

Role of Metalloproteinases in Tendon Pathophysiology

Diego Sbardella^{1,2}, Grazia R. Tundo^{1,2}, Giovanni Francesco Fasciglione¹, Magda Gioia^{1,2}, Salvatore Bisicchia¹, Elena Gasbarra¹, Ernesto Ippolito¹, Umberto Tarantino^{1,3}, Massimo Coletta^{1,2,3} and Stefano Marini^{1,2,3*}

¹Department of Clinical Sciences and Translational Medicine, University of Roma Tor Vergata, Via Montpellier 1, I-00133 Roma, Italy; ²Interuniversity Consortium for the Research on Chemistry of Metals in Biological Systems, Via C. Ulpiani 27, I-70126 Bari, Italy; ³Center for Space Biomedicine, University of Roma Tor Vergata, Via Montpellier 1, I-00133 Roma, Italy

Abstract: Tendons play a crucial role in musculoskeletal functioning because they physically connect bones and muscles making the movement of articular joints possible. The molecular composition of tendons mostly include collagen I fibrils, which aggregate together to form fibers to form a fascicle. A complex network composed of resident cells (*i.e.*, tenocytes) and extracellular matrix macromolecules (glycosaminoglycans, proteoglycans, glycoproteins and other non collagenous proteins) interact and define the structure of tendons and their properties. Development, renewal and remodeling of tendons composition occur at all ages of living organisms so the homeostasis of proteolytic systems is a critical issue. A major role is played by Metalloproteinases, a family of Zn²⁺-dependent endopeptidases involved in the catabolism of several components of the extracellular matrix, such as collagens, proteoglycans, fibronectin and many others. Among these, two main classes are mostly involved in tendon pathophysiology, namely the Matrix Metalloproteinases (MMPs) and a Disintegrin-like and Metalloproteinase domain with Thrombospondin motifs (ADAMTSs). This study analyses the various aspects of the roles played by Metalloproteinases in the physiological and pathological processes of tendons.

Keywords: Review, Matrix metalloproteinases, A Disintegrin-like and Metalloproteinase domain with Thrombospondin-1 motifs, Tendon Pathophysiology, Repairing Process, Therapeutical Approach.

INTRODUCTION

Tendons play a crucial role in musculoskeletal apparatus because they physically connect bones and muscles making the movement of articular joints possible. Therefore, the development, remodeling and healing of tendons are a strictly regulated processes at all ages in living organisms.

The molecular composition of tendons includes collagen fibrils and several other Extracellular Matrix (ECM) components secreted by tenocytes, the only resident cell which stems from tenoblast differentiation. Within the extracellular matrix network, tenoblasts and tenocytes represent the predominant cell histotype with a minor contribution of chondrocytes at the bone insertion and capillary endothelial cells and smooth muscle cells of arterioles.

The tendons macromolecules assemble together to form a complex which is a spatially defined network that guarantees the physical/mechanical properties of the tendon, such as tensile strength [1]. Furthermore, in higher organisms (such as mammals), tendon activity is constantly monitored by neurotendinous proprioceptive receptors, which inform the nervous system about elongation and release, thus preserving

tendons and muscle integrity from excessive and/or unnatural strains through activation of reflex arcs.

TENDONS BIOCHEMICAL COMPOSITION AND ULTRASTRUCTURE

From an ultrastructural point of view, in tendons, collagen fibrils are the most abundant type, with collagen I being the major component [2]. Collagen is arranged in hierarchical levels of increasing complexity, beginning with tropocollagen, a triple-helix poly-peptide chain, which unites to form fibrils; fibers (primary bundles); fascicles (secondary bundles); tertiary bundles; and the tendon itself. The collagen I monomer consists of two α -1 chains and one α -2 chain intertwined into a triple helix, each monomer being staggered from its molecular neighbor by a multiple of 67 nm in the direction of the helix [3-5]. Laterally, the helices are arranged almost hexagonally to each other within the microfibril [6]. A right-handed microfibril is composed of five collagen molecules packed together: a higher-order supramolecular structure that resembles a helix and that interdigitates with neighboring microfibrils to form the basis of the fibrils [7]. Fibrils tend to aggregate together to form fibers, which then make up fascicles, which can be observed surrounding tenoblasts and tenocytes (Fig. 1). Fascicles are predominantly aligned along the longitudinal axis of the tendon, being responsible for its tensile strength. In addition to collagen, a variety of glycosaminoglycans, proteoglycans

*Address correspondence to this author at the Department of Clinical Sciences and Translational Medicine, University of Roma Tor Vergata, Via Montpellier 1, I-00133 Roma, Italy; Tel: +39-06-72596354; Fax: +39-07-72596353; E-mail: stefano.marini@uniroma2.it

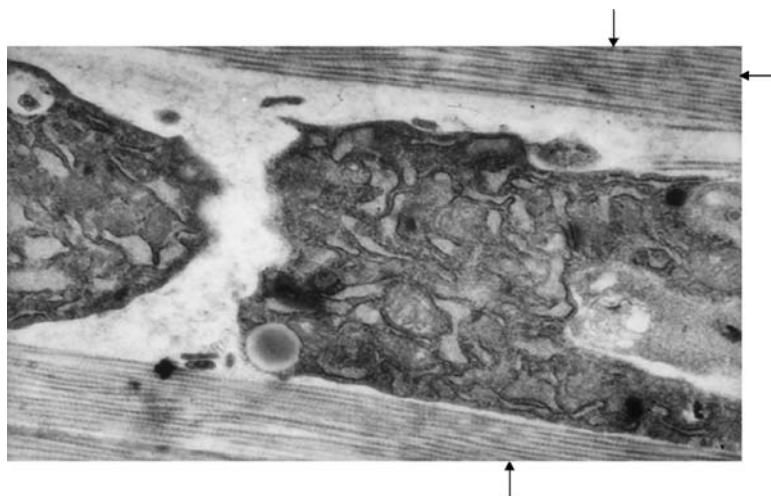


Fig. (1). Electron microscopy image (20,000 X) of a tenoblast: numerous collagen I filaments (black arrows) can be observed outside the cell.

(large and small), glycoproteins and other non collagenous proteins, in particular tenascin-C, are secreted by tenocytes and orderly inserted into the collagen fascicle network [8-11]. Tenascin-C is a molecule containing a number of repeating fibronectin type-III domains which undergo stress-induced unfolding thus allowing the whole macromolecule to behave as an elastic protein. Tenascin-C is distributed, according to its role in collagen fibril organization, maintaining the interface between fibrils and adjacent structures. Furthermore, tenascin-C, which is abundant in the tendon body and at the osteotendinous and myotendinous junctions, is assumed to assist in structural and spatial arrangement of the collagen fibers [12]. In fact, this structural arrangement and the tenocyte interrelationships are also affected by age [13], clearly showing a different tendon structure in newborn, young and adult individuals (Fig. 2) [14].

Proteoglycans are heavily glycosylated and strongly hydrophilic proteins made up by different core proteins and one or more covalently attached glycosaminoglycan (GAGs) chains. GAGs, which can be found both as side chains of proteoglycans or free complex polysaccharides, are composed of linear disaccharide repeating units, each unit composed of a N-acetylated or N-sulfated hexosamine and either a uronic acid (glucuronic acid or iduronic acid) or galactose [10, 11]. The Glycosaminoglycans (GAGs) which are covalently bound to the different protein cores of the proteoglycans are mostly represented by chondroitin sulfate (both chondroitin 4-sulphate and chondroitin 6-sulphate), dermatan sulfate, keratan sulfate and heparan sulphate (also known as mucopolysaccharides). Through their SO_4^{2-} groups, and then the presence of extensive negative charges at their surface, GAGs bind Na^+ ions which, as a matter of fact, attract water via osmosis, contributing to keep the tendon hydrated, hence conferring resistance to compression, rapid diffusion of water-soluble molecules and the migration of cells [15].

The major proteoglycan present in the proximal/tensional regions of the tendon is decorin [16-18], even though lower amounts of other proteoglycans, such as biglycan, aggrecan

and versican, have also been detected [19, 20]. Decorin and biglycan belong to the small leucine-rich family of proteoglycans (characterized by leucine-rich repeat structures) which have been proposed to be directly involved in the binding of proteoglycans to collagen [21]. In particular, decorin plays multiple roles, such as (i) maintenance and regulation of collagen fibril structure, (ii) regulation, mainly through inhibition, of cell proliferation and spreading, and (iii) stimulation of immune responses. Then, decorin is considered to be a key regulator of matrix assembly because it limits collagen fibril formation and thus directs tendon remodeling due to tensile forces [22]. Biglycan (which is expressed mainly in tendon, cartilage and bone as well as in dermis and blood vessels) also interacts with type I collagen, even though the nature of this interaction is not well understood and, similarly to decorin, it can also bind to TGF beta. Therefore, it has the potential to participate in the modulation of cell proliferation [23, 24].

