FAK Inhibition Attenuates Increased Tendon Cell Nuclear Aspect Ratio with Applied Mechanical Strain *In Situ*

Thomas P. Leahy, BS[1]
Nathaniel A. Dyment, PhD[1]
Louis J. Soslowsky, PhD[1]

[1]McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA

Introduction

Tendons carry tensile loads via their dense extracellular matrix (ECM), which transmits mechanical strain to the resident cells. Regulatory mechanical cues maintain tendon homeostasis, as unloading and overloading both result in reduced ECM organization and mechanical integrity as well as loss of tendon cell phenotype, including changes in cell and nuclear shape and reduced tenogenic gene expression.[1–3] Focal adhesion kinase (FAK) is an intracellular protein kinase that plays a critical role in regulating cell-ECM attachment as well as turnover of the mechanosensitive actin network that transmits mechanical cues from the plasma membrane to the nucleus. While FAK activation is required for tenogenic gene expression in response to stimulation by growth factors and mechanical stretching,[4–7] the mechanism by which FAK activity regulates the cell's ability to sense mechanical stretching within the *in situ* tendon environment is unknown. Therefore, the objective of this study was to evaluate the effects of FAK inhibition on tendon cell nuclear response to mechanical strain within the *in situ* tendon ECM. We hypothesized that increases in nuclear aspect ratio (nAR) in response to applied macroscale strain would be attenuated in tendons treated with a FAK inhibitor compared to untreated tendons.

Methods

Study Design

Flexor digitorum longus (FDL) tendons from male WT adult mice were freshly dissected and maintained in DMEM supplemented with 5% FBS and 25 mM HEPES. Tendons were randomized to untreated and FAK-inhibited (FAK-I) groups (n = 5-6 tendons per group). For FAK-I tendons, media was supplemented with 10 M PF-573228 (Tocris; Minneapolis, MN) for 1 hour at 37C, while the untreated tendons were maintained for 1 hour at 37°C. Following treatment, cell nuclei were stained with DRAQ5 (1:1000) for 30 minutes, mounted within a custom mechanical loading device, and imaged with confocal microscopy at 0, 5, and 10% applied strain (Figure 1A-B). Nuclei were segmented with FIJI, nAR computed, and manually tracked between strain levels (n = 12-36 nuclei per tendon).[8] Live/dead staining was performed with calcein-AM and ethidium homodimer-1 (ThermoFisher; Waltham, MA) to confirm tissue viability within the loading system.

Mechanical Loading Device

We developed a custom mechanical loading device to apply strain to a tendon sample while being imaged on an inverted confocal microscope. The device consists of 2 linear actuators that apply mechanical strain to the tendon, along with a 20 lb. (88.96 N) load cell to monitor load. Manual stages center the tendon in the x and z directions over the objective and place it within the objective working distance. Custom LabView software was developed to operate the device, including centering the tendon over the objective in the y direction and applying mechanical strain based on the gauge length.

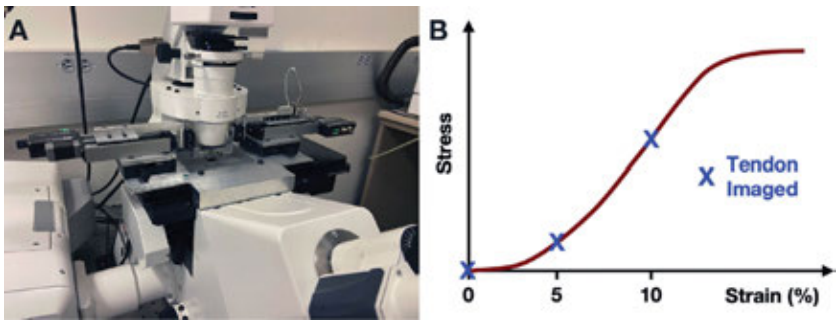

Figure 1. Custom Mechanical Loading Device. **(A)** Fabricated device mounted on a Zeiss LSM 710 inverted confocal microscope. **(B)** Stress-strain curve indicating image capture locations at 0, 5, and 10% strain.

