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Abstract

Matrix metalloproteinases (MMPs) constitute a group of over 20 - structurally-related proteins which include a Zn^{++} ion binding site that is essential for their proteolytic activities. These enzymes play important role in extracellular matrix turnover in order to maintain a proper balance in its synthesis and degradation. MMPs are associated to several physiological and pathophysiological processes, including diabetes mellitus (DM). The mechanisms of DM and its complications is subject of intense research and evidence suggests that MMPs are implicated with the development and progression of diabetic microvascular complications such as nephropathy, cardiomyopathy, retinopathy and peripheral neuropathy. Recent data has associated DM to changes in the tendon structure, including abnormalities in fiber structure and organization, increased tendon thickness, volume and disorganization obtained by image and a tendency of impairing biomechanical properties. Although not fully elucidated, it is believed that DM-induced MMP dysregulation may contribute to structural and biomechanical alterations and impaired process of tendon healing.

Keywords

MMPs • Diabetic alterations • Extracellular matrix • Tendon

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List of Abbreviations

AGE	advanced glycation end products
ADAMTS	Disintegrin and Metalloproteinase domain with Thrombospondin-1 motifs
CTGF	connective tissue growth factor
DM	diabetes mellitus

ECM	extracellular matrix
IL	interleukin cytokine family
iNOS	inducible nitric oxide synthase
MMP(s)	matrix metalloproteinase(s)
PGE2	prostaglandin-E2
RAGE(s)	receptor(s) for advanced glycation end products
ROS	reactive oxygen species
TGF- β 1	transforming growth factor beta 1
TIMP(s)	tissue inhibitor(s) of metalloproteinase
TNF- α	tumor necrosis factor alpha
STZ	Streptozotocin.

MMPs Structure, Function and Regulation

Matrix metalloproteinases (MMPs) constitute a group of over 20 structurally-related proteins which include a Zn^{++} ion binding site that is essential for their proteolytic activities. These enzymes play important role in extracellular matrix (ECM) turnover in order to maintain a proper balance in its synthesis and degradation [1]. Thus, MMPs substrates are mainly collagens, but also many other ECM proteins, including fibronectin, laminin, tenascin, aggrecan and osteonectin [2].

MMPs can be classified in four groups accordingly to their domain organization, sequence similarities and their substrate specificity. The group of (i) gelatinases comprises MMP-2 and MMP-9 and is characterized by an additional domain, called collagen binding domain, which represents the preferential binding domain for fibrillar collagen I [3]. MMP-2 is generally expressed in endothelial cells, fibroblasts, keratinocytes and chondrocytes; while MMP-9 is present in alveolar macrophages, trophoblasts, osteoclasts and polymorphonuclear leukocytes [4, 5]. The group of (ii) matrilysins is composed of MMP-7 and MMP-26 which are able to process collagen IV but not collagen I [6], and are involved in degradation of ECM constituents in the uterus at postpartum and implantation of

embryos [7]. The (iii) typical MMPs group comprises three subgroups, namely stromelysins (MMP-3,-10 and-11), which participate of ECM turnover but are not able to cleave fibrillar collagen I; Collagenases (MMP-1,-8 and -13), which are responsible for degrading several native collagen (type I-V, XI) and other MMPs such as MMP-12, MMP-19, MMP-20 and MMP-27 [4, 8]. The last group is referred as (iv) furin-activated MMPs and consists of membrane type MMPs (MT-1,-2,-3,-4,-5-MMPs), transmembrane MMPs type II and secreted MMPs [4]. With a different structural arrangement from that of MMPs, the disintegrin and metalloproteinase domain with thrombospondin-1 motifs (ADAMTS) is another family of metalloproteinases and consists of 19 members which specifically process proteoglycans, in particular aggrecan, and pro-collagen [9, 10].

The MMPs are synthesized as inactive zymogens with a pro-peptide domain that must be withdrawn by several proteases, such as plasmin, thrombin, chimase, and MT-MMPs for protease activation. Activated MMPs can be either cell-secreted or membrane-bound exhibiting catalytic activity at the cell surface, intracellularly and extracellularly [5]. Due to its high proteolytic potential, MMPs are subjected to tight control and restrictive regulatory mechanisms that maintain homeostasis at the intracellular and extracellular level. One of the regulation mechanisms of the MMPs activity is by their endogenous inhibitors, tissue inhibitors of MMPs (TIMPs), which comprise a family of four members (TIMP-1 to 4) [8]. TIMPs are expressed in a tissue specific pattern and can operate through direct MMPs inhibition or by controlling their activation [11].