Aggrecan and versican are members of the large aggregating family of proteoglycans (also called lecticans) and are characterized by a number of domains (e.g., G1, which binds to hyaluronan, and G3) [25]. Aggrecan, which is present at low concentrations in tensional parts of tendons and at higher levels in compressed regions (mostly in the fibrocartilage), is bound to hyaluronan, making tendons more resilient. Versican binds to hyaluronan and collagen I in tensional parts of tendons and in compressed regions, increasing the viscoelasticity and maintaining the cell shape [25, 26].

This complex protein to protein organization requires a fine tuning of the modeling and turnover in order to optimize the tendons role in the musculo-skeletal system. In this regard, the functional modulation of proteolytic systems of the extracellular matrix is very important both from a physiological and pathological standpoint, a major role being played by lysyl oxidase [27] and by Matrix MetalloProteinases (MMPs). These latter enzymes are able to enzymatically process most of the molecular components

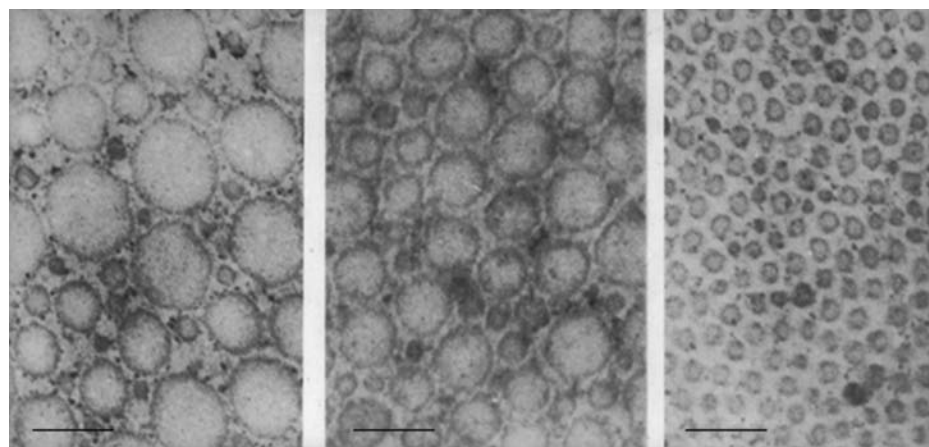


Fig. (2). Cross section of tendon matrix (48,000 X) in newborn (*right panel*), young (*central panel*) and old tendon (*left panel*). It is noteworthy to observe the different diameter of collagen fibers, which ranges between 180 and 550 Å in the newborn tendon, whereas in the young tendon the diameter varies between 180 and 1660 Å and in the old tendon between 180 and 2030 Å. Scale bars correspond to 0.2 μm.

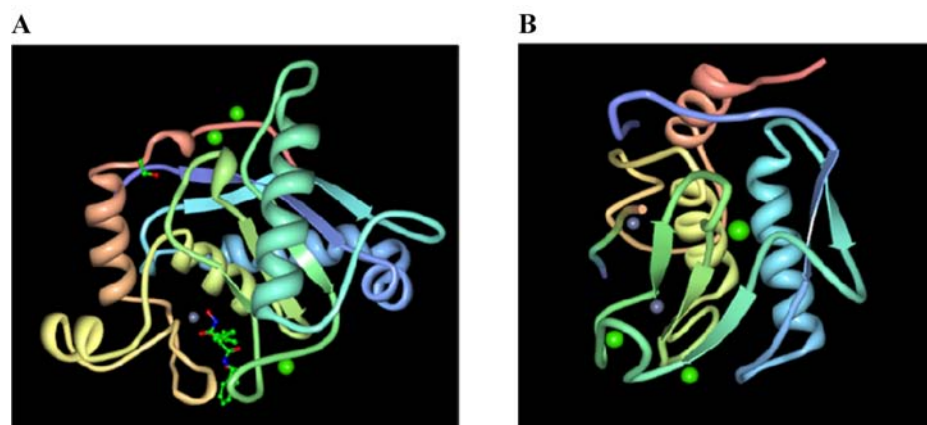


Fig. (3). Catalytic domains of human ADAMTS-5 (*panel A*) complexed with an amino 2-indol compound and of human MMP-2 (*panel B*). Some major differences in the architecture of the catalytic pockets between ADAMTS and MMPs are shown: they mainly concern the S2 and the S1 pockets. These structural features allow the aggrecanases to adapt their conformations to the substrate, while MMPs display a rather more fixed conformation. In both cases, Zn²⁺ ions are represented by grey spheres and Ca²⁺ ions by green spheres.

of the connective tissue and to modulate the bio-availability of soluble factors, which eventually leads to tendinopathy [28].

Metalloproteinases are a family of Zn²⁺-dependent endopeptidases involved in the proteolytic processing of several components of the extracellular matrix, such as collagens, proteoglycans, fibronectin and many others [29]. Among these, two main classes are mostly involved in tendon pathophysiology, namely the Matrix Metalloproteinases (MMPs) and a Disintegrin and Metalloproteinase domain with Thrombospondin-1 motifs (ADAMTSs) (Fig. 3).

MMPs have been implicated in several physiological and pathological processes, such as skeletal growth and remodelling, wound healing, cancer, arthritis, multiple sclerosis [30-37] and tendinopathies [38]. MMPs-domain composition usually consists of an amino-terminal prodomain (which is removed upon activation of the enzyme), a catalytic domain (where the active Zn²⁺ is located) and a carboxy-terminal hemopexin-like domain, which is separated

from the catalytic domain by a proline-rich linker peptide (also called hinge region) of variable length [29, 39-41]. MMPs can be classified into five main groups, namely (i) collagenases (*i.e.*, MMP-1, MMP-8 and MMP-13), which are able to cleave fibrillar collagen and bind collagen preferentially through the hemopexin-like domain [42-44], (ii) gelatinases (*i.e.*, MMP-2 and MMP-9), which are characterized by an additional domain, called collagen binding domain, which is made up of three fibronectin II-like repeats located in their catalytic domain; this domain represents the preferential binding domain for fibrillar collagen I [45-46], (iii) stromelysins (*i.e.*, MMP-3, MMP-10 and MMP-11), which are unable to cleave fibrillar collagen I, (iv) matrilysins (*i.e.*, MMP-7 and MMP-26), which lack the hemopexin-like domain and are able to process collagen IV but not collagen I [47], and (v) membrane-bound MMPs (*i.e.*, MMP-14, MMP-15, MMP-16 and MMP-17), whose carboxy-terminal domain contains an intermembrane region (or segment), followed by a short cytoplasmic tail and/or a glycosylphosphatidyl inositol anchor. MMP-14, MMP-15

and possibly MMP-16 are membrane-bound MMPs which have been shown to be able to cleave fibrillar collagen I [48, 49]. The proteolytic activity of MMPs is regulated *in vivo* by their interaction with natural inhibitors (called Tissue Inhibitors of Metalloproteinases, TIMPs), whose spatial and temporal distribution is generally related to MMPs activity in living tissues [50]. Therefore the relative levels of expression of MMPs and TIMPs are often a signal of the intrinsic regulatory mechanism of MMPs activity.

The ADAMTS family of metalloproteinases consists of 19 members, whose hallmark is an ancillary domain containing one or more thrombospondin type 1 repeats [51]. Their structural arrangement is drastically different from that of MMPs (Fig. 4) and these enzymes play an important role in the turnover of extracellular matrix proteins in various tissues, their altered regulation being implicated in diseases such as cancer, arthritis and atherosclerosis [52-54]. In particular, ADAMTS5 is expressed by specific cell lineages during embryonic development, such as arterial smooth muscle cells, concomitantly with the appearance in the neuromuscular system of some of its substrates, namely versican and aggrecan [55]. Unlike other metalloproteinases, ADAMTS members demonstrate a marked substrate selectivity due to the various exosites located in the C-terminal regions of the enzymes, which influence protein recognition, matrix localization and correct tissue compartmentalization. Proteoglycans, in particular aggrecan, and pro-collagen are specific substrates of ADAMTSs [56-58].