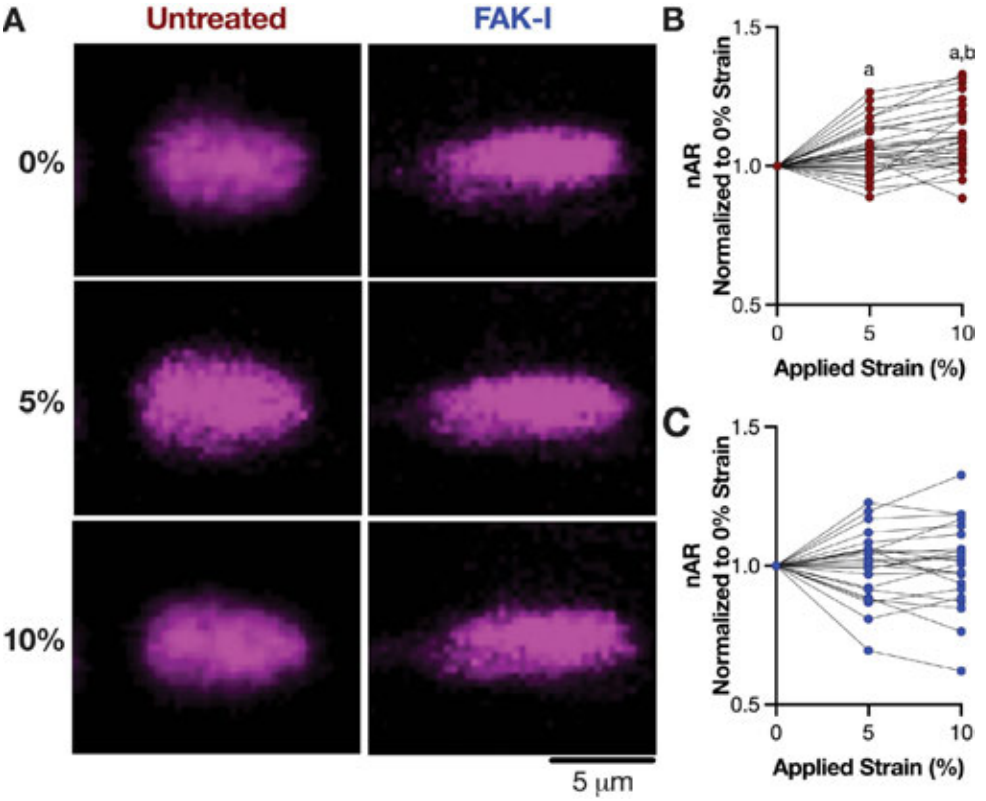

Figure 2. Increases in nAR with increasing applied strain are attenuated by FAK inhibition. **(A)** Representative images for untreated and FAK-I nuclei at the indicated applied strain value. nAR plotted across strain values for **(B)** untreated and **(C)** FAK-I samples. Statistical comparison performed on non-normalized data (data not shown) between strain levels using repeated measures one-way ANOVA with Tukey's post-hoc tests (significance at p 0.05). a, significant increase relative to 0% strain; b, significant increase relative to 5% strain.

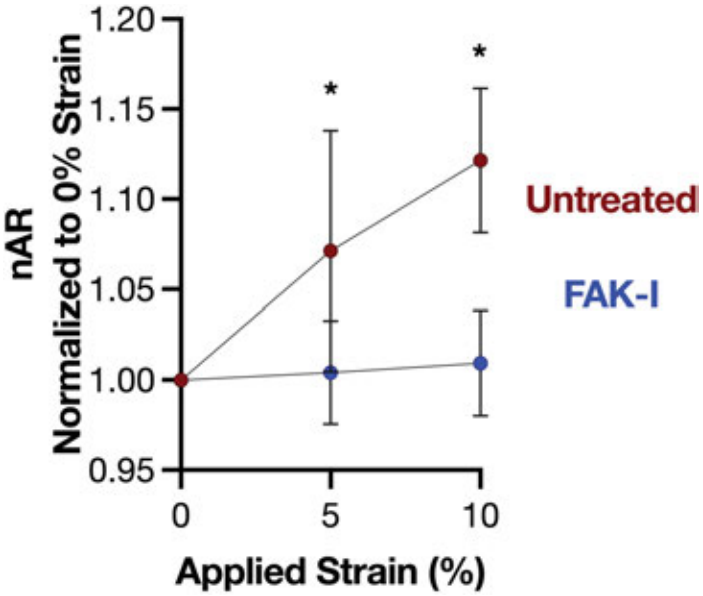

Figure 3. Increases in nAR with increasing applied strain were consistently attenuated by FAK inhibition across all samples. Data is represented as mean normalized nAR ± standard de-viation for each group. Statistical comparison performed using t-tests to compare treatment groups at 5% and 10% strain (significance at p 0.05). *, significant difference between groups at strain value.

Confocal Imaging

Confocal imaging was performed with a Zeiss LSM 710 confocal microscope while the tendon was mounted and maintained at the desired strain level. Imaging was performed with a 633nm excitation laser and 10x objective by imaging through the maximum light penetration depth at a z-stack interval of 5 m.

Results

Live/dead staining indicated that the tissue was viable with no differences between untreated and FAK-I tendons (data not shown). Nuclei tracked across strain levels in untreated tendons became increasingly elongated with applied strain (Figure 2A-B), while the nuclei from FAK-I treated tendons did not elongate across strain levels (Figure 2A,C). Across tendons, normalized nAR was decreased at both 5% and 10% strain in FAK-I tendons relative to untreated tendons (Figure 3).

Discussion

Consistent with our hypothesis, FAK-I treatment attenuated the increases in nAR with applied strain observed in the untreated tendons both within tendons (Figure 2) and across all tendons measured (Figure 3). These results indicate that FAK regulates ECM to nucleus strain transmission in tendon cells. Previous studies demonstrated that intact actin networks are required for maintenance of tendon cell fate, collagen fibril deposition, collagen crosslinking, and re-tensioning the ECM.[9–11] Given FAK's role in establishing focal adhesions to tether actin networks to the ECM, it is not surprising that FAK is required for tenogenic gene expression.[4–7] Results from the present study suggest that the dependence on FAK for tenogenic gene expression may due to its role in regulating nuclear mechanosensitivity. The rapid effect of FAK inhibition on nuclear response to strain suggests that tendon cells regularly turn over their actin networks and reestablish focal adhesions to actively probe their local mechanical environment. This result is particularly interesting in mature

tendon cells encased in established ECMs, where the local mechanical environment is presumably stable.

Significance

This study evaluated the effects of FAK inhibition on nuclear response to mechanical strain within *in situ* tendon ECM. We found that inhibition of FAK attenuated increases in nAR with applied strain, which suggests that FAK is required for tendon cell sensation of its surrounding mechanical environment.

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