

Metalloproteases and tendinopathy

Angelo Del Buono¹
 Francesco Oliva²
 Leonardo Osti³
 Nicola Maffulli^{4,5}

¹ Department of Orthopaedic and Trauma Surgery, Campus Biomedico University of Rome, Italy

² Department of Orthopaedic and Trauma Surgery, University of Rome Tor Vergata, Rome, Italy

³ Unit of Arthroscopy and Sports Trauma Surgery, Hesperia Hospital, Modena, Italy

⁴ Head of Department Centre for Sports and Exercise Medicine, Queen Mary University of London, Barts and The London School of Medicine and Dentistry, Mile End Hospital, London, U.K.

⁵ Head of Department of Physical and Rehabilitation Medicine, University of Salerno, Azienda Ospedaliera San Giovanni Di Dio e Ruggi d'Aragona

Corresponding author:

Nicola Maffulli

Head of Department Centre for Sports and Exercise Medicine, Queen Mary University of London, Barts and The London School of Medicine and Dentistry, Mile End Hospital, London, U.K.

Head of Department of Physical and Rehabilitation Medicine, University of Salerno, Azienda Ospedaliera San Giovanni Di Dio e Ruggi d'Aragona

Largo Città di Ippocrate

84131 Salerno, Italy

e-mail: n.maffulli@qmul.ac.uk

Summary

Matrix metalloproteinases (MMP) are involved in the development of tendinopathy. These potent enzymes completely degrade all components of the connective tissue, modify the extracellular matrix (ECM), and mediate the development of painful tendinopathy. To control the local activity of activated proteinases, the same cells produce tissue inhibitors of metalloproteinases (TIMP). These latter bind to the enzyme and prevent degradation. The balance between the activities of MMPs and TIMPs regulates tendon remodeling, whereas an imbalance produces a collagen dis-regulation and disturbances in tendons. ADAMs (a disintegrin and metalloproteinase) are cell membrane-linked enzymes with proteolytic and cell signaling functions.

ADAMTSs (ADAM with thrombospondin motifs) are secreted into the circulation and constitute a heterogeneous family of proteases with both anabolic and catabolic functions. Further studies are needed to better define the mechanism of action, and whether these new strategies are safe and effective in larger models.

KEY WORDS: metalloproteases, tendinopathy, tendon healing.

Introduction

Tendinopathy is a spectrum of disorders varying from an asymptomatic condition of ultrasound (US) changes to severe pain, discomfort, and frank functional impairment. Biomechanical factors, functional alterations, aging, and metabolic disorders may predispose to it. Diagnosis is usually clinical but, at times, it needs to be confirmed by US and MRI. However, the definitive verdict comes from histology. This is a “failed healing response” to overuse or stress tendon injury, with haphazard proliferation of tenocytes, intracellular abnormalities in tenocytes, disruption of collagen fibers, and a subsequent increase in non-collagenous matrix^{1,2}. These intratendinous changes may be part of normal ageing^{3,4}; however, the exact pathophysiologic mechanism is unclear.

Many molecular changes occur within tendinopathic tendon, but much attention has to be paid to better understand the role of matrix metalloproteinases (MMP) in tendinopathy^{5,6}. MMPs are a family of 24 zinc-dependent endopeptidases activated after proteolysis of an inactive pro-form. They degrade the extracellular matrix⁷⁻¹², but they are also involved in many normal and pathological processes: embryonal development, ovulation, wound healing, periodontitis, tumor invasion and metastasis, and soft tissue remodeling after injury^{7,13-15}. In addition, MMPs are implicated in the degeneration process of the intervertebral disc^{16,17} and of loosening after hip arthroplasty^{18,19}. On the other hand, they are reversibly inhibited by tissue inhibitors of metalloproteinase (TIMPs), natural endogenous inhibitors of the MMPs^{12,20,21}. The aim of this review is to describe the mechanism by which MMPs and TIMPs are involved in tendon remodelling, collagen dysregulation, and development of tendinopathy^{22,23}.

Enzymes of the extracellular matrix

The turnover of the extracellular matrix is a dynamic process in which synthesis and degradation are finely

balanced^{24,25}. The matrix metalloproteinases (MMPs) are potent molecules able to completely digest and degrade the connective tissue and change the properties of the extracellular matrix (ECM)²⁶. They take also part regulation to the inflammatory response mediated by chemokine and cytokine signaling and by neoepitopes expressed from the ECM²⁷. There are 4 main groups of MMPs, organized based on the substrate preference of these enzymes. The most well known enzyme is the collagenase, synthesized by connective tissue fibroblasts²⁸. Collagenases MMP-1, -8, and -13 cleave all subtypes of collagen, especially the triple helix fibrillar collagens types I, II, and III which confer mechanical strength to tissues⁷⁻¹². Collagenase 4 (MMP-18) is often omitted from the list of human MMPs, but it has been found in human ligaments²⁹. MMP-2 and MMP-14 play collagenolytic activities.

MMP-2 and -9 are gelatinases which degrade smaller collagen fragments released, and cleave denatured collagens and type IV collagen. Membrane-type MMPs (MT-MMPs) are cell membrane-linked proteases engaged in many activities. Stromelysins degrade proteoglycans, fibronectin, casein, collagen types III, IV, and V. Stromelysins (MMP-3 and -10) and matrilysin (MMP-7) are broad-spectrum proteinases engaged in the activation of other MMPs³⁰.

