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Regulation of gene expression in human tendinopathy

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Abstract

Background: Chronic tendon injuries, also known as tendinopathies, are common among professional and recreational athletes. These injuries result in a significant amount of morbidity and health care expenditure, yet little is known about the molecular mechanisms leading to tendinopathy.

Methods: We have used histological evaluation and molecular profiling to determine gene expression changes in 23 human patients undergoing surgical procedures for the treatment of chronic tendinopathy.

Results: Diseased tendons exhibit altered extracellular matrix, fiber disorientation, increased cellular content and vasculature, and the absence of inflammatory cells. Global gene expression profiling identified 983 transcripts with significantly different expression patterns in the diseased tendons. Global pathway analysis further suggested altered expression of extracellular matrix proteins and the lack of an appreciable inflammatory response.

Conclusions: Identification of the pathways and genes that are differentially regulated in tendinopathy samples will contribute to our understanding of the disease and the development of novel therapeutics.

Background

Chronic injuries to the Achilles, patellar, extensor carpi radialis brevis, and supraspinatus tendons remain a common problem for both elite and recreational athletes, as well as for individuals engaging in repetitive activities. These overuse type injuries account for 30-50% of all sports injuries and result in a significant amount of morbidity and health care expenditure [1].

Histologic studies have shown that the primary pathology is not inflammation as implied by the commonly used term "tendonitis." Instead, samples of diseased tendons show collagen degeneration, fiber disorientation, mucoid ground substance, hypercellularity, vascular ingrowth, and relative absence of inflammatory cells under light microscopy [2-4]. Tendinopathy (or tendinosis) is now the term most commonly used to describe the clinical entity and histologic findings. Interestingly, these findings are common to all tendinopathies, suggesting a similar etiology and pathophysiology.

The etiology of tendinopathy remains unclear, but most believe that a combination of extrinsic and intrinsic factors is responsible. The extrinsic theory suggests that direct mechanical contact leads to tendon fiber micro-damage and subsequent injury of the tendon that eventually results in weakness and pain. An example is impingement of the acromion on the supraspinatus tendon, which serves as the rationale behind acromioplasty surgery [5]. The intrinsic theory suggests that the tendon itself becomes inherently degenerative, probably as a result of microscopic fiber failure leading to accumulation of damage due to inability of the tendon to self-repair. Local ischemia may also be a contributory factor. Studies on the supraspinatus tendon have shown that its mid-portion is relatively hypovascular [6]. This lack of perfusion may result in the formation of oxygen free radicals or other molecules that initiate the pathological process.

Several observations have been made about the molecular mediators of tendinopathy. Tenocyte apoptosis or "programmed cell death" has been shown to occur at an increased frequency in tendinopathy specimens [7]. Free radicals as well as cyclic loading may induce the activation of molecules that lead to apoptosis [8,9]. In addition, animal studies have shown that various cytokines

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and matrix metalloproteinases (MMPs) may be disproportionately expressed in tendinopathy specimens. The application of cyclic strain has been shown to increase the production of prostaglandin E2 (PGE2), interleukin-6 (IL6), and IL1β [10,11]. IL1β in turn increases the production of MMP1, MMP3, and PGE2 [12]. Alfredson et al. studied samples from patients with Achilles tendinopathy and found downregulation of *MMP3* mRNA and upregulation of *MMP2* and vascular endothelial growth factor compared with control samples [13]. Riley et al. reported decreased *MMP3* and *MMP2* mRNA activity, with an increase in *MMP14* [14]. These studies show that an imbalance in cytokines and MMPs exists in diseased tendons and probably contributes to the pathophysiology; however, inconsistencies in the expression of specific molecules in various studies indicate that more research needs to be done.

Currently, the only non-surgical therapies available to patients who suffer from chronic tendinopathies are physical therapy, activity modification, non-steroidal anti-inflammatory medications (NSAIDs), and steroid or platelet-rich plasma injections. These therapies offer unpredictable results, and in the case of steroids, can lead to serious side effects and more rapid degeneration of the tendon. By understanding the molecular mediators that lead to tendinopathy, novel therapeutic targets could potentially be identified for drug development. This will result in more effective treatments while minimizing side effects.

Microarray analysis has become a powerful tool in drug development. Microarrays allow researchers to screen samples of tissue for the expression of thousands of genes encoded in the human genome. This "shotgun" approach can identify which genes are active in a given sample by quantifying the production of specific mRNAs. Identification of the products of these mRNAs and their functions can serve to direct future research and development.

The purpose of this study was to investigate whether there is selective regulation of certain cytokines, matrix metalloproteinases, and protein kinases in human samples of tendinopathy compared with normal tendons, as evidenced by microarray analysis. Specifically, we were interested in determining which specific cytokines and degenerative enzymes are upregulated, and which are downregulated, in diseased samples compared with normal control tendons from the same patients. This information may contribute to the development of drugs that can selectively modulate the disease process.

Methods
Subjects
All protocols were approved by the Institutional Review Board at the Hospital for Special Surgery and informed consent was obtained from each participant. A prospective study was initiated to collect tissue from patients undergoing surgery as standard of care for tendinopathy. Biopsies (~3 mm^3) of diseased tendons and a section of grossly normal appearing tendon were collected from 35 patients. Written informed consent was obtained from all patients prior to any study-related procedure. Information on patient demographics, diagnosis, prior treatments, current procedures, and operative findings was collected and is presented in Table 1. Patients with a diagnosis of inflammatory arthritis (such as rheumatoid arthritis or lupus) or those who had prior surgery on the involved tendon were excluded. Patients who received prior corticosteroid injection were included and this information was recorded.

