Review Article

Involvement of Proteoglycans in Tendinopathy

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Abstract

A major feature of chronic tendinopathy is a change in the nature and organisation of the extracellular matrix of tendon. Increased levels of proteoglycans have been shown in the extracellular matrix of tendinopathic tendons and these appear to influence the increased hydration and swelling of the tissue that is a feature of this condition. There is a paucity of knowledge about proteoglycans in normal and tendinopathic tendons. This review sets out to describe the nature, function and metabolism of proteoglycans present in normal tendon and in tendinopathy and outlines how changes in proteoglycan metabolism may contribute to the development and progression of this disease.

Keywords: Proteoglycans, Tendinopathy, Tendon

Introduction

The unique structure of the extracellular matrix of tendon not only allows for the transmission of force generated by a muscle onto a bone, but also gives the tissue its viscoelastic properties. Tendons are heterogeneous structures in that the composition of the extracellular matrix varies along their length allowing tendon to meet specific mechanical requirements[1]. In tendinopathy the extracellular matrix is compromised, resulting in swelling and pain that affects an individual's ability to function and in chronic cases their quality of life[2,3]. Tendons commonly affected by tendinopathy include the patellar, Achilles, and rotator cuff-tendons[4-6].

Various theories have been proposed as to the cause of tendinopathy but none have explained all aspects of the disease[7]. From the literature there does not appear to be inflammatory cell infiltration in later stage tendinopathy[8-11] dismissing the term 'tendinitis'. However, inflammation cannot be completely ruled out at earlier stages of the disease process[12]. Degenerative and failed healing processes have also been described in the literature[7,13].

In tendinopathy all tendons exhibit similar pathological characteristics including increased levels of proteoglycans, water content and cellularity as well as collagen disorganisation and fibril separation[13-21]. Various aspects of tendinopathy investigated in these studies have used tendon samples from people with chronic symptoms that represent late stages of the condition. The role of proteoglycans in normal and tendinopathic tendon is poorly understood and this review aims to evaluate proteoglycan functions and interactions in normal tendons and in tendinopathy and to discuss changes in the synthesis and catabolism of proteoglycans in tendinopathy that may influence the progression of this disease.

Proteoglycans in the Extracellular Matrix of Tendon

Normal Tendon

Type I collagen fibres form the majority of the tendon extracellular matrix providing tendon with its tensile strength[4,22,23] (Figure 1). Other minor collagens such as Type III collagen are also present in the extracellular matrix of tendon[7]. Proteoglycans in the extracellular matrix are often associated with collagen fibrils or hyaluronan. Non-collagenous proteins (elastin, link protein, cartilage oligomeric matrix protein, tenascin-C, fibronectin, thrombospondin) are also present in the extracellular matrix of tendon and are involved in matrix-matrix, cell-matrix organisation or cell-matrix signalling. Embedded between collagen fibrils are tendon cells (tenocytes) that are responsible for the synthesis and maintenance of the extracellular matrix of the tissue (Figure 1).

