HEAT SHOCK PROTEIN AND APOPTOSIS IN SUPRASPINATUS TENDINOPATHY

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Introduction: Tendinopathy and tears of the rotator cuff are common, however, the molecular and biochemical pathophysiology of these disorders is incompletely understood.

Excessive apoptosis has been found in torn supraspinatus tendon1 and mechanically loaded tendon cells2. Following oxidative and other forms of stress, one family of proteins that is often unregulated are Heat Shock Proteins (HSPs). The purpose of this study was to determine if HSPs were unregulated in human and rat models of tendinopathy and to determine if this was associated with increased expression of regulators of apoptosis (c-FLIP, Caspase 3 & 8, HSP 27 & 70). 

Materials and Methods: Methods

In the first part of this study, the expression of 5760 genes were evaluated in a running rat tendinopathy model. In the second part of the study, the expression of apoptotic regulatory genes that were unregulated in the running model were evaluated in human rotator cuff tendon.

Rat model

Twelve Sprague-Dawley rats underwent a daily treadmill running regime to model tendon overload resulting in degeneration as previously described3. After four weeks of running, rats were sacrificed by CO2 inhalation and both supraspinatus tendons were collected. Twelve non-exercised rats were used as controls.

Microarray analysis

Custom microarrays consisting of 5760 rat oligonucleotides in duplicate were used. All genes were represented by at least two independent targets on each microarray, and the signal from each target was used to calculate an average for each gene.

Human tendon model

Following ethics approval, seventeen supraspinatus tendon samples were obtained from patients with full thickness rotator cuff tears undergoing shoulder surgery. This included 7 males and 10 females with a mean age of 59 ± 3.2 SEM. The rotator cuff repair surgery was carried out using a standard arthroscopic technique. Samples of the subscapularis tendon in which there was no evidence of damage were included.

As independent controls ten samples (6 males, 4 female mean age 35 ± 3 ) of subscapularis tendon was collected from patients undergoing arthroscopic surgery for shoulder stabilization. The absence of rotator cuff tears was confirmed at the time of surgery.

Gene expression

All specimens were immediately placed in RNA later solution (Ambion Life Technologies) and stored at -20°C until RNA extraction. Total RNA was isolated from tendon tissue using Trizol Reagent (Ambion Life Technologies) as per manufactures instructions. The expression of genes of interest was evaluated by semi-quantitative RT-PCR and confirmed at the protein level by immunohistochemistry. Positive and negative control specimens were included.

Histology and Immunohistochemistry

Tendinopathy was assessed on a basic histological scale (Grade 4 = marked degeneration, 3 = advanced degeneration, 2 = moderate degeneration 1 = mild degeneration, 0 = normal tendon) by two independent assessors.

Paraffin sections were dewaxed and antigen retrieval was achieved using DAKO Target Retrieval Solution as per the manufactures instructions. Endogenous peroxidase activity was scavenged with 3% H2O2, and non-specific antibody blocked with 10% milk in TBS buffer. Tissue slides were incubated with primary antibody (c-FLIP, Caspase 3 & 8, HSP 27 & 70) diluted 1:1000 in 1% BSA/TBS at room temperature for 60 minutes. Positive and negative control specimens were included, in addition to the surgical specimens for each individual antibody staining technique.

Results: Rat Microarray analysis: Upregulation of HSP 27 (x3.4) &70 (x2.5) and cFLIP (x2.2) receptor was noted in degenerative rat supraspinatus tendon subjected to daily treadmill running for 14 days compared to tendons of animals subject to cage activity only.

Histological analysis: All torn human supraspinatus tendon showed marked tendinopathy with mucoid change. Matched subscapularis tendon showed moderate-advanced degenerative change with mucoid ground substance. All control samples showed fibrotendinous tissue with large distinct collagen fibres.

Apoptosis mRNA expression: The expression levels of caspase 3 & 8 and HSPs 27 & 70 were significantly higher in the torn edges of supraspinatus when compared to matched subscapularis tendon and control tendon (p<0.01). cFLIP showed significantly greater (p<0.001) expression in matched subscapularis compared to supraspinatus and control tendon.

Immunohistochemical analysis: cFLIP, Caspase 3 & 8 and HSP 27 and 70 was confirmed in all samples of torn supraspinatus tendon. Significantly increased immunoactivity of Caspase 3&8 and HSP 27 & 70 were found in torn supraspinatus (p<0.001) compared to matched and normal subscapularis. The proteins were localized to tendon cells.

Discussion: The finding of significantly increased levels of Heat Shock Proteins in human and rat models of tendinopathy with the co-expression of other regulators of apoptosis suggests that Heat Shock Proteins play a role in the cascade of stress activated-programmed cell death and degeneration in tendinopathy. Our finding of marked tendinopathy in matched subscapularis tendon may also provide a useful human tendinopathy model.

References:


Figure A

Relative expression of apoptosis genes compared to house-keeping gene, β-actin in human tendon samples. Data displayed as mean ± SEM, n=17 for supraspinatus and matched subscapularis, n=10 for control group. (*p<0.05, **p<0.01, ***p<0.001)