MMPs and TIMPs are associated to several physiological processes such as embryogenesis, wound healing, proliferation, cell motility, remodeling, angiogenesis and key reproductive events [4, 8]. On the other hand, recent evidence has shown that pathologically increased MMP activity or imbalance between MMP and TIMP expression can exacerbate a variety of diseases [12]. Indeed, MMPs are correlated to the

pathogenesis of abnormalities of the growth plate and wound healing, heart failure, arthritic synovial joints, cancer, periodontal disease, ischemic brain injury [13] and even tendinopathies [14]. This chapter aims to relate MMPs changes in the diabetes mellitus (DM) pathogenesis with special focus on the tendon tissue.

MMPs and Pathogenesis of Diabetic Complications

DM is a group of metabolic disorders considered one of the major health problems worldwide [15, 16]. Chronic hyperglycemia resulting from defects in the secretion and/or insulin action is the main feature of the disease and may lead to vascular damage in vessels, nerves and many internal organs including the eyes, kidneys and heart.

Currently, the development mechanism of DM and its complications is subject of intense research. It has been observed the involvement of the MMPs in the development and progression of various complications of DM [17] and proteases are speculated to have importance for diabetes pathogenesis [18]. Previous studies have demonstrated higher concentrations of MMPs in the serum from type 1 DM [19], type 2 DM patients [20] and in a cell culture model for type 2 DM [21]. By using cultures of endothelial cells, Death and colleagues [22] demonstrated that hyperglycemia enhanced activity and expression of MMP-1,-2, and macrophage-derived MMP-9; while MMP-3 and TIMP-1 had expression and protein levels decreased and unaffected, respectively. These results indicate augmented ECM degradation in hyperglycemic conditions and suggest that MMPs can be useful markers for risk and/or progression of diabetic complications such as diabetic nephropathy and microangiopathy [23].

High levels of blood glucose are also associated to dysfunction of several intracellular signal transduction cascades including generation of reactive oxygen species (ROS), modulation of protein kinases and accumulation of advanced glycation end products (AGEs) (see

Chap. 18) [24]. The receptor for AGE (RAGE) represents the main binding site for AGEs on resident and/or inflammatory cells and this interaction is capable to stimulate the production, expression and activity of MMPs in specific cell lines [25]. RAGEs are mainly located on the surface of macrophages, where they bind to the ECM proteins.

It is recognized that AGEs are able to affect the production and structure of ECM proteins, mainly due to formation of collagen cross-links. Aside from altered mechanical properties of collagen, AGEs can modify the interaction of collagen with other molecules such as proteoglycans (PGs), MMPs and cell integrins [26]. This collagen modification by AGEs have been correlated to inhibited wound repair and exacerbated inflammation in a rotator cuff tendon-bone [27] and medial collateral ligament [28] healing models. If the mechanical effects of AGEs in the collagenous tissue is not well understood, it is known that AGE cross-links are involved in reduced remodeling capacity due to reduced sensitivity to collagenases [29, 30], possibly via alterations in collagen solubility and augmented collagen resistance to protease breakdown [26, 31].

In some tissues, matrix-glycation products and AGEs formation can attract and stimulate cells to release cytokines (e.g., IL-1 and IL-6), MMPs and growth factors (e.g., TNF- α) during wound repair, which in turn accelerates the inflammatory response [32]. It should be noted that certain growth factors (e.g., TGF- β and CTGF) seem to have a role in the pathogenesis of long-term diabetic complications since they have increased concentrations and are associated to ECM accumulation in diabetic nephropathy [17] and tenocyte death and scar formation in tendons [33]. In addition, elevated levels of inducible nitric oxide synthase (iNOS) and prostaglandin E2 (PGE2), disturbance of the insulin secretion/action and its key signalling proteins, genetic polymorphism of MMPs and participation of oxidative stress have all been attributed to play a role in the elevated MMP expression during hyperglycemia in diabetic patients and models [25, 34].