LEGEND

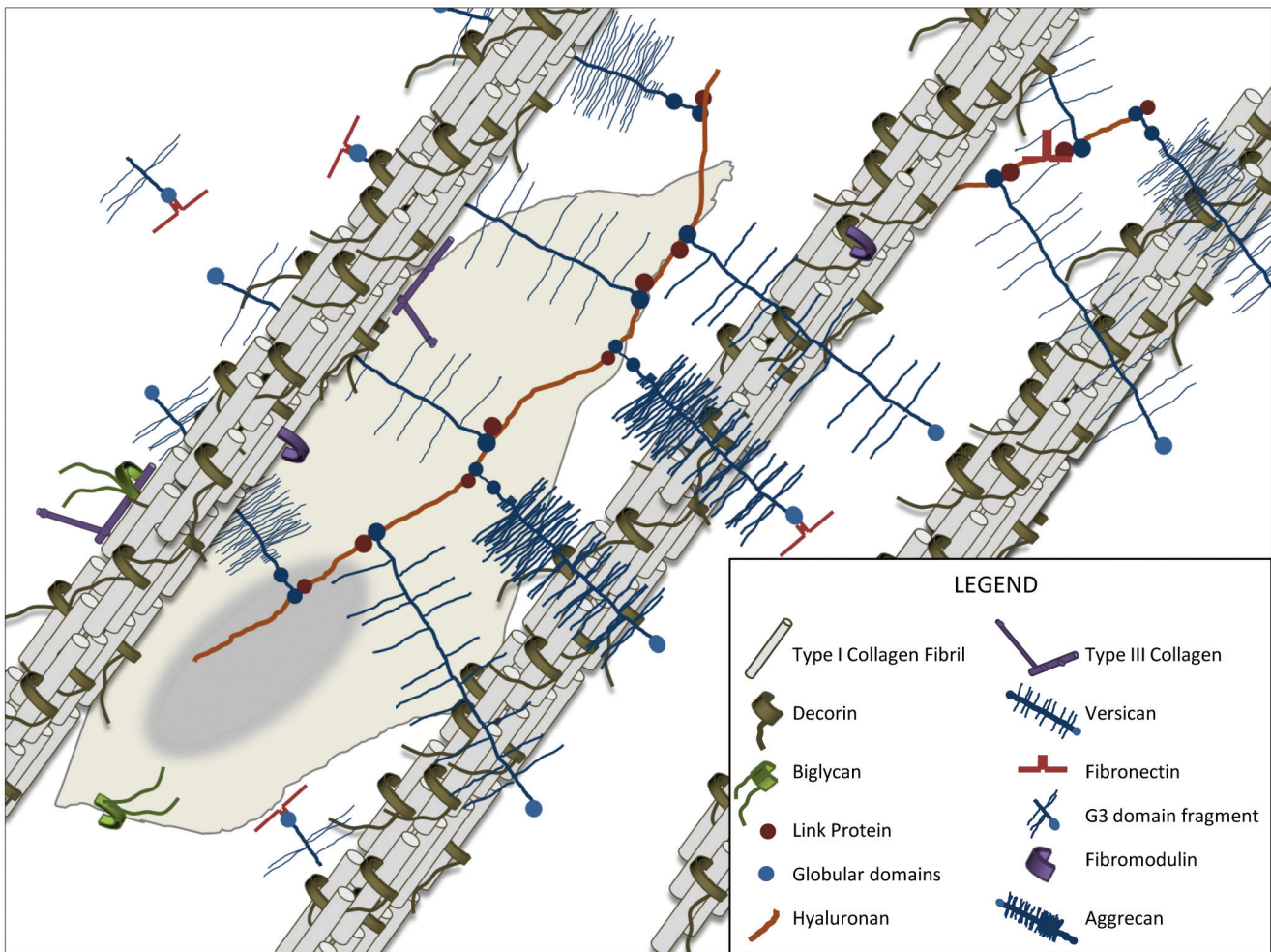|			
Type I Collagen Fibril		Type III Collagen	
Decorin		Versican	
Biglycan		Fibronectin	
Link Protein		G3 domain fragment	
Globular domains		Fibromodulin	
Hyaluronan		Aggrecan	

Figure 1. Interactions of proteoglycans and other matrix macromolecules within the extracellular matrix of the tensile regions of normal tendon.

The function of the different proteoglycans is determined by the structure of their protein core and glycosaminoglycan (GAG) chains. Although proteoglycans make up approximately 1% of the dry weight of tensile regions of most tendons[23], they are likely to contribute to the biomechanical and structural properties of the extracellular matrix of tendon[24,25]. The proteoglycans found in tendon are classified into two groups: 1) the small leucine-rich proteoglycans (SLRPs) and 2) the large aggregating proteoglycans.

Small Leucine-Rich Proteoglycans

The major proteoglycan present in tendon (based on levels of the respective core proteins) is the SLRP decorin, which constitutes approximately 80% of the total proteoglycan content in the tensional region of tendon[26]. Also present are other SLRPs including biglycan, fibromodulin and lumican as well as the recently observed keratocan[27]. The prevalence of this group of proteolgycans in the tissue is reflective of the collagenous nature of tendon. The core proteins of the SLRPs are horseshoe shaped and

are characterised by leucine-rich repeats[28], which are involved in the binding of the SLRPs to collagen[28-31] (Figure 1). They have been shown to bind to collagen fibrils and regulate collagen fibrillogenesis[32]. Decorin, for example, acts to modulate collagen fibril formation through its attachment to collagen fibres every 64-68 nm[32,33]. Furthermore, collagen fibrils in tendons of SLRP knockout mice (decorin, biglycan, fibromodulin and lumican) have smaller diameter and abnormal morphology, resulting in weaker tendons with decreased stiffness. This demonstrates the importance of these SLRPs in tendon extracellular matrix structure and function especially the organisation of collagen fibrils[34-39].