In this review we deal with some physiological aspects of the activity of MMPs and ADAMTSs regarding tendons, such as during development, as well as with some pathological aspects (as in the case of rotator cuff disease, patellar tendinopathy and Achilles tendon rupture).

ROLE OF MMPs AND ADAMTSs IN SKELETAL AND TENDON DEVELOPMENT

During tendon development, collagen fibrils are initially released as short, small diameter intermediates. These represent the building blocks of the mature fibril [59-63]. These intermediates are then incorporated into fibers, and within a narrow period of time, they form the characteristic long fibrils of variable length of the mature, mechanically functional tendon. This assembly leads to growth and relevant enhancement of the tensile strength of the tendon. The formation of collagen fibrils is critically regulated by the proteolytic cleavage of procollagen I to mature collagen I, an event undertaken initially by *N*- and *C*-proteinases, with the active participation of ADAMTSs, which remove the respective propeptides from procollagen, within the secretory pathway in tendon fibroblasts [64]. Furthermore, tendon fibrillogenesis is modulated through the interaction of the fibril surface with decorin (and other closely related leucine-rich proteoglycans, such as biglycans) as well as with fibril-associated collagens (*e.g.*, type XII and type XIV collagens) [65-67]. These molecules indeed are good substrates for MMPs, in particular for MMP-2. In this regard, a marked enhancement of MMP-2 activity can be observed during this stage of the

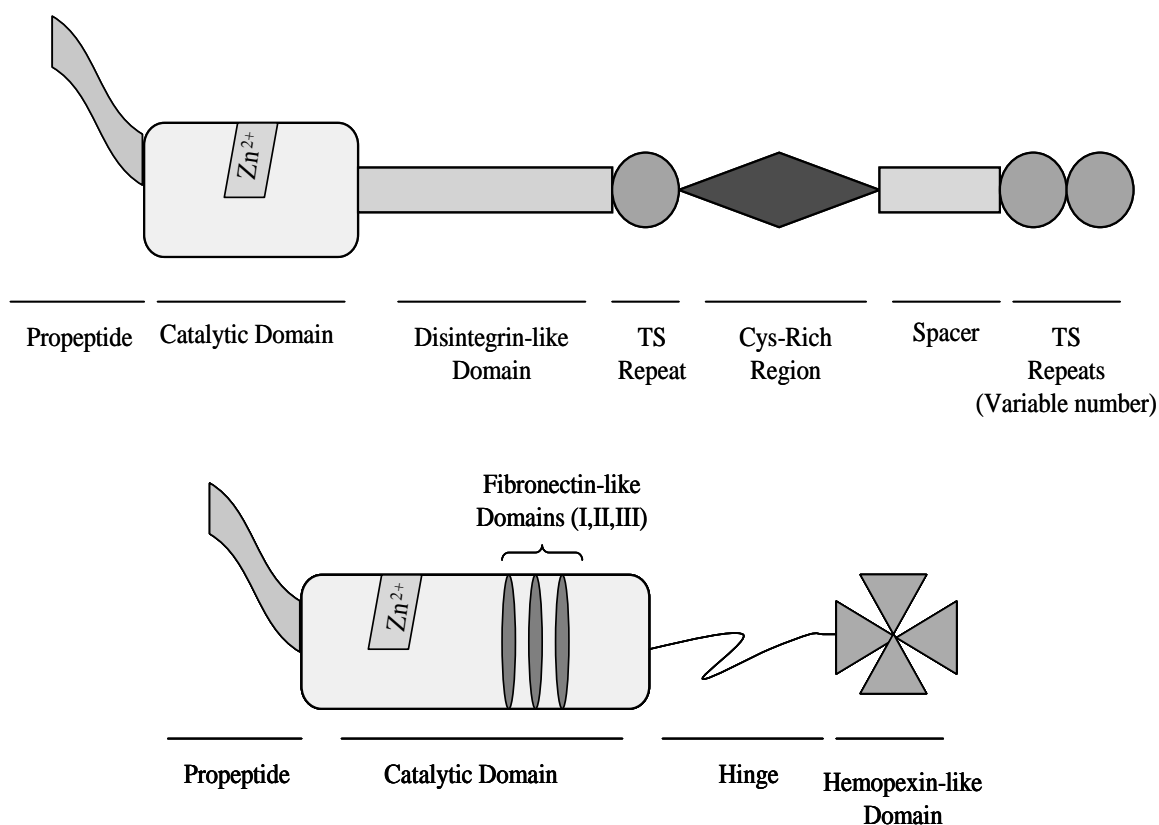


Fig. (4). Comparison between domain organization of ADAMTS (upper panel) and Gelatinases (*i.e.*, MMP-2 and MMP-9) (lower panel).

development pathway, mostly associated to an increased extent of pro-MMP-2 activation induced by an overexpression of MMP-16, which is known to be a gelatinase activator [29, 68]. In addition, during the development of the musculoskeletal system, a considerable enhancement can also be observed for collagenases, interstitial collagenase in particular (*i.e.*, MMP-1), being mostly evident in the metaphyses and diaphyses of long bones [69]. This effect, which is mainly detected in hypertrophic chondrocytes and osteoblastic cells localized along the newly formed bone trabeculae, may turn out to be very important, since MMP-1 is very efficient in the enzymatic processing of native collagen I, II, III and X, which are also components of the tendon fibers. In this regard, it is important to note how the concerted action of MMP-2 and MMP-1 could bear a critical relevance in tendon fiber remodeling. Therefore, we know that the proteolytic activity of MMP-2 occurs through faster kinetics whenever the triple-helix arrangement of collagen I is preliminary unrolled by MMP-1 activity [45, 70-72].

In addition, tendon function crucially depends on aggrecan localization, since the accumulation of pericellular and interfibrillar aggrecan impairs tendon function. Thus, aggrecan removal in the pericellular matrix by tendon fibroblasts is a strictly regulated process which is probably carried out by ADAMTS4, ADAMTS5 and ADAMTS15. Notably, mice which are knockout for ADAMTS5 display severe impairment of tendon functions, with larger cross-sectional area and increased collagen fibril area fraction in the Achilles tendon, which leads to a higher tensile modulus and a weakened enthesis of the tendon [73]. Although ADAMTS-4 and ADAMTS-5 display similarities in substrate selectivity, the latter is considered to bear the main aggrecan-degrading activity under physiological condition [74], while ADAMTS-4 is a suitable candidate to exert a pivotal role in aggrecan turnover during pathological conditions, like as osteoarthritis, since its expression and secretion by synoviocytes is triggered by pro-inflammatory stimuli, mostly by Il-1 β [75]. Finally, the ADAMTS15 abundant expression in ligaments and tendons of the developing knee joint and on the articular surface was recently documented and a contribution to aggrecan cleavage *in vitro* assessed [76, 77].

Conversely, aggrecan is considered to be resistant to MMPs cleavage. Furthermore, it is interesting to outline that the ECM turnover of the tendon is influenced by physical activity, since it has been shown that the level of collagen synthesis and of MMPs increase with mechanical loading [78-80]. In particular, strain brings about fibroblast responses, such as changes in differentiation and migration patterns [79], which are then accompanied by spontaneous self-assembly of collagen into highly-organized arrays and preferential incorporation of collagen into loaded fibrils [81]. Physical activity is also linked to an increased activity of MMP-2 and MMP-9 in the calcaneal tendon, thus inducing a sort of "vicious cycle" [82], wherefore mechanical overstimulation of the tendon affects the enzymatic degradation and repair phenomena, which leads to chronic tendinopathy (see below). In particular, after repeated stressing physical activity an increased thickness, cellularity and blood vessel volume fraction of the peritendinous sheath has been

observed, without any inflammatory process. This tissue modification is accompanied by a marked enhancement of the active MMP-2, clearly indicating an increase in pro-MMP-2 activation [83]. The net result is a strain-controlled remodeling of collagen fibrils, which also becomes more resistant to enzymatic degradation by collagenases compared to unstrained fibrils [84]. This enhanced resistance also casts some light on the general aspects of matrix phenomena, such as development and adaptation, and tissue engineering.

ROLE OF MMPs AND ADAMTSs IN TENDON PATHOLOGICAL PROCESSES

Tendons are frequently affected by chronic pain and rupture. Many causative factors have been implicated in the pathology, which until recently was under-researched and poorly understood. There is now greater knowledge regarding the molecular basis of tendon disease.