Once secreted into the extracellular space in the form of proenzymes (pro-MMP), some MMPs are stored within the cells (e.g. MMP-9 in neutrophil granules), others bind cellular membranes (e.g. MT1-MMP). These pro-MMPs are activated in the extracellular space by proteolytic cleavage³¹⁻³³. The baseline production of MMPs is low, but their synthesis may be induced by cytokines (interleukin -1, -4, -6, and -10, and tumor necrosis factor- α), growth factors, extracellular MMP inducer (EMMPRIN) and cell-cell or cell-matrix interactions. These are stimulator mediated by signal intracellular pathways including the mitogen-activated protein kinase pathway³⁴⁻³⁸.

Therefore, the structure and properties of the ECM vary based on the balance of tissue formation and breakdown. The remodeling process is followed by a repair response in which collagen fibers are involved³⁹. Even though it is not properly defined how the new connective tissue is formed, it is known that new collagen is synthesized, some collagen fibers are destructed (turnover) of, part of fibers are weaved, and new collagen takes place in the existing structure of the tendon⁴⁰. This regulation of all this is at levels of genetic transcription, pro-MMP activation, and inhibition of active enzymes.

The local activity of such activated proteinases is controlled by tissue inhibitors of metalloproteinases (TIMP). Released by the same cells which produce MMPs the inhibitors are bind to the enzyme, and prevent degradation⁴¹. The fine balance between MMPs and endogenous inhibitors is crucial to maintain the dynamic homeostasis and integrity of the extracellular matrix, and to control tendon remodeling. On the other hand, when there is some imbalance, collagen formation and tendon homeostasis undergo dis-regulation²².

MMPs and TIMPs are also engaged in the development, morphogenesis, reproduction, tissue remodeling, apoptosis, and evolution of rheumatoid arthritis and osteoarthritis^{42,7,13,10}. When referring to osteoarthritis and rheumatoid arthritis²⁰, it is somewhat oversimplified to consider MMPs as being solely tissue-degrading enzymes. Four main TIMPs reversibly inhibit all MMPs based on a 1:1 interaction with the zinc-binding site³⁶. TIMP-1, -2, and -4 may be in various tissues, including the vascular system; TIMP-3 is sequestered within the ECM⁴³. They also regulate angiogenesis and cellular proliferation⁴⁴. Other factors such as the soluble α -1-antitrypsin and α -2-macroglobulin⁴⁵ and cell membrane-linked MMP inhibitors⁴³ may also work as additional endogenous inhibitors of MMPs. MMPs may also have anti-inflammatory action reasonably mediated by anti-inflammatory cytokines and chemokines^{46,47}.

ADAMs and ADAMTS

Two other groups of proteases are related to the MMPs. ADAMs (disintegrin and metalloproteinase) are cell membrane-linked enzymes involved in proteolysis and cellular signalling. ADAMTSs (ADAM with thrombospondin motifs) belong to a family of proteases with anabolic and catabolic activities, but their functions are only partially known. ADAMs bind to the cell membrane; ADAMTS are released into the peri-cellular space and in circulation. Thirty-three ADAMs have been identified, involved in the proteolysis of other membrane-bound proteins (e.g. growth factors in precursor state), cell signalling, and cell adhesion processes⁴⁸. The well known types of ADAMTS are 19, distinguished into 4 groups. The aggrecanases (ADAMTS-1, -4, -5, -8, -9, -15, and -20) have proteoglycanolytic action, regulate angiogenesis and degradation of other proteins. ADAMTS-2, -3, and -14 belong to the second group, all with anabolic function and acting as procollagen N-propeptidases for collagen types I, II, and III. An ADAMTS-2 mutation has been discovered in Ehlers-Danlos syndrome type VII C (a condition with fragile skin, joint laxity, and hernias); ADAMTS-13, as cleavage factor of the von Willebrand factor, is supposed to be involved in thrombotic thrombocytopenic purpura. The function of the fourth group (ADAMTS -6, -7, -10, -12, -16, -17, -18, and -19) is still unclear⁴⁹. TIMP-3 is the main TIMP which inhibits activity against some of the ADAMs and ADAMTS⁴⁸.

Other MMPs inhibitors

Tetracycline antibiotics are pharmacological inhibitors of MMPs. They usually bind to the zinc site of the MMP enzymes, and block their activity. Doxycycline is probably the most potent MMP inhibitor, by inhibiting MMPs -1, -2, -7, -8, -9, -12, and -13^{50,51}. They also inhibit MMP gene expression level, and reduce the activation via the inflammatory cascade and through reactive

oxygen species. Chemically modified tetracyclines may prevent unwanted effects on the endogenous microbial flora. For instance, low-dose doxycycline, 20 mg twice a day instead of the standard dose of 100 mg twice a day, still retains its MMP-inhibitory efficacy^{52,53}, but may reduce effects on the microbial flora of the vagina or the gut⁵⁴. Synthetic MMP inhibitors are a large heterogeneous group of compounds, modified to produce increased inhibitory potency and increased specificity against particular MMPs⁵⁵. Bisphosphonates, inhibitors of osteoclastic bone resorption, also have potent MMP-inhibitory properties, probably through cation-chelation of zinc^{56,36}.

