Investigating YAP/TAZ Dynamics in Tensional Homeostasis and Tendinopathy Onset: A Two-Pronged Approach

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Introduction

Tendons, as collagenous connective tissues, function to transmit sustained tensile forces from muscle to bone so as to enable musculoskeletal movement [1]. Through repetitive routine, tendons undergo loading cycles from muscle contraction and thus, maintain a constant residual tension [2]. Loss of tensional homeostasis (by transection, immobility, microdamage, or overuse) alters the architecture and diminishes load transmission in endogenous tendon fibroblasts (tenocytes) [3]. It has long been established that tendinopathic phenotypes exhibit catabolic gene programming and increases in matrix metalloproteinases (MMPs) that degrade extracellular matrix (ECM) [3]; however, the mechanics that mediate early cellular events are relatively unknown. Studying the mechanotransductive response of tenocytes can better elucidate the onset of tensional homeostasis loss.

Yes-Associated Protein (YAP) and Transcriptional coactivator with PDZ binding motif (TAZ) are transcriptional regulators that are activated by growth factors and mechanical triggers. YAP and TAZ enter the nucleus, where they bind to transcription factors to co-activate/repress gene expression [4]. Recently, YAP and TAZ were implicated in mediating cellular response to tenocyte detensioning after tendon injury [5]. Specifically, small interfering RNA (siRNA) YAP/TAZ knockout phenocopied the effects of tenocyte detensioning, increasing levels of catabolic MMPs, while YAP overexpression repressed MMP expression [5]. In this study, we attempt to establish a model to examine mechanisms by which YAP and TAZ regulate downstream gene expression. Our orthogonal approach is designed to determine the roles of YAP and TAZ in transcription factor co-activation and downstream transcription. Mechanistic understanding can result in non-invasive treatment strategies to maintain tensional homeostasis.

Materials and Methods

Cell Culture: Human tenocytes (HTen) (cAP-0041, Angio-Proteomie), were seeded onto a flask coated with Quick Coating Solution (Angio-Proteomie). Passaging requires careful rinsing of cells using Hanks Salt Based Solution (HBSS) (RT) twice, and lifting using Trypsin/EDTA (RT). Trypsin neutralization buffer was added after 2 minutes and cell solution was spun down at 800g for 5 minutes. Supernatant was aspirated and cell pellet was resuspended. Cells were counted for reseeding in Tenocyte Growth Medium (cAP-0040, Angio-Proteomie).

Biochemical Treatment: MGH-CP1, YAP/TEAD inhibitor, was administered to cells in serum absent Alpha-MEM + 1% Pen/Strep for 2 hours. After serum starving (2 hours), HTen media was added to cells for 6 hours before lysis for RNA.

RNAi Knockdown: RNAi using human Yap1 SMARTpool: ON-TARGETplus siRNA (Dharmacon). Non-targeting scramble siRNA served as a control. HTen were transfected using Lipofectamine RNAiMAX (Thermo Fisher Scientific) in Opti-MEM (Thermo Fisher Scientific) for 24 hours, replated at 7,500 cells/cm^2, and lysed for RNA after 72 hours.

RNA Isolation, cDNA Synthesis, and qRT-PCR: Cells were lysed directly in RNA lysis buffer supplied in RNeasy Mini Kit (Qiagen). mRNA isolation was carried out using the kit and concentration was quantified by a NanoDrop spectrophotometer. cDNA was synthesized using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qRT-PCR was quantified using SYBR Green (Thermo Fisher Scientific) and analyzed using QuantStudio 6 Pro (Applied Biosystems). Analysis was completed using the delta delta Ct method with RRN18S as a housekeeping gene and a 2 sample t-test assuming equal variance.

Results and Discussion

First, we wished to verify the effects of siRNA knockdown of YAP and TAZ. We expect that YAP/TAZ depletion will decrease YAP/TAZ abundance and suppress expression of downstream target genes including Cyr61 (cell adhesion/differentiation) and Ctgf (cell proliferation) [8].

Second, we sought to characterize a biochemical inhibitor of YAP/TAZ signaling in tenocytes using MGH-CP1, a YAP-TEAD interaction inhibitor. YAP and TAZ do not have a DNA-binding domain and must bind to a transcription factor partner like TEAD to elicit transcriptional effects [6]. Loss of the palmitate on TEAD (by MGH-CP1) inhibits solely YAP translocation into the nucleus [7].

