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Gene expression and matrix turnover in overused and damaged tendons

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Chronic, painful conditions affecting tendons, frequently known as tendinopathy, are very common types of sporting injury. The tendon extracellular matrix is substantially altered in tendinopathy, and these changes are thought to precede and underlie the clinical condition. The tendon cell response to repeated minor injuries or "overuse" is thought to be a major factor in the development of tendinopathy. Changes in matrix turnover may also be effected by the cellular response to physical load, altering the balance of matrix turnover and changing the structure and composition of the tendon. Matrix turnover is relatively high in tendons exposed to high mechanical demands, such as the supraspinatus and Achilles, and this is thought to represent either a repair or tissue maintenance function. Metalloproteinases are a large family of enzymes capable of degrading all of the tendon matrix components, and these are thought to play a major role in the degradation of matrix during development, adaptation and repair. It is proposed that some metalloproteinase enzymes are required for the health of the tendon, and others may be damaging, leading to degeneration of the tissue. Further research is required to investigate how these enzyme activities are regulated in tendon and altered in tendinopathy. A profile of all the metalloproteinases expressed and active in healthy and degenerate tendon is required and may lead to the development of new drug therapies for these common and debilitating sports injuries.

Tendon injuries and chronic tendon pain are extremely common in athletes, as well as in the general population. Despite their prevalence, these conditions are poorly understood and often do not respond well to treatment. There are many articles, which discuss the potential causes and treatment of tendon pathology (tendinopathy), but few have investigated the molecular processes underlying the condition. This review summarizes recent work investigating the molecular composition of the human tendon extracellular "matrix", and how this is altered in chronic tendinopathy. These studies have shown that the cells resident within the tendon, known as "tenocytes", are capable of synthesizing and degrading the tendon matrix, a process of "turnover" that is important in the tissue response to exercise, mechanical strain and injury. The pattern of loading (whether tensile or compressive, for example) as well as its magnitude and duration, is fundamental in the regulation of the tenocyte remodelling response. This review will highlight the role of matrix-degrading enzymes in tendon matrix turnover, and discuss their role in tendon health and disease.

The etiology of tendinopathy

Most tendinopathy is associated with multiple factors such as increased age, reduced vascular perfusion, anatomical variation (e.g. leg-length discrepancy), occupation and the level and type of sporting activity (Leadbetter, 1992; Józsa & Kannus, 1997a; Kannus, 1997; Riley, 2004a). The pattern and duration of physical stress experienced by the tendon is generally thought to be one of the most important factors, since tendons at sites exposed to high mechanical demands are more often affected. These include the supraspinatus tendon in the shoulder, the extensor carpi radialis brevis tendon at the elbow, the patellar tendon at the knee and the Achilles at the ankle. However, most cases of tendinopathy occur in the absence of any single traumatic episode, and are associated with repeated low-level loading of the muscle–tendon unit, frequently described as "overuse" injuries (Józsa & Kannus, 1997a).

Although once considered almost inert, the activity of the tendon cell (tenocyte) is thought to play a pivotal role in the pathology of tendinopathy. Tenocytes synthesize and degrade the tendon extracellular

matrix, a process of matrix turnover that is normally well-regulated and essential for maintaining the structural properties of the tissue. An imbalance of matrix turnover is implicated in other degenerative conditions such as osteoarthritis (Clark & Parker, 2003), which suggests that reduced matrix synthesis or increased matrix degradation may be targets for future drug therapy of tendinopathy.

Histopathology of tendinopathy

The structure and histology of tendon and tendinopathy have been described in detail elsewhere and only general features are discussed in this review – the reader is recommended to refer to the following sources for more information (Kannus & Józsa, 1991; Åström & Rausing, 1995; Jarvinen et al., 1997; Józsa & Kannus, 1997a; Józsa & Kannus, 1997b; Benjamin, 2004).

Tendon is a dense fibrous connective tissue, consisting of relatively few fibroblasts (tenocytes) embedded in a collagen-rich matrix. Haematoxylin and eosin (H&E) preparations of human Achilles tendon show the highly organized collagen fibril bundles running longitudinally through the matrix and the elongated tenocyte nuclei squeezed between them (See Fig. 1a). The cells are generally widely dispersed, although they have been shown to communicate with each other via gap junctions at the end of long cellular processes, both longitudinally and laterally throughout the tendon (McNeilly et al., 1996).

A typical feature of painful Achilles tendinopathy, shared with other tendon lesions at various sites, is an infiltration of blood vessels and increased cellularity, a so-called "angiofibroblastic" response (Leadbetter, 1992; Jarvinen et al., 1997) (See Fig. 1b). The majority of cells at the site of the lesion are fibroblasts, often rounded or ovoid, and there is rarely any evidence of inflammatory cells within the tendon, or in the surrounding paratenon (Åström & Rausing, 1995). The proteoglycan content is increased compared with normal tendon, as shown by toluidine blue staining of the matrix (Fig 1c and d). These degenerative changes are thus thought to represent an active, cell-mediated process that results in substantial changes to the structure and composition of the tendon matrix.

The biochemistry of tendinopathy

Several studies have addressed the major biochemical changes in tendon matrix composition in human tendinopathy (Riley et al., 1994a, b, 1996a, b, 2002; Bank et al., 1999; Ireland et al., 2001). Predominantly consisting of collagen, there are many other matrix constituents, including proteoglycans and non-collagen glycoproteins, many of which are poorly characterized (Aumailley & Gayraud, 1998). For example, there are 27 different collagen molecules now identified, although little is known about the tissue distribution and function of the less abundant "minor" collagens, particularly types XX to XXVII which were discovered relatively recently (Fitzgerald & Bateman, 2001; Koch et al., 2001; Hashimoto et al., 2002; Sato et al., 2002; Banyard et al., 2003; Boot-Handford et al., 2003; Koch et al., 2003; Pace et al., 2003; Myllyharju & Kivirikko, 2004).

A normal tendon contains mostly type I collagen, estimated to represent almost 95% of the total collagen, with smaller amounts of collagen types
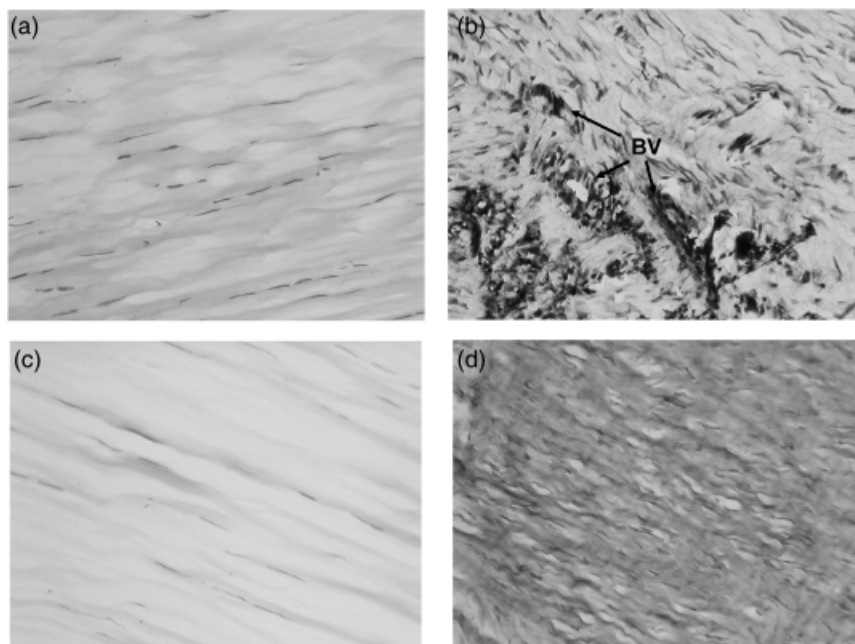

Fig. 1. Histopathology of tendinopathy. Normal Achilles tendon from a cadaver (a, c) and a surgical specimen from a patient with tendinopathy (b, d) were fixed, frozen and prepared for standard histology. (a) and (b) were stained with haemotoxylin and eosin, (c) and (d) were stained with toluidine blue to show matrix proteoglycans/glycosaminoglycans. (a) Normal tendon histology, with long thin fibroblasts dispersed in the ordered fibrillar matrix. (b) Blood vessel (BV) infiltration and cellular proliferation within the degenerate tendon lesion, with no inflammatory cells visible. (c) Relative absence of proteoglycans in the normal tendon matrix. (d) A substantial increase in proteoglycan throughout the degenerate tendon matrix.

III, V, VI, XII and XIV, at levels which are difficult to quantitate by current methodologies (Riley, 2004b). In addition, collagen types II, IX, X and XI are present at specific sites of "fibrocartilage, found at the bone insertion and where the tendon is subject to shear forces or compression (Fukuta et al., 1998; Waggett et al., 1998). These "minor" collagens, although a very small proportion of the total, are implicated in a number of important processes including collagen fibril formation, regulating the ultimate diameter of the fibrils and mediating interactions with the surrounding cells and matrix (Aumailley & Gayraud, 1998).

Most is known about the major fibrillar collagens of tendon, types I and III, since these are more amenable to analysis, most abundant and more readily extracted from the tissue, which becomes increasingly insoluble with age (Riley et al., 1994a). We have previously shown that degenerate tendons, such as the supraspinatus in the shoulder, have a small but significant reduction in the total collagen content relative to the tissue dry weight (Riley et al., 1994a). This was partly because of an increase in the non-collagen glycoprotein content, as well as increases in matrix proteoglycan (Riley et al., 1994b). The type and distribution of collagen also changed, with an increase in the proportion of type III collagen and a corresponding decrease in the concentration of type I collagen (Riley et al., 1994a). The type III collagen was found associated with the type I collagen fibril bundles by immunohistochemistry, thought to be intercalated into the fibrils, suggesting that the original fibrils had been extensively remodelled, resulting in a greater proportion of small diameter and randomly organized fibrils. The collagen was more readily extracted from degenerate tendon, although the level of mature collagen cross-links was elevated compared with normal (Bank et al., 1999). Thus it appeared that the newly synthesized collagen had been resident in the tissues at least long enough for the maturation of collagen cross-links, after whatever stimulus had driven the change in collagen synthesis.

The levels of the glycation cross-link, pentosidine, a marker of the molecular age of the matrix, was also lower than expected for the age of the individual, confirming that much of the original fiber network had been replaced by new collagen (Bank et al., 1999). This finding was confirmed by analysis of the racemization of aspartate, another marker of protein residence time (Riley et al., 2002). Macroscopically normal supraspinatus tendons showed relatively high levels of matrix turnover compared with normal biceps brachii tendons (which showed very little if any matrix turnover), although less than degenerate supraspinatus. These data are consistent with the hypothesis that increased matrix turnover is associated with the sub-clinical matrix degeneration that precedes the clinical condition. Similar biochemical evidence now exists for the Achilles, suggesting that remodelling of the tendon is a general feature of tendinopathy (Eriksen et al., 2002). Whether this remodelling weakens the tendon is a moot point, since many cases of painful tendinopathy persist for months if not years, and do not go on to rupture.

Very little is known about the changes in proteoglycan in tendinopathy, apart from the generalized increase in sulfated glycosaminoglycan, the majority of which was chondroitin sulfate (Riley et al., 1994b). There are several proteoglycan species in tendon, including the large hyalectans, aggrecan and versican, and the small leucine-rich proteoglycans (SLRP), decorin, biglycan, fibromodulin and lumican (Berenson et al., 1996; Waggett et al., 1998). Versican is thought to be the major hyalectan in the tendon mid-substance and aggrecan is more abundant in fibrocartilage. Of the SLRPs, decorin is the most abundant and the biglycan content is increased in fibrocartilaginous regions. Thus the proteoglycan content is an indicator of the "mechanical history" of tendon, with regions exposed to predominantly tensile load rich in versican and decorin, and regions that are compressed or subject to shear forces (such as the insertion) are rich in aggrecan and biglycan. The proteoglycans turn over much more rapidly than fibrillar collagen, as part of the normal tissue maintenance, although hyalectans have a greater rate of turnover than the SLRP (Rees et al., 2000; Samiric et al., 2004a, b). Apart from acting to hold water in the tissues, the proteoglycans have roles in the resistance of compression, lubricating movement of adjacent fiber bundles, mediating cell–matrix interactions and the sequestration of growth factors and enzymes in the matrix (Hardingham & Fosang, 1992). It is uncertain what role, if any, fibrocartilage plays in tendinopathy, although pathology is frequently found in fibrocartilaginous regions of tendon, which are avascular and therefore potentially less capable of repair. For example, it has been postulated that fibrocartilage may develop in tendon in response to excessive shear or compressive forces, and may be a precursor of pathology (Cawston et al., 1996). On the other hand, fibrocartilage formation may be part of the normal adaptive process, which functions to protect tendon against potentially harmful patterns of loading (Vogel & Koob, 1989).

Non-collagen glycoproteins in tendon include tenascin-C, cartilage oligomeric matrix protein (COMP), fibronectin, elastin, fibrillin, laminin and link protein (Józsa & Kannus, 1997a; Kannus, 2000; Riley, 2004b). Again, relatively little is known about changes in glycoprotein content in tendinopathy. Fibronectin and tenascin-C are up-regulated after injury, and are thought to play a role in modulating

cell migration and activity (Mackie et al., 1988; Lehto et al., 1990; Amiel et al., 1991). There are changes in the expression of tenascin-C in chronic tendinopathy, with changes in the splice variants expressed as well as increased abundance, predominantly associated with rounded cells in the degenerate matrix (Riley et al., 1996b).

In summary, the biochemical changes in tendinopathy are still relatively poorly characterized, although the evidence to date is consistent with the hypothesis that accumulated "micro-trauma" affect the cellular expression of many different matrix components. The question remains whether this represents a response to injury (i.e. damage to the matrix structure) or modulation of the tenocyte activity by mechanical strain, directly affecting the turnover of tendon matrix components.

Adaptation of tendon and the response to mechanical load

Studies with animals have shown that changes in matrix quantity and quality can be induced by altering the mechanical strain experienced by the tendon (Gillard et al., 1979; Woo et al., 1981, 1982; Tipton et al., 1986; Akeson et al., 1987; Curwin et al., 1988; Hannafin, 1995; Yasuda & Hayashi, 1999). The most dramatic changes can be induced by immobilization or stress-shielding, resulting in a fairly rapid loss of tendon strength and a reduction in the matrix (collagen) mass (Tipton et al., 1986; Akeson et al., 1987; Yasuda & Hayashi, 1999). There is a reduction in the total area of collagen fibrils in the tendon cross-section, and an increased number of thin and immature fibrils (Yasuda & Hayashi, 1999). The changes in tensile properties were dependent on the presence of a viable cell population, and could be reversed by the application of cyclic tensile loading *in vitro* (Hannafin, 1995). Similar results were reported for *in vivo* studies, with the tendon slowly returning to normal after the resumption of loading, although the insertion responds more slowly compared with the mid-substance (Woo et al., 1987). Mechanical load is important for improving the strength of healing tendon after injury (Gelberman et al., 1981), although the response of normal tendon to exercise is more equivocal. Small increases in the material properties of exercised tendons have been reported, although these were studies of relatively immature animals, and may not reflect the situation in adult tendon (Woo et al., 1981, 1982; Curwin et al., 1988). Indeed, studies of equine tendons have suggested that exercise may have only a deleterious effect on the adult tendon matrix, and only immature tendons are capable of an adaptive response (Patterson-Kane et al., 1997; Smith et al., 1999; Smith et al.,

2002). However, studies of human tendon using dialysis catheters placed adjacent to the tendon have shown that acute exercise in healthy volunteers will stimulate collagen synthetic activity, in addition to increasing tendon blood flow, metabolic activity and the release of certain inflammatory mediators and matrix-degrading matrix metalloproteinases (MMP) (Langberg et al., 1998, 1999, 2001; Kjaer et al., 2000; Heinemeier et al., 2003; Koskinen et al., 2004). Since isolated tendon cells can be stimulated to produce interleukin (IL)-1β, cyclooxygenase 2 (COX 2) and MMP (MMP-1 and MMP-3) by mechanical load and fluid-induced shear stress *in vitro*, an affect that may be modulated by negative feedback from extracellular ATP, this provides a theoretical basis for the induction of tendinopathy by repeated cyclical loading below the injury threshold (Archambault et al., 2002; Tsuzaki et al., 2003a).

Mediators of matrix degradation – the MMPs

Proteolytic activity is an essential component of tissue growth, maintenance, adaptation and repair. After injury, proteolysis is required to remove any damaged matrix and remodel the newly formed scar so that it more closely resembles the normal tissue. Some collagen in tendon is probably degraded intracellularly after phagocytosis, with fibroblasts and macrophages engulfing collagen molecules which are then digested by lysosomal enzymes (Everts et al., 1996; Creemers et al., 1998). This is a major activity in the rapidly remodelling peridontal ligament, although few studies have investigated the relative importance of this route in tendon. Most studies have focused on collagen degradation occurring in the extracellular environment and mediated by secreted enzymes known as MMP.

Comprehensive reviews of the MMP have been published elsewhere and only salient points are reviewed here (Matrisian, 1990; Murphy et al., 1994; Nagase, 1994; Birkedal-Hansen, 1995; Cawston, 1995; McCawley & Matrisian, 2001; Clark & Parker, 2003). MMP are members of the "MB" clan of metallopeptidases, generically referred to as "metzincins" since they contain zinc at the active site and a conserved methionine eight residues downstream. They possess activity at neutral pH against a broad spectrum of different matrix substrates. Although important in matrix degradation, MMP also have activity against cell surface receptors and growth factor precursors (McCawley & Matrisian, 2001). Consequently these enzymes also have an important role in the regulation of numerous cellular activities including cell proliferation, cell death (apoptosis), cell migration and chemotaxis (McCawley & Matrisian, 2001).

There are 23 MMPs found in human, which can be subdivided into collagenases, gelatinases, stromelysins and membrane-type MMP, based on their structures and substrate specificities (Nagase, 1994; Cawston, 1995; Clark & Parker, 2003) (See Fig. 2). Collagenases are some of the few enzymes capable of cleaving the intact type I collagen molecule in the extracellular environment, which occurs at a specific locus in the triple helix between residues 775 and 776 (Cawston, 1995). The initial cleavage by a collagenase is the rate-limiting step in fibrillar collagen turnover, generating $\frac{3}{4}$ and $\frac{1}{4}$ length fragments that are susceptible to other enzymes such as the gelatinases. MMPs with collagenase activity include MMP-1 (collagenase-1; EC 3.4.24.7), MMP-8 (neutrophil collagenase; EC 3.4.24.34), MMP-13 (collagenase-3; MEROPS ID M10.013), MMP-2 (gelatinase A; EC 3.4.24.24) and MMP-14 (MT1-MMP; MEROPS ID M10.014). The collagenases differ in their activities against the various fibrillar collagens, although precisely which enzymes are implicated in the physiological and pathological turnover of connective tissues such as tendon is still the subject of research.

The activities of MMPs are normally tightly controlled *in vivo*, with regulation at the levels of transcription, translation, activation and inhibition. In general, expression and activity of the MMPs is stimulated by pro-inflammatory cytokines such as IL-1 and tumour necrosis factor (TNF), and inhibited by growth factors such as transforming growth factor-β (TGF-β). MMPs are potently inhibited by α 2 macroglobulin in the serum, and also by a family of

specific inhibitors produced by cells within the tissues known as tissue inhibitors of metalloproteinases (TIMPs) (Murphy et al., 1994; Cawston, 1995; Murphy & Willenbrock, 1995). There are four TIMPs that have been characterized to date, and these may be constitutively expressed, such as TIMP-2, or stimulated by growth factors such as TGF-β, such as TIMP-1 and TIMP-3. Each TIMP will bind to active MMPs in a stoichiometric (1:1) ratio, resulting in a stable, inactive complex.

Mediators of matrix proteoglycan degradation – the "aggrecanases"

Proteoglycans are turned over much more rapidly than the fibrillar collagens (Birkedal-Hansen, 1995; Cawston, 1995). Although some members of the MMP family such as MMP-3 (stromelysin-1) can degrade proteoglycans such as aggrecan *in vitro*, most activity *in vivo* is associated with a related but distinct group of metallo-endopeptidases, commonly known as "aggrecanases".

Aggrecanases were first identified on the basis of their ability to cleave aggrecan at specific Glu–Xaa bonds, with a major site in the interglobular (IGD) domain of the aggrecan core protein (Glu^{373}–Ala^{374}), resulting in the loss of the glycosaminoglycan-rich portion of the molecule from the tissue (Sandy et al., 1991). This activity was associated with the loss of cartilage proteoglycan that accompanies osteoarthritis (Sandy et al., 1992). Aggrecanases were subsequently identified as members of the a disintegrin and metalloproteinase (ADAM)-thrombospondin (TS) family, a sub-group of ADAM with TS type I motifs (Kaushal & Shah, 2000; Cal et al., 2002).

To date, 19 mammalian ADAMTS enzymes have been identified, many of which remain to be fully characterized (Kaushal & Shah, 2000; Cal et al., 2002) (See Fig. 3). ADAMTS-2, ADAMTS-3 and ADAMTS-14 are pro-collagen peptidases, and function as key regulators of collagen fibril assembly (Colige et al., 1999; Fernandes et al., 2001; Colige et al., 2002). ADAM-TS4 (aggrecanase 1) and ADAM-TS5 (aggrecanase 2) were the first aggrecanases to be identified (Abbaszade et al., 1999; Tortorella et al., 1999; Kaushal & Shah, 2000) although activity has since been also attributed to the phylogenetically related enzymes ADAMTS-1, ADAMTS-8, ADAMTS-9 and ADAMTS-15 (Kuno et al., 2000; Yamaji et al., 2000; Somerville et al., 2003; Collins-Racie et al., 2004). Best known for their activity against aggrecan, ADAMTS1 and ADAMTS-4 are also capable of cleaving other matrix proteoglycans such as versican and brevican (Sandy et al., 2001), and glycoproteins such as COMP (Dickinson et al., 2003), at least *in vitro*. Although inhibition of
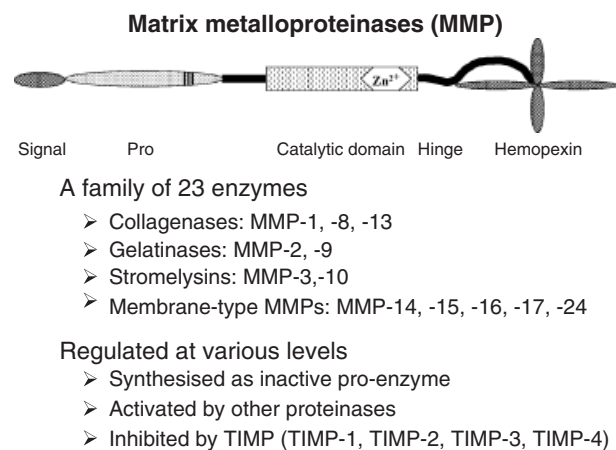

Matrix metalloproteinases (MMP)

Signal   Pro   Catalytic domain   Hinge   Hemopexin

A family of 23 enzymes
➢ Collagenases: MMP-1, -8, -13
➢ Gelatinases: MMP-2, -9
➢ Stromelysins: MMP-3,-10
➢ Membrane-type MMPs: MMP-14, -15, -16, -17, -24

Regulated at various levels
➢ Synthesised as inactive pro-enzyme
➢ Activated by other proteinases
➢ Inhibited by TIMP (TIMP-1, TIMP-2, TIMP-3, TIMP-4)

Fig. 2. The matrix metalloproteinase family. Diagram of the general domain structure of matrix metalloproteinases (MMP). Most MMP are synthesized with a pro-domain, which is enzymatically removed to activate the enzyme. The catalytic domain contains zinc at the active site. The hemopexin domain is essential for the substrate specificity of collagenases. There are variations in structure between the different family numbers, some of which lack the hemopexin domain and others also possess a trans-membrane domain.
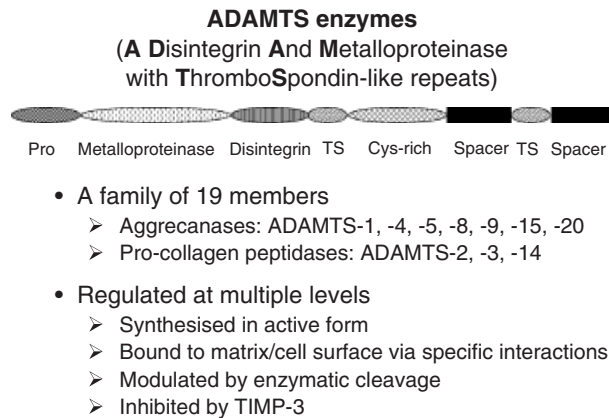
ADAMTS enzymes
(**A D**isintegrin **A**nd **M**etalloproteinase
with **T**hrombo**S**pondin-like repeats)

Pro Metalloproteinase Disintegrin TS Cys-rich Spacer TS Spacer

- A family of 19 members
  - Aggrecanases: ADAMTS-1, -4, -5, -8, -9, -15, -20
  - Pro-collagen peptidases: ADAMTS-2, -3, -14
- Regulated at multiple levels
  - Synthesised in active form
  - Bound to matrix/cell surface via specific interactions
  - Modulated by enzymatic cleavage
  - Inhibited by TIMP-3

Fig. 3. The ADAMTS family of enzymes. Diagram of the general domain structure of the ADAMTS enzymes. Several members of the family are able to cleave proteoglycans such as aggrecan and versican and are given the generic title "aggrecanases". Other members of the family are pro-collagen peptidases, involved in the processing of collagen molecules prior to the formation of fibrils. The functions of the remaining family members are currently poorly understood.

ADAMTS-4 and -5 can prevent cartilage degradation in tissue culture models (Tortorella et al., 2001), the enzymes responsible for proteoglycan degradation in osteoarthritis and other diseases of connective tissues have yet to be identified.

Aggrecanase activity is thought to be regulated at multiple levels, although the mechanisms are currently poorly understood. Differential regulation of ADAMTS mRNA has been deduced from analysis of their expression in cell and explant cultures, albeit with considerable variation between studies (Caterson et al., 1999; Tortorella et al., 2001; Vankemmelbeke et al., 2001; Koshy et al., 2002; Tsuzaki et al., 2003b). A study of human tendon cells recently reported small and variable effects of IL-1 on ADAMTS-4 expression (Tsuzaki et al., 2003b).

In addition to regulation of gene transcription, the activities of ADAMTS enzymes are also subject to post-translational regulation. The non-catalytic ancillary domains of ADAMTS-4 are required for both catalytic activity and substrate specificity (Flannery et al., 2002; Kashiwagi et al., 2003). Full-length enzyme is sequestered in the matrix via GAG-binding sequences in the spacer domain, and sulfated GAGs attached to the aggrecan core protein are required for ADAMTS-4 activity (Kashiwagi et al., 2003). Deletion of the C-terminal spacer domain increased the efficiency of hydrolysis of aggrecan at Glu^{373}–Ala^{374} bonds, and revealed new activities against fibromodulin, decorin and a general protein substrate (Kashiwagi et al., 2003). Several short forms of ADAMTS-4 are found in cartilage, thought to be generated by autocatalytic C-terminal truncation, potentially contributing to the degradation of a

broad range of protein substrates in addition to PGs (Flannery et al., 2002; Kashiwagi et al., 2003). The enzymes are thought to be secreted in an active form after cleavage of the pro-domain within the cell by furin, which may be followed by C-terminal truncation by MMP-17 at the cell surface (Gao et al., 2003; Wang et al., 2004). Active ADAMTS are inhibited by the endogenous inhibitor TIMP-3, but not by other TIMPs (Kashiwagi et al., 2001).

Matrix degradation in tendinopathy

Several MMP have been implicated in matrix degradation of tendon. Specimens of human rotator cuff tendons placed in culture were shown to synthesize collagenase (MMP-1) and TIMP-1, and there was no significant difference between normal and degenerate tendons (Dalton et al., 1995). Thus degenerate tendons were shown to be capable of remodelling the tendon matrix, even at a late stage of the disease, although the factors regulating this activity *in vivo* were not identified (Dalton et al., 1995). A comparison of human tendons showed substantial differences in collagen turnover between tendons from different sites. There was very little collagen turnover in normal biceps brachii tendons, which contained no significant levels of metalloproteinase activity and the linear accumulation of pentosidine with increasing age (Riley et al., 2002). In contrast, supraspinatus tendons obtained from normal shoulders showed relatively high levels of collagen turnover, with lower levels of pentosidine than expected for the age of the tissue, and there were correspondingly high levels of MMP-1, MMP-2 and MMP-3 activity (Riley et al., 2002). These enzyme activities were thought to represent either a repair or maintenance function, occurring in the normal supraspinatus as a result of the high mechanical demands placed upon the tendon in the shoulder. It may also be associated with an underlying degenerative process, which was common in asymptomatic shoulders, potentially caused by repeated minor injuries, mechanical strain or "overuse". In ruptured supraspinatus tendon there was increased activity of MMP-1, reduced activity of MMP-2 and MMP-3, and evidence of increased turnover of the collagen network (Riley et al., 2002). The potential role of MMPs in (or after) tendon rupture was also demonstrated by a study of synovial fluids from patients with rotator cuff tears. There were high levels of expression of both MMP-1 and MMP-3, with no change in the levels of TIMP-1, and the levels of enzyme correlated with the size of tear (Yoshihara et al., 2001). Glycosaminoglycan levels were also higher in fluids from massive tears compared with partial tears, consistent with increased turnover of matrix proteoglycans

(Yoshihara et al., 2001). Immunolocalization of ruptured supraspinatus showed MMP-1 expressed in the tendon at the edge of the tear (Gotoh et al., 1997). Tendon degeneration was thus shown to be an active, cell-mediated process that may result from a failure to regulate specific MMP activities in response to repeated injury or mechanical strain. It is most likely to be mediated by the resident tenocyte population, although some contribution from surrounding tissues and infiltrating cells cannot be definitively excluded in pathological situations. It has also been demonstrated that matrix turnover is substantially higher in tendons exposed to high mechanical demands (Riley et al., 2002), and this is thought to represent either a repair or maintenance function.

Relatively little is currently known about proteoglycan turnover in tendinopathy. Since levels of proteoglycan are increased in the degenerate tendon lesion (unlike degenerate cartilage) it will be interesting to determine whether this is caused by an increase in proteoglycan synthesis or a decrease in proteoglycan degradation mediated by aggrecanases (or both). These studies are currently hampered by the limited availability of sensitive and specific aggrecanase activity assays, although technical developments in this area are expected soon.

Gene expression in tendinopathy

Analysis of gene expression in human tendon specimens has not been widely attempted, although techniques of molecular biology such as cDNA arrays and real time reverse transcriptase-polymerase chain reaction (RT-PCR) are capable of providing at least a semi-quantitative analysis of many different gene targets in very small tissue samples, such as can be obtained from tendon biopsies. These techniques are highly sensitive, specific and can provide useful information about cell activities in the tissue.

There are two published studies describing the use of cDNA arrays to investigate gene expression in Achilles tendinopathy (Ireland et al., 2001; Alfredson et al., 2003). A number of genes were found to show either increased or decreased expression in degenerate tendon, however in several cases apparent changes in gene expression were not confirmed by RT-PCR analysis of a greater number of tissue specimens (Alfredson et al., 2003; Corps et al., 2004). This serves to highlight inherent drawbacks of the technique, which requires relatively large amounts of RNA and is expensive, and is often used to compare a relatively small number of specimens.

Recently, we have investigated the expression of several matrix and enzyme genes in human Achilles tendon specimens using real time semi-quantitative

RT-PCR. We obtained macroscopically normal tendons ($n = 14$) from cadavers and tissue specimens obtained during surgery. The surgical specimens were either from patients with a history of chronic pain ($n = 10$), or had suffered an acute tendon rupture ($n = 8$). All specimens were obtained after informed consent and with the approval of the Cambridge Local Research Ethical Committee. RT-PCR analysis of two collagen genes (COL1A1 and COL3A1) showed relatively high but variable levels of expression in normal tendon, and significantly increased expression of both genes in painful tendinopathy (Riley GP, unpublished observations). These findings are consistent with biochemical analyses, which showed changes in collagen synthesis prior to tendon rupture (Eriksen et al., 2002). An analysis of versican mRNA, the major hyalectan found in tendon (see above), showed that two splice variants (V0 and V1) were most abundant, and there was a significant decrease in versican mRNA in both painful and ruptured tendons (Corps et al., 2004). Of the MMP genes that have been investigated to date, there was little detectable expression of MMP-1 and MMP-13 in any of the specimens, whether normal or degenerate (Riley GP, unpublished observations). Since both these collagenases are stimulated by inflammatory cytokines, this observation is consistent with the non-inflammatory nature of the lesion. There was a small but significant reduction in MMP-2 mRNA, but the most dramatic change was in the level of MMP-3 mRNA, which was substantially lower in painful and ruptured tendons. This study confirmed earlier observations made using cDNA arrays and biochemical analysis, which suggested that MMP-3 was potentially important for the maintenance of the normal tendon matrix (Ireland et al., 2001). However the substrate for MMP-3 in tendon has not been identified, and since most of the enzyme is in the inactive pro-form, the physiological relevance is uncertain. Finally, an analysis of the expression of three aggrecanases, ADAMTS-1, ADAMTS-4 and ADAMTS-5, showed expression of all three enzymes in the tissue specimens (Riley GP, unpublished observations). When expressed relative to the housekeeping gene GAPDH, there was found to be no significant change in ADAMTS-4, although ADAMTS-1 was reduced in ruptured tendon and ADAMTS-5 was reduced in both painful and ruptured tendons (unpublished). These observations require confirmation and their potential significance is uncertain, but it is possible to speculate that a reduction in aggrecanase expression may underlie the increase in proteoglycan found in degenerate tendon.

There are several caveats that must be taken into consideration in any study of gene expression in surgical and post-mortem tissues. Levels of gene expression may not correlate with levels of protein

expression or enzyme activity. This is particularly relevant with respect to the MMP, which are regulated at multiple levels and not just at the level of synthesis. The analysis of small biopsies may not be representative of the whole tendon, which generally shows highly focal changes in cellularity and matrix organization. The specimens are homogenized, and expression may be localized at specific sites within the tendon. The tendon specimens were also obtained from a heterogeneous group of patients, varying widely in several parameters such as age, levels of physical activity and the duration of disease. The analysis also provides only a "snap-shot" of cell activity, representing only the time of sampling, and changes in gene expression may be relatively transient. Finally, surgery, drug treatment, tissue storage and post-mortem changes can all profoundly affect the levels of expression in the tissue specimens, as well as reduce the quality of mRNA. With these caveats in mind, analyses of gene expression are able to provide some insight into the metabolic activity of human tendon, and will provide an important adjunct to biochemical techniques.

Perspectives

The evidence presented suggests that tenocytes are actively synthesizing and degrading the matrix in normal tendon, and that these activities are substantially altered in tendinopathy. Some matrix turnover is beneficial for the health of the tendon, a contention that is supported by the observation that drugs which affect the expression or activity of MMP, such as broad-spectrum MMP inhibitors and fluoroquinolone antibiotics, can induce tendon lesions in patients being treated for other conditions (Pierfitte & Royer, 1996; Brown, 1999; Corps et al., 2002). A large number of enzymes are potentially implicated in this process, since there are at least 23 MMPs and 19 ADAMTS enzymes, and studies to date have focused on relatively few of these. Some of these enzymes are likely to be required for normal tendon maintenance or repair, whilst others may be damaging to the tendon and responsible for matrix degeneration. Consequently it is important that the complete profile of enzyme expression and activity in human tendon is characterized, so that specific targets for future drug therapy can be identified.

Key words: matrix metalloproteinase, MMP, aggrecanase, ADAMTS.

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