Metalloproteasis in tendinopathy

MMPs degrade the extracellular matrix and may predispose to painful tendinopathy and tendon rupture^{57,6}. Associations between variants of the MMP-3 gene and painful Achilles tendinopathy have been found⁵⁸. Although enzyme inhibitors have not been extensively evaluated in the treatment of tendinopathy, 2 trials have showed that patients with patellar and Achilles tendinopathy may be well responsive to peritendinous injections of aprotinin, a general protease inhibitor^{59,60}. In a randomized trial of patients with Achilles tendinopathy, combined aprotinin and eccentric exercises did not significantly improve outcomes when compared to placebo, but results were satisfactory in both groups⁶¹. Aprotinin is no longer available in most countries to use as an injectable MMP inhibitor (in small doses), even though it had been used in Europe for tendinopathy since the 1970s⁶⁰. The major indication for aprotinin was to reduce bleeding in major surgery (esp. cardiac) using large intravenous doses. Recent data have recently shown that the risk profile outweighs the benefit for this indication⁶¹. Aprotinin also has been shown to predispose to anaphylactic reactions in some cases^{62,63} without any superiority to other injectables products such as PRP (platelet rich plasma) or glucose prolotherapy. Tranexamic acid, which inhibits MMPs indirectly, through plasmin inhibition⁶⁴, could theoretically be used in tendinopathy, but laboratory and clinical evidences would be required.

The neurotransmitter substance P (SP) is a pain mediating neurotransmitter^{65,66} which may regulate the gene expression of MMPs and TIMPs in fibroblasts⁶⁷, and would be responsible for altered regulation profile of MMPs and TIMPs. When SP is administered exogenously, it seems to enhance proliferation of fibroblasts and tendon healing^{68,69}. Achilles tendinopathy could be a model tendinopathy and, probably it will be a model for treatment studies, but the molecular profiles of other tendinopathies, such as supraspinatus tendinopathy, should be taken into account⁷⁰.

There is evidence of rotator cuff disease associated with altered matrix composition. Specifically, total collagen concentration is reduced whereas the proportion of type III collagen is significantly increased⁷¹. Changes in colla-

gen composition have been also detected in macroscopically normal tendons before rupture. This suggests that an altered pattern of collagen synthesis and turnover, probably related to the aging process, may precede the occurrence of chronic tendinopathy and, over time, tendon rupture. It has been shown that human cuff tendons may produce MMPs and TIMPs when placed in an organ culture²². In torn rotator cuff tendons, both mRNA and protein levels of MMP-13 are increased. Rotator cuff tendons contain type I collagen^{72,73}. MMP-13, MMP-1 and MMP-8 degrade type I collagen. In both tearing and remodeling of rotator cuff tendons⁷⁴, MMP-13 cleaves gelatin much more efficiently than MMP-1 and MMP-8^{9,10,12,74}. MMP-13 induce excessive degradation of the extracellular matrix, and is play a main role in pathological conditions such as osteoarthritis, rheumatoid arthritis, cutaneous and intestinal ulcers, and periodontal inflammation^{11,75,12}. However, degradation is important for healing and remodeling of connective tissues¹³. The increased MMP-13 levels may be expression of active tissue remodeling or of a healing response after injury; it is unknown whether these levels are secondary to the effects of rotator cuff tearing itself or responsible of the pathogenesis of rotator cuff tearing⁷⁰. The fact that MMP-3 mRNA (stromelysin) levels are decreased in torn rotator cuffs suggests that MMP-3 may be the result of failed matrix remodeling and inadequate homeostasis of tendons^{70,71,76}.

No significant difference between MMP-1 mRNA levels of normal and torn tendons have been found⁷⁰, but Yoshihara et al.⁷⁷ and Zhen et al.⁷⁸ demonstrated elevated levels of MMP-1, MMP-3, and glycosaminoglycans in the synovial fluid in patients with massive rotator cuff tears. The MMP-2 activation during the healing process after supraspinatus tendon tearing may induce extracellular matrix degradation in both the tendon edge and reparative tissue^{70,79}.

Metalloproteasis in tendon healing

Data from unloaded healing rat flexor tendons have suggested that MMP-9 and MMP-13 mediate tissue degradation during the early phase of healing, whereas MMP-2, -3, and -14 mediate both tissue degradation and later remodeling⁸⁰. As observed in an experimental study, systemic treatment with the MMP inhibitor doxycycline weakens rat Achilles tendons during healing, highlighting the key role of MMPs in tendon healing⁸¹. Specific MMPs may be deleterious for tendon healing whereas MMP inhibitors could enhance it. For instance, MMP-7 levels inversely correlate with tendon strength in humans⁸², whereas MMP-13 expression, strongly up-regulated in rotator cuff rupture⁷⁰, could be responsible of tendon degradation and stress-deprivation⁸³. In rabbits undergoing anterior cruciate ligament reconstruction, intra-articular injections of endogenous MMP inhibitor α -2-macroglobulin improve strength and histological features of tendon to bone healing⁸⁴. In tendon rupture, transient reduction of the tendon strength may occur immediately after

