

Ddr2 is Upregulated in Response to Mechanical Load in Tendons

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INTRODUCTION: Tendons can undergo profound structural and biological changes in response to mechanical loading, either normal physiological load or abnormal overload¹. Changes in the mechanical environment is perceived by resident cells and through the constituents of the extracellular matrix. Fibrillar collagens may bind to discoidin domain receptor 2 (Ddr2) to activate cellular responses. Tendons are comprised of predominantly fibrillar collagen type I, but whether the changes in the mechanical load in a tendon induce cellular responses related to Ddr2 is yet unknown. The purpose of this study was to investigate the involvement of Ddr2 in changes in the mechanical environment in a tendon. We hypothesized that overloading of the plantaris tendon by ablation of the synergist Achilles tendon would increase Ddr2 expression resulting in tendon hypertrophy.

METHODS: This study has been approved by the Weill Cornell Institutional Animal Care and Use Committee. Six 12-week old male C57BL/6J mice underwent synergist ablation. Gait analysis was performed by the DigiGait system pre-op and 2 weeks post-op (n = 5). Tissue morphology of the synergist ablated plantaris tendons at 2 weeks post-op was assessed by gross inspection and hematoxylin and eosin staining (n = 3). Tissue expression of Ddr2 in the synergist ablated plantaris tendons at 2 weeks post-op was assessed by immunofluorescence (n = 3). Achilles-plantaris tendon complexes from age-matched unoperated mice were used as controls (n = 3). mRNA expression of *Ddr2* and *Colla1* in synergist ablated plantaris tendons at 2 weeks post-op was measured by real-time quantitative polymerase chain reaction and compared to the contralateral plantaris tendon from the operated mice (n = 3). Changes in gene expression were assessed via fold change. Statistical significance was set at $p < 0.05$. Due to small sample size, a Mann-Whitney U test was used for statistical testing.

RESULTS: Gait analysis showed changes in the mechanical environment of the operated limb (Figure 1). Overloading of the plantaris tendon was confirmed by hypertrophy at gross inspection from 2 weeks post-op. Hematoxylin and eosin staining showed increased cross sectional area of synergist ablated plantaris tendons compared to un-operated plantaris tendons (Figure 2). Immunofluorescence showed increased Ddr2 in the synergist ablated plantaris tendon compared to the contralateral Achilles-plantaris tendon complex. Ddr2 expression was limited along the cell membrane in un-operated tendons. In synergist ablated plantaris tendons, Ddr2 was expressed along the cell membrane in the original tendon in a manner similar to an un-operated tendon. The increased Ddr2 expression was predominantly in the hypertrophied neo-tendon surrounding the original tendon. This was prominent especially at the tendon periphery (Figure 3). mRNA expression of *Ddr2* appeared to be increased in the synergist ablated compared to the contralateral plantaris tendons. mRNA expression of *Colla1* was increased in the synergist ablated compared to the contralateral plantaris tendon as well.

DISCUSSION: Synergist ablation changed the mechanical environment of the plantaris tendon, resulting in tendon hypertrophy. This was confirmed by increased tendon cross section area assessed by histology, and indicated overloading of the plantaris tendon. Immunofluorescence showed increased Ddr2 expression in the overloaded tendon that was most marked at the neo-tendon, suggesting the role of Ddr2 in response to mechanical load. This was also indicated by the predominant expression of Ddr2 at the neo-tendon periphery as tendon hypertrophy develops by the growth of new tendon tissues surrounding the old tissues². mRNA expression of *Ddr2* as well as *Colla1* appeared to be increased in the ablated compared to the contralateral plantaris tendons.

SIGNIFICANCE/CLINICAL RELEVANCE: Increased expression of Ddr2 in synergist ablated neo-tendon tissues indicate the involvement of Ddr2 in changes in the mechanical environment of a tendon. Further mechanistic studies on Ddr2 may suggest Ddr2 as a therapeutic target to optimize tendon biological and structural responses to mechanical loading.

REFERENCES:

1. Wang J H.-C., Mechanobiology of tendons. *Journal of Biomechanics*. 2006;39(9):1563-1582.
2. Gumucio JP, Phan AC, Ruehlmann DG. Synergist ablation induces rapid tendon growth through the synthesis of a neotendon matrix. *J Appl Physiol*. 2014;117(11):1287-91.

FIGURES

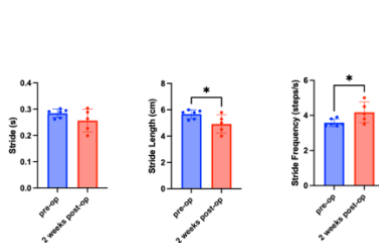


Figure 1. Gait analysis at 2 weeks post-op of the operated limb.

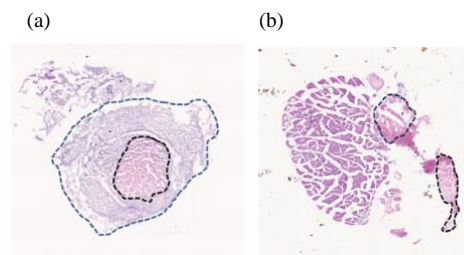


Figure 2. Representative histology image of (a) synergist ablated plantaris tendon. Original tendon matrix outlined in black; neotendon outlined in blue. (b) Representative histology image of contralateral Achilles plantaris tendon complex from the same mouse at 2 weeks post-op. Achilles-plantaris tendon complex is outlined in black.

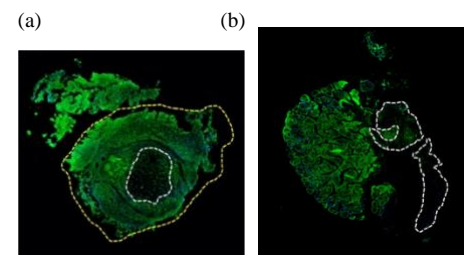


Figure 3. Representative image of Ddr2 immunofluorescence in (a) synergist ablated plantaris tendon. Original tendon matrix outlined in white; neotendon outlined in yellow. (b) Representative image of Ddr2 immunofluorescence in contralateral Achilles-plantaris tendon complex from the same mouse at 2 weeks post-op. Achilles-plantaris tendon complex is outlined in white.