The Mechanosensor Focal Adhesion Kinase Regulates Cell Shape and Tendon Development

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Introduction

Throughout development and postnatal growth, resident tendon cells respond to mechanical cues from the nascent tendon extracellular matrix (ECM) to regulate tissue properties. Focal adhesion kinase (FAK, gene: *Ptk2*) is an intracellular protein kinase that regulates the actin cytoskeleton and cell-ECM adhesions. In tendon cells, FAK activity is required for tenogenic gene expression in response to growth factor stimulation and mechanical stretching.[1–3] In addition, pharmacological inhibition of FAK in explanted tendons significantly attenuates ECM to nuclei strain transmission.[4] Despite these known roles for FAK in tendon, the mechanism by which FAK activity regulates cell mechanotransduction as well as the role of FAK-dependent mechanotransduction in tendon development remain unknown. Therefore, the objective of this study was to evaluate the regulatory role of FAK in (1) tendon cell-ECM mechanical interactions and in (2) tendon development. We hypothesized that (1) FAK activity regulates tendon cell *in vitro* focal adhesion morphology and cell spreading behavior, and that (2) reduced FAK expression will negatively impact tendon development.

Methods

In Vitro Cell Culture

Tail tendon cells were isolated from P30 WT male and female mice (3 mice in 2 independent experiments; n = 30 cells/treatment/mouse). Cells were cultured on fibronectin-coated coverslips and treated with a FAK inhibitor (10μM PF-573228; FAK-I) or vehicle (DMSO) control. Immunofluorescence staining was performed to quantify cell morphology and pFAK localization 6 hours post-treatment.

In Vivo Mouse Model

Tendon targeted FAK knockout (Scx-Cre;$FAK^{F/F}$; FAK-KO) mice were generated.[5] Achilles tendons (ATs), flexor digitorum longus tendons (FDLs), and patellar tendons (PTs) from P30 male and female FAK-KO and WT littermate controls were used for gene expression analysis, paraffin histology, and viscoelastic mechanical testing. *Gene Expression:* RNA was isolated from tendons to evaluate *Ptk2* expression using Taqman assays, with *Abl1* as a housekeeping control (n=6/genotype/sex). *Paraffin Histology:* Whole ankle and knee joints were fixed, decalcified, paraffin embedded, and sectioned in the sagittal plane (n=5/genotype/sex). Hoechst nuclear staining was used to quantify cell density and nuclear aspect ratio (nAR). Overall tissue morphology was evaluated via toluidine blue staining. *Viscoelastic Mechanics:* Tendon cross-sectional areas (CSAs) were measured (n=7-9/genotype/sex), and tendons were subjected to a viscoelastic mechanical testing protocol (preconditioning, viscoelastic stress relaxation and dynamic frequency sweep, and a quasi-static ramp to failure).

Results

In Vitro Cell Culture

FAK-I treated cells developed pronounced cell protrusions compared to DMSO treated cells (Figure 1A). While cell area was not different between groups (Figure 1B), FAK-I treated cells had significantly higher cell compactness values relative to DMSO treated cells (Figure 1C), which is indicative of the increased protrusion phenotype. Focal adhesions were closer to the cell periphery and colocalized less with pFAK staining in FAK-I treated cells relative to DMSO treated cells (Figure 1D-E).

In Vivo Mouse Model

Ptk2 expression was reduced in all FAK-KO tendons relative to WT tendons, thereby validating our conditional knockout mouse model (Figure 2). FAK-KO tendons were not remarkably distinct from WT tendons histologically, and there were no differences between groups in quantified cell density or nAR (data not shown). Interestingly, FAK-KO tendons were consistently smaller compared to WT tendons (Figure 3A), while there was

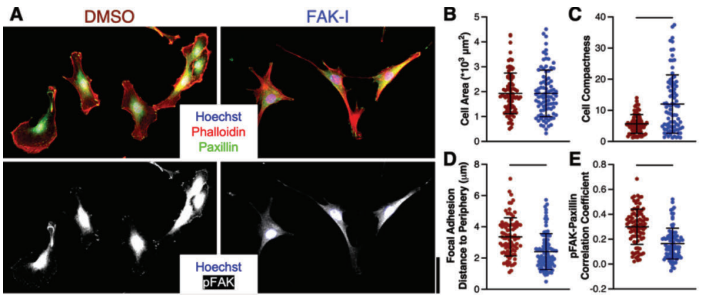

Figure 1. (A) Representative images of DMSO and FAK-I treated tail tendon cells. Scale: 50 μm; **(B)** Cell area; **(C)** cell compactness, **(D)** focal adhesion distance to cell periphery, and **(E)** pFAK-Paxillin correlation coefficient quantifications of DMSO and FAK-I treated tail tendon cells. Data was analyzed with t-tests comparing groups. Bars represent sig. diff. between groups ($p < 0.05$).