surgical repair^{85,86}. At that stage, MMPs may induce degradation close to the sutures, making the tendon tissue around the suture weak⁸⁷. Even if MMPs inhibition could improve tendon suture-holding capacity, doxycycline-coated sutures could be used to promote early tendon suture-holding capacity in a rat model⁸⁸. TIMP-2, TIMP-3, and TIMP-4 mRNA levels are decreased in suffering tendons⁷. High levels of TIMP-3 are associated with cells undergoing apoptosis both *in vitro* and *in vivo*^{43,89-92}. Because there is an increased number of apoptotic cells tendon tears compared to controls, decreased TIMP-3 mRNA levels suggest that TIMP-3 may not play a role in apoptosis in tendon tearing⁷⁰. On the other hand, the expression of TIMP-1 controls and inhibits the excessive degradation of the matrix by MMP-2⁷⁹. Local administration of α -2-macroglobulin, an endogenous MMP inhibitor, at the greater tuberosity footprint induces histological changes at the healing enthesis after rotator cuff repair⁹³, with a statistically significant reduction in local collagen degradation 2 and 4 weeks after the operation. The reduction of MMP activity was associated with increased formation of fibrocartilage 2 weeks after the operation, and improved collagen organization after 4 weeks. The local administration of an MMP inhibitor in the peri-operative period may favor the tendon-bone healing⁶⁵.

Membrane type 1 matrix metalloproteinase (MT1-MMP, also called MMP-14) is a membrane-bound matrix metalloproteinase involved in the embryologic development of musculoskeletal tissues⁹⁴. Gulotta et al., in a recent study on rats, have hypothesized that this gene involved in the formation of tendon-to-bone insertion sites during embryogenesis, could induce regeneration⁹⁵. They found significantly improved outcome in tendon-to-bone healing after application of adenoviral MT1-MMP transduced MSCs compared to application of MSCs alone. In rotator cuff surgery, the over-expression of MT1-MMP leads to improved biomechanical strength over the tendon-bone interface after 4 weeks from the index surgery. The exact role of MT1-MMP in this process is unknown, but it is involved in cell surface activation of MMP-2, and proteolytic activity⁹⁶. Tendon to bone healing would be induced by 2 mechanisms. Membrane type 1 matrix metalloproteinase may digest unwanted scar tissue and restore an environment similar to the native insertion site. The second mechanism would be based on COX-2 inhibition⁹⁷. MT1-MMP probably up-regulates COX-2, with beneficial effects on tendon healing, by inducing bone and cartilage formation in the fibrovascular scar tissue. Doxycycline-mediated inhibition of interstitial collagenase (MMP-13) favorably influences early healing after tendon repair: collagen organization, biomechanical and histologic parameters are significantly improved⁹⁸. The exact mechanism by which tetracycline antibiotics inhibit MMP 13 remains to be defined⁹⁸.

Conclusions

Biologic modulation of endogenous MMP activity to basal levels may reduce pathologic tissue degradation

and favorably influence healing after tendon disease^{70, 77-79}. Further studies are needed to better define the mechanism of action, and whether these new strategies are safe and effective in larger models.

References

- Maffulli N, Del Buono A. Platelet plasma rich products in musculoskeletal medicine: Any evidence? *The Surgeon* 2012; 10:148-150.
- Longo UG, Ronga M, Maffulli N. Achilles tendinopathy. *Sports Med Arthrosc* 2009; 17: 112-126.
- Del Buono A, Battery L, Denaro V, Maccauro G, Maffulli N. Tendinopathy and inflammation: some truths. *Int J Immunopathol Pharmacol* 2011; 24(1 Suppl 2):45-50.
- Del Buono A, Papalia R, Denaro V, Maccauro G, Maffulli N. Platelet rich plasma and tendinopathy: state of the art. *Int J Immunopathol Pharmacol* 2011; 24 (1 Suppl 2):79-83.
- Fu SC, Chan BP, Wang W, Pau HM, Chan KM, Rolf CG. Increased expression of matrix metalloproteinase 1 (MMP1) in 11 patients with patellar tendinosis. *Acta Orthop Scand* 2002; 73:658-662.
- Riley G. The pathogenesis of tendinopathy. A molecular perspective. *Rheumatology (Oxford)* 2004; 43:131-142.
- Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* 1993; 4:197-250.
- Brown PD. Matrix metalloproteinase inhibitors in the treatment of cancer. *Med Oncol* 1997; 14:1-10.
- Kahari VM, Saarialho-Kere U. Matrix metalloproteinases in skin. *Exp Dermatol* 1997; 6: 199-213.
- Nagase H, Woessner JF, Jr. Matrix metalloproteinases. *J Biol Chem* 1999; 274:21491-21494.
- Ravanti L, Kahari VM. Matrix metalloproteinases in wound repair (review). *Int J Mol Med* 2000; 6:391-407.
- Vincenti MP. The matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) genes. Transcriptional and posttranscriptional regulation, signal transduction and cell-type-specific expression. *Methods Mol Biol* 2001; 151:121-148.
- Hellio Le Graverand MP, Eggerer J, Sciore P, Reno C, Vignon E, Otterness I, Hart DA. Matrix metalloproteinase-13 expression in rabbit knee joint connective tissues: influence of maturation and response to injury. *Matrix Biol* 2000; 19:431-441.
- Kawahara E, Okada Y, Nakanishi I, Iwata K, Kojima S, Kumagai S, Yamamoto E. The expression of invasive behavior of differentiated squamous carcinoma cell line evaluated by an *in vitro* invasion model. *Jpn J Cancer Res* 1993; 84:409-418.
- Matrisian LM. Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet* 1990; 6:121-125.
- Goupille P, Jayson MI, Valat JP, Freemont AJ. Matrix metalloproteinases: the clue to intervertebral disc degeneration? *Spine (Phila Pa 1976)* 1998; 23:1612-1626.
- Matsui Y, Maeda M, Nakagami W, Iwata H. The involvement of matrix metalloproteinases and inflammation in lumbar disc herniation. *Spine (Phila Pa 1976)* 1998; 23:863-868; discussion 868-869.
- Del Buono A, Denaro V, Maffulli N. Genetic susceptibility to aseptic loosening following total hip arthroplasty: a systematic review. *Br Med Bull* 2012;101:39-55.
- Takagi M, Santavirta S, Ida H, Ishii M, Mandelin J, Konttinen YT. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in loose artificial hip joints. *Clin Orthop*

