Pathologic innervation in models of tendon overuse

Manyu Zhu1, Xin Xing1, Qizhi Qin1, Mingxin Xu1, Zhao Li1, Stanley Chun Ming Wu1, Masnсен Cherief1, Mary Archer1, Ziyi Wang1, Aaron W James1
1. Department of Pathology, Johns Hopkins University, Baltimore, USA, 21205
2. Department of Biomedical Engineering, Johns Hopkins University, Baltimore, USA, 21218

Email of Presenting Author: mzhu45@jh.edu

Disclosures: Manyu ZHU(N), Xin Xing(N), Qizhi Qin(N), Zhao Li(N), Mingxin Xu(N), Stanley Chun Ming Wu(N), Masnсен Cherief(N), Mary Archer(N), Ziyi Wang(N), Aaron W. James (N) (Scientific advisory board, Novadip LLC; Consultant, Lifesprout LLC and Novadip LLC; Editorial Board, Bone Research, American Journal of Pathology)

INTRODUCTION: Peripheral afferent neurons terminate at the tendon surface, transmitting nociceptive and mechanoreceptive signals. Nerve ingrowth within the tendon proper has been found as a feature of tendinopathy, indicating pro-inflammatory, nociceptive, and hypertrophic tissue responses. Chronic tendinopathy, marked by enduring tendon pain, impaired function, and structural alterations, constitutes a substantial health care challenge capable of exerting profound effects on both the well-being of individuals and athletic prowess. Overuse triggers chronic tendinopathy by subjecting the tendon to repetitive mechanical stress, which can lead to microtrauma and impaired tissue healing. Our prior work suggested after acute tendon injury, peripheral afferent nerves innervate the damaged tendon site and induce a regenerative response. However, whether and how peripheral nerves play a role in the chronic tendinopathy remains uncertain. To this end, our objective is to investigate the aberrant innervation patterns in chronic tendinopathy through mouse tendon overuse models and propose potential therapeutic insights for human chronic tendinopathy.

METHODS: All animals experiments were conducted under approval of the Johns Hopkins Animal Care and Use Committee. Human biceps tendon biopsies were identified at Johns Hopkins University surgical pathology archives under IRB approval. The slides were interpreted using the semiquantitative grading scale of Movin. We applied dorsiflexion immobilization (DI) model, in which ankles of mice were fixed, to mimic the overstrain of Achilles tendons. Immobilization devices were crafted from TempAssure 0.5-ml PCR tubes by removing caps and bottoms, drilling dual holes, and affixing with iron wire, inducing dorsiflexion and tules calcaneus positioning. 8-week-old scleras (Scx)-GFP and nerve growth factor (NGF)-eGFP were used. The devices were applied on left feet of mice to maintain dorsiflexion 6 hr/day, 5 day/week, alongside non-immobilized contralateral limb for control. In addition, a four-week intensive treadmill running (ITR) model was conducted on 10-week-old Scx-GFP mice to mimic Achilles tendon overuse. Routine histological staining was used to assess tendinopathy. Nerve distribution and vasculogenesis within Achilles tendons was documented using immunohistochemistry for Beta III Tubulin (TUBB3) and CD31. Unpaired two-tailed Student t-test was used for a two-group comparison.

RESULTS: The Achilles tendon was examined for chronic tendinopathy at 4 weeks post DI. Histological analysis revealed heightened safranin O-stained glycosaminoglycan, pronounced collagen fiber disorientation and remodeling, shown as reduced collagen fiber length (36.0% reduction) and increased collagen width (9.4% increase) in Picrosirius red (PSR) staining (Fig.1A, B). PSR result indicated subtle alterations in collagen fiber orientation 4 weeks post ITR, as well as disturbances in width. Significant increase in TUBB nerve innervation (9.5-fold increase) and CD31+ vasculatization (4.5-fold increase) in DI animals. A similar upregulation was observed in animals after ITR. Furthermore, nerves and blood vessels ingrowth into the tendon body was observed in animals underwent DI (Fig.1A). Substantial upregulation of NGF reporter activity in NGF-eGFP animals was observed 1 week after DI (37.2% increase) (Fig. 2A, B). Finally, histological analysis of human biceps tendons revealed increasing innervation within the tendon body and peritenon with increasing degree of tendinopathy (Fig. 3A, B).

DISCUSSION: Approximately half of sports injuries result from overuse, with a notable focus on tendon-related issues. Achilles tendinopathy affects roughly 30% of athletes, with an annual incidence of 7-9%. Overuse-related tendon injuries present a significant hurdle in sports medicine, currently lacking effective treatments. Our study suggested that the severity of tendinopathy exhibited a positive correlation with innervation. In mouse DI and ITR models, we revealed abnormal innervation patterns under pathological conditions. Abnormal nerve sprouting in tendinopathy amplified inflammatory and metabolic pathways during tendon regeneration, facilitated by neurotrophic factors like NGF. Continued research aims to investigate nerve modulation as a potential therapeutic strategy for addressing chronic tendinopathy.

SIGNIFICANCE/Clinical Relevance: This study presents a comprehensive investigation of innervation within the framework of tendon overuse model, suggesting future therapeutic relevance for neuromodulation in human chronic tendinopathy.