AMPK signaling is dysregulated in tendinopathy, altering ECM-specific cell adhesion and matrix organization

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INTRODUCTION: Tendinopathy, a disorder that results in pain, swelling, and impaired tendon function, is a clinical problem that affects ~3.5 million people in the US. Tendinopathy is caused by failure of tendon to self-repair and is characterized by degenerative extracellular matrix (ECM), decreased cell viability, and poor biomechanical function. AMP-activated protein kinase (AMPK), an energy stress sensor that maintains intracellular metabolism, homeostasis and autophagy, has recently been identified as a potential regulator of ECM remodeling in musculoskeletal tissues.1,2 For example, cartilage-specific loss of both Prkca1 and Prkca2, genes that encode AMPKα1 & 2, respectively, resulted in ECM degeneration, increased matrix metalloproteinase (MMP) expression, and cell death.2 Conversely, activation of AMPK via metformin prevented ECM degeneration, decreased MMP expression, and decreased cellular senescence in a mouse osteoarthritis model.2 Additionally, loss of AMPK α1 subunit activity, the formation of centrally located active β1-integrin and cell spreading.3 We have recently shown that in vivo loss of AMPKα1 in tendon fibroblasts (TFs) utilizing a Prkca1αβ1-Cre (AMPKcKO) mouse model results in decreased cell viability, accelerated age-dependent ECM degeneration, and impaired biomechanical properties. While our preliminary data strongly supports the necessity of AMPK for maintenance of tendon homeostasis, it remains unknown how AMPK drives cell attachment and matrix interaction in TFs. In this study we tested the hypothesis that AMPK signaling is downregulated in tendinopathy and furthermore loss of AMPK regulates cell matrix interactions and ECM organization.

METHODS: Human study procedures and protocols were approved by Institutional Review Boards (REC 11/S0704/7, HUM00196928). Bulk RNAseq was performed on tendon samples from tendinopathic and healthy hamstrings. TFs were cultured on ECM array slides (36 conditions × 9 technical replicates per condition) prepared using standard techniques and stained with picrosirius red to enhance polarized light microscopy. P1 TFs were plated on ECM array slides (36 conditions × 9 technical replicates per condition) fixed, imaged using fluorescence microscopy, and segmented & counted in Fiji/ImageJ using the StarDist plugin. To test if there is differential adhesion between the WT and AMPKcKO TFs, between different substrates, or preferential adhesion of the strains. We compared the fit of a range of Bayesian regression models.

RESULTS: We found 83 and 252 genes to be up and downregulated with tendinopathy, respectively. We identified enrichment of AMPK signaling, metabolism, and focal adhesion pathways in the tendinopathic samples compared with healthy tendons (Fig 1a). AMPK signaling pathway was driven by 7 differentially expressed genes (DEG), of which 6 were downregulated with tendinopathy (Fig 1b). Using ECM arrays, we found the negative binomial response models fit better than the Poisson response models, suggesting either shared spot level variation, substantial growth dynamics, and/or synergistic adhesion through cell-to-cell interaction. For the baseline models, where genotype and matrix were not allowed to interact, we found that the AMPKcKO strain was less adherent than the WT and that COL1, COL6, fibronecin and vitronectin were more permissive and COL3 to be less permissive for adhesion (data not shown). The interaction modeling (genotype and matrix interact) suggests there was a modest preference of AMPKcKO cell adhesion for COL4 and decreased preference for COL1 and laminin relative than what would be expected from the strain-substrate effects by themselves (Fig 2). Loss of AMPK increased tendon organization at 1 month only (Fig 3). DYSREGULATION: We found that AMPK signaling is dysregulated in tendinopathic patients. Furthermore, we observed that loss of AMPK disrupts tendon fibroblast function including adhesion and primary mouse tendon cells to specific ECM proteins but increases matrix organization earlier in life. Future work will define metabolic and transcriptional changes in AMPKcKO tendon cells as well as ECM remodeling. Our long-term goal is to identify targets of AMPK-dependent ECM remodeling for therapeutic intervention of tendon disease.

SIGNIFICANCE/CLINICAL RELEVANCE: Tendinopathy has few nonsurgical treatment options.1 Elucidating metabolic targets for druggable therapy will improve current clinical limitations.


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