MMPs and Diabetic Tendon Disorders: Current Findings

A growing body of evidence has associated DM to changes in the tendon structure, including abnormalities in fiber structure and organization [35], increased tendon thickness, volume and disorganization obtained by image findings [36, 37], and a tendency of impairing biomechanical properties [38, 39]. Interestingly, these tendon alterations may represent features of the ECM, which is in a constant state of dynamic equilibrium between synthesis and degradation [40].

Besides the relevance of MMPs in the ECM turnover and their role in the tendon pathophysiology [41], data linking these proteases to the development and progression of diabetic tendon disorders is still scarce. In a recent review, Shi and colleagues [39] highlighted the participation of the MMPs in the tendon alterations based in two studies. The first work, from Ahmed and co-workers [42], aimed to investigate the mechanical properties and expressional changes of genes involved in the tendon healing process in a diabetic rodent model. The authors found strong expression of MMP-13 and MMP-3 in tenocytes both in diabetic and healthy tendons; whereas MMP-13 expression was upregulated and MMP-3 expression decreased in the tendon healing model. Moreover, Tsai and others [43] found upregulation of MMP-9 and MMP-13 and increased enzymatic activity of MMP-9 in a tendon cells culture treated with high glucose concentration. Overall, these results indicate that hyperglycemia might induce collagen degradation by increasing MMPs activity and lead to a low-quality tendon structure [39].

MMPs are also implicated in the development of diabetic fibrosis, a pathological finding characterized by ECM accumulation with or without changes in the ECM composition. Cardiac fibrosis, for example, is a major feature of diabetic cardiomyopathy and results in cardiac dysfunction [44]. Fibrosis is often a result of an imbalance between MMPs and TIMPs activity after tissue insults such as hyperglycemia, dyslipidemia and hypertension [17], but

increased production of collagen and other ECM components may be also involved. To date, it is not clear if DM results in fibrosis in uninjured or healing tendons. Previously, a streptozotocin (STZ)-induced diabetes study in Achilles tendons of rats demonstrated areas of higher density of collagen I and collagen fibers disorganization when compared to the controls [45]. It was suggested that increased vascularization associated with cell proliferation and possible migration caused hypercellularity and accounted for over-deposition of collagen in the diabetic group [45]. In fact, recruitment of inflammatory cells have been connected to dysregulation of tenocyte homeostasis followed by secretion of ECM proteins, which results in an increased turnover and remodeling of the tendon ECM [41, 46]. Moreover, MMPs are able to increase release of TGF- β 1 (resident in the tendon's matrix) which in turn may lead to an increased fibroblasts proliferation and collagen I deposition [47]. In this manner, a relationship between MMP/TIMP system and pro-fibrotic growth factors might contribute to fibrosis development due to ECM turnover dysregulation [17].

As previously mentioned, DM is known to produce a poor wound healing due to complications in the connective tissue metabolism, and higher expression of gelatinases in diabetes might be due to the prolonged inflammatory period [48]. In a recent work with STZ-induced diabetes in rats, Mohsenifar et al [49] showed impaired mechanical properties and altered inflammatory response in the diabetic group after Achilles tenotomy. The authors also found lower collagen content in the diabetic group at 15 days after surgery. Taken together, these results corroborate with other studies [42, 50] that point out for a delayed healing process in tendons of diabetic animals. It is noteworthy to mention that different MMPs operate in tendons, participating not only of the ECM degradation but also promoting micro-trauma healing and maintaining normal tendon function [41]. In this manner, besides augmented collagen turnover, other mechanism associated to the pathogenesis of tendinopathies may involve a "failed healing response" via decreased MMP-3

activity, for example [41]. Thus, although not fully elucidated, it is reasonable to highlight the participation of regulators of the ECM turnover (such as MMPs and others) in the tendon's delayed healing response.

Conclusion

MMPs play a role in the mechanisms of several diabetic complications. Although little studied, evidence suggests that diabetes-induced MMP dysregulation may contribute to structural and biomechanical alterations and impair the healing process in tendons.

