effects on chondrogenic or protease mRNA levels (Fig.3).

DISCUSSION: Our findings demonstrate that tendon overload alters the interaction between collagen fibrils and cells which may lead to cellular understimulation. Cellular understimulation leads to F-actin depolymerization which directly alters gene expression. Our findings provided partial support for our hypothesis that actin depolymerization regulates gene expression through MRTF. While F-actin depolymerization regulates a certain subset of genes (tenogenic genes) via MRTF, F-actin depolymerization regulates chondrogenic and protease gene expression independent of MRTF.

REFERENCES: ¹Bloom et al., J. Biomech. Eng (2023); ²Inguito et al., Mol. Biol. Cell (2022)

SIGNIFICANCE/CLINICAL RELEVANCE: A greater understanding of the regulation of molecular expression during tendinosis by actin may allow for new therapeutic opportunities against disease progression.

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IMAGES:

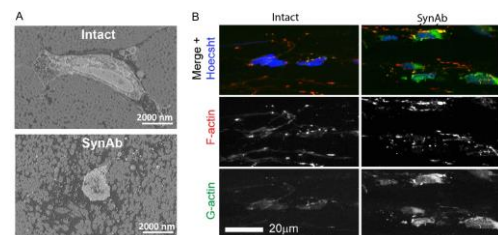


Figure 1. Effect of SynAb on plantaris tendon. (A) SBF-SEM showing increased space between cell and collagen fibrils. (B) Confocal image showing decrease in F/G-actin.

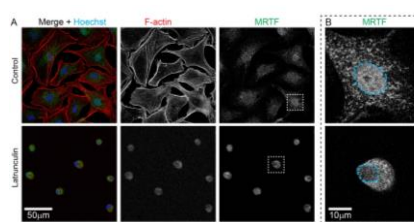


Figure 2. Effect of F-actin depolymerization on MRTF localization. (A) Low magnification image of cells demonstrating loss of F-actin. (B) High magnification image of cells demonstrating MRTF nuclear clearing. Nuclei are outlined in blue dash lines.

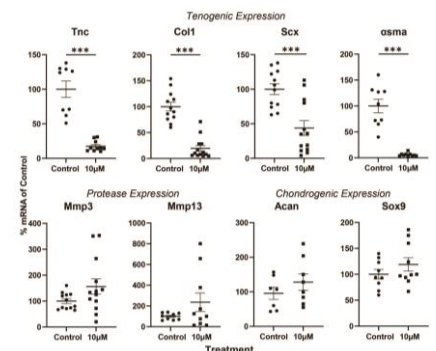


Figure 3. Effects of MRTF inhibitor (CCG1423) on cultured tenocyte mRNA levels. CCG1423 alters tenogenic (top row) but not protease and chondrogenic expression (bottom row) mRNA levels.