INSTRUCTIONAL REVIEW: UPPER LIMB

A systematic review of the histological and molecular changes in rotator cuff disease

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Introduction
The pathogenesis of rotator cuff disease (RCD) is complex and not fully understood. This systematic review set out to summarise the histological and molecular changes that occur throughout the spectrum of RCD.

Methods
We conducted a systematic review of the scientific literature with specific inclusion and exclusion criteria.

Results
A total of 101 studies met the inclusion criteria: 92 studies used human subjects exclusively, seven used animal overuse models, and the remaining two studies involved both humans and an animal overuse model. A total of 58 studies analysed supraspinatus tendon exclusively, 16 analysed subacromial bursal tissue exclusively, while the other studies analysed other tissue or varying combinations of tissue types including joint fluid and muscle. The molecular biomarkers that were altered in RCD included matrix substances, growth factors, enzymes and other proteins including certain neuropeptides.

Conclusions
The pathogenesis of RCD is being slowly unravelled as a result of the significant recent advances in molecular medicine. Future research aimed at further unlocking these key molecular processes will be pivotal in developing new surgical interventions both in terms of the diagnosis and treatment of RCD.

Key messages
- The pathogenesis of RCD is complex and multifactorial
- The progressive histological changes in RCD are of a characteristic pattern
- The levels of several molecular biomarkers are altered in RCD

Strengths and limitations
- The studies of RCD are heterogeneous in several ways, including subject type and disease characteristics
- The supraspinatus tendon is highly variable morphologically in terms of loading patterns, and this has a consequent effect on the local molecular biomarker levels
- Understanding the changes in molecular biomarkers is paramount in guiding the future research and treatment of RCD

Article focus
To determine the key histological and molecular changes in rotator cuff disease (RCD) by systematically reviewing the scientific literature

Keywords: Molecular, Biomarkers, Degenerative, Rotator cuff disease, Shoulder, Tendinopathy, Ageing
structures was popularised by Neer in 1972, and the term ‘impingement’ was coined. The pathogenesis of cuff disease is multifactorial and likely results from a combination of intrinsic, extrinsic and environmental factors.

The rotator cuff insertion onto the humeral tuberosities is broad, continuous, multilayered and interwoven. The supraspinatus and infraspinatus tendons fuse 1.5 cm proximal to their insertions. Tears in the supraspinatus tendon (SST) are the most common and they are most frequently found near to the tendon’s bony insertion; as SST tears become larger they are more likely to involve infraspinatus due to their common insertion. In this context the anatomy of the SST’s insertion is of key relevance in terms of its extracellular matrix composition and has been categorised into four transition zones. The first zone is proper tendon, made up of largely type I collagen and small amounts of decorin. The second zone is fibrocartilage and consists of largely types II and III collagen, with small amounts of types I, IX and X collagen. The third zone is mineralised fibrocartilage and consists of type II collagen, with significant amounts of type X collagen and aggrecan. The fourth zone is bone and is largely type I collagen with a high mineral content. This effective bone-tendon attachment is achieved through a functional grading in mineral content and collagen fibre orientation. The SST enthesis is a highly specialised inhomogeneous structure that is subjected to both tensile and compressive forces; this appears important in both the development and propagation of cuff tears.

Tendon homeostasis and its failure in degenerative disease is a complex process that involves the interplay between a variety of cells, matrix components, enzymes, cytokines, growth factors and proteins. The roles of the different anatomical structures involved (the SST itself, the subacromial bursa (SAB) and the glenohumeral joint capsule (GHC)) are yet to be fully determined. The purpose of this systematic review was to summarise the cellular and molecular changes in rotator cuff disease and explain their possible significance in terms of the disease pathogenesis and future research.

Materials and Methods

This systematic review used the PRISMA-Statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) as a guideline in the development of the study protocol and the report of the current study. The inclusion criteria and methods of analysis were specified in advance and documented in a protocol.

Information sources and search strategy. Studies were identified by searching the PubMed and Cochrane electronic databases. The search was undertaken in April 2012. The following search terms were used in PubMed: shoulder nerve growth factor, shoulder NGF, shoulder neuronal regulation, shoulder neuropeptide Y, shoulder NPY, shoulder noradrenaline, shoulder VIP, shoulder Acetylcholine, shoulder substance P, shoulder TGF, shoulder CGRP, shoulder IB4, shoulder galanin, shoulder receptin shoulder opio*, shoulder histological, shoulder molecule*, shoulder somatostatin, shoulder encephalin, shoulder endorphin, shoulder neurokinin, shoulder histamine, shoulder prostagland*, shoulder NMDA, shoulder AMPA, shoulder glutam*, shoulder collagen, shoulder matrix, shoulder GAG and Glycosaminoglycan*, shoulder proteoglycan, shoulder apoptosis, shoulder cytokines, shoulder chemokines, shoulder growth factor*, shoulder VEGF, shoulder Interleuk*, shoulder TIMP, shoulder metalloprot*, shoulder MMP, shoulder ADAMT, shoulder TNF, shoulder tendinopathy*, shoulder degenerative disease.

All searches were repeated with the word ‘shoulder’ being substituted by ‘rotator cuff’. Additional studies were located by searching reference lists of short listed articles. Hand searches were undertaken on the British and American editions of the Journal of Bone and Joint Surgery and the Journal of Shoulder and Elbow Surgery. Study selection. The citations identified from the searches were combined and duplicates excluded. All citations for papers clearly referring to a topic other than the shoulder were excluded, as were others whose title clearly showed that the paper was not relevant to the current study.

Full copies of the remaining papers were obtained and assessed. Papers concerning the cellular or molecular changes in degenerative RCD were included. Degenerative RCD included patients with asymptomatic age-related degeneration (ARD) and symptomatic patients including the diagnoses of subacromial bursitis, impingement syndrome (IS), rotator cuff tendinopathy, rotator cuff tear (RCT), calcifying tendinopathy (CT), long head of biceps (LHB) tendinopathy and cuff tear arthropathy.

Papers that studied cuff tear models and in vitro studies were excluded. Papers relating to animal overuse models and animal impingement models were included. Papers relating to non-degenerative conditions (such as frozen shoulder) were excluded unless the results for the patients with degenerative disease could be separated. Papers describing solely macroscopic changes, molecular changes that had no control groups for comparison, or relating to studies of any tissue or fluid not located in the region of the shoulder were excluded.

Data collection process. The descriptive histological results and molecular changes were recorded and have been summarised in Tables I, II and III; where the molecular change was within different RCD subgroups this was documented in the results.

Results

Study selection and characteristics. The search strategy revealed a total of 6145 results (Fig. 1). After removal of duplicate entries, 3956 unique papers remained. Screening of the titles and abstracts revealed 190 papers eligible for inclusion. Further assessment of eligibility, based on full-text papers, led to the exclusion of 89 papers. This left 101 papers meeting our criteria for inclusion.
A total of 92 studies used exclusively human subjects, 12 of which used cadavers. Seven studies used exclusively animal overuse models (six rat and one dog model), and two studies used both human subjects and a model of animal overuse. The SST alone was analysed in 58 studies, SAB tissue alone in 16, while the other studies analysed other tissue or varying combinations of tissue types. All studies analysed RCD in humans or the effects of overuse in an animal model. A total of 43 studies were exclusively histological, 36 studies exclusively molecular, while the remaining 22 analysed both histological and molecular changes. All studies on molecular changes had control groups or performed sub-group analyses based on specific subject characteristics. The types of control included those undergoing surgery for other reasons (instability/trauma), cadaveric specimens and subscapularis samples. A wide variety of techniques were used, including immunohistochemistry (IHC), reverse transcription polymerase chain reaction (RT-PCR) and enzyme linked immunosorbent assays (ELISAs).

**Table I. Histological changes in rotator cuff disease (RCD)**

<table>
<thead>
<tr>
<th>Age-related changes (ARD)</th>
<th>Rotator cuff tendinopathy/impingement syndrome (IS)/Calcific tendinopathy (CT)</th>
<th>Rotator cuff tears (RCTs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular changes</td>
<td>Rounding of tenocyte nuclei&lt;sup&gt;16,71,101&lt;/sup&gt;</td>
<td>Rounding of tenocyte nuclei&lt;sup&gt;12,52,65,69,71&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Increased cellularity&lt;sup&gt;86&lt;/sup&gt;</td>
<td>Plump mesenchymal cells present&lt;sup&gt;81&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Increased apoptosis&lt;sup&gt;54,76&lt;/sup&gt;</td>
<td>Increased cellular proliferation&lt;sup&gt;68&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Bursal inflammatory cell infiltrate&lt;sup&gt;12,15,76&lt;/sup&gt;</td>
<td>More variable cellularity&lt;sup&gt;32&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>No bursal inflammatory cell infiltrate&lt;sup&gt;76&lt;/sup&gt;</td>
<td>Increased cellularity&lt;sup&gt;62,54,65,88&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Macrophages and multinucleate cells located around areas of resorption in CT&lt;sup&gt;76,95,96&lt;/sup&gt;</td>
<td>Increased apoptosis&lt;sup&gt;12,54,18,106&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Chondrocyte type cells&lt;sup&gt;76,95,96&lt;/sup&gt;</td>
<td>(a increasing degeneration&lt;sup&gt;102&lt;/sup&gt;)</td>
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<tr>
<td>Extracellular matrix</td>
<td>Loss of matrix organisation&lt;sup&gt;49,71,101&lt;/sup&gt;</td>
<td>Loss of matrix organisation&lt;sup&gt;75,30,31,52-54,56,65,71,77,85,102&lt;/sup&gt;</td>
</tr>
<tr>
<td>changes</td>
<td>Increased mechanoreceptor numbers&lt;sup&gt;18&lt;/sup&gt;</td>
<td>Thinning of collagen fibres&lt;sup&gt;51,92&lt;/sup&gt;</td>
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<tr>
<td>Vitrocartilagenous</td>
<td>Fatty degeneration/infiltration&lt;sup&gt;78&lt;/sup&gt;</td>
<td>Fatty degeneration/infiltration&lt;sup&gt;75,30,31,43,42,78&lt;/sup&gt;</td>
</tr>
<tr>
<td>change†</td>
<td>Fibrocartilagenous change&lt;sup&gt;1,16,85&lt;/sup&gt;</td>
<td>Focal areas of tissue necrosis&lt;sup&gt;23,30,83,92&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mucoid/myoid degeneration</td>
<td>Fatty degeneration/infiltration&lt;sup&gt;15&lt;/sup&gt;</td>
<td>Mucoid/myoid degeneration&lt;sup&gt;51,33,37,60,106&lt;/sup&gt;</td>
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<tr>
<td>Fatty degeneration/</td>
<td>Fibrocartilagenous change around areas of calcification&lt;sup&gt;78,95,96&lt;/sup&gt;</td>
<td>Calcified deposition&lt;sup&gt;70,31,33&lt;/sup&gt;</td>
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<tr>
<td>infiltration†</td>
<td></td>
<td>Procollagen type I at tear margins&lt;sup&gt;12&lt;/sup&gt;</td>
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<tr>
<td>Increased GAG</td>
<td></td>
<td>Amyloid deposition&lt;sup&gt;17&lt;/sup&gt;</td>
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<tr>
<td>Calcified deposit</td>
<td></td>
<td>Focal areas of increased vascularity&lt;sup&gt;22,69,85&lt;/sup&gt; (a increasing degeneration&lt;sup&gt;102&lt;/sup&gt;)</td>
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<tr>
<td>Vascularity</td>
<td>Increased vascularity&lt;sup&gt;20&lt;/sup&gt;</td>
<td>Increased vascularity&lt;sup&gt;45,52,65&lt;/sup&gt;</td>
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<td></td>
<td>Bursal vascular proliferation&lt;sup&gt;33&lt;/sup&gt;</td>
<td>Vascular proliferation&lt;sup&gt;31,43,54&lt;/sup&gt;</td>
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<tr>
<td>Overall change</td>
<td>Increased general degeneration&lt;sup&gt;94&lt;/sup&gt;</td>
<td>Unchanged overall vascularity&lt;sup&gt;24&lt;/sup&gt;</td>
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<td></td>
<td>Chondroid metaplasia&lt;sup&gt;12&lt;/sup&gt;</td>
<td>Increased bursal vascularity (RCT vs non-RCT&lt;sup&gt;16&lt;/sup&gt;) (IRCT vs pIRCT&lt;sup&gt;24&lt;/sup&gt;)</td>
</tr>
<tr>
<td></td>
<td>Hyaline degeneration&lt;sup&gt;77&lt;/sup&gt;</td>
<td>Increased LHB vascularity in RCT&lt;sup&gt;96&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Reduced cellularity&lt;sup&gt;50&lt;/sup&gt;</td>
<td>Increased general degeneration&lt;sup&gt;54,57,60,71,106&lt;/sup&gt;</td>
</tr>
<tr>
<td>Failed tendon healing†</td>
<td>Increased bursal fibrosis&lt;sup&gt;23,55,70&lt;/sup&gt;</td>
<td>Chondroid metaplasia&lt;sup&gt;12,22,23,33,55,58-60&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glenohumeral joint</td>
<td>Increased LHB degeneration&lt;sup&gt;19&lt;/sup&gt;</td>
<td>Hyaline degeneration&lt;sup&gt;71,52,65,71&lt;/sup&gt;</td>
</tr>
<tr>
<td>degeneration increased</td>
<td>Degeneration of acromion under surface/CAL&lt;sup&gt;27,95&lt;/sup&gt;</td>
<td>Fibrocartilagenous/granulation tissue at tear edge&lt;sup&gt;24,25,27,30,43,45,52&lt;/sup&gt;</td>
</tr>
<tr>
<td>with RCT&lt;sup&gt;21,54&lt;/sup&gt;</td>
<td>Degeneration of acromion under surface/CAL&lt;sup&gt;22,67,81&lt;/sup&gt;</td>
<td>Increased bursal fibrosis&lt;sup&gt;15&lt;/sup&gt;</td>
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<td></td>
<td>Lower number of axons innervated LHBT&lt;sup&gt;84&lt;/sup&gt;</td>
<td>Increased bursal reaction&lt;sup&gt;56,78,99,104&lt;/sup&gt; (IRCT &gt; pIRCT&lt;sup&gt;24&lt;/sup&gt;)</td>
</tr>
<tr>
<td></td>
<td>Glenohumeral joint synovial inflammation&lt;sup&gt;28&lt;/sup&gt;</td>
<td>Glenohumeral joint synovial inflammation&lt;sup&gt;28&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Increased subscapularis tendon degeneration&lt;sup&gt;20&lt;/sup&gt;</td>
<td>Increased subscapularis tendon degeneration&lt;sup&gt;20&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Degeneration of acromion under surface/CAL&lt;sup&gt;77,93&lt;/sup&gt;</td>
<td>Degeneration of acromion under surface/CAL&lt;sup&gt;77,93&lt;/sup&gt; (RCT &gt; non-RCT&lt;sup&gt;67,95&lt;/sup&gt;)</td>
</tr>
<tr>
<td></td>
<td>Supraspinatus muscle fatty infiltration/degeneration&lt;sup&gt;87&lt;/sup&gt;</td>
<td>Supraspinatus muscle fatty infiltration/degeneration&lt;sup&gt;87&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* GAG, glycosaminoglycan; CAL, coracoacromial ligament
† LHBT, long head of biceps; LHBT, long head of biceps tendinopathy
‡ ft, full-thickness; pt, partial thickness
The histological changes in RCD are summarised in Table I. The significant molecular changes are summarised in Tables II and III.

A reduction in overall collagen content and were seen in RCD; type II and III collagen content were increased in multiple studies. Overall glycosaminoglycan (GAG) levels were increased, while certain proteoglycans levels were decreased in RCD. The general in RCD was towards a fibrocartilagenous phenotype.

The collagenases matrix metalloproteinase (MMP)-1 and -13 were increased in RCD, while the gelatinases MMP-2 and MMP-9 were also increased in RCD. MMP-3 levels were altered in seven studies, being increased in three and decreased in four. Tissue inhibitor of metalloproteinases (TIMP)-1, -2 and -3 have all been shown to be decreased in RCD, while no change in TIMP-4 was demonstrated. Overall there is a clear catabolic trend in RCD.

The changes in terms of cytokines were generally pro-inflammatory. Several members of the Interleukin family were increased in RCD (Interleukin-1α, 1β, -6, 11, 15, 18 and IL1-receptor antagonist). Tumour necrosis factor (TNF)-α, stromal derived factor-1α and the small inducible cytokines were all increased. The cyclo-oxygenases (1 and 2), Cathepsin D and nitric oxide synthase were all increased in RCD. Several growth factors were increased in RCD including vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β, fibroblast growth factor (FGF), bone morphogenetic protein (BMP) 2 and BMP 7. Insulin-like growth factor (IGF)-1 was decreased in RCD.

Several proteins associated with apoptosis are increased in RCD, including p53, poly(ADP-ribose) polymerase, caspases 3/8, B-cell lymphoma (BCL)-2, BNIP3, type II angiotensin receptor, cFLIP and cFLIP receptor. Peroxiredoxin 5 and the heat shock proteins 27/70 were increased, while there was no obvious trend in hypoxia-inducible factor (HIF)-1α levels; these substances may all play a protective role for cells at times of high stress. In terms of neuropeptides, increases in substance P and β-endorphin were seen in RCD. The increase in PGP9.5 and GAP43 is likely to represent neoinnervation in RCD.

**Discussion**

The results of this systematic review must be seen in the context of the heterogeneity of the studies and of RCD in general. RCD includes a whole spectrum of changes to the histological and molecular characteristics of the tissue. Different studies analysed the tissue of patient groups that were highly variable in terms of disease stage, symptomatology and patient demographics. It has been shown that significant molecular differences are found depending on if the sampled tendon is from an over-stressed or stress-shielded region. Several studies used
gresses have been well described. Small tears of the rotator cuff show changes consistent with an attempt to heal such as increased fibroblast cellularity, blood vessel proliferation and the presence of a significant inflammatory component. These features of attempting healing diminish as the size of the tear and the amount of tendon degeneration increase. The progressive tendon degeneration is characterised by thinning of the collagen fibres, a loss of collagen structure, myxoid degeneration, hyaline degeneration, chondroid metaplasia and fatty infiltration. The overall picture is one of pathological chondroplasia in which tissue which normally exhibits a cartilage-like phenotype is replaced by tissue of a fibro-chondroplasia in which tissue which normally exhibits a cartilage-like phenotype.23

The cellular changes that occur as cuff disease progresses have been well described. Small tears of the rotator cuff may account for some of the apparent discrepancies in the study findings. All these factors may account for some of the apparent discrepancies in the study findings. All these factors

<table>
<thead>
<tr>
<th>Cytokines/growth factors</th>
<th>Apoptosis/cell cycle related</th>
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<tbody>
<tr>
<td>IL-1α ↑14,49</td>
<td>HIF-1α ↑11,43,56 ↓59,60,61</td>
</tr>
<tr>
<td>IL-1ra ↑11,26-28</td>
<td>BNIIP-3 ↑11</td>
</tr>
<tr>
<td>IL-1β ↑13,14,26-28,42,75,82,99</td>
<td>BCL-2 ↑58</td>
</tr>
<tr>
<td>IL-2 ↓60</td>
<td>Caspase 3 ↑59,60</td>
</tr>
<tr>
<td>IL-6 ↑13,14,26-28,42,75,82,99</td>
<td>Caspase 8 ↑59,61</td>
</tr>
<tr>
<td>IL-11 ↑60,61</td>
<td>Heat shock protein 27 ↑59-61</td>
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<tr>
<td>IL-15 ↑60</td>
<td>Heat shock protein 70 ↑59-61</td>
</tr>
<tr>
<td>Stromal derived factor-1α (SDF-1α) ↑↑11,41</td>
<td>poly(ADP-ribose) polymerase ↑50,61</td>
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<tr>
<td>TNFα ↑14,42,75,82,99</td>
<td>type-2 angiotensin II receptor ↓50,61</td>
</tr>
<tr>
<td>VEGF ↑43,46,58,68,82 ↑ (associated with motion pain104)</td>
<td>cFLIP receptor ↑60</td>
</tr>
<tr>
<td>IGF-1 ↓7,18</td>
<td>p-53 induced gene I, cell division cycle 25A, Max protein, meiotic recombination 11 homolog A ↑51</td>
</tr>
<tr>
<td>TGF-β ↑50-75</td>
<td>Peroxiredoxin 5 ↑50</td>
</tr>
<tr>
<td>bFGF ↑40,67,75</td>
<td>P53 ↓56,61</td>
</tr>
<tr>
<td>FGF 18 ↑61</td>
<td>P53 inhibitors ↓54</td>
</tr>
<tr>
<td>BMP2 and BMP7 ↑67</td>
<td>NF-κB ↓54</td>
</tr>
<tr>
<td>Small inducible cytokines ↑14</td>
<td>Receptor activator of NF-κB ↑60</td>
</tr>
<tr>
<td>Macrophage inhibitory factor (MIF) ↑60</td>
<td>Others</td>
</tr>
<tr>
<td>Heparin affinity regulatory peptide (HARP) ↑</td>
<td>Ubiquitin proteasome pathway UBE2A and UBE3A ↑ (massive tears vs small/controls49)</td>
</tr>
<tr>
<td>Five-lipoxygenase activating protein ↑68</td>
<td>Calpain (CAPN1) and CTSB (lysosomal enzyme) ↑ (massive tears vs small/controls49)</td>
</tr>
<tr>
<td>Hepatocyte growth factor ↓61</td>
<td>vWF ↑68</td>
</tr>
<tr>
<td>Neuronal factors</td>
<td>T-cell receptor variable β chain ↑60</td>
</tr>
<tr>
<td>Substance P ↑40 (higher in non-perforated RCTs vs perforated25)</td>
<td>Ig heavy chain, T cell receptor α chain ↓60</td>
</tr>
<tr>
<td>β-endorphin ↑40</td>
<td>GATA binding protein, PAF acetylhydrolase, Attractin, IgG-2b chain ↑60,61</td>
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<tr>
<td>Anti-NGF30 ↑60,61</td>
<td>Insulin induced gene 1, FGF11, nuclear receptor coactivator 2, G protein coupled receptor S4, Ephrin A1, Thyrotroph embryonic factor, Odd Oz/ten-m homolog 2, POU domain, TNF 11, TGF-β binding protein 3, T cell receptor β chain, cytochrome b-245, CD3 γ chain, polyprotein 1-microglobulin, Fc receptor IgG, solute carrier family 2, adenosine deaminase, integrin-linked kinase ↑61</td>
</tr>
<tr>
<td>PGP9.5, GAP43 ↑103</td>
<td>Dynein, nuclear receptor subfamily 2 group F member 1, Homeobox A1, FGF receptor 3, MHC class I-like sequence, T-cell receptor β chain, killer cell lectin-like receptor, strain T-cell receptor ↓61</td>
</tr>
<tr>
<td>glutamate receptor 5, glutamate receptor metabotropic 6, glutamate receptor inotropic 3A, GABA receptor α1 ↑61</td>
<td>T-cell receptor ↓40,61</td>
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<tr>
<td>AMPA1, glutamate receptor interacting protein 1/2 ↓61</td>
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</table>

animal overuse models which are hypothesised to mimic the pathophysiology of RCD, however there are likely to be some significant differences between the molecular changes found in these models and RCD in humans. There was also significant diversity between studies in terms of what was measured. Some studies measured molecular biomarker levels directly, some measured the gene expression of molecular biomarkers using mRNA and some used both of these techniques. All these factors may account for some of the apparent discrepancies in the study findings.
The regulation of matrix turnover involves several cell types and numerous cytokines. The tenocyte is the resident fibroblast present in tendon and is arguably the most pivotal cell type; other cells involved include extrinsic fibroblasts and inflammatory cells such as the macrophage. Tenocytes have been shown to produce a number of cytokines in response to increased strain such as Interleukin-1β (IL-1β), Interleukin-6 (IL-6), VEGF, HIF-1α, TGF-β and prostaglandin-E2 (PG-E2). Our results show that numerous cytokines and growth factors including IL-1β, IL-6, VEGF, bFGF, TGF-β, TNF-α, HIF-1α, cyclooxygenase (COX)-1, COX-2 and nitric oxide synthase (NOS) are all increased in RCD. VEGF, IGF-1, TGF-β and FGF are all increased during normal tendon healing and their increase in RCD is demonstrative of tendon attempting to heal. An increased IL-1β production has been hypothesised to promote cell survival in a high stress environment. Numerous complex interactions occur between these cytokines, the extracellular matrix synthesis, catabolic mediators and cytoskeleton assembly. Pro-inflammatory cytokines affect extracellular matrix homeostasis, accelerate remodelling, amplify biomechanical adaptivity and promote tenocyte apoptosis. The trend towards a pro-inflammatory state in RCD is indicative of the imbalance that occurs between the catabolic and anabolic systems, the cytokines being key regulatory factors of these. As RCD progresses and cuff tears become increasingly sizeable there is a clear increase in apoptosis, as evidenced by increases in several apoptosis related markers including BNIP-3, BCL-2, the caspases and the heat shock proteins. The increase in p53 activity in RCD may also be important in promoting apoptosis.

Tendon is highly mechanically adaptive and a characteristic feature of RCD is the progressive mechanical failure of tendon to meet the physical demands placed upon it. Total collagen content decreases, while there is a significant increase in the proportion of type II and III collagen relative to type I collagen. This change in collagen makeup goes hand in hand with a transformation of the matrix from larger organised fibrils to smaller disorganised fibrils with decreasing mechanical properties. The mature and hydroxylysine cross-links are significantly increased which may be a feature of the incomplete remodelling found in scar tissue. The increase in the glycoproteins tenasin-C and fibronectin is consistent with a wound healing process occurring in degenerate tendon. The changes to several different proteoglycans in RCD are varied but the overall picture appears to be of fibrocartilagenous change; this is characterised by increased aggrecan and biglycan, with decreased decorin. Therefore overall the matrix changes are consistent with the degenerate tendon attempting to heal, with a progressively mechanically weak scar tissue being laid down as part of this failing remodelling process.

Higher levels of matrix remodelling and turnover have been linked with RCD and the tissue-degrading enzymes of the metalloproteinase family are important in this process. The family includes the MMPs, their close relatives ‘a disintegrin and metalloproteinase’ (ADAMs) and ‘a disintegrin and metalloproteinase with thrombospondin motifs’ (ADAMTS). The MMP family consists of 24 known MMPs including the collagensases (MMP1, -8 and -13), the gelatinases (MMP-2 and -9), membrane-type MMPs (MT-MMPs), the stromelysins (MMP-3 and -10 and the matrilysins (MMP-7 and -26). The collagensases, as well as MMP-2 and -14, have important collagenolytic activity. The ADAMTS are divided into four groups, of which ADAMTS -1,-4,-5,-8,-9,-15,-20 are the aggrecanases and ADAMTS -2,-3,-14 are the procollagen N-proteinases. The TIMPs are endogenous inhibitors of the metalloproteinases and there are four in humans; they reversibly inhibit all MMPs by a 1:1 interaction with the zinc binding site. The MMPs do not solely degrade tissue; they may have anti-inflammatory actions by processing certain cytokines and chemokines.

The increased collagen turnover in RCD is consistent with the increase in two collagensases (MMP-1 and -13) and two gelatinases (MMP-2 and -9). MMP-3 is thought to be important in the regulation of matrix turnover and is reduced in the degenerate SST; this is consistent with tendinopathy resulting from a failure in tendon repair or matrix maintenance. The decreases in TIMP-1,-2, and -3 in RCD are also consistent with this catabolic picture of increased matrix degradation and failing remodelling. The roles of the ADAMs and ADAMTS have yet to be determined in RCD. Older tendon is more susceptible to mechanically induced failure involving MMP activity and this may be related to age-related change in tenocytes. The role of tendon stem cells (TSCs) remains to be determined but their responses to differing mechanical stimuli hint towards an important role. TSCs have...
been shown to proliferate and produce collagen in response to exercise, while they have been shown to differentiate into non-tenocytes if excessively mechanically loaded. A recent report suggested that an extracellular matrix rich niche, organised partly by biglycan and fibromodulin, controls the self-renewal and differentiation of TSCs. The self-renewal capacity and differentiation capability of TSCs reduces with increasing age and this is likely to be important in explaining the age-related nature of RCD.

This review has summarised just how much progress has been made in recent years, particularly in the advent of modern molecular medical techniques. Intrinsically, extrinsic and environmental factors all have an important role to play in the disordered tendon homeostasis of RCD which can lead to progressive mechanical failure. Among the key questions that remain to be answered include why some patients’ tendons degenerate, while others do not, and why some patients experience pain, while others with the same amount of macroscopic tendon degeneration do not. Certainly there is still a great deal to be understood as regards the pathogenesis of RCD and undoubtedly, unlocking these secrets could pave the way for some very exciting new treatments in the future.

Supplementary material
A table giving details of each of the 101 studies included in this review is available with this article on our website www.bjr.boneandjoint.org.uk

References
Cytokines and apoptosis


A SYSTEMATIC REVIEW OF THE HISTOLOGICAL AND MOLECULAR CHANGES IN ROTATOR CUFF DISEASE


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