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Actin Depolymerization of Tenocytes Promotes a Tendinosis-like Gene Expression

Kameron Inguito, BA
Thomas Jefferson University, kameronmel.inguito@students.jefferson.edu

Valerie West
University of Delaware

Karl Matthew Ebron
University of Delaware

Justin Parreno, PhD
University of Delaware

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Kameron Inguito, BA[1], Valerie West[2], Karl Matthew Ebron[3], Justin Parreno, PhD[3*]

[1]Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA

[2]Department of Biomedical Engineering, University of Delaware, DE

[3]Department of Biological Sciences, University of Delaware, DE

Introduction

Optimal cellular mechanotransduction is essential for tendon matrix homeostasis. We recently developed an in vivo rat model of tendinosis, where the plantaris tendon are overloaded through ablation of the synergistic Achilles tendon. Using this model we determined that tissue overload disrupts matrix-cell interactions, which results in under-stimulation of tendon cells (tenocytes) (Fig.1).

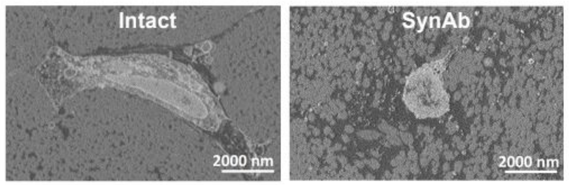

Figure 1. Serial Block Face SEM showing collagen fibrils and tenocytes, 8 months post-surgery.

Using an ex vivo model of tendon stress deprivation by maintaining tail tendon fascicles in floating culture we showed that tenocyte under-stimulation results in destabilization of filamentous (F-)actin (Fig.2). F-actin destabilization coincides with tendinosis-like gene expression: downregulation of tenogenic genes (Col1, Tnc, asma, Scx), upregulation of chondrogenic (Acan, Sox9) and matrix metalloproteinases (Mmp-3, Mmp-13).[2]

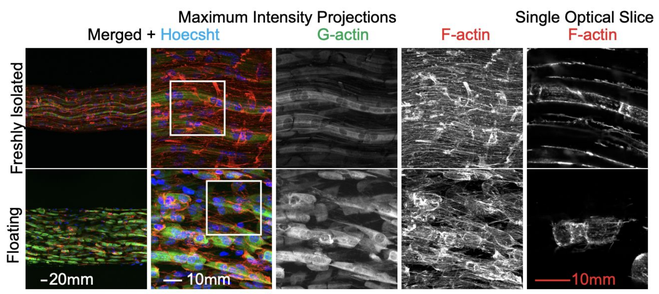

Figure 2. Whole mount confocal images of tail tendons stained for G- and F-actin (DNAse-I and Phallodin, respectively) The mechanisms regulating gene expression by F-actin depolymerization are unknown in tendon. However, we have shown in other cell types (chondrocytes, lens epithelial cells) that F-actin depolymerization regulates gene expression by a G-actin binding transcription factor, myocardin related transcription factor.[3]

Hypothesis

In this study, we test the hypothesis that actin depolymerization regulates gene expression through, G-actin binding, myocardin related transcription factor (MRTF).

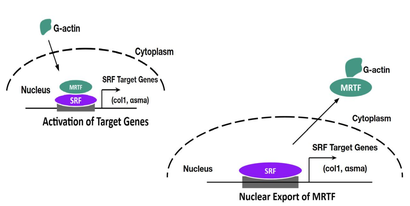

Figure 3. Schematic of MRTF-mediated gene regulation

Results

Latrunculin reduces nuclear MRTF and promotes tendinosis like gene expression

To test the effects of direct F-actin dynamics perturbation on tenocyte MRTF localization and gene expression, we exposed isolated tenocytes to Latrunculin A. Latrunculin A binds to G-actin monomers which prevents F-actin assembly, resulting in disassembly of F-actin stress fibers and net actin depolymerization. This coincides with nuclear export of MRTF (Fig.4A) and tendinosis like gene expression changes (Fig.4B).

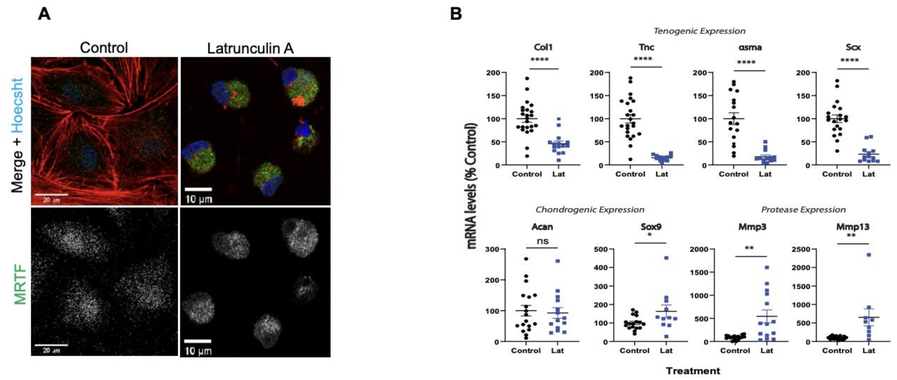

Figure 4. Effects of latrunculin A treatment (2uM) on tenocytes. (A) Tenocytes stained for F-actin (Phalloidin; Red), MRTF (Green), and Nuclei (Hoecsht; Blue). (B) Relative Real-time PCR for tenogenic, chondrogenic, and protease gene expression.

Pharmalogical inhibition of nuclear MRTF specifically downregulates tenogenic genes

To investigate the specific effects of MRTF in regulating gene expression, we treated isolated tenocytes with pharmacological inhibitor, CCG1423, which prevents accumulation of MRTF in cells. Exposure of Tenocytes to CCG1423 reduces nuclear accumulation of MRTF (Fig.5A, B) and represses tenogenic mRNA levels (Figure 5C). Chondrogenic and protease mRNA levels were not affected.

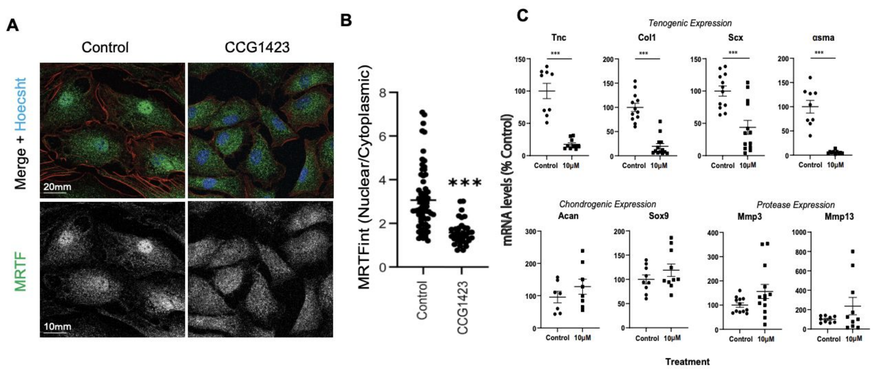

Figure 5. Effects of CCG1423 treatment on tenocytes. (A) Immunostaining for MRTF shows nuclear clearing by treatment with CCG1423. (B) MRTF nuclear quantification demonstrates a decrease in MRTFint with treatment of MRTF inhibitor, CCG1423. (C) Relative Real-time PCR for tenogenic, chondrogenic, and protease expression.

Results

MRTF inhibition reduces tenogenic molecule protein levels

To determine if CCG1423 represses tenogenic protein levels, we extracted protein lysates from tenocytes exposed to CCG1423. We performed WES Capillary Electrophoresis to investigate protein expression and found that aSMA and COL1A1 protein levels are reduced by CCG1423 (Fig.6).

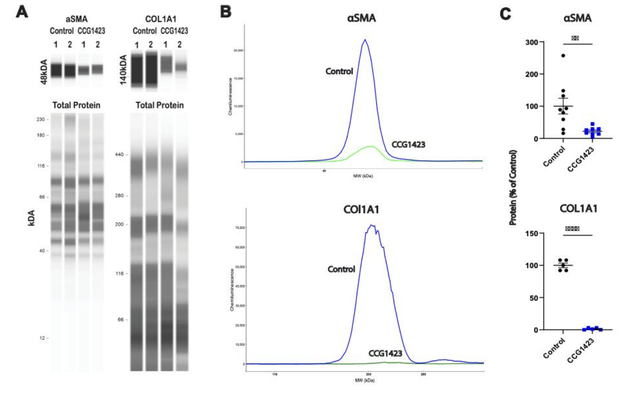

Figure 6. (A) Representative WES electrophoresis data showing pseudo-Western blots with corresponding (B) electropherograms for αSMA and Col1a1. (C) αSMA and Col1a1 protein after normalization to total protein. Protein levels are expressed as a percentage of untreated controls.

Conclusions

This study confirmed that gene expression is regulated by actin depolymerization. This was made evident by the tendinosis-like gene modulation experienced by inhibition of actin polymerization by Latrunculin.

MRTF inhibition did not affect the expression of proteases, suggesting that the regulation of these genes by actin are via MRTF independent pathways. WES also verified MRTF's role in regulating tenogenic molecules at the protein level, showing decreases in alpha-smooth muscle actin and collagen-1.

In conclusion, actin depolymerization is a regulator of gene expression in tendon cells, partially through regulation of MRTF. Further understanding the regulation of gene expression during tendinosis by actin may lead to new therapeutic opportunities against disease progression.

Acknowledgements

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References

[1]Bloom et al., J Biomech Eng (2023); [2]Inguito et al., MBoC (2022); [3]Parreno et al., IOVS (2020), 4 Parreno et al., FEBS Let (2014)