The most prevalent tendon pathologies, such as tendinopathy, are associated with degeneration. This is considered to be an active, cell-mediated process, even though it does not usually develop as a typical flogistic process and recruitment of immune cells plays not a major role. Effectively, it has been proven that prolonged mechanical stimuli induce the production of cytokines and inflammatory prostaglandins, which may be mediators of early phases of tendinopathy [85, 86] MMPs activation by prostaglandins and inflammatory cytokines, in turn, increases degradation and remodeling of the extracellular matrix network.

Notably, activation of MMPs is expected to trigger a sort of vicious cycle wherefore further matrix degrading enzyme, in particular ADAMTS-4 and ADAMTS-5, are released in the injured microenvironment following MMPs activation. For instance, MMPs mediate the release of soluble TGF- β (trapped in ECM) which, in turn, is a main promoter of ADAMTS-4 and ADAMTS-5 expression [87].

Application of cyclic strain increases production of prostaglandin E2 (PGE2) in human patellar tenocytes [85], and it increases interleukin-6 (IL-6) secretion [86] and IL-1 β gene expression in human flexor tenocytes [88]. The recruitment of immune cells is generally replaced by a dysregulation of tenocyte homeostasis accompanied by secretion of ECM components and enzymes, which results in an increased turnover and remodeling of the tendon extracellular matrix [89].

In particular, chronic tendinopathies appear to be characterized by an increased collagen turnover with a significant decrease of collagen content, type I collagen in particular [90], and an enhanced secretion of large proteoglycans. The alteration of tendon micro-architecture seems to be related instead to an altered metabolism of tenocytes and inappropriate retention of these macromolecules [15]. One of the outdated hypothesis that is currently being reconsidered suggests that non-uniform stress within tendons, due to intrinsic factors such as misalignments and biomechanical faults, may have a causative role in tendon disorders by producing abnormal load concentrations and frictional forces between fibrils thus causing localized fiber damage [91]. Under maximal tensile load, in particular in

misalignments, tendons may undergo ischemia. During relaxation, when reperfusion occurs, oxygen free radicals can be produced [92, 93] which, in turn, can damage both cells and tissues. Peroxiredoxin 5 is an anti-oxidant enzyme that protects cells against damage from such reactive oxygen species found also in human tenocytes. Its expression is increased in tendinopathies, a finding that supports the view that oxidative stress may play a role [94]. Hypoxia alone may also result in degeneration, given that tendons rely on oxidative energy metabolism to maintain cellular homeostasis. Under these stress conditions, an increased production of prostaglandins has also been observed [95], mostly of PGE₂, which induces stem cells to differentiate in non-tenocytes which, associated with the turnover of physiological tenocytes, reduces the pool of tenocytes able to repair tendons injured by mechanical loading.

Surprisingly, no significant increase in the overall content of MMPs and ADAMTSs [96] is generally observed in the micro-environment of the chronically damaged tendon: MMP-2, MMP-9 and TIMP-1 are the only molecules for which, to date, in most cases, a consistent up-regulation has been reported [15, 97]. However, it must be considered that the hall-mark of this pathological process might be the excessive activation of pro-MMPs instead of a gene up-regulation, a fact that has been demonstrated in other inflammatory processes [35].

In the supraspinatus tendons, after tendon rupture, it has been shown that there is an increased activity of MMP-1 and a reduced activity of MMP-2 and MMP-3, [98]. A decreased activity of the gelatinase MMP-2 and of the stromelysin MMP-3 associated with a larger activity of the collagenase MMP-1 do, in fact, lead to an enhanced collagen turnover and further deterioration in the quality of the collagen network. Down-regulation of MMP-3 mRNA has also been reported in Achilles tendinopathy [97, 99]. This evidence may be correlated with a reduced repair capability, often associated with a decrease of MMP-3 activity, which is able to maintain the tendon matrix in response to the mechanical strain and repeated micro-trauma [100]. Therefore, it appears quite evident that the role of MMPs in these chronic pathologies of the tendon is not univocal, since some MMPs, such as MMP-2 and MMP-1, are involved in collagen catabolism, thus facilitating tendon degradation and eventually its rupture, whereas other ones, such as MMP-3, may be involved in repair mechanisms ranging from micro-traumas to the maintenance of the normal tendon function. This different role appears to be associated with tendon retraction following a tear in the rotator cuff, since an elevated expression and enzymatic activity can be detected for MMP-1 and MMP-9. This evidence appears to be associated to the extent of the tendon retraction, being accompanied by a marked decrease of MMP-3 activity compared to healthy tendons [101]. Therefore, a tendinopathy seems to be characterized by an imbalance between collagen fibril cleavages by some MMPs, such as collagenases and gelatinases, and a "failed healing response", due to reduced stromelysin activity. This imbalance brings about a proliferation of tenocytes with intracellular abnormalities and enhancement of collagen disruption, accompanied by the subsequent increase of non collagenous matrix [102]. In this regard, it is

interesting to observe that tendon cells secrete a fair amount of all collagenases, while during rest, this amount is significantly reduced [103], which improves tendon functionality. Under the same conditions, no reduction is observed for stromelysin MMP-3 secretion, suggesting that this molecule may either do nothing or even facilitate the recovery of the damaged tendon and stressed ligament. However, collagen metabolism alteration during tendinopathy is usually also associated with an increased synthesis of large proteoglycans, such as versican and aggrecan, as well as an enhancement of their disruption and loss (likely related to the up-regulation of ADAMTS4, which is able to enzymatically process large proteoglycans), whereas small leucine-rich proteoglycans, such as decorin, seem to remain unaltered [15].

In addition, tendon integrity depends crucially on the extracellular matrix metabolism, and thus on the regulation of proteolytic activity. A differential temporal sequence of MMP expression may occur, and MMP or other matrix-molecule expression may differ between tendinopathic and ruptured tendons. As an example, the rupture of the Achilles tendon has been found to be closely related to a marked increase in the gene expression of proteoglycan-core proteins, such as decorin and versican, as well as to an enhanced enzymatic activity of MMP-2 and MMP-9 [104]. This evidence is accompanied by a marked decrease in carbohydrate content in the rupture area, where little evidence exists for the presence of glycosaminoglycans in the tendon. As a whole, it suggests there is a gross rearrangement of the tendon connective tissue, which is likely to be connected to the gelatinase activity in the rupture area. Finally, activities of MMP-2 and MMP-9 in tenocytes increase with age. This might further provide a mechanistic explanation of how MMPs and aging contributes to tendinopathy or tendon rupture [105].

Finally, up-regulated activity of MMPs might play a critical contribute also in fibrotic degeneration of tendons in advanced phases of the pathological condition. The increased bioavailability of TGF- β upon MMPs up-regulation might lead to an increased fibroblasts proliferation and collagen I deposition [106]. In addition, the previously discussed contribution of TGF- β to ADAMTS-4 and ADAMTS-5 expression might represent a further critical issue in fibrotic degeneration; thus, through their catalytic activities, these ADAMTS reduce the overall content of bylgican and decorin, which are thought to play an important role in TGF- β balance (with particular reference to decorin), by binding and sequestering it, as it has been demonstrated in other human tissues [107].

A THERAPEUTICAL APPROACH TO TENDON PATHOLOGY: FINAL CONSIDERATIONS

MMPs levels are finely-tuned during tendon healing [99, 108]. In a rat flexor tendon rupture model, the expression of MMP-9 and MMP-13 peaked between the second week after the surgery while in another experimental model [109], MMP-2, MMP-3, and MMP-14 levels increased after surgery and remained high until the fourth week. These findings suggest that MMP-9 and MMP-13 may participate in collagen degradation only, whereas MMP-2, MMP-3 (as

previously suggested), and MMP-14 participate both in collagen degradation and in collagen remodeling.

On the basis of the above-mentioned observations on the role of Metalloproteinases in tendon pathology, it is evident that the local use of metalloproteinase inhibitors may be helpful in facilitating the healing repair phenomena although it must be said that a broad and not-specific inhibition may not necessarily lead to significant healing. In this respect, even though the differences between the catalytic site of the MMPs and ADAMTS (Fig. 3) might allow to design specific synthetic and/or peptidomimetic inhibitors, the main reason of the unsuccessful approach of specific metalloenzymes inhibition in human diseases likely relies in the complexity of their biological behaviors. Thus, it has been proven that specifically inhibited MMPs, through their extra-catalytic domains, still exert some effects on their substrate (like as triple helix unwinding in collagen IV), thus facilitating their cleavage by other enzymes [29].