- Relat Res 1998;35-45.
20. Bramono DS, Richmond JC, Weitzel PP, Kaplan DL, Altman GH. Matrix metalloproteinases and their clinical applications in orthopaedics. *Clin Orthop Relat Res* 2004;272-285.
 21. Gomez DE, Alonso DF, Yoshiji H, Thorgeirsson UP. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur J Cell Biol* 1997; 74: 111-122.
 22. Dalton S, Cawston TE, Riley GP, Bayley IJ, Hazleman BL. Human shoulder tendon biopsy samples in organ culture produce procollagenase and tissue inhibitor of metalloproteinases. *Ann Rheum Dis* 1995; 54:571-577.
 23. Jhingan S, Perry M, O'Driscoll G, Lewin C, Teatino R, Malliaras P, Maffulli N, Morrissey D. Thicker Achilles tendons are a risk factor to develop Achilles tendinopathy in elite professional soccer players. *Muscles, Ligaments and Tendons Journal* 2011; 1:51-56.
 24. Del Buono A, Oliva F, Longo U, Rodeo S, Orchard J, Denaro V, Maffulli N. Metalloproteases and rotator cuff disease. *J Shoulder Elbow Surg* 2012; 21:200-208.
 25. Merolla G, Paladini P, Saporito M, Porcellini G. Conservative management of rotator cuff tears: literature review and proposal for a prognostic Prediction Score. *Muscles, Ligaments and Tendons Journal* 2011; 1:12-19.
 26. Mandal M, Mandal A, Das S, Chakraborti T, Sajal C. Clinical implications of matrix metalloproteinases. *Mol Cell Biochem* 2003; 252:305-329.
 27. Pearce WH, Shively VP. Abdominal aortic aneurysm as a complex multifactorial disease: interactions of polymorphisms of inflammatory genes, features of autoimmunity, and current status of MMPs. *Ann N Y Acad Sci* 2006; 1085:117-132.
 28. Gross J, Lapiere CM. Collagenolytic activity in amphibian tissues: a tissue culture assay. *Proc Natl Acad Sci USA* 1962; 48:1014-1022.
 29. Foos MJ, Hickox JR, Mansour PG, Slauterbeck JR, Hardy DM. Expression of matrix metalloprotease and tissue inhibitor of metalloprotease genes in human anterior cruciate ligament. *J Orthop Res* 2001; 19:642-649.
 30. Al-Sadi O, Schulze-Tanzil G, Kohl B, Lohan A, Lemke M, Ertel W, John T. Tenocytes, pro-inflammatory cytokines and leukocytes: a relationship? *Muscles, Ligaments and Tendons Journal* 2011; 1:68-76.
 31. Dollery CM, Libby P. Atherosclerosis and proteinase activation. *Cardiovasc Res* 2006; 69: 625-635.
 32. van Meurs J, van Lent P, Stoop R, Holthuysen A, Singer I, Bayne E, Mudgett J, Poole R, Billingham C, van der Kraan P, Buma P, van den Berg W. Cleavage of aggrecan at the Asn341-Phe342 site coincides with the initiation of collagen damage in murine antigen-induced arthritis: a pivotal role for stromelysin 1 in matrix metalloproteinase activity. *Arthritis Rheum* 1999; 42:2074-2084.
 33. Wildemann B, Klatt F. Biological aspects of rotator cuff healing. *Muscles, Ligaments and Tendons Journal* 2011; 1:161-168.
 34. Corps AN, Curry VA, Buttle DJ, Hazleman BL, Riley GP. Inhibition of interleukin-1beta-stimulated collagenase and stromelysin expression in human tendon fibroblasts by epigallocatechin gallate ester. *Matrix Biol* 2004; 23:163-169.
 35. Gabison EE, Hoang-Xuan T, Mauviel A, Menashi S. EMM-PRIN/CD147, an MMP modulator in cancer, development and tissue repair. *Biochimie* 2005; 87:361-368.
 36. Hidalgo M, Eckhardt SG. Development of matrix metalloproteinase inhibitors in cancer therapy. *J Natl Cancer Inst* 2001; 93:178-193.
 37. Kossakowska AE, Edwards DR, Prusinkiewicz C, Zhang MC, Guo D, Urbanski SJ, Grogan T, Marquez LA, Janowska-Wieczorek A. Interleukin-6 regulation of matrix metalloproteinase (MMP-2 and MMP-9) and tissue inhibitor of metalloproteinase (TIMP-1) expression in malignant non-Hodgkin's lymphomas. *Blood* 1999; 94:2080-2089.
 38. Meller D, Li DQ, Tseng SC. Regulation of collagenase, stromelysin, and gelatinase B in human conjunctival and conjunctivochalasis fibroblasts by interleukin-1beta and tumor necrosis factor-alpha. *Invest Ophthalmol Vis Sci* 2000; 41:2922-2929.
 39. Giai Via A, Frizziero A, Oliva F. Biological properties of mesenchymal Stem Cells from different sources. *Muscles, Ligaments and Tendons Journal* 2012; 2:154-162.
 40. Laurent GJ. Dynamic state of collagen: pathways of collagen degradation in vivo and their possible role in regulation of collagen mass. *Am J Physiol* 1987; 252:C1-9.
 41. Cawston TE, Murphy G, Mercer E, Galloway WA, Hazleman BL, Reynolds JJ. The interaction of purified rabbit bone collagenase with purified rabbit bone metalloproteinase inhibitor. *Biochem J* 1983; 211:313-318.
 42. Ahonen M, Baker AH, Kahari VM. Adenovirus-mediated gene delivery of tissue inhibitor of metalloproteinases-3 inhibits invasion and induces apoptosis in melanoma cells. *Cancer Res* 1998; 58:2310-2315.
 43. Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. *J Cell Sci* 2002; 115:3719-3727.
 44. Chirco R, Liu XW, Jung KK, Kim HR. Novel functions of TIMPs in cell signaling. *Cancer Metastasis Rev* 2006; 25:99-113.
 45. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* 2006; 69:562-573.
 46. Gueders MM, Balbin M, Rocks N, Foidart JM, Gosset P, Louis R, Shapiro S, Lopez-Otin C, Noel A, Cataldo DD. Matrix metalloproteinase-8 deficiency promotes granulocytic allergen-induced airway inflammation. *J Immunol* 2005; 175:2589-2597.
 47. Owen CA, Hu Z, Lopez-Otin C, Shapiro SD. Membrane-bound matrix metalloproteinase-8 on activated polymorphonuclear cells is a potent, tissue inhibitor of metalloproteinase-resistant collagenase and serpinase. *J Immunol* 2004; 172:7791-7803.
 48. Mochizuki S, Okada Y. ADAMs in cancer cell proliferation and progression. *Cancer Sci* 2007; 98:621-628.
 49. Jones GC, Riley GP. ADAMTS proteinases: a multi-domain, multi-functional family with roles in extracellular matrix turnover and arthritis. *Arthritis Res Ther* 2005; 7:160-169.
 50. Golub LM, Lee HM, Ryan ME, Giannobile WV, Payne J, Sorsa T. Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. *Adv Dent Res* 1998; 12:12-26.
 51. Peterson JT. Matrix metalloproteinase inhibitor development and the remodeling of drug discovery. *Heart Fail Rev* 2004; 9:63-79.
 52. Emingil G, Atilla G, Sorsa T, Luoto H, Kirilmaz L, Baylas H. The effect of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid matrix metalloproteinase-8 levels in chronic periodontitis. *J Periodontol* 2004; 75:106-115.
 53. O'Dell JR, Elliott JR, Mallek JA, Mikuls TR, Weaver CA, Glickstein S, Blakely KM, Hausch R, Leff RD. Treatment of early seropositive rheumatoid arthritis: doxycycline plus methotrexate versus methotrexate alone. *Arthritis Rheum* 2006; 54:621-627.
 54. Walker C, Preshaw PM, Novak J, Hefti AF, Bradshaw M, Powala C. Long-term treatment with sub-antimicrobial dose doxycycline has no antibacterial effect on intestinal flora. *J Clin Periodontol* 2005; 32:1163-1169.
 55. Hu J, Van den Steen PE, Sang QX, Opendakker G. Matrix metalloproteinase inhibitors as therapy for inflammatory