Large Aggregating Proteoglycans

The large aggregating proteoglycans found in the tensional region of tendon are aggrecan and versican[40-43] (Figure 1). A characteristic feature of the large aggregating proteoglycans is the presence of globular domains separated by a GAG attachment region[44].

The aggrecan monomer consists of a large core protein con-

taining three globular domains (G1, G2 and G3)[44]. At the amino-terminus of the core protein is the G1 domain that specifically binds hyaluronan, forming large multimolecular aggregates[45] (Figure 1). The G1 domain of aggrecan is homologous to link protein[46] and is separated from the G2 domain by an interglobular domain[47,48]. The G2 domain of aggrecan appears to have a role in the secretion of the proteoglycan from cells[49]. Aggrecan also contains a carboxyl-terminal G3 domain which has lectin-like structures and therefore has the potential to interact with other matrix macromolecules such as tenascin-R, fibulin-1 and -2 and fibrilin-1[50-54]. Versican contains an amino-terminal G1 domain that is homologous to the G1 domain of aggrecan and also binds hyaluronan and link protein[55] and a carboxyl-terminal G3 domain but does not contain a G2 domain[24,56]. Since aggrecan and versican have a high number of negatively charged GAG chains attached to their core protein, this gives these proteoglycans the ability to attract and bind water and in tendon it is likely that they may contribute to the biomechanical properties of normal tissue[57].

The distal region of tendon (where the tissue wraps around bone) has a fibrocartilagenous structure and contains increased levels of aggrecan and biglycan in comparison to the tensional region[58]. The distal region also contains increased levels of proteoglycan 4; a large proteoglycan which has been shown to have a lubricating role[59-61].

We have investigated the nature of proteoglycans present in the extracellular matrix of the tensile regions of normal tendons. It appears that the core proteins of the SLRPs are mostly intact indicating that there is little accumulation of degraded SLRPs[20]. This is in contrast with the large proteoglycans that are present in the extracellular matrix of normal tendon as macromolecules with predominantly degraded core proteins. Furthermore, a number of aggrecan and versican catabolic fragments contain the G3 domain indicating that the G3 domain may be interacting with other components of the extracellular matrix of tendon[20]. Fragments of large proteoglycans may also be trapped within the extracellular matrix of tendon entangled with each other or other matrix macromolecules.

Tendinopathy

Few studies have investigated the detailed nature of proteoglycan species associated with tendinopathy. Histochemical analysis of pathologic tendons have reported increased GAG content of the extracellular matrix of the tissue compared to normal tendons[7,57]. Our work showed a 5-fold increase in GAG content in tendinopathic tendons relative to normal tendons, which are reflected in increased levels of the large proteoglycans aggrecan and versican[19,20].

As the large proteoglycans bind water, increased levels of these proteoglycans are likely to contribute to tissue swelling that is a common feature of the condition. In patellar tendon sections excised from human subjects, we have shown a 16% increase in water content based on wet weight in tendinopathic tissue compared to corresponding sections of normal tendons[20]. This increased water content is consistent with a previous study of other pathological tendons, such as supraspinatus and subscapularis ten-

dons, which contained significantly higher water content compared to normal tendons[22]. In addition, this change in water content may also be in part due to disruption of the collagen network[62]. It is very likely that there may be a concomitant increase in collagen network disruption and proteoglycan levels[20,62].

In tendinopathic tendon we have also shown an increase in the levels of the SLRPs fibromodulin and biglycan[20]. Since the interactions between collagens and SLRPs are critical for the correct assembly of collagen fibrils, changes in the levels of fibromodulin and biglycan in tendinopathy may contribute to the degeneration of the collagen network.

The nature of the SLRPs and large proteoglycans are similar in normal and tendinopathic tendons in that the majority of SLRPs exist in the extracellular matrix with intact core proteins whereas the majority of the large proteoglycans have mainly fragmented core proteins[20].

Metabolism of Proteoglycans in Tendon

Normal Tendon

Tenocytes are responsible for the metabolism of proteoglycans in normal tendons. Under steady state conditions, there is a balance between the synthesis and catabolism of proteoglycans. Tenocytes respond to mechanical loading with the expression of proteoglycans and other extracellular matrix macromolecules[63].