Some relevant beneficial effect has been recently observed for the tendon-to-bone healing in animal models of rotator cuff repair. In particular, doxycycline, which has been found to inhibit collagenases and gelatinases very efficiently [110], may improve collagen organization [111, 112]. Reducing the inflammatory burst by using antisteroidal or anti-inflammatory drugs could be helpful in improving healing. In fact, nonsteroidal anti-inflammatory drugs are often widely used to treat sports-related tendon injuries or tendinopathies. However, even in these cases, more information to complete the picture is necessary. In fact, a recent study has shown that ibuprofen greatly upregulates the expression of both collagenases (*i.e.*, MMP-1, MMP-8 and MMP-13) and gelatinase (*i.e.*, MMP-9) expression by tendon cells, with a consequent enhancement of collagen disruption. This is not accompanied by an increase in collagen synthesis, thus bringing about a severe impairment of the healing repair mechanism [113]. An additional danger concerns the possibility that in some cases exceedingly prolonged or frequent use of antibiotics, such as Ciprofloxacin, might induce the insurgence of tendinopathy. This could be associated to the Ciprofloxacin- induced up-regulation of MMP-2 expression in tendon cells, with a consequent massive degradation of type I collagen [114], because it has been demonstrated that this MMP is able to digest collagen I fibers [45].

In conclusion, it is evident that MMPs are deeply involved in tendon pathologies even though, to date, the use of their inhibitors in therapy remains in their early stages.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The authors want to express their gratitude to Profs. Maria Grazia Ascenzi and G. Opdenakker for very fruitful discussions. The financial support from the Italian Ministry of University and Research (MiUR PRIN 200993WWF9_003 to M.C.) and the Italian Space Agency

(ASI SMEMCO n. DC-DTE-2011-033 to U.T.) is gratefully acknowledged.

REFERENCES

- [1] Xu, Y.; Murrell, G. The basic science of tendinopathy. *Clin. Orthop. Relat. Res.*, **1977**, *466*, 1528-1538.
- [2] Tresoldi, I.; Oliva, F.; Benvenuto, M.; Fantini, M.; Masuelli, L.; Bei, R.; Modesti, A. Tendon's ultrastructure. *Muscles Ligaments Tendons J.*, **2013**, *3*, 2-6.
- [3] Rich, A.; Crick, F.H. The molecular structure of collagen. *J. Mol. Biol.*, **1961**, *3*, 483-506.
- [4] Okuyama, K.; Takayanagi, M.; Ashida, T.; Kakudo, M. A new structural model for collagen. *Polymer J.*, **1971**, *9*, 341-343.
- [5] Kramer, R.Z.; Bella, J.; Brodsky, B.; Berman, H.M. The crystal and molecular structure of a collagen-like peptide with a biologically relevant sequence. *J. Mol. Biol.*, **2001**, *311*, 131-147.
- [6] Hulmes, D.J.; Miller, A. Quasi-hexagonal molecular packing in collagen fibrils. *Nature*, **1979**, *282*, 878-880.
- [7] Orgel, J.P.; Irving, T.C.; Miller, A.; Wess, T.J. Microfibrillar structure of type I collagen *in situ*. *Proc. Natl. Acad. Sci. USA.*, **2006**, *103*, 9001-9005.
- [8] Kastelic, J.; Galeski, A.; Baer, E. The multicomposite structure of tendon. *Connect. Tissue Res.*, **1978**, *6*, 11-23.
- [9] Ippolito, E.; Natali, P.G.; Postacchini, F.; Accinni, L.; De Martino, C. Ultrastructural and immunohistochemical evidence of actin in the tendon cells. *Clin. Orthop.*, **1977**, *126*, 282-284.
- [10] Samiric, T.; Ilic, M.Z.; Handley, C.J. Characterization of proteoglycans and their catabolic products in tendon and explants cultures of tendon. *Matrix Biol.*, **2004**, *23*, 127-140.
- [11] Thorpe, C.T.; Birch, H.L.; Clegg, P.D.; Screen, H.R. The role of the non-collagenous matrix in tendon function. *Int. J. Exp. Pathol.*, **2013**, *94*, 248-259.
- [12] Sharma, P.; Maffulli, N. Biology of tendon injury: healing, modeling and remodeling. *J. Musculoskelet. Neuronal Interact.*, **2006**, *6*, 181-190.
- [13] Ippolito, E.; Natali, P.G.; Postacchini, F.; Accinni, L.; De Martino, C. Morphological, immunochemical and biochemical study of rabbit Achilles tendon at various ages. *J. Bone Joint Surg.*, **1980**, *62*, 583-598.
- [14] Klatte-Schulz, F.; Pauly, S.; Scheibel, M.; Greiner, S.; Gerhardt, C.; Schmidmaier, G.; Wildemann, B. Influence of age on the cell biological characteristics and the stimulation potential of male human tenocyte-like cells. *Eur. Cell Mater.*, **2012**, *24*, 74-89.
- [15] Parkinson, J.; Samiric, T.; Ilic, M.Z.; Cook, J.; Feller, J.A.; Handley, C.J. Change in proteoglycan metabolism is a characteristic of human patellar tendinopathy. *Arthr. & Rheum.*, **2010**, *62*, 3028-3035.
- [16] Dunkman, A.A.; Buckley, M.R.; Mienaltowski, M.J.; Adams, S.M.; Thomas, S.J.; Satchell, L.; Kumar, A.; Pathmanathan, L.; Beason, D.P.; Iozzo, R.V.; Birk, D.E.; Soslowsky, L.J. Decorin expression is important for age-related changes in tendon structure and mechanical properties. *Matrix Biol.*, **2013**, *32*, 3-13.
- [17] Dunkman, A.A.; Buckley, M.R.; Mienaltowski, M.J.; Adams, S.M.; Thomas, S.J.; Satchell, L.; Kumar, A.; Pathmanathan, L.; Beason, D.P.; Iozzo, R.V.; Birk, D.E.; Soslowsky, L.J. The tendon injury response is influenced by Decorin and Biglycan. *Ann. Biomed. Eng.*, **2014**, *42*, 619-630.
- [18] Vogel, K.G.; Meyers, A.B. Proteins in the tensile region of adult bovine deep flexor tendon. *Clin. Orthop.*, **1999**, *367*, S344-S355.
- [19] Rees, S.G.; Flannery, C.R.; Little, C.B.; Hughes, C.E.; Caterson, B.; Dent, C.M. Catabolism of aggrecan, decorin, and biglycan in tendon. *Biochem. J.*, **2000**, *350*, 181-188.
- [20] Carrino, D.A.; Onnerfjord, P.; Sandy, J.D.; Cs-Szabo, G.; Scott, P.G.; Sorrell, J.M.; Heinegård, D.; Caplan, A.I. Age-related changes in the proteoglycans of the human skin. Specific cleavage of decorin to yield a major catabolic fragment in adult skin. *J. Biol. Chem.*, **2003**, *278*, 17566-17572.
- [21] Neill, T.; Schaefer, L.; Iozzo, R.V. Decorin: a guardian from the matrix. *Am. J. Pathol.*, **2012**, *181*, 380-387.
- [22] Nastase, M.V.; Young, M.F.; Schaefer, L. Biglycan: a multivalent proteoglycan providing structure and signals. *J. Histochem. Cytochem.*, **2012**, *60*, 963-975.

