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10.1016/j.matbio.2015.02.005

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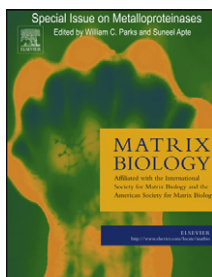
Role of gelatinases in pathological and physiological processes involving the dystrophin–glycoprotein complex

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http://dx.doi.org/10.1016/j.matbio.2015.02.005
Edited by W.C. Parks and S. Apte

Abstract

Dystrophin is a cytosolic protein belonging to a membrane-spanning glycoprotein complex, called dystrophin–glycoprotein complex (DGC) that is expressed in many tissues, especially in skeletal muscle and in the nervous system. The DGC connects the cytoskeleton to the extracellular matrix and, although none of the proteins of the DGC displays kinase or phosphatase activity, it is involved in many signal transduction pathways. Mutations in some components of the DGC are linked to many forms of inherited muscular dystrophies. In particular, a mutation in the dystrophin gene, leading to a complete loss of the protein, provokes one of the most prominent muscular dystrophies, the Duchenne muscular dystrophy, which affects 1 out of 3500 newborn males. What is observed in these circumstances, is a dramatic alteration of the expression levels of a multitude of metalloproteinases (MMPs), a family of extracellular Zn^{2+}-dependent endopeptidases, in particular of MMP-2 and MMP-9, also called gelatinases. Indeed, the enzymatic activity of MMP-2 and MMP-9 on dystroglycan, an important member of the DGC, plays a significant role also in physiological processes taking place in the central and peripheral nervous system. This mini-review discusses the role of MMP-2 and MMP-9, in physiological as well as pathological processes involving members of the DGC.

Introduction

Dystrophin is a cytosolic protein associated to a glycoprotein complex, the dystrophin–glycoprotein complex (DGC), composed by the intracellular α and β-syntrophin, α-dystrobrevin and neuronal nitric oxide synthase (nNOS), the transmembrane β-dystroglycan, α-, β-, γ- and δ-sarcoglycan and sarcospan, and the extracellular α-dystroglycan [1,2]. The DGC is expressed in a wide variety of tissues, especially in skeletal muscle and in the nervous system, and provides a strong contribution to the sarcolemma stability. The DGC represents an important link between the cytoskeleton and the extracellular matrix, in several respects. It is directly and indirectly involved in different signal transduction pathways. As an example, nNOS, an enzyme activated by muscle contraction, produces nitric oxide from L-arginine, which in turn triggers the production of cGMP catalyzed by the guanylyl cyclase; the second messenger cGMP stimulates vasodilatation favoring the blood influx into the contracting muscle [3–5]. Furthermore, some DGC members, including dystrobrevin [6], syntrophins [7] and β-dystroglycan [8], serve as platforms to recruit phosphatases and kinases involved in signal transduction pathways. In addition to their indirect involvement in signal transduction pathways, the two dystroglycan subunits, α- and β, which represent the DGC inner core, interact in a non-covalent fashion through the C-terminal domain of α-dystroglycan and the β-dystroglycan ectodomain [9], playing a key role in maintaining the connection

between the cytoskeleton and the extracellular matrix through formation of a multitude of interactions [10].

This network of interactions contributes to the plasma membrane stability in normal conditions, on the other hand, the connection between the cytosol and the extracellular matrix needs to be interrupted when tissue remodeling takes place. In such circumstances, an overexpression of some matrix–metalloproteinases (MMPs) is often observed. These proteins belong to a family of Zn^{2+}-dependent extracellular endopeptidases involved in many