References

- Klein T, Bischoff R (2011) Physiology and pathophysiology of matrix metalloproteinases. *Amino Acids* 41(2):271–290
- Stamenkovic I (2003) Extracellular matrix remodelling: the role of matrix metalloproteinases. *J Pathol* 200:448–464
- Gioia M, Monaco S, Fasciglione GF et al (2007) 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* 368(4):1101–1113
- Piperi C, Papavassiliou AG (2012) Molecular mechanisms regulating matrix metalloproteinases. *Curr Top Med Chem* 12(10):1095–1112
- Fanjul-Fernandez M, Folgueras AR, Cabrera S et al (2010) Matrix metalloproteinases: evolution, gene regulation and functional analysis in mouse models. *Biochim Biophys Acta* 1803(1):3–19
- Wilson CL, Matrisian LM (1996) Matrilysin: an epithelial matrix metalloproteinase with potentially novel functions. *Int J Biochem Cell Biol* 28(2):123–136
- Qiu W, Bai SX, Zhao MR et al (2005) Spatiotemporal expression of matrix metalloproteinase-26 in human placental trophoblasts and fetal red cells during normal placentation. *Biol Reprod* 72(4):954–959
- Amălinei C, Căruntu ID, Bălan RA (2007) Biology of metalloproteinases. *Rom J Morphol Embryol* 48(4):323–334
- Tortorella M, Pratta M, Liu RQ et al (2000) The thrombospondin motif of aggrecanase-1 (ADAMTS-4) is critical for aggrecan substrate recognition and cleavage. *J Biol Chem* 275(33):25791–25797
- Colige A, Ruggiero F, Vandenberghe I et al (2005) 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* 280(41):34397–34408
- Brew K, Nagase H (2010) The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. *Biochim Biophys Acta* 1803(1):55–71
- Tsioufis C, Bafakis I, Kasiakogias A et al (2012) The role of matrix metalloproteinases in diabetes mellitus. *Curr Top Med Chem* 12(10):1159–1165
- Malemud CJ (2006) Matrix metalloproteinases (MMPs) in health and disease: an overview. *Front Biosci* 11:1696–1701
- Del Buono A, Oliva F, Osti L et al (2013) Metalloproteinases and tendinopathy. *Muscles Ligaments Tendons J* 3(1):51–57
- Wild S, Roglic G, Green A et al (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27(5):1047–1053
- Meetoo D, McGovern P, Safadi R (2007) An epidemiological overview of diabetes across the world. *Br J Nurs* 16(16):1002–1007
- Ban CR, Twigg SM (2008) Fibrosis in diabetes complications: pathogenic mechanisms and circulating and urinary markers. *Vasc Health Risk Manag* 4(3):575–596
- The Diabetes Control and Complications Trial Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986
- Gharagozlian S, Svennevig K, Bangstad HJ et al (2009) Matrix metalloproteinases in subjects with type 1 diabetes. *BMC Clin Pathol* 9:7
- Tayejee MH, Lip GY, MacFadyen RJ (2005) What role do extracellular matrix changes contribute to the cardiovascular disease burden of diabetes mellitus? *Diabet Med* 22(12):1628–1635
- Uemura S, Matsushita H, Li W et al (2001) Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. *Circ Res* 88(12):1291–1298
- Death AK, Fisher EJ, McGrath KC et al (2003) High glucose alters matrix metalloproteinase expression in two key vascular cells: potential impact on atherosclerosis in diabetes. *Atherosclerosis* 168(2):263–269
- Derosa G, Avanzini MA, Geroldi D et al (2005) Matrix metalloproteinase 2 may be a marker of microangiopathy in children and adolescents with type 1 diabetes mellitus. *Diabetes Res Clin Pract* 70(2):119–125
- King GL, Wakasaki H (1999) Theoretical mechanisms by which hyperglycemia and insulin resistance could cause cardiovascular diseases in diabetes. *Diabetes Care* 22:C31–C37
- Ryan ME, Ramamurthy NS, Sorsa T et al (1999) MMP-mediated events in diabetes. *Ann N Y Acad Sci* 878:311–334
- Snedeker JG, Gauthier A (2014) The role of collagen crosslinks in ageing and diabetes – the good, the bad, and the ugly. *Muscles Ligaments Tendons J* 4(3):303–308

27. Bedi A, Fox AJ, Harris PE et al (2010) Diabetes mellitus impairs tendon-bone healing after rotator cuff repair. *J Shoulder Elbow Surg* 19(7):978–988
28. Frank C, McDonald D, Wilson J (1995) Rabbit medial collateral ligament scar weakness is associated with decreased collagen pyridinoline crosslink density. *J Orthop Res* 13(2):157–165
29. Reddy GK, Stehno-Bittel L, Enwemeka CS (2002) Glycation-induced matrix stability in the rabbit Achilles tendon. *Arch Biochem Biophys* 399(2):174–180
30. Reddy GK (2004) Cross-linking in collagen by non-enzymatic glycation increases the matrix stiffness in rabbit Achilles tendon. *Exp Diabetes Res* 5(2):143–153
31. Monnier VM, Mustata GT, Biemel KL et al (2005) Cross-linking of the extracellular matrix by the maillard reaction in aging and diabetes: an update on “a puzzle nearing resolution”. *Ann N Y Acad Sci* 1043:533–544
32. Nesto RW, Rutter MK (2002) Impact of the atherosclerotic process in patients with diabetes. *Acta Diabetol* 2:S22–28
33. Sharir A, Zelzer E (2011) Tendon homeostasis: the right pull. *Curr Biol* 21:025
34. Kadoglou NP, Daskalopoulou SS, Perrea D et al (2005) Matrix metalloproteinases and diabetic vascular complications. *Angiology* 56(2):173–189
35. Grant WP, Sullivan R, Sonenshine DE et al (1997) Electron microscopic investigation of the effects of diabetes mellitus on the Achilles tendon. *J Foot Ankle Surg* 36(4):272–278
36. Papanas N, Courcoutsakis N, Papatheodorou K et al (2009) Achilles tendon volume in type 2 diabetic patients with or without peripheral neuropathy: MRI study. *Exp Clin Endocrinol Diabetes* 117(10):645–648
37. Abate M, Schiavone C, Pelotti P et al (2010) Limited joint mobility in diabetes and ageing: recent advances in pathogenesis and therapy. *Int J Immunopathol Pharmacol* 23(4):997–1003
38. de Oliveira RR, Lemos A, de Castro Silveira PV (2011) Alterations of tendons in patients with diabetes mellitus: a systematic review. *Diabet Med* 28(8):886–895
39. Shi L, Rui YF, Li G et al (2015) Alterations of tendons in diabetes mellitus: what are the current findings? *Int Orthop* 39(8):1465–1473
40. Jones GC, Corps AN, Pennington CJ et al (2006) Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human Achilles tendon. *Arthritis Rheum* 54(3):832–842
41. Sbardella D, Tundo GR, Fasciglione GF et al (2014) Role of metalloproteinases in tendon pathophysiology. *Mini Rev Med Chem* 14(12):978–987
42. Ahmed AS, Schizas N, Li J et al (2012) Type 2 diabetes impairs tendon repair after injury in a rat model. *J Appl Physiol* (1985) 113(11):1784–1791
43. Tsai WC, Liang FC, Cheng JW et al (2013) High glucose concentration up-regulates the expression of matrix metalloproteinase-9 and -13 in tendon cells. *BMC Musculoskelet Disord* 14:255
44. Asbun J, Villarreal FJ (2006) The pathogenesis of myocardial fibrosis in the setting of diabetic cardiomyopathy. *J Am Coll Cardiol* 47(4):693–700
45. de Oliveira RR, Martins CS, Rocha YR et al (2013) Experimental diabetes induces structural, inflammatory and vascular changes of Achilles tendons. *PLoS One* 8(10), e74942
46. Riley GP, Harrall RL, Constant CR, Chard MD et al (1994) Tendon degeneration and chronic shoulder pain: changes in the collagen composition of the human rotator cuff tendons in rotator cuff tendinitis. *Ann Rheum Dis* 53(6):359–366
47. Mendias CL, Gumucio JP, Davis ME et al (2012) Transforming growth factor-beta induces skeletal muscle atrophy and fibrosis through the induction of atrogen-1 and scleraxis. *Muscle Nerve* 45(1):55–59
48. Arul V, Kartha R, Jayakumar R (2007) A therapeutic approach for diabetic wound healing using biotinylated GHK incorporated collagen matrices. *Life Sci* 80(4):275–284
49. Mohsenifar Z, Feridoni MJ, Bayat M et al (2014) Histological and biomechanical analysis of the effects of streptozotocin-induced type one diabetes mellitus on healing of tenotomised Achilles tendons in rats. *Foot Ankle Surg* 20(3):186–191
50. Egemen O, Ozkaya O, Ozturk MB et al (2012) The biomechanical and histological effects of diabetes on tendon healing: experimental study in rats. *J Hand Microsurg* 4(2):60–64