- [23] Gonçalves, A.I.; Rodrigues, M.T.; Lee, S.J.; Atala, A.; Yoo, J.J.; Reis, R.L.; Gomes, M.E. Understanding the role of growth factors in modulating stem cell tenogenesis. *PLoS One*, **2013**, *8*, e83734.
- [24] Wight, T.N. Versican: a versatile extracellular matrix proteoglycan in cell biology. *Curr. Opin. Cell Biol.*, **2002**, *14*, 617-623.
- [25] Yoon, J.H.; Halper, J. Tendon proteoglycans: biochemistry and function. *J. Musculoskelet. Neuronal Interact.*, **2005**, *5*, 22-34.
- [26] Wu, Y.J.; La Pierre, D.P.; Wu, J.; Yee, A.J.; Yang, B.B. The interaction of versican with its binding partners. *Cell Res.*, **2005**, *15*, 483-494.
- [27] Marturano, J.E.; Xylas, J.F.; Sridharan, G.V.; Georgakoudi, I.; Kuo, C.K. Lysyl oxidase-mediated collagen crosslinks may be assessed as markers of functional properties of tendon tissue formation. *Acta Biomater.*, **2014**, *10*, 1370-1379.
- [28] Rees, J.D.; Stride, M.; Scott, A. Tendons – time to revisit inflammation. *Br. J. Sports Med.*, **2014**, *48*, 1553-1557.
- [29] Sbardella, D.; Fasciglione, G.F.; Gioia, M.; Ciaccio, C.; Tundo, G.R.; Marini, S.; Coletta, M. Human matrix metalloproteinases: an ubiquitous class of enzymes involved in several pathological processes. *Mol. Aspects Med.*, **2012**, *33*, 119-208.
- [30] Matrisian, L.M.; Bowden, G.T.; Krieg, P.; Furstenberger, G.; Briand, J.P.; Leroy, P.; Breathnach R. The mRNA coding for the secreted protease transin is expressed more abundantly in malignant than in benign tumors. *Proc. Natl. Acad. Sci. USA.*, **1986**, *83*, 9413-9417.
- [31] Hirose, T.; Riefe, R.A.; Smith, G.N.jr; Stevensm, R.M.M.; Mainardim, C.L.; Hasty, K.A. Characterization of type V collagenase (gelatinase) in synovial fluid of patients with inflammatory arthritis. *J. Rheumatol.*, **1992**, *19*, 593-599.
- [32] Wysocki, A.B.; Staiano-Coico, L.; Grinnell, F. Wound fluid from chronic leg ulcers contains elevated levels of metalloproteinases MMP-2 and MMP-9. *J. Invest. Dermatol.*, **1993**, *101*, 64-68.
- [33] Bafetti, L.M.; Young, T.N.; Itoh, Y.; Stack, M.S. Intact vitronectin induces matrix metalloproteinase-2 and tissue inhibitor of metalloproteinases-2 expression and enhanced cellular invasion by melanoma cells. *J. Biol. Chem.*, **1998**, *273*, 143-149.
- [34] Vu, T.H.; Shipley, J.M.; Bergers, G.; Berger, J.E.; Helms, J.A.; Hanahan, D.; Shapiro, S.D.; Senior, R.M.; Werb, Z. MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell*, **1998**, *93*, 411-422.
- [35] Maiotti, M.; Monteleone, G.; Tarantino, U.; Fasciglione, G.F.; Marini, S.; Coletta, M. Correlation between osteoarthritic cartilage damage and levels of proteinases and proteinase inhibitors in synovial fluid from the knee joint. *Arthroscopy*, **2000**, *16*, 522-526.
- [36] Marini, S.; Fasciglione, G.F.; Monteleone, G.; Maiotti, M.; Tarantino, U.; Coletta, M. A correlation between knee cartilage degradation observed by arthroscopy and synovial proteinases activities. *Clin. Biochem.*, **2003**, *36*, 295-304.
- [37] Opendakker, G.; Nelissen, I.; van Damme, J. Functional roles and therapeutic targeting of gelatinase B and chemokines in multiple sclerosis. *Lancet Neurol.*, **2003**, *2*, 747-756.
- [38] Del Buono, A.; Oliva, F.; Osti, L.; Maffulli, N. Metalloproteases and tendinopathy. *Muscles Ligaments Tendons J.*, **2013**, *3*, 51-57.
- [39] Nagase, H.; Woessner, J.F. Matrix metalloproteinases. *J. Biol. Chem.*, **1999**, *274*, 21491-21494.
- [40] Sternlicht, M.D.; Werb, Z. How matrix metalloproteinases regulate cell behavior. *Annu. Rev. Cell Dev. Biol.*, **2001**, *17*, 463-516.
- [41] Aureli, L.; Gioia, M.; Cerbara, I.; Monaco, S.; Fasciglione, G.F.; Marini, S.; Ascenzi, P.; Topai, A.; Coletta, M. Structural bases for substrate and inhibitor recognition by matrix metalloproteinases. *Curr. Med. Chem.*, **2008**, *15*, 2192-2222.
- [42] Murphy, G.; Allan, J.A.; Willenbrock, F.; Cockett, M.I.; O'Connell, J.P.; Docherty, A.J.P. The role of the C-terminal domain in collagenase and stromelysin specificity. *J. Biol. Chem.*, **1992**, *267*, 9612-9618.
- [43] Hirose, T.; Patterson, C.; Pourmotabbed, T.; Mainardi, C.L.; Hasty, K.A. Structure-function relationship of human neutrophil collagenase: identification of regions responsible for substrate specificity and general proteinase activity. *Proc. Natl. Acad. Sci. USA.*, **1993**, *90*, 2569-2573.
- [44] Gioia, M.; Fasciglione, G.F.; Marini, S.; D'Alessio, S.; De Sanctis, G.; Diekmann, O.; Pieper, M.; Politi, V.; Tschesche, H.; Coletta M. Modulation of the catalytic activity of neutrophil collagenase MMP-8 on bovine collagen I. Role of the activation cleavage and of the hemopexin-like domain. *J. Biol. Chem.*, **2002**, *277*, 23123-23130.
- [45] Gioia, M.; Monaco, S.; Fasciglione, G.F.; Coletti, A.; Modesti, A.; Marini, S.; Coletta, M. Characterization of the mechanisms by which gelatinase A, neutrophil collagenase, and membrane-type metalloproteinase MMP-14 recognize collagen I and enzymatically process the two alpha-chains. *J. Mol. Biol.*, **2007**, *368*, 1101-1113.
- [46] Steffensen, B.; Wallon, U.M.; Overall, C.M. Extracellular matrix binding properties of recombinant fibronectin type II-like modules of human 72-kDa gelatinase/type IV collagenase. High affinity binding to native type I collagen but not native type IV collagen. *J. Biol. Chem.*, **1995**, *270*, 11555-11566.
- [47] Wilson, C.L.; Matrisian, L.M. Matrilysin: an epithelial matrix metalloproteinase with potentially novel functions. *Int. J. Biochem. Cell Biol.*, **1996**, *28*, 123-136.
- [48] Ohuchi, I.; Imai, K.; Fujii, Y.; Sato, H.; Seiki, M.; Okada, Y. Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. *J. Biol. Chem.*, **1999**, *272*, 2446-2451.
- [49] Hotary, K.; Allen, E.; Punturieri, A.; Yana, I.; Weiss, S.J. Regulation of cell invasion and morphogenesis in a three-dimensional type I collagen matrix by membrane-type matrix metalloproteinases 1, 2, and 3. *J. Cell Biol.*, **2000**, *149*, 1309-1323.
- [50] Brew, K.; Nagase H. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. *Biochim. Biophys. Acta.*, **2010**, *1803*, 55-71.
- [51] Apte, S.S. A disintegrin-like and metalloprotease (reprolysin-type) with thrombospondin type 1 motif (ADAMTS) superfamily: functions and mechanisms. *J. Biol. Chem.*, **2009**, *84*, 31493-31497.
- [52] Turner, S.L.; Blair-Zajdel, M.E.; Bunning, R.A. ADAMs and ADAMTSs in cancer. *Br. J. Biomed. Sci.*, **2009**, *66*, 117-128.
- [53] Lin, E.A.; Liu, C.J. The role of ADAMTS in arthritis. *Protein Cell*, **2010**, *1*, 33-47.
- [54] Salter, R.C.; Ashlin, T.G.; Kwan, A.P.; Ramji, D.P. ADAMTS proteases: key roles in atherosclerosis? *J. Mol. Med.*, **2010**, *88*, 1203-1211.
- [55] McCulloch, D.R.; Le Goff, C.; Bhatt, S.; Dixon, I.J.; Sandy, J.D.; Apte, S.S. Adamts5, the gene encoding a proteoglycan-degrading metalloprotease, is expressed by specific cell lineages during mouse embryonic development and in adult tissues. *Gene Expr. Patterns.*, **2009**, *9*, 314-323.
- [56] Kuno, K.; Matsushima, K. ADAMTS-1 protein anchors at the extracellular matrix through the thrombospondin type I motifs and its spacing region. *J. Biol. Chem.*, **1998**, *273*, 13912-13917.
- [57] Tortorella, M.; Pratta, M.; Liu, R.Q.; Abbaszade, I.; Ross, H.; Burn, T.; Arner, E. The thrombospondin motif of aggrecanase-1 (ADAMTS-4) is critical for aggrecan substrate recognition and cleavage. *J. Biol. Chem.*, **2000**, *275*, 25791-25797.
- [58] Colige, A.; Ruggiero, F.; Vandenberghe, I.; Dubail, J.; Kesteloot, F.; Van Beeumen, J.; Beschin, A.; Brys, L.; Lapière, C.M.; Nusgens, B. Domains and maturation processes that regulate the activity of ADAMTS-2, a metalloproteinase cleaving the aminopropeptide of fibrillar procollagens types I-III and V. *J. Biol. Chem.*, **2005**, *280*, 34397-34408.
- [59] Cetta, G.; Tenni, R.; Zanaboni, G.; De Luca, G.; Ippolito, E.; De Martino, C.; Castellani, A.A. Biochemical and morphological modifications in rabbit Achilles tendon during maturation and ageing. *Biochem. J.*, **1982**, *204*, 61-67.
- [60] Birk, D.E.; Trelstad, R.L. Extracellular compartments in tendon morphogenesis: collagen fibril, bundle, and macroaggregate formation. *J. Cell Biol.*, **1986**, *103*, 231-240.
- [61] Birk, D.E.; Zycband, E.I.; Winkelmann, D.A.; Trelstad, R.L. Collagen fibrillogenesis in situ: fibril segments are intermediate in matrix assembly. *Proc. Natl. Acad. Sci. USA.*, **1989**, *86*, 4549-4553.
- [62] Kadler, K.E.; Holmes, D.F.; Trotter, J.A.; Chapman, J.A. Collagen fibril formation. *Biochem. J.*, **1996**, *316*, 1-11.
- [63] Graham, H.K.; Holmes, D.F.; Watson, R.B.; Kadler, K.E. Identification of collagen fibril fusion during vertebrate tendon morphogenesis. The process relies on unipolar fibrils and is regulated by collagen-proteoglycan interaction. *J. Mol. Biol.*, **2000**, *295*, 891-902.
- [64] Canty-Laird, E.G.; Lu, Y.; Kadler, K.E. Stepwise proteolytic activation of type I procollagen within the secretory pathway of tendon fibroblasts in situ. *Biochem. J.*, **2012**, *441*, 707-717.