- and vascular diseases. *Nat Rev Drug Discov* 2007; 6:480-498.
56. Heikkila P, Teronen O, Moilanen M, Kontinen YT, Hanemaaijer R, Laitinen M, Maisi P, van der Pluijm G, Bartlett JD, Salo T, Sorsa T. Bisphosphonates inhibit stromelysin-1 (MMP-3), matrix metalloelastase (MMP-12), collagenase-3 (MMP-13) and enamelysin (MMP-20), but not urokinase-type plasminogen activator, and diminish invasion and migration of human malignant and endothelial cell lines. *Anti-cancer Drugs* 2002; 13:245-254.
 57. de Mos M, van El B, DeGroot J, Jahr H, van Schie HT, van Arkel ER, Tol H, Heijboer R, van Osch GJ, Verhaar JA. Achilles tendinosis: changes in biochemical composition and collagen turnover rate. *Am J Sports Med* 2007; 35:1549-1556.
 58. Raleigh SM, van der Merwe L, Ribbans WJ, Smith RK, Schwellnus MP, Collins M. Variants within the MMP3 gene are associated with Achilles tendinopathy: possible interaction with the COL5A1 gene. *Br J Sports Med* 2009; 43:514-520.
 59. Magra M, Maffulli N. Matrix metalloproteases: a role in overuse tendinopathies. *Br J Sports Med* 2005; 39:789-791.
 60. Orchard J, Massey A, Brown R, Cardon-Dunbar A, Hofmann J. Successful management of tendinopathy with injections of the MMP-inhibitor aprotinin. *Clin Orthop Relat Res* 2008; 466:1625-1632.
 61. Brown R, Orchard J, Kinchington M, Hooper A, Nalder G. Aprotinin in the management of Achilles tendinopathy: a randomised controlled trial. *Br J Sports Med* 2006; 40:275-279.
 62. Beierlein W, Scheule AM, Dietrich W, Ziemer G. Forty years of clinical aprotinin use: a review of 124 hypersensitivity reactions. *Ann Thorac Surg* 2005; 79:741-748.
 63. Dietrich W, Spath P, Zuhlsdorf M, Dalichau H, Kirchhoff PG, Kuppe H, Preiss DU, Mayer G. Anaphylactic reactions to aprotinin reexposure in cardiac surgery: relation to anti-aprotinin immunoglobulin G and E antibodies. *Anesthesiology* 2001; 95:64-71; discussion 5A-6A.
 64. Maffulli N, Longo UG, Denaro V. Novel approaches for the management of tendinopathy. *J Bone Joint Surg Am*; 92:2604-2613.
 65. Alfredson H. The chronic painful Achilles and patellar tendon: research on basic biology and treatment. *Scand J Med Sci Sports* 2005; 15:252-259.
 66. Schubert TE, Weidler C, Lerch K, Hofstadter F, Straub RH. Achilles tendinosis is associated with sprouting of substance P positive nerve fibres. *Ann Rheum Dis* 2005; 64: 1083-1086.
 67. Cury PR, Canavez F, de Araujo VC, Furuse C, de Araujo NS. Substance P regulates the expression of matrix metalloproteinases and tissue inhibitors of metalloproteinase in cultured human gingival fibroblasts. *J Periodontol Res* 2008; 43:255-260.
 68. Burssens P, Steyaert A, Forsyth R, van Ovost EJ, Depaeppe Y, Verdonk R. Exogenously administered substance P and neutral endopeptidase inhibitors stimulate fibroblast proliferation, angiogenesis and collagen organization during Achilles tendon healing. *Foot Ankle Int* 2005; 26:832-839.
 69. Steyaert AE, Burssens PJ, Vercruyse CW, Vanderstraeten GG, Verbeeck RM. The effects of substance P on the biomechanical properties of ruptured rat Achilles' tendon. *Arch Phys Med Rehabil* 2006; 87:254-258.
 70. Lo IK, Marchuk LL, Hollinshead R, Hart DA, Frank CB. Matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase mRNA levels are specifically altered in torn rotator cuff tendons. *Am J Sports Med* 2004; 32:1223-1229.
 71. Riley GP, Harrall RL, Constant CR, Chard MD, Cawston TE, Hazleman BL. Glycosaminoglycans of human rotator cuff tendons: changes with age and in chronic rotator cuff tendinitis. *Ann Rheum Dis* 1994; 53:367-376.
