Role of Endoplasmic Reticulum Stress and the Interaction Between the Endoplasmic Reticulum and Mitochondria in the Development of Tendinopathy

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INTRODUCTION:

Chronic tendinopathy increases yearly, primarily due to long-term and repeated mechanical damage to the tendons. Recent research into the pathological mechanisms of tendons has made significant strides. However, a comprehensive understanding of the pathological processes and molecular mechanisms underlying tendinopathy remains insufficient. The most common clinical cause of supraspinatus (SST) tendinopathy is identified as rotator cuff impingement syndrome. Cong et al. introduced a mouse model of rotator cuff impingement-induced tendinopathy that effectively simulates the clinical model of shoulder impingement syndrome. This model differs from the previously standard chemical tendinitis model established by collagenase tendon injection. Additionally, a study from the Hospital for Special Surgery (HSS) in the U.S. demonstrated that oxidative stress plays a crucial role in the onset and progression of tendinopathy. When the tendon is injured, the levels of reactive oxygen species (ROS) increase while the levels of superoxide dismutase (SOD) decrease, leading to significant mitochondrial damage and degradation. Studies have shown that mitochondria and the endoplasmic reticulum (ER) are the prominent organelles responsible for synthesizing ATP and proteins in cells. They are interconnected and function synergistically in various diseases. Previous studies on heart, liver, and lung diseases have highlighted the key roles of mitochondria and the ER in regulating cell metabolism, apoptosis, and autophagy. The ER is essential for calcium storage and directly influences cell apoptosis through its calcium transport mechanisms in conjunction with mitochondria. The ER-mitochondrial calcium transport complex, composed of IP3R, Grp75, and VDAC1, is a critical target in endoplasmic reticulum stress (ERS) and plays a significant role in various disease-related phenotypes. This study aims to investigate the role of ER stress, particularly the coupling of ER and mitochondrial calcium, in the pathogenesis of rotator cuff tendinopathy. It will also examine potential targets related to ERS in the pathophysiology of tendinopathy, providing a reference for early clinical intervention.

METHODS:

A total of 65 male wild-type C57BL/6 mice were utilized for this study. Fifty-two mice were used to establish a supraspinatus tendinopathy model induced by subacromial impingement. After anesthesia, the subjects underwent bilateral shoulder surgery where titanium microchips were implanted beneath the bilateral subacromial processes and firmly fixed to the periosteum on the acromion surface. The subjects in the supraspinatus tendon (SST) impingement model were divided into four groups based on the time interval after impingement: 2 weeks, 4 weeks, 6 weeks, and 8 weeks. Sixteen normal mice were included as a control group for normal SST. The mice in each group were sacrificed at predetermined times, and the supraspinatus tendon specimens were obtained. These specimens were then thoroughly analyzed and evaluated using multiple imaging techniques, including biomechanics, histology, immunohistochemistry, quantitative polymerase chain reaction (qPCR), and electron microscopy.

RESULTS:

The mean ultimate tensile strength (UTS) and stiffness of the tendinopathy specimens exhibited a considerable decrease of approximately 50% ($P<0.001$) when compared to normal tendons (refer to Figure 2). Histological analysis revealed a significant elevation in Bonar scores following impact (Figure 1G). In the surgical group, there was a notable increase in the positive area of endoplasmic reticulum stress (ERS)-related molecules, demonstrating a consistent trend post-impact. Furthermore, genes associated with ERS were upregulated in the tendinopathy group, with a significant increase in the expression of the inositol triphosphate receptor (IP3R). Immunofluorescence staining indicated heightened molecules involved in ER-mitochondrial calcium coupling (Figure 3A-D). Transmission electron microscopy (TEM) provided evidence of substantial alterations in ERS morphology across all surgical groups (Figure 4). Additionally, semiquantitative analysis corroborated these morphological changes in ERS and underscored the augmented contacts between the endoplasmic reticulum and mitochondria in tendinopathy specimens.

DISCUSSION AND CONCLUSION:

The endoplasmic reticulum (ER) and mitochondria are pivotal in tendinopathy's progression and underlying mechanisms. These organelles are involved in the regulation of excessive apoptosis and autophagy in tendocytes.

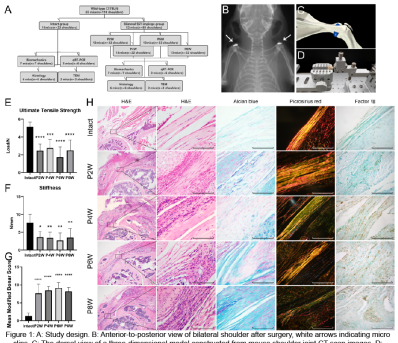

Figure 1: A: Study design. B: Anterior-to-posterior view of bilateral shoulder after surgery, white arrows indicating micro clips. C: The dorsal view of a three-dimensional model constructed from mouse shoulder joint CT scan images. D: Ultimate tensile strength test set up. E: Ultimate Tensile Strength (UTS). F: Stiffness (n=7). G: Bonar scoring of supraspinatus tendon histology(n=6). H: Histological images show increased cellularity, extracellular matrix accumulation, disordered collagen alignment, and vascular infiltration post-impingement (scale bars: 100 μm). Mean Bonar scores are shown with H&E and Alcian Blue staining. Significance levels: **** P < 0.0001, *** P < 0.001, ** P < 0.01, * P < 0.05 vs. intact tendon. Values are means with standard deviation (SD) error bars.

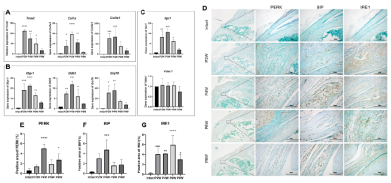

FIGURE 2 qPCR analysis of tendon function, ERS and ER-Mito Ca2+ coupling related gene expression in mice supraspinatus tendon with subacromial clip insertion and representative immunohistochemistry of supraspinatus tendon (each group n= 6 shoulders). A: Genes related to tendon function and maturation; B: Genes related to ERS, the increase of which indicating the occurrence of ERS; C: Gene expression related to ER-Mito Ca2+ coupling. D: PERK, BIP, IRE1 immunohistochemical staining in the intact and impingement groups. PERK, BIP and IRE1 are all markers indicating the activation of UPR/ERS. Scale bars depict 100μm. B-D: The positive area rate of PERK, BIP, IRE1 in each group. **** P <0.0001, *** P <0.001, ** P <0.01, * P <0.05.

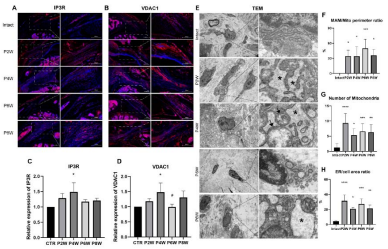

Figure 3 Representative immunofluorescence and TEM images of supraspinatus tendon. A: IP3R immunofluorescence staining in the intact and impingement groups. B: VDAC1 immunofluorescence staining in the intact and impingement groups. Scale bars depict 100μm. C-D: The positive area rate of IP3R and VDAC1. E: TEM images of SST from different time points. Asterisk marks indicating the edema, dilation, cavity degeneration and granolysis of ER, arrows indicating the swelling of mitochondria. F: Rate of the MAM length comparing to the mitochondria perimeter, indicates the alteration of communications between ER and mitochondria. G: Numbers of Mitochondria of each tenocytes. H: Rate of ER area comparing to whole tenocyte, indicates the dilation of ER and the occurrence of ERS. **** P <0.0001, *** P <0.001, ** P <0.01, * P <0.05.