- [65] Birk, D.E.; Mayne, R. Localization of collagens type I, III and V during tendon development. Changes in collagen type I and III are correlated with changes in fibril diameter. *Eur. J. Cell. Biol.*, **1997**, *72*, 352-361.
- [66] Iozzo, R.V. The biology of the small leucine-rich proteoglycans. Functional network of interactive proteins. *J. Biol. Chem.*, **1999**, *274*, 18843-18846.
- [67] Zhang, G.; Young, B.B.; Ezura, Y.; Favata, M.; Soslowsky, L.J.; Chakravarti, S.; Birk, D.E. Development of tendon structure and function: regulation of collagen fibrillogenesis. *J. Musculoskel. Neuronal Interact.*, **2005**, *5*, 5-21.
- [68] Jung, J.C.; Wang, P.X.; Zhang, G.; Ezura, Y.; Fini, M.E.; Birk, D.E. Collagen fibril growth during chicken tendon development: Matrix metalloproteinase-2 and its activation. *Cell Tissue Res.*, **2009**, *336*, 79-89.
- [69] Gack, S.; Vallon R, Schmidt J, Grigoriadis A, Tuckermann J, Schenkel J, Weiher H, Wagner EF, Angel P. Expression of interstitial collagenase during skeletal development of the mouse is restricted to osteoblast-like cells and hypertrophic chondrocytes. *Cell Growth Differ.*, **1995**, *6*, 759-767.
- [70] Perumal, S.; Antipova, O.; Orgel, J.P.R.O. Collagen fibril architecture, domain organization and triple-helical conformation govern its proteolysis. *Proc. Natl. Acad. Sci. USA.*, **2008**, *105*, 2824-2829.
- [71] Van den Steen, P.E.; Proost, P.; Brand, D.D.; Kang, A.H.; Van Damme, J.; Opdenakker, G. Generation of glycosylated remnant epitopes from human collagen type II by gelatinase B. *Biochemistry*, **2004**, *43*, 10809-10816.
- [72] Rosenblum, G.; Van den Steen, P.E.; Cohen, S.R.; Grossmann, J.G.; Frenkel, J.; Sertchook, R.; Slack, N.; Strange, R.W.; Opdenakker, G.; Sagi, I. Insights into the structure and domain flexibility of full-length pro-matrix metalloproteinase-9/gelatinase B. *Structure*, **2007**, *15*, 1227-1236.
- [73] Wang, V.M.; Bell RM, Thakore R, Eyre DR, Galante JO, Li J, Sandy JD, Plaas A. Murine tendon function is adversely affected by aggrecan accumulation due to the knockout of ADAMTS5. *J. Orthop. Res.*, **2012**, *30*, 620-626.
- [74] Tsuzaki, M.; Guyton, G.; Garrett, W.; Archambault, J.M.; Herzog, W.; Almekinders, L.; Bynum, D.; Yang, X.; Banes, A.J. IL-1 beta induces COX2, MMP-1, -3 and -13, ADAMTS-4, IL-1 beta and IL-6 in human tendon cells. *J. Biol. Chem.*, **2013**, *288*, 37267-37276.
- [75] Gendron, C.; Kashiwagi, M.; Lim, N.H.; Enghild, J.J.; Thøgersen, I.B.; Hughes, C.; Caterson, B.; Nagase, H. Proteolytic activities of human ADAMTS-5: comparative studies with ADAMTS-4. *J. Orthop. Res.*, **2003**, *21*, 256-264.
- [76] Dancevic, C.M.; Fraser, F.W.; Smith, A.D.; Stupka, N.; Ward, A.C.; McCulloch, D.R. Biosynthesis and expression of a disintegrin-like and metalloproteinase domain with thrombospondin-1 repeats-15: a novel versican-cleaving proteoglycanase. *J. Biol. Chem.*, **2007**, *282*, 18294-18306.
- [77] Kelwick, R.; Wagstaff, L.; Decock, J.; Roghi, C.; Cooley, L.S.; Robinson, S.D.; Arnold, H.; Gavrilović, J.; Jaworski, D.M.; Yamamoto, K.; Nagase, H.; Seubert, B.; Krüger, A.; Edwards, D.R. Metalloproteinase-dependent and -independent processes contribute to inhibition of breast cancer cell migration, angiogenesis and liver metastasis by a disintegrin and metalloproteinase with thrombospondin motifs-15. *Int. J. Cancer.*, **2014**, in the press.
- [78] Blain, E.J.; Gilbert, S.J.; Wardale, R.J.; Capper, S.J.; Mason, D.J.; Duance, V.C. Up-regulation of matrix metalloproteinases expression and activation following cyclical compressive loading of articular cartilage *in vitro*. *Arch. Biochem. Biophys.*, **2001**, *396*, 49-55.
- [79] Altman GH, Horan RL, Martin I, Farhadi J, Stark PR, Volloch V, Richmond JC, Vuniak-Novakovic G, Kaplan DL. Cell differentiation by mechanical stress. *FASEB J.*, **2002**, *16*, 270-272.
- [80] Koskinen, S.O.; Heinemeier, K.M.; Olesen, J.L.; Langberg, H.; Kjaer, M. Physical exercise can influence local levels of matrix metalloproteinases and their inhibitors in tendon-related connective tissue. *J. Appl. Physiol.*, **2004**, *96*, 861-864.
- [81] Giraud-Guille, M.M.; Besseau, L.; Martin, R. Liquid crystalline assemblies of collagen in bone and *in vitro* systems. *J. Biomech.*, **2003**, *36*, 1571-1579.
- [82] Cousineau-Pelletier, P.; Langelier, E. Relative contributions of mechanical degradation, enzymatic degradation and repair of the extracellular matrix on the response of tendons when subjected to under- and over- mechanical stimulations *in vitro*. *J. Orthop. Res.*, **2010**, *28*, 204-210.
- [83] De Mello Malheiro, O.C.; Giacomini, C.T.; Justulin, L.A.jr; Delella, F.K.; Dal-Pai Silva, M.; Felisbino, S.L. Calcaneal tendon regions exhibit different MMP-2 activation after vertical jumping and treadmill running. *Anat. Rec. (Hoboken)*, **2009**, *292*, 1656-1662.
- [84] Flynn, B.P.; Bhole, A.P.; Saeidi, N.; Liles, M.; DiMarzio, C.A.; Ruberti, J.W. Mechanical strain stabilizes reconstituted collagen fibrils against enzymatic degradation by mammalian collagenase matrix metalloproteinase 8 (MMP-8). *PLoS One*, **2010**, *5*, e12337.
- [85] Wang, J.H.; Jia, F.; Yang, G.; Yang, S.; Campbell, B.H.; Stone, D.; Woo, S.L. Cyclic mechanical stretching of human tendon fibroblasts increases the production of prostaglandin E2 and levels of cyclooxygenase expression: a novel *in vitro* model study. *Connect Tissue Res.*, **2003**, *44*, 128-133.
- [86] Skutek, M.; van Griensven, M.; Zeichen, J.; Brauer, N.; Bosch, U. Cyclic mechanical stretching enhances secretion of Interleukin 6 in human tendon fibroblasts. *Knee Surg. Sports Traumatol. Arthrosc.*, **2001**, *9*, 322-329.
- [87] Yamanishi, Y.; Boyle, D.L.; Clark, M.; Maki, R.A.; Tortorella, M.D.; Arner EC, Firestein GS. Expression and regulation of aggrecanase in arthritis: the role of TGF-beta. *J. Immunol.*, **2002**, *168*, 1405-1412.
- [88] Riley, G.P. Chronic tendon pathology: molecular basis and therapeutic implications. *Expert Rev. Mol. Med.*, **2005**, *24*, 1-25.
- [89] Riley, G.P.; Harrall, R.L.; Constant, C.R.; Chard, M.D.; Cawston, T.E.; Hazleman, B.L. Tendon degeneration and chronic shoulder pain: changes in the collagen composition of the human rotator cuff tendons in rotator cuff tendinitis. *Ann. Rheum. Dis.*, **1994**, *53*, 359-366.
- [90] Arndt, A.N.; Komi, P.V.; Bruggemann, G.P.; Lukkariemi, J. Individual muscle contributions to the *in vivo* achilles tendon force. *Clin. Biomech.*, **1998**, *13*, 532-541.
- [91] Goodship, A.E.; Birch, H.L.; Wilson, A.M. The pathobiology and repair of tendon and ligament injury. *Vet. Clin. North Am. Equine Pract.*, **1994**, *10*, 323-349.
- [92] Bestwick, C.S.; Maffulli, N. Reactive oxygen species and tendon problems: review and hypothesis. *Sports Med. Arthroscopy Rev.*, **2000**, *8*, 6-16.
- [93] Wang, M.X.; Wei, A.; Yuan, J.; Clippe, A.; Bernard, A.; Knoop, B.; Murrell, G.A. Antioxidant enzyme peroxiredoxin 5 is upregulated in degenerative human tendon. *Biochem. Biophys. Res. Commun.*, **2001**, *284*, 667-673.
- [94] Zhang, J.; Wang, J.H.C. Production of PGE₂ increases in tendons subjected to repetitive mechanical loading and induces differentiation in tendon stem cells into non-tenocytes. *J. Orthop. Res.*, **2010**, *28*, 198-203.
- [95] Bell, R.; Li, J.; Shewman, E.F.; Galante, J.O.; Cole, B.J.; Bach, B.R.Jr; Troy, K.L.; Mikecz, K.; Sandy, J.D.; Plaas, A.H.; Wang, V.M. ADAMTS5 is required for biomechanically-stimulated healing of murine tendinopathy. *J. Orthop. Res.*, **2013**, *10*, 1540-1548.
- [96] Alfredson, H.; Lorentzon, M.; Backman, S.; Backman, A.; Lerner, U.H. cDNA-arrays and real-time quantitative PCR techniques in the investigation of chronic Achilles tendinosis. *J. Orthop. Res.*, **2003**, *21*, 970-975.
- [97] Riley, G.P.; Curry, V.; DeGroot, J.; van El, B.; Verzijl, N.; Hazleman, B.L.; Bank, R.A. Matrix metalloproteinase activities and their relationship with collagen remodelling in tendon pathology. *Matrix Biol.*, **2002**, *21*, 185-195.
- [98] Tsuzaki, M.; Bynum, D.; Almekinders, L.; Yang, X.; Faber, J.; Banes, A.J. ATP modulates load-inducible IL-1beta, COX 2, and MMP-3 gene expression in human tendon cells. *J. Cell Biochem.*, **2003**, *89*, 556-562.
- [99] Ireland, D.; Harrall, R.; Curry, V.; Holloway, G.; Hackney, R.; Hazleman, B.; Riley, G. Multiple changes in gene expression in chronic human Achilles tendinopathy. *Matrix Biol.*, **2001**, *20*, 159-169.
- [100] Birch, H.L.; Worboys, S.; Eissa, S.; Jackson, B.; Strassburg, S.; Clegg, P.D. Matrix metabolism rate differs in functionally distinct tendons. *Matrix Biol.*, **2008**, *27*, 182-189.
- [101] Lakemeier, S.; Braun, J.; Efe, T.; Foelsch, C.; Archontidou-Aprin, E.; Fuchs-Winkelmann, S.; Paletta, J.R.; Schofer, M.D. Expression of matrix metalloproteinases 1, 3 and 9 in differing extents of