 72. Blevins FT, Djurasovic M, Flatow EL, Vogel KG. Biology of the rotator cuff tendon. *Orthop Clin North Am* 1997; 28:1-16.
 73. Fan L, Sarkar K, Franks DJ, Uthoff HK. Estimation of total collagen and types I and III collagen in canine rotator cuff tendons. *Calcif Tissue Int* 1997; 61:223-229.
 74. Knauper V, Lopez-Otin C, Smith B, Knight G, Murphy G. Biochemical characterization of human collagenase-3. *J Biol Chem* 1996; 271:1544-1550.
 75. Vaalamo M, Mattila L, Johansson N, Kariniemi AL, Karjalainen-Lindsberg ML, Kahari VM, Saarialho-Kere U. Distinct populations of stromal cells express collagenase-3 (MMP-13) and collagenase-1 (MMP-1) in chronic ulcers but not in normally healing wounds. *J Invest Dermatol* 1997; 109:96-101.
 76. Riley GP, Curry V, DeGroot J, van El B, Verzijl N, Hazleman BL, Bank RA. Matrix metalloproteinase activities and their relationship with collagen remodelling in tendon pathology. *Matrix Biol* 2002; 21:185-195.
 77. Yoshihara Y, Hamada K, Nakajima T, Fujikawa K, Fukuda H. Biochemical markers in the synovial fluid of glenohumeral joints from patients with rotator cuff tear. *J Orthop Res* 2001; 19:573-579.
 78. Zhen EY, Brittain IJ, Laska DA, Mitchell PG, Sumer EU, Karsdal MA, Duffin KL. Characterization of metalloprotease cleavage products of human articular cartilage. *Arthritis Rheum* 2008; 58:2420-2431.
 79. Choi HR, Kondo S, Hirose K, Ishiguro N, Hasegawa Y, Iwata H. Expression and enzymatic activity of MMP-2 during healing process of the acute supraspinatus tendon tear in rabbits. *J Orthop Res* 2002; 20:927-933.
 80. Oshiro W, Lou J, Xing X, Tu Y, Manske PR. Flexor tendon healing in the rat: a histologic and gene expression study. *J Hand Surg Am* 2003; 28:814-823.
 81. Pasternak B, Fellenius M, Aspenberg P. Doxycycline impairs tendon repair in rats. *Acta Orthop Belg* 2006; 72:756-760.
 82. Pasternak B, Schepull T, Eliasson P, Aspenberg P. Elevation of systemic matrix metalloproteinases 2 and 7 and tissue inhibitor of metalloproteinase 2 in patients with a history of Achilles tendon rupture: pilot study. *Br J Sports Med*; 44:669-672.
 83. Arnoczky SP, Lavagnino M, Egerbacher M, Caballero O, Gardner K. Matrix metalloproteinase inhibitors prevent a decrease in the mechanical properties of stress-deprived tendons: an in vitro experimental study. *Am J Sports Med* 2007; 35:763-769.
 84. Demirag B, Sarisozen B, Ozer O, Kaplan T, Ozturk C. Enhancement of tendon-bone healing of anterior cruciate ligament grafts by blockage of matrix metalloproteinases. *J Bone Joint Surg Am* 2005; 87:2401-2410.
 85. Wada A, Kubota H, Miyanishi K, Hatanaka H, Miura H, Iwamoto Y. Comparison of postoperative early active mobilization and immobilization in vivo utilising a four-strand flexor tendon repair. *J Hand Surg Br* 2001; 26:301-306.
 86. Yildirim Y, Kara H, Cabukoglu C, Esemeli T. Suture holding capacity of the Achilles tendon during the healing period: an in vivo experimental study in rabbits. *Foot Ankle Int* 2006; 27:121-124.
 87. McDowell CL, Marqueen TJ, Yager D, Owen J, Wayne JS. Characterization of the tensile properties and histologic/biochemical changes in normal chicken tendon at the site of suture insertion. *J Hand Surg Am* 2002; 27:605-614.
 88. Pasternak B, Missios A, Askendal A, Tengvall P, Aspenberg P. Doxycycline-coated sutures improve the suture-holding capacity of the rat Achilles tendon. *Acta Orthop* 2007; 78:680-686.