In normal bovine tendon, the newly synthesised large proteoglycans have a $T_{1/2}$ of approximately two days[43]. Contrasting the rapid turnover of the large proteoglycans, the rate of loss of newly synthesised SLRPs was shown to be longer with a $T_{1/2}$ of greater than 20 days[43,64]. It was proposed that the slow loss of newly synthesised SLRPs is due to their close association with tendon matrix components, in particular the collagen network and it is a possibility that the turnover of decorin may be associated with the turnover of collagen[65].

The catabolism of large proteoglycans in tendon is likely to be mediated by the aggrecanases. Aggrecanases are members of the ADAMTS (A Disintegrin-like and Metalloprotease with Thromospondin motifs) family of proteases. The aggrecanases were first discovered in articular cartilage by their ability to cleave aggrecan at specific sites, with the major site being in the interglobular domain of the aggrecan core protein between residues Glu_{373}-Ala_{374}[66-68]. Other aggrecanase sites include the chondroitin sulphate domains of the aggrecan core protein[67]. Cleavage at these sites results in the loss of the GAG attachment regions of aggrecan in cartilage but not in tendon where such fragments are retained by the tissue[26,66,68,69].

We have identified aggrecanase- and matrix metalloproteinase- (MMP) products of the catabolism of large proteoglycans in bovine tendon[26]. Although the mechanisms involved in the catabolism of versican are unclear, most of the versican fragments present in the tendon matrix lack the G1 domain and are most likely generated from versican variants V_0, V_1 and V_2[26]. The recombinant aggrecanases, ADAMTS-1 and -4, have been shown to cleave versican at a specific site adjacent to the G1 domain between residues Glu_{441}-Ala_{442} and such cleavage has been observed *in vivo*[70].

Tendinopathy

The rate of synthesis of ^{35}S-labelled proteoglycans is 25-fold greater in tendinopathy than in normal tendons[21]. This increase in the rate of synthesis has been attributed to increased synthesis of the large proteoglycans aggrecan and versican in tendinopathic tissue[21]. The increase in proteoglycan synthesis is likely to reflect in part the increase in cellularity in tendinopathic tissue[71]. This increased rate of synthesis of proteoglycans in tendinopathy may also be a result of altered mechanical loads that increase local growth factor levels such as transforming growth factor-β[72,73].

Our work has shown no significant change in the gene expression of specific proteoglycan species between normal and tendinopathic human patellar tendons[20]. There was however a trend for a change in mRNA levels concomitant with the up regulation of versican and down regulation of fibromodulin[20]. A problem with using mRNA expression analysis as a measure of protein production is that it is unknown whether the reported up or down regulation of mRNA for specific genes results in the translation and survival of the specific protein[74,75]. For example, the trend towards a decrease in fibromodulin expression in tendinopathic tendon did not cause a reduction of protein levels in the extracellular matrix[20]. Instead, tendinopathic tendons contained higher levels of fibromodulin than normal tendons indicating an accumulation of fibromodulin in the extracellular matrix of tendinopathic tendons suggesting a change in the metabolism of fibromodulin. This may also be true for the accumulation of other proteoglycans in tendinopathy. In contrast to this observation, the trend towards an increase in the gene expression of versican correlated with an increase in versican at the protein level[20]. Other studies have shown an increase in aggrecan and biglycan gene expression in human Achilles tendinopathy with variability in the expression of total versican and versican variants[75,76]. In contrast, decorin gene expression showed no significant difference[77] or a reduction in decorin mRNA[76] in tendinopathic Achilles tendons compared to normal Achilles tendons.

The rate of loss of ^{35}S-labelled proteoglycans from tendinopathic tendon is significantly higher compared to normal tendons so that over a ten day period 40% of the radiolabelled proteoglycans remained in the matrix of tendinopathic tendon compared to 90% in normal tendon[21]. This may be due to changes in either tendon metabolism or to increased cellularity or both. The differences in the rate of loss of radiolabelled proteoglycans between normal and tendinopathic tendon explant cultures is likely to be due to increased synthesis of large proteoglycans[21], since the large radiolabelled proteoglycans are lost more rapidly than the radiolabelled SLRPs and they represent the major proportion of radiolabelled proteoglycan loss in tendinopathic tendon explants[21,43].