- tendon retraction in the torn rotator cuff. *Knee Surg. Sports Traumatol. Arthrosc.*, **2011**, *19*, 1760-1765.
- [102] Del Buono, A.; Oliva, F.; Longo, U.G.; Rodeo, S.A.; Orchard, J.; Denaro, V.; Maffulli, N. Metalloproteases and rotator cuff disease. *J. Shoulder Elbow Surg.*, **2012**, *21*, 200-208.
- [103] Harper, J.; Amiel, D.; Harper, E. Collagenases from periarticular ligaments and tendon: enzyme levels during the development of joint contracture. *Matrix*, **1989**, *9*, 200-205.
- [104] Karousou, E.; Ronga, M.; Vigetti, D.; Passi, A.; Maffulli, N. Collagens, proteoglycans, MMP-2, MMP-9 and TIMPs in human Achilles tendon rupture. *Clin. Orthop. Relat. Res.*, **2008**, *466*, 1577-1582.
- [105] Yu, T.Y.; Pang, J.H.; Wu, K.P.; Chen, M.J.; Chen, C.H.; Tsai, W.C. Aging is associated with increased activities of matrix metalloproteinase-2 and -9 in tenocytes. *BMC Musculoskelet. Disord.*, **2013**, *14*, 2.
- [106] Mendias, C.L.; Gumucio, J.P.; Davis, M.E.; Bromley, C.W.; Davis, C.S.; Brooks, S.V. Transforming growth factor-beta induces skeletal muscle atrophy and fibrosis through the induction of atrogen-1 and scleraxis. *Muscle Nerve*, **2012**, *45*(1), 55-59.
- [107] Kolb, M.; Margetts, P.J.; Sime, P.J.; Gauldie, J. Proteoglycans decorin and biglycan differentially modulate TGF-beta-mediated fibrotic responses in the lung. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **2001**, *280*(6), 1327-1334.
- [108] Vu, T.H.; Werb, Z. Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev.*, **2000**, *14*, 2123-2133.
- [109] Oshiro, W.; Lou, J.; Xing, X.; Tu, Y.; Manske, P.R. Flexor tendon healing in the rat: a histologic and gene expression study. *J. Hand Surg.*, **2003**, *28*, 814-823.
- [110] Paemen, L.; Martens, E.; Norga, K.; Masure, S.; Roets, E.; Hoogmartens, J.; Opdenakker, G. The gelatinase inhibitory activity of tetracyclines and chemically modified tetracycline analogues as measured by a novel microtiter assay for inhibitors. *Biochem. Pharmacol.*, **1996**, *52*, 105-111.
- [111] Bedi, A.; Fox, A.J.; Kovacevic, D.; Deng, X.H.; Warren, R.F.; Rodeo, S.A. Doxycycline-mediated inhibition of matrix metalloproteinases improves healing after rotator cuff repair. *Am. J. Sports Med.*, **2010**, *38*, 308-317.
- [112] Bedi, A.; Kovacevic, D.; Hettrich, C.; Gulotta, L.V.; Ehteshami, J.R.; Warren, R.F.; Rodeo, S.A. The effect of matrix metalloproteinase inhibition on tendon-to-bone healing in a rotator cuff repair model. *J. Shoulder Elbow Surg.*, **2010**, *19*, 384-391.
- [113] Tsai, W.C.; Hsu, C.C.; Chang, H.N.; Lin, Y.C.; Lin, M.S.; Pang, J.H. Ibuprofen upregulates expressions of matrix metalloproteinase-1, -8, -9, and -13 without affecting expressions of types I and III collagen in tendon cells. *J. Ortop. Res.*, **2010**, *28*, 487-491.
- [114] Tsai, W.C.; Hsu, C.C.; Chen, C.P.; Chang, H.N.; Wong, A.M.; Lin, M.S.; Pang, J.H. Ciprofloxacin up-regulates tendon cells to express matrix metalloproteinase-2 with degradation of type I collagen. *J. Ortop. Res.*, **2011**, *29*, 67-73.