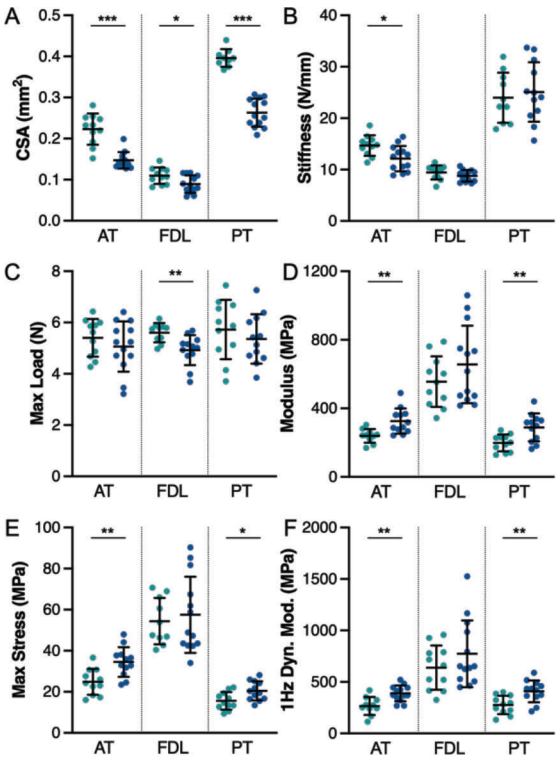

Figure 2. *Ptk2* expression for ATs, FDLs, and PTs from WT and FAK-KO tendons. Data was analyzed with t-tests comparing groups. Bars represent sig. diff. between groups ($p < 0.05$).

no difference in animal body weight (body weight data not shown). Despite the decreased size in all FAK-KO tendons, stiffness was only decreased in PTs (Figure 3B), and modulus was generally comparable and, in fact, superior in ATs (Figure 3C). Failure properties of FAK-KO tendons demonstrated decreased maximum load in the AT and PT, while maximum stress was increased in the FDL (Figure 3D-E). Viscoelastic stress relaxation was not different between groups (data not shown), though dynamic modulus was increased in all FAK-KO tendons relative to WT tendons at all frequencies evaluated (1Hz dynamic modulus data shown as representative in Figure 3F).

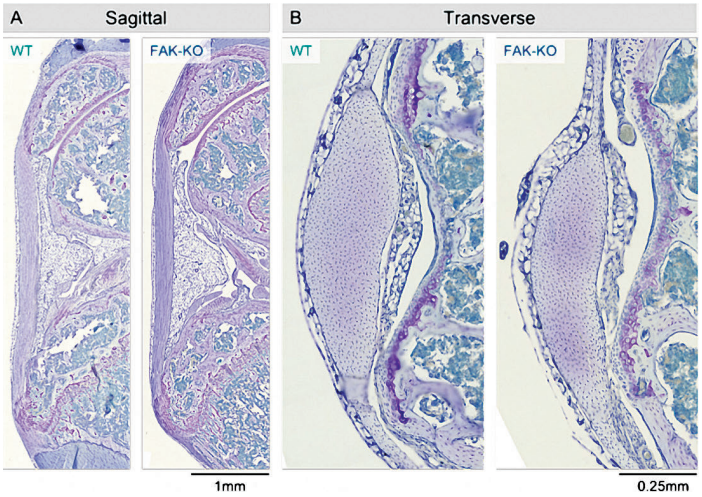

Figure 3. (A) Cross-sectional area (CSA); **(B)** stiffness; **(C)** modulus; **(D)** maximum load; **(E)** maximum stress; and **(F)** 1Hz dynamic modulus measurements for WT and FAK-KO tendons. Data was analyzed with t-tests comparing groups. Bars represent sig. diff. between groups ($p < 0.05$).

Discussion

Consistent with our hypothesis, inhibition of FAK activity in tendon cells significantly affected focal adhesion morphology and cell spreading behavior. Taken together with our previous results demonstrating attenuated ECM to nuclear strain transmission with reduced FAK activity,[4] these findings help explain FAK's regulatory role on tenogenic gene expression.[1–3] In our in vivo model, all tendons from FAK-KO mice were smaller, which is consistent with our hypothesis and indicates that FAK plays an essential role in tendon development. Interestingly, structural properties were not consistently reduced, and material properties were comparable or increased in FAK-KO tendons relative to WT tendons, potentially indicating changes in matrix assembly.

Significance

Due to the mechanical role and mechano-responsiveness of tendons, defining the key transductive pathways that regulate cell and tissue properties will be critical to better understand disease and to develop improved therapies nmnm . Our results indicate that FAK-dependent tendon cell mechanotransduction may drive tissue assembly during growth and development.

Acknowledgements

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