89. Baker AH, Zaltsman AB, George SJ, Newby AC. Divergent effects of tissue inhibitor of metalloproteinase-1, -2, or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation, and death in vitro. TIMP-3 promotes apoptosis. *J Clin Invest* 1998; 101:1478-1487.
90. Bond M, Murphy G, Bennett MR, Newby AC, Baker AH. Tissue inhibitor of metalloproteinase-3 induces a Fas-associated death domain-dependent type II apoptotic pathway. *J Biol Chem* 2002; 277:13787-13795.
91. Smith MR, Kung H, Durum SK, Colburn NH, Sun Y. TIMP-3 induces cell death by stabilizing TNF-alpha receptors on the surface of human colon carcinoma cells. *Cytokine* 1997; 9:770-780.
92. Wallace JA, Alexander S, Estrada EY, Hines C, Cunningham LA, Rosenberg GA. Tissue inhibitor of metalloproteinase-3 is associated with neuronal death in reperfusion injury. *J Cereb Blood Flow Metab* 2002; 22:1303-1310.
93. Bedi A, Kovacevic D, Hettrich C, Gulotta LV, Ehteshami JR, Warren RF, Rodeo SA. The effect of matrix metalloproteinase inhibition on tendon-to-bone healing in a rotator cuff repair model. *J Shoulder Elbow Surg*; 19:384-391.
94. Apte SS, Fukai N, Beier DR, Olsen BR. The matrix metalloproteinase-14 (MMP-14) gene is structurally distinct from other MMP genes and is co-expressed with the TIMP-2 gene during mouse embryogenesis. *J Biol Chem* 1997; 272:25511-25517.
95. Gulotta LV, Kovacevic D, Montgomery S, Ehteshami JR, Packer JD, Rodeo SA. Stem cells genetically modified with the developmental gene MT1-MMP improve regeneration of the supraspinatus tendon-to-bone insertion site. *Am J Sports Med*; 38:1429-1437.
96. Kinoh H, Sato H, Tsunozuka Y, Takino T, Kawashima A, Okada Y, Seiki M. MT-MMP, the cell surface activator of proMMP-2 (pro-gelatinase A), is expressed with its substrate in mouse tissue during embryogenesis. *J Cell Sci* 1996; 109(Pt 5):953-959.
97. Cohen DB, Kawamura S, Ehteshami JR, Rodeo SA. Indomethacin and celecoxib impair rotator cuff tendon-to-bone healing. *Am J Sports Med* 2006; 34:362-369.
98. Bedi A, Fox AJ, Kovacevic D, Deng XH, Warren RF, Rodeo SA. Doxycycline-mediated inhibition of matrix metalloproteinases improves healing after rotator cuff repair. *Am J Sports Med*; 38:308-317.