Together, we anticipate that our orthogonal approach, featuring genetic depletion and pharmacologic inhibition, will enable study of the transcriptional dynamics of mechanotransductive regulation of matrix anabolic and catabolic genes (including Cyr61/CTGF, MMPs) in tenocyte tensional homeostasis.

Conclusions

In vitro manipulation of primary patient-derived tenocytes is challenging. This study provides a two-pronged toolbox to study the role of YAP/TAZ signaling in tenocyte mechanobiology. Biochemical YAP inhibition presented various challenges, which we are overcoming. Serum deprivation was incorporated to first drive down endogenous YAP/TAZ

activity. This starve effectively mimics an onset of detensioning. Serum replacement after 2 hours rekindles activity to show a clear difference between the control and knockdown groups. I am currently testing different concentrations of serum to examine its effects on modulating YAP/TAZ activity. Deletion of YAP/TAZ has previously been seen to elevate catabolic gene expression of MMPs, similar to gene expression after cytoskeletal detensioning [5]. Inhibition of tensional homeostasis by two approaches of YAP/TAZ knockdown/inhibition is expected to elevate matrix degradation and remodeling genotypes. Late stage tendinopathy is characterized by overuse-induced microdamage and subsequent remodeling. Here, we develop a model of acute detensioning to elucidate the catabolic gene programs regulated by YAP/TAZ mechanotransduction. This model could inform mechanisms that direct early tendinopathy.

References

[1] J. H-C. Wang, Q. Guo, and B. Li, “Tendon Biomechanics and Mechanobiology—A Minireview of Basic Concepts and Recent Advancements,” *Journal of Hand Therapy*, vol. 25, no. 2, pp. 133–141, Apr. 2012, doi: https://doi.org/10.1016/j.jht.2011.07.004.

[2] D. L. Korvick, J. F. Cummings, E. S. Grood, J. P. Holden, S. M. Feder, and D. L. Butler, “The Use of an Implantable Force Transducer to Measure Patellar Tendon Forces in Goats,” *Journal of Biomechanics*, vol. 29, no. 4, pp. 557–561, Apr. 1996, doi: https://doi.org/10.1016/0021-9290(95)00036-4.

[3] S. P. Arnoczky, M. Lavagnino, and M. Egerbacher, “The Mechanobiological Aetiopathogenesis of tendinopathy: Is It the over-stimulation or the under-stimulation of Tendon cells?,” *International Journal of Experimental Pathology*, vol. 88, no. 4, pp. 217–226, Aug. 2007, doi: https://doi.org/10.1111/j.1365-2613.2007.00548.x.

[4] C. D. Kegelman, J. M. Collins, M. P. Nijsure, E. A. Eastburn, and J. D. Boerckel, “Gone caving: Roles of the Transcriptional Regulators YAP and TAZ in Skeletal Development,” *Current Osteoporosis Reports*, vol. 18, no. 5, pp. 526–540, Oct. 2020, doi: https://doi.org/10.1007/s11914-020-00605-3.

[5] D. L. Jones *et al.*, “Mechanoepigenetic Regulation of Extracellular Matrix Homeostasis via Yap and Taz,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 120, no. 22, p. e2211947120, May 2023, doi: https://doi.org/10.1073/pnas.2211947120.

[6] A. V. Pobbati and W. Hong, “A Combat with the YAP/TAZ-TEAD Oncoproteins for Cancer Therapy,” *Theranostics*, vol. 10, no. 8, pp. 3622–3635, 2020, doi: https://doi.org/10.7150/thno.40889.

[7] Y. Sun *et al.*, “Pharmacological Blockade of TEAD–YAP Reveals Its Therapeutic Limitation in Cancer Cells,” *Nature Communications*, vol. 13, no. 1, Nov. 2022, doi: https://doi.org/10.1038/s41467-022-34559-0.

[8] D. Shome, T. von Woedtke, K. Riedel, and K. Masur, “The HIPPO Transducer YAP and Its Targets CTGF and Cyr61 Drive a Paracrine Signaling in Cold Atmospheric Plasma-Mediated Wound Healing,” *Oxidative Medicine and Cellular Longevity*, vol. 2020, p. e4910280, Feb. 2020, doi: https://doi.org/10.1155/2020/4910280.