RNA Preparation and Hybridization
RNA extraction and isolation were performed as previously described [15]. Fifty nanograms of RNA was labeled using the WT-Ovation FFPE System (Nugen, San Carlos, CA). Labeled cDNA was hybridized to GeneChip® Human Genome U133 2.0 arrays according to the manufacturer's protocol (Affymetrix, Santa Clara, CA). For each array, all probe sets were normalized to a mean signal intensity value of 100. The default GeneChip Operating Software statistical values were used for all analyses. The raw microarray data has been deposited in the Gene Expression Omnibus (GEO) database http://www.ncbi.nlm.nih.gov/projects/geo/ as accession number GSE26051.

Identification of Differentially Expressed Genes
Expression values for all probe sets were subjected to locally weighted scatterplot smoothing (LOWESS) transformation. Correlation analysis and hierarchical clustering verified that all samples had similar patterns of expression. Only transcripts that were called as present in 65% of the samples and expressed greater than 35 signal units in either the normal or diseased samples were used for further analysis. Expression values were log2 transformed and transcripts were considered differentially regulated if the p-value based on a paired t-test analysis was < 0.01 and the average fold change was greater than 1.5. Extensive clinical information was collected for each patient. Analysis of variance models were constructed to determine whether any differences in expression could be explained by differences in histological disease score, disease duration, gender, steroid use, or NSAID use. Values presented are expressed as mean ± standard error of the mean. Differences were considered statistically significant for $p < 0.01$.

Identification of Significantly Regulated Gene Sets
Significantly regulated biological pathways were identified using a modified version of the sigPathway

Table 1 Demographics and patient clinical diagnosis

ID	Age	Sex	Diagnosis	Duration of Sx (months)	Num of Inject*	Pathology Sample	Control Sample
5	45	M	Distal Biceps Rupture	0.3		Distal Biceps	Brachialis
9	48	M	ECRB tear	12		ECRB	EDC
10	41	M	Patella Tendon Rupture	0.1		Patella	Quad tendon
12	62	M	Flexor/Pronator	18	1	flexor/pronator	Distal Flexor/Pronator
13	62	F	RTC tear	48	2	Supraspinatus	Subscap
15	32	M	Patella Tendon Tear	3		Patella tendon	Nl Patellar Tendon
16	61	F	RTC tear	24	2	Supraspinatus	Subscap
17	59	M	RTC tear	6	1	Supraspinatus	Subscap
19	45	M	Flexor/Pronator	18	3	flexor/pronator	Distal Flexor/Pronator
20	63	F	RTC tear	9	1	Supraspinatus	Subscap
21	65	F	RTC tear	3	1	Supraspinatus	Biceps
23	64	F	RTC tear	8	1	Supraspinatus	Subscap
24	50	M	RTC tear	18	1	Supraspinatus	Biceps
26	55	M	ECRB tear	4		ECRB	Distal ECRB
27	50	M	RTC tear	4		Supraspinatus	Normal RTC
28	41	M	Supra tear (PT), OA, Bankart	>24		Supraspinatus	Biceps Tendon
29	46	M	RTC tear, Adhesive Capsulitis	4		Supraspinatus	Biceps Tendon
30	52	M	ECRB tear	12		ECRB	ECRL
31	66	M	RTC tear	6		Supraspinatus	Subscap
32	46	F	RTC tear	2	1	Supraspinatus	Teres minor
33	44	M	RTC tear	6	1	Supraspinatus	Subscap
34	59	F	RTC tear	1	3	Supraspinatus	Biceps
35	49	F	RTC tear	24		Supraspinatus	Subscap

*Number of corticosteroids injections.
ECRB Extensor Carpi Radialis Brevis.
RTC Rotator Cuff.
OA Osteoarthitis.
EDC extensor digitorum comunis.

algorithm [16] incorporating a modified normalization routine and using gene sets collections C1 to C5 defined by the Molecular Signatures Database (MSigDB) [17]. A gene set was considered significant when $q1 \leq 0.05$ and $q2 \leq 0.05$, where q1 or q2 are the permutation-based false-discovery rates for the Q1 or Q2 hypotheses (see [18] for an explanation of Q1 and Q2). These cut-offs were met by 1761 gene sets, which can be considered as differentially expressed.

Histology

Tissue samples were frozen and sectioned for histological analysis. Sections were processed using standard procedures, including Alcian blue (AB) staining. Sections were scored on four categories: intratendinous cellular proliferation, intratendinous vascular proliferation, intratendinous glycosaminoglycan (GAG) accumulation, and intratendinous fiber disorientation. Each category was scored based on a 5-point scale, from 0 for normal, 3 for moderate, and 5 for severe, by two independent histologists.

Results

Analysis of Tendinopathy Tissue Samples

Diseased and normal tendons were collected from 35 patients with rotator cuff tendinopathy, lateral epicondylitis (tennis elbow), patellar tendinopathy, or chronic Achilles tendinopathy who were undergoing surgery as standard of care treatment for tendinopathy. Tendinopathy was confirmed both macroscopically and microscopically. The diseased tendon appeared dull, yellow, and soft compared with normal tissue, which is shiny, white, and firm. Normal tendon tissue had relatively few cells, contained little vasculature, and had collagen fibers aligned in the direction of force. All diseased tendons had a similar loss of normal fibrillar structure and changes in cell orientation, which are histological characteristics of tendinopathy [2,19,20]. All diseased tissue had an increased number of fibroblasts, increased vasculature, and increased glycosaminoglycan staining (GAG content) although the degree of increase in each of the parameters was variable between patients (Figure 1, Table 2). There was little evidence of any inflammatory cells in either the tendon or the surrounding paratenon.
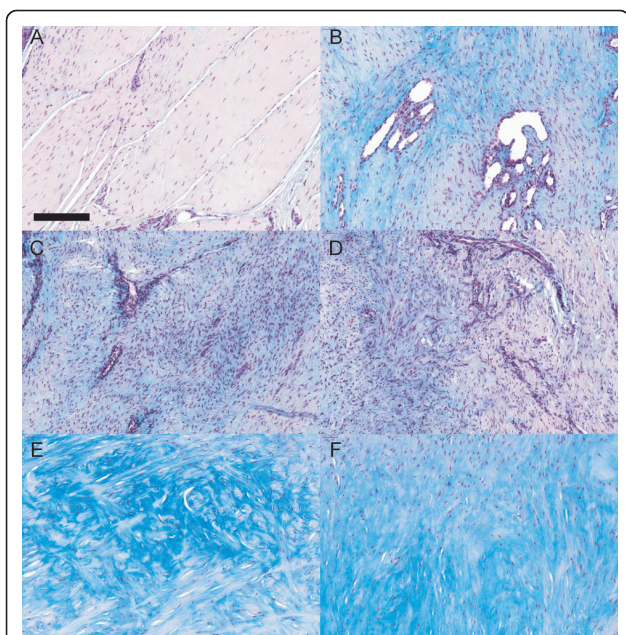
Figure 1 Histological evaluation of human tendinopathy.
Representative diseased tendons were stained with Alcian blue to
visualize the proteoglycans in patient 12 (A), patient 10 (B), patient
15 (C), patient 24 (D), patient 25 (E), and patient 32 (F). Only a few
bundles from patient 12 appear normal. The remaining sections
show highly disorganized tissues with increased cell proliferation,
increased vasculature, and/or increased GAG content. Scale bar is
200 μM.

Analysis of Gene Expression

Global transcriptional analysis using Affymetrix genome-
wide U133 2.0 Plus arrays identified 20055 transcripts
expressed in 23 pairs of normal and diseased tendons
that passed quality control filters. Of these, 983 tran-
scripts were differentially regulated based on a paired t-
test (p < 0.01, fold change ≥1.5. Among the top regu-
lated genes were a disintegrin and metalloprotease 12
(*ADAM12*), tenascin C (*TNC*), periostin (*POSTN*), and
interleukin 13 receptor alpha 2 (*IL13RA2*; Figure 2,
Table 3). An analysis of covariance (ANCOVA) showed
little relationship between the age of the patient and the
level of gene expression for most transcripts. Further-
more, ANCOVA analysis suggested that < 1.2% of the
transcripts showed any relationship between gene
expression and histological disease score, disease dura-
tion, steroid use, NSAID use, gender, or duration of
symptoms.

Global pathway analysis, based on a modified sigPath-
way algorithm [16], identified increased expression of
genes involved in the extracellular matrix, including
genes involved in focal adhesion, integrin signaling, and
collagen synthesis. In addition, expression of genes
involved in cell cycle progression, TGFβ signaling, and
NFκB signaling was increased (Table 4). This global

pathway analysis did not suggest that cytokines as a
whole were differentially regulated. However, of the 426
transcripts annotated by Gene Ontology as cytokines,
cytokine signaling molecules, or related to cytokines, six
were differentially regulated in diseased tendons, includ-
ing chemokine-like factor super family 4 (*CMTM4*),
interleukin 13 receptor alpha 2 (*IL13RA2*), interleukin
17D (*IL17D*), and interleukin 4 receptor (*IL4R*) (Table 5).

Consistent with a role in increased matrix turnover,
tendonopathy tissue exhibited increased expression of
genes encoding MMPs (Table 6) and genes encoding
collagens (Table 7). The majority of collagens interro-
gated showed a modest 2-fold increase in expression in
diseased tendons. Diseased tendons also showed
increased expression of several enzymes that mediate
collagen breakdown, including *MMP2*, *MMP9*, *MMP14*,
MMP13, and *MMP19*. While many MMP genes were
induced in diseased tendons, not all transcripts encoding
MMPs were upregulated; in fact, *MMP3* and *MMP24*
were significantly downregulated in diseased tissue. Of
the four known tissue inhibitors of metalloproteases
(TIMPs), only expression of *TIMP1* was increased. No
ADAMTS (a disintegrin and metalloprotease with
thrombospondin motifs) family members were differen-
tially regulated based on our cut-offs.

Diseased tendons showed increased expression of a
number of relevant and important signaling pathways;
for example the fibroblast growth factors *FGFR1* (1.7-
fold) and *FGFR2* (2.7-fold), and genes in the Notch sig-
naling pathway, including *JAG1* (3.1-fold) and *Notch3*
(3.6-fold). There was also evidence for increased WNT
signaling. Among the highest regulated transcripts, four
are involved in WNT signaling: *WISP1* (4.2-fold), *DKK3*
(3.6-fold), *WNT3* (3.6-fold), and *LEF1* (4.1-fold). Addi-
tionally, tendinopathy tissue showed differential expres-
sion of other WNT pathway genes, including increased
expression of *CTNNB* (1.5-fold), *WNT5a* (2.7-fold), and
FZD1 (1.7-fold) and decreased expression of *LRP5* (0.59-
fold) and *FRZB* (0.35-fold).

We found little evidence for endochondral ossification
even though this has been reported in Achilles and
patella tendinopathy [21]. Histological evaluation failed
to identify any gross areas of ossification in any of the
patients and RNA analysis did not show increased
expression of the bone markers osteocalcin or osterix.
However, expression of *CBFA1*, a transcription factor
associated with osteoblast differentiation, was increased
1.8-fold and that of *RANKL*, a key factor for osteoclast
differentiation and activation, was increased 4.0-fold in
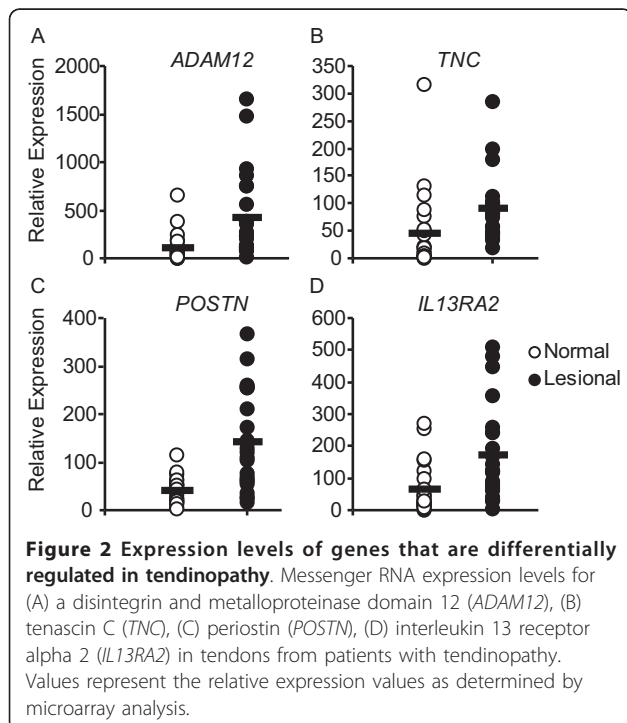diseased tendons.

In addition to the global pathway analysis, we specifi-
cally investigated the regulation of kinases in diseased
tendons (Table 8). The most statistically significant
regulated kinase is diseased tendons is the thousand and

Table 2 Intratendinous tendinopathy score[a]

Patient[b]	Cellular Proliferation	Vascular Proliferation	GAG Accumulation	Fiber Disorientation	Total Score
5	3.5	1.5	1.5	5	11.5
9	5	2	5	5	17
10	4.5	5	5	5	19.5
12	4	5	1	5	15
13	5	1	3.5	5	14.5
15	5	5	5	4.5	19.5
16	3.5	2.5	2	5	13
17	5	4.5	3	5	17.5
19	4.5	5	3	5	17.5
20	5	3	3	5	16
21	5	1	2	5	13
23	4.5	3	4	5	16.5
24	5	5	3	5	18
26	5	3	5	5	18
27	4.5	3	1	5	13.5
28	5	1.5	5	5	16.5
29	5	5	5	5	20
30	4	4.5	3	4.5	16
31	4.5	1.5	4	5	15
32	4.5	1.5	5	5	16
33	5	0.5	4	5	14.5
34	5	0.5	3	5	13.5
35	5	5	3	5	18

[a]Average of two independent histologist evaluation.
[b]Normal tendon were scored 0 in all fields.

Figure 2 Expression levels of genes that are differentially regulated in tendinopathy. Messenger RNA expression levels for (A) a disintegrin and metalloproteinase domain 12 (*ADAM12*), (B) tenascin C (*TNC*), (C) periostin (*POSTN*), (D) interleukin 13 receptor alpha 2 (*IL13RA2*) in tendons from patients with tendinopathy. Values represent the relative expression values as determined by microarray analysis.

one amino acid kinase (*TAOK1*), which showed a 1.7-fold increase in expression. TAOK1 activates the p38 MAP kinase pathway and tendonopathy tissue also showed increased expression of multiple MAP kinases, including *MAP3K2* (1.8-fold), *MAP4K5* (1.9-fold), and *MAPK8* (2.0-fold).

Discussion

We have evaluated gene expression and histological changes associated with human tendinopathy. While gene expression changes in human tendinopathy have previously been described [13,22,23], this study represents the largest and most complete study to date. However, our study has several limitations. First, control tendons were taken from the same joints as the diseased tendons. These control tendons appeared normal macroscopically but their proximity to the diseased tendon may have some effect on the control tendon. Furthermore, the control tendon was an anatomically distinct tendon exposed to different loads and strains and it is not known how this may affect the gene expression profile. Moreover, the tendon specimens were collected from a heterogeneous population with differences in age, gender, symptoms, duration of disease, and physical activity (Table 1). While changes in

Table 3 Top regulated transcripts in human tendinopathy as determined by microarray analysis

Gene Symbol	Description	p-value	q-value	Fold Change
ADAM12	A disintegrin and metalloproteinase domain 12	1.57E-05	2.09E-04	5.6
AP3M1	Adaptor-related protein complex 3, mu 1 subunit	1.24E-03	1.91E-03	1.7
ARL7	ADP-ribosylation factor-like 7	1.43E-03	2.11E-03	4.0
ARPC5	Actin related protein 2/3 complex, subunit 5	3.63E-05	2.10E-04	1.8
ASXL1	Additional sex combs like 1 (Drosophila)	1.11E-03	1.78E-03	1.8
ATP6V1A	ATPase, H+ transporting, lysosomal 70 kDa	4.18E-05	2.10E-04	2.5
BACE2	Beta-site APP-cleaving enzyme 2	3.17E-06	8.01E-05	1.9
BNC2	Basonuclin 2	1.08E-04	3.15E-04	2.9
CA12	Carbonic anhydrase XII	3.57E-05	2.10E-04	2.8
CTHRC1	Collagen triple helix repeat containing 1	5.20E-04	1.10E-03	4.4
DDEF1	Development and differentiation enhancing factor	2.83E-05	2.10E-04	2.1
DOCK10	Dedicator of cytokinesis 10	7.91E-03	8.11E-03	3.3
EDIL3	EGF-like repeats and discoidin I-like domains 3	4.80E-03	5.34E-03	2.9
FOXP1	Forkhead box P1	4.67E-05	2.10E-04	1.7
GPR161	G protein-coupled receptor 161	2.76E-04	6.89E-04	1.9
IGFBP3	Insulin-like growth factor binding protein 3	7.29E-03	7.67E-03	3.5
IL13RA2	Interleukin 13 receptor, alpha 2	2.80E-03	3.50E-03	3.5
ILK	Integrin-linked kinase	1.00E-04	3.15E-04	1.6
IQGAP1	IQ motif containing GTPase activating protein 1	4.73E-05	2.10E-04	1.5
ITGB1	Integrin, beta 1	3.52E-04	8.29E-04	1.7
ITPKB	Inositol 1,4,5-trisphosphate 3-kinase B	7.37E-04	1.34E-03	2.5
JAG1	Jagged 1 (Alagille syndrome)	1.90E-03	2.52E-03	3.1
KCNE4	Potassium voltage-gated channel 4	8.19E-03	8.19E-03	3.4
LAMA4	Laminin, alpha 4	6.73E-04	1.31E-03	3.4
LRRC15	Leucine rich repeat containing 15	3.50E-03	4.24E-03	3.2
LRRC17	Leucine rich repeat containing 17	1.74E-03	2.40E-03	2.8
MARCKS	Myristoylated alanine-rich kinase C substrate	3.93E-03	4.50E-03	2.8
NOTCH3	Notch homolog 3 (Drosophila)	1.65E-03	2.36E-03	3.6
PDIR	protein disulfide isomerase-related	4.86E-04	1.08E-03	2.1
POSTN	Periostin, osteoblast specific factor	1.10E-04	3.15E-04	3.5
RAP1GDS1	RAP1, GTP-GDP dissociation stimulator 1	1.01E-03	1.68E-03	2.1
S100A10	S100 calcium binding protein A10	1.95E-03	2.52E-03	2.8
SIN3A	SIN3 homolog A, transcription regulator (yeast)	9.51E-05	3.15E-04	1.7
SLC2A13	Solute carrier family 2 13	5.41E-03	5.85E-03	2.8
TAOK1	TAO kinase 1	1.51E-04	4.03E-04	1.7
TJP1	Tight junction protein 1 (zona occludens 1)	6.87E-04	1.31E-03	1.6
TNC	Tenascin C (hexabrachion)	9.20E-05	3.15E-04	5.3
UBE2E3	Ubiquitin-conjugating enzyme E2E 3	8.72E-04	1.52E-03	3.7
WDR1	WD repeat domain 1	4.01E-06	8.01E-05	1.6
WISP1	WNT1 inducible signaling pathway protein 1	3.93E-03	4.50E-03	3.4

gene expression were not related to any of these differences, they all contribute to variations in the data. Given the vast variation in the human population, we reasoned that control samples from the same patients would be a better control for interpatient variability than tendons from control patients without any tendon symptoms.

Progression of tendinopathy is dependent on extracellular matrix integrity and remodeling of the tendon is a common feature of tendinopathy [24]. The extracellular matrix of normal tendons consists of many of structural proteins (collagens) and proteoglycans. Collagen is the main component of tendons and type I collagens account for 65% to 80% of the total tendon mass [25]. mRNA levels for type I and type III collagens (*COL1A1*, *COL1A2*, and *COL3A1*) were increased in the diseased tendons. Tendons also contain several proteoglycans, although none of the proteoglycans analyzed (including aggrecan, versican, decorin, biglycan, fibromodulin, and lumican) showed increased expression in diseased

Table 4 Genesets regulated in human tendinopathy as determined by sigpathway

	NTk*
Up regulated	
HSA04110_CELL_CYCLE	6.52
RNA_PROCESSING	6.44
SPINDLE	6.25
HSA04510_FOCAL_ADHESION	6.10
TGFBETA_LATE_UP	6.02
CELL_CYCLE_KEGG	6.00
CELL_CYCLE	5.94
ST_INTEGRIN_SIGNALING_PATHWAY	5.40
COLLAGEN	5.32
RUIZ_TENASCIN_TARGETS	5.24
I_KAPPAB_KINASE_NF_KAPPAB_CASCADE	5.23
MITOSIS	5.03
INOS_ALL_UP	4.89
Down Regulated	
ION_TRANSMEMBRANE_TRANSPORTER_ACTIVITY	-4.99
G_PROTEIN_COUPLED_RECEPTOR_ACTIVITY	-5.01
ELECTRON_TRANSPORT_CHAIN	-5.21
MOOTHA_VOXPHOS	-6.01
ION_CHANNEL_ACTIVITY	-6.20
SUBSTRATE_SPECIFIC_CHANNEL_ACTIVITY	-6.27

*As defined in Tian et. al [18].

tendons. In contrast, expression of the non-collagen glycoproteins fibronectin, tenascin C, fibrillin, and laminin was increased in diseased tendons. Consistent with active remodeling, increased MMP activity, particularly of MMP2 and MMP9, has been associated with ruptured Achilles tendon [26]. Diseased tendons showed increased expression of several matrix associated proteins including *MMP19, MMP9, MMP13, MMP14*, and *MMP2*. In contrast, *MMP3* expression was decreased in tendinopathy in both our study and in others [13,22].

We can begin to speculate how MMP activity is regulated in tendinopathy tissue. Diseased tissues showed increased expression of components of the JAK/STAT pathway (*STAT3*, 1.9-fold, and *JAK3*, 1.8-fold). The JAK/STAT pathway is known to activate *IL4R* [27], which induces expression of *IL13RA2* [28]. Expression of both *IL4R* and *IL13RA2* was elevated in diseased tissue (3.2- and 3.5-fold, respectively). IL13, secreted by T helper type 2 cells, has anti-inflammatory properties and through interaction with its receptor IL13RA2 induces certain MMPs [29] including *MMP9* and *MMP14* [30]. Together, these findings suggest that activation of the JAK/STAT pathway may lead to increased MMP activation.

Chard et al. previously suggested that tendon pathology is linked to formation of excessive fibrocartilage [31]; however, other prior studies [22] and our current study found little evidence of chondrocyte-like cells. Tendinopathy tissue showed decreased expression of a number of cartilage markers including *COMP*, but expression of other cartilage markers such as aggrecan was not altered. In contrast, expression of type I collagen was increased, further supporting the absence of increased chondrocytic cells in diseased tendons. This is in contrast to an overuse animal model of tendinopathy [32,33] that suggested increased formation of fibrocartilage associated with overuse. This discrepancy can possibly be explained by differences in disease severity. The human samples were collected during surgery following tendon tear or rupture and represent late stage disease in which surgical intervention became necessary, while the animal models represent earlier development of disease. Furthermore, the human tendon samples represent

Table 5 Chemokines regulated in human tendinopathy

Gene Symbol	Description	p-value	q-value	Fold Change
BMP1	Bone morphogenetic protein 1	0.000	0.005	1.69
BMP8B	Bone morphogenetic protein 8b	0.002	0.011	1.79
CCL2	Chemokine (C-C motif) ligand 2	0.005	0.014	1.74
CMTM4	Chemokine-like factor super family 4	0.008	0.017	1.98
CMTM6	Chemokine-like factor super family 6	0.014	0.023	1.37
IFNGR1	Interferon gamma receptor 1	0.027	0.034	1.42
IL13RA2	Interleukin 13 receptor, alpha 2	0.003	0.011	3.48
IL17D	Interleukin 17D	0.008	0.017	0.54
IL6R	Interleukin 6 receptor	0.026	0.034	0.73
LEPR	Leptin receptor	0.028	0.034	0.49
OSMR	Oncostatin M receptor	0.038	0.043	1.81
SP110	SP110 nuclear body protein	0.010	0.019	1.51
STAT2	Signal transducer and activator of transcription 2	0.051	0.054	1.4
STAT3	Signal transducer and activator of transcription 3	0.004	0.013	1.87
TXLNA	Taxilin	0.001	0.006	1.39

Table 6 Change in matrix metallopeptidase gene expression in human tendinopathy

Gene Symbol	Description		p-value	q-value	Fold Change
Regulated					
MMP2	Matrix metallopeptidase 2	Expressed	7.42E-03	4.82E-02	1.8
MMP3	Matrix metallopeptidase 3	Expressed	5.50E-02	2.38E-01	0.3
MMP14	Matrix metallopeptidase 14	Expressed	1.79E-03	1.66E-02	1.9
MMP19	Matrix metallopeptidase 19	Expressed	1.91E-03	1.66E-02	3.4
Not Regulated					
MMP28	Matrix metallopeptidase 28	Expressed	0.09	0.30	1.4
TIMP1	TIMP metallopeptidase inhibitor 1	Expressed	0.10	0.30	1.6
TIMP2	TIMP metallopeptidase inhibitor 2	Expressed	0.37	0.50	1.1
TIMP3	TIMP metallopeptidase inhibitor 3	Expressed	0.26	0.49	0.9
TIMP4	TIMP metallopeptidase inhibitor 4	Expressed	0.71	0.80	0.9
Expressed below limited of detection					
MMP1	Matrix metallopeptidase 1	-	0.96	0.96	1.0
MMP7	Matrix metallopeptidase 7	-	0.34	0.49	0.8
MMP8	Matrix metallopeptidase 8	-	0.81	0.84	0.8
MMP9	Matrix metallopeptidase 9	-	0.00	0.02	3.2
MMP10	Matrix metallopeptidase 10	-	0.08	0.30	0.8
MMP12	Matrix metallopeptidase 12	-	0.79	0.84	0.9
MMP13	Matrix metallopeptidase 13	-	0.03	0.14	2.6
MMP15	Matrix metallopeptidase 15	-	0.15	0.35	0.5
MMP16	Matrix metallopeptidase 16	-	0.57	0.67	0.6
MMP17	Matrix metallopeptidase 17	-	0.56	0.67	0.8
MMP20	Matrix metallopeptidase 20	-	0.53	0.67	0.7
MMP21	Matrix metallopeptidase 21	-	0.13	0.33	1.5
MMP23A/B	Matrix metallopeptidase 23A/B	-	0.34	0.49	0.8
MMP24	Matrix metallopeptidase 2	-	0.29	0.49	0.6
MMP25	Matrix metallopeptidase 25	-	0.18	0.40	0.7
MMP26	Matrix metallopeptidase 26	-	0.34	0.49	0.8
MMP27	Matrix metallopeptidase 27	-	0.33	0.49	0.8

small biopsies, mostly from the midsubstance. One might expect any increase in fibrocartilage to occur near the bone insertion site, an area from which samples were not collected.

Global pathway analysis identified novel pathways that are differentially regulated in human tendinopathy including regulation of genes involved in WNT signaling. Tendinopathy is characterized by increased cellular infiltration and proliferation. Increases in WNT signaling can promote proliferation and maintain cells in an undifferentiated state [34] and the regulation of multiple WNT signaling genes suggests that this pathway is activated in tendinopathy. Pathway analysis also suggested an increased regulation of genes involved in integrin signaling. Integrins are receptors involved in cell adhesion to the extracellular matrix and transmit extracellular signals into the cell to regulate gene expression. While many of the genes encoding integrins were only slightly upregulated, expression of integrin beta 1 (fibronectin receptor) was significantly increased in tendinopathy tissue.

We found little direct evidence to support an inflammatory response in established human tendinopathy. Histological evaluation did not identify a significant number of inflammatory cells and analysis of the gene expression of inflammatory cytokines identified only a handful of cytokine genes that were differentially regulated in diseased tendons, although a number of these are implicated in tendon function and tendon healing. Expression of the proinflammatory cytokine *IL17D* was reduced in diseased tendons. IL17 increases the turnover of type I collagen through both inhibiting its synthesis and promoting its breakdown [35], and members of the IL17 cytokine family members are inhibitors of human hematopoietic progenitor proliferation [36]. The oncostatin M receptor was significantly upregulated in diseased tendons. Oncostatin M contributes to the release of proteoglycans and the breakdown of collagens [37]. IL6 is a pro-inflammatory cytokine important for tendon healing; lack of IL6 prevents proper tendon healing [38]. Evidence for aberrant regulation of the IL6 pathway in damaged tendons

Table 7 Change in collagen gene expression in human tendinopathy

Gene Symbol	Description		p-value	q-value	Fold Change
Regulated					
COL1A1	Collagen, type I, alpha 1	Expressed	9.37E-03	3.96E-02	1.7
COL1A2	Collagen, type I, alpha 2	Expressed	4.45E-03	3.16E-02	1.6
COL3A1	Collagen, type III, alpha 1	Expressed	1.50E-02	4.28E-02	2.0
COL4A1	Collagen, type IV, alpha 1	Expressed	1.91E-02	4.53E-02	2.7
COL4A2	Collagen, type IV, alpha 2	Expressed	6.09E-03	3.16E-02	2.3
COL5A1	Collagen, type V, alpha 1	Expressed	1.20E-02	4.28E-02	2.0
COL5A2	Collagen, type V, alpha 2	Expressed	3.99E-03	3.16E-02	2.1
COL5A3	Collagen, type V, alpha 3	Expressed	3.02E-03	3.16E-02	2.0
COL6A1	Collagen, type VI, alpha 1	Expressed	2.93E-02	6.18E-02	1.8
COL6A2	Collagen, type VI, alpha 2	Expressed	3.62E-02	6.89E-02	1.8
COL6A3	Collagen, type VI, alpha 3	Expressed	5.45E-03	3.16E-02	1.7
COL8A1	Collagen, type VIII, alpha 1	Expressed	2.49E-02	5.57E-02	2.0
COL8A2	Collagen, type VIII, alpha 2	Expressed	1.48E-02	4.28E-02	1.8
COL10A1	Collagen, type X, alpha 1	Expressed	1.69E-02	4.28E-02	2.1
COL12A1	Collagen, type XII, alpha 1	Expressed	4.10E-03	3.16E-02	2.1
COL18A1	Collagen, type XVIII, alpha 1	Expressed	6.65E-03	3.16E-02	2.2
COL27A1	Collagen, type XXVII, alpha 1	Expressed	1.20E-03	3.16E-02	2.5
Not Regulated					
COL7A1	Collagen, type VII, alpha 1	Expressed	0.96	0.96	1.0
COL11A1	Collagen, type XI, alpha 1	Expressed	0.58	0.71	1.2
COL15A1	Collagen, type XV, alpha 1	Expressed	0.51	0.64	1.2
COL16A1	Collagen, type XVI, alpha 1	Expressed	0.12	0.19	1.4
COL21A1	Collagen, type XXI, alpha 1	Expressed	0.34	0.45	1.4
Expressed below limited of detection					
COL2A1	Collagen, type II, alpha 1	-	0.21	0.31	0.5
COL4A3	Collagen, type IV, alpha 3	-	0.15	0.23	0.6
COL4A4	Collagen, type IV, alpha 4	-	0.90	0.96	1.0
COL4A5	Collagen, type IV, alpha 5	-	0.71	0.84	0.9
COL4A6	Collagen, type IV, alpha 6	-	0.94	0.96	1.0
COL9A1	Collagen, type IX, alpha 1	-	0.94	0.96	1.0
COL9A2	Collagen, type IX, alpha 2	-	0.05	0.08	1.9
COL11A2	Collagen, type XI, alpha 2	-	0.12	0.19	0.7
COL13A1	Collagen, type XIII, alpha 1	-	0.08	0.13	2.0
COL14A1	Collagen, type XIV, alpha 1	-	0.02	0.04	1.8
COL17A1	Collagen, type XVII, alpha 1	-	0.33	0.45	1.3
COL19A1	Collagen, type XIX, alpha 1	-	0.02	0.04	0.5
COL22A1	Collagen, type XXII, alpha 1	-	0.03	0.07	0.5
COL23A1	Collagen, type XXIII, alpha 1	-	0.75	0.84	1.0
COL24A1	Collagen, type XXIV, alpha 1	-	0.33	0.45	1.3
COL25A1	Collagen, type XXV, alpha 1	-	0.76	0.84	0.9

includes decreased expression of the *IL6* receptor. *IL6* can also signal through *STAT3*, which was upregulated in diseased tendons. *STAT3* expression has also been identified in ruptured rotator cuff. However, activation of STAT3 is mainly induced by proliferating vessels [39], and since diseased tendons have increased vasculature, many of the observed changes in cytokine expression may simply be due to this change in vasculature. Therefore, whether these cytokines play a direct role in tendonopathy requires further study. In addition to the lack of evidence for direct regulation of many pro-inflammatory cytokines, there is also indirect evidence for the absence of inflammatory cytokine activity. The lack of expression of *MMP1* and *MMP13*, which are known to be induced by inflammatory cytokines [40], further supports the proposal that pro-inflammatory cytokines do not play a major role in tendinopathy at this late stage of disease.

Table 8 Kinases regulated in human tendinopathy

Gene Symbol	Description	pValue	q-value	Fold Change
ABL2	v-abl Abelson leukemia viral oncogene homolog 2	4.79E-03	7.83E-03	31.0
BMPR2	Bone morphogenetic protein receptor, type II	4.10E-03	7.83E-03	27.0
CAV2	Caveolin 2	1.22E-02	1.44E-02	43.0
CDK6	Cyclin-dependent kinase 6	1.37E-02	1.53E-02	24.0
CSNK1D	Casein kinase 1, delta	5.21E-03	8.13E-03	19.0
CSNK1G1	Casein kinase 1, gamma 1	8.18E-03	1.03E-02	40.0
DCLK1	Doublecortin-like kinase 1	3.78E-03	7.83E-03	28.0
EML4	Echinoderm microtubule associated protein like 4	9.69E-04	3.15E-03	41.0
EPS8	Epidermal growth factor receptor substrate 8	1.96E-04	1.67E-03	9.0
FGFR1	Fibroblast growth factor receptor 1	5.64E-03	8.46E-03	1.7
ILK	Integrin-linked kinase	1.00E-04	1.67E-03	1.6
JAK3	Janus kinase 3	4.62E-03	7.83E-03	1.8
KIAA0220	PI-3-kinase-related kinase SMG-1	2.70E-03	7.02E-03	1.6
KIAA0999	KIAA0999 protein	1.62E-03	4.86E-03	2.1
MAP3K2	Mitogen-activated protein kinase kinase kinase 2	4.82E-03	7.83E-03	1.8
MAP4K5	Mitogen-activated protein kinase kinase kinase 5	1.31E-02	1.50E-02	1.9
MAPK8	Mitogen-activated protein kinase 8	1.42E-02	1.54E-02	2.0
NAGK	N-acetylglucosamine kinase	4.16E-03	7.83E-03	1.8
PALM2-AKAP2	A kinase (PRKA) anchor protein 2	2.31E-02	2.37E-02	1.9
PANK2	Pantothenate kinase 2	6.39E-04	2.67E-03	1.6
PAPSS1	3'-phosphoadenosine 5'-phosphosulfate synthase 1	2.35E-04	1.67E-03	1.8
PDLIM5	PDZ and LIM domain 5	8.94E-03	1.09E-02	1.9
PFTK1	PFTAIRE protein kinase 1	4.74E-03	7.83E-03	1.6
PGAM1	Phosphoglycerate mutase 1 (brain)	4.81E-04	2.35E-03	1.6
PI4K2B	Phosphatidylinositol 4-kinase type 2 beta	7.80E-03	1.02E-02	1.7
PIK3C2A	Phosphoinositide-3-kinase, class 2, alpha	2.28E-02	2.37E-02	1.5
PIP4K2A	Phosphatidylinositol-5-phosphate 4-kinase, IIa	7.52E-04	2.67E-03	1.5
PRKAA1	Protein kinase, AMP-activated, alpha 1	5.32E-02	5.32E-02	1.7
PRKD1	Protein kinase D1	7.86E-03	1.02E-02	1.8
PTK9	Twinfilin, actin-binding protein, homolog 1	1.62E-04	1.67E-03	1.7
RIPK2	Receptor-interacting serine-threonine kinase 2	1.84E-03	5.11E-03	1.6
RIPK5	Receptor interacting protein kinase 5	5.91E-03	8.53E-03	1.7
SGMS2	Sphingomyelin synthase 2	3.38E-03	7.76E-03	2.3
SHC1	Src homology 2 domain containing protein 1	2.99E-04	1.67E-03	1.7
SOCS3	Suppressor of cytokine signaling 3	3.07E-03	7.48E-03	1.7
SSA2	TROVE domain family, member 2	2.72E-04	1.67E-03	1.6
TAOK1	TAO kinase 1	1.51E-04	1.67E-03	1.7
TRIM27	Tripartite motif-containing 27	6.94E-04	2.67E-03	1.6
TTN	Titin	6.67E-03	9.29E-03	0.2

Conclusions

In this paper we describe the global transcriptome of human tendinopathy. Although we have identified a number of genes that are differentially regulated, the ultimate roles of these genes and pathways in tendon disease are yet to be determined. However, we have provided a resource that we and other investigators can use to explore the molecular changes associated with tendinopathy.

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Authors' contributions

Competing interests

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