The influence of growth factors and cytokines in tendinopathy is yet to be established[78]. They are likely to promote the activation of proteinases such as the ADAMTSs and MMPs, causing the breakdown and disorganisation of the tendon extracellular matrix seen in tendinopathy. Indeed changes in the levels of gene expression of ADAMTS, MMP, and tissue inhibitor of metalloproteinases (TIMP) have been reported in tendinopathy[75,78,79]. However, our work showed no difference in the expression levels of ADAMTS-1, -4 and -5 or TIMP-2, -3 and -4 in tendinopathic tendon compared to normal tendons[21]. Jones and colleagues[79] showed no difference in the expression of ADAMTS-1 and -4 and TIMP-1, -2 and -4 but showed a down-regulation of ADAMTS-5 and TIMP-3 in painful Achilles tendons compared to normal Achilles tendons. Indeed, in another study the expression of ADAMTS-4 showed no significant change between normal Achilles tendons and tendons with chronic pain[80]. In the same study, Corps and colleagues (2008) analysed the nature of ADAMTS-4 in the matrix showing an increase in the processed active form of this proteinase in painful Achilles tendons. These studies indicate that in painful tendinopathy ADAMTS activity is increased by activation of the proteinases and not by elevated gene expression.

The overall gene expression of the MMPs has been shown to be relatively low in normal tendons[79], as is required for the healthy remodelling of tendons, but in pathological tendons the levels of MMP gene expression can change greatly, possibly resulting in degradation of the tendon[74]. Our work has shown that the gene expression of MMP-9 and TIMP-1 was significantly up regulated in tendinopathy[21]. This finding is supported by a study of ruptured Achilles tendons where MMP-9 and TIMP-1 gene expression was also upregulated[79]. In pathologies of the Achilles tendon MMP-3 gene expression was found to be significantly down regulated compared to normal Achilles tendons[74,77,79]. Although only MMP-9 was up regulated, the significant loss of proteoglycans suggests an increase in the levels of proteinases in the tendon matrix. It is therefore important to determine the protein levels and activities of these proteinases in tendinopathy.

Perspectives/Discussion

Evidence suggests that proteoglycans must play a key role in the structure and function of the extracellular matrix of tendon in health and disease. Our work has shown that proteoglycan metabolism by tendon is dynamic in that these macromolecules are actively synthesised and catabolised by both normal and tendinopathic tissue[21,26,43]. This change in the metabolism of proteoglycans that occurs in tendinopathy is likely to be the result of increased levels of cytokines and growth factors. Further studies are needed to establish the role of growth factors and cytokines in the activation of matrix proteinases such as the MMPs and ADAMTSs that cause the breakdown and disorganisation of the tendon extracellular matrix seen in tendinopathy.

Tendons respond to changes in their environment by directly altering the metabolism of large proteoglycans[81-84]. In normal tendons, the synthesis of large proteoglycans is increased in response to compressive loading whereas the synthesis of SLRPs continues at a similar rate irrespective of mechanical loading[81-84]. This change in proteoglycan metabolism leads to the accumulation of large proteoglycans within the area of compression and when the compressive load is removed, tissue

levels of large proteoglycans returns to normal[82]. It has been shown that in individuals with tendinopathy who have undergone strength training there is an immediate increase in Achilles tendon volume[85]. This swelling is likely to result from tendon responding to increased loading by increasing the synthesis of large proteoglycans that accumulate within the tissue. It is possible that normal tendons respond in a similar manner to increased loading by synthesising proteoglycans that result in the short-term in increased tissue levels of large proteoglycans that after a period of rest return to normal levels. However, further work is required to establish these changes.

These changes in the metabolism of large proteoglycans complements a proposed model of tendinopathy that suggests the condition is a continuum consisting of three stages; reactive, disrepair and degeneration[86]. The development of the reactive stage may result from the inability of tendon to modulate the metabolism of large proteoglycans, resulting in accumulation within the tissue as a response to increased loading. It is not until the tendon disrepair phase during which the tendon attempts to regenerate the extracellular matrix, which ultimately fails, and results in collagen disorganisation. This suggests that a change in large proteoglycan metabolism precedes any change in collagen organisation[86].

This model allows for early intervention during the reactive tendinopathy phase using imaging of the affected tendon that may allow clinicians an opportunity to implement a regime to reverse the condition before the tendon disrepair/tendon degeneration phase through load reduction, pain management and the use of selective pharmacological treatments aimed at reducing tissue levels of large proteoglycans[86,87].

Proteoglycans therefore appear to play a major role in the function of normal tendon but an aberration in their metabolism is likely to drive the pathogenesis of tendon where damage to collage fibrils and overall collagen integrity through swelling pressure occurs at all stages of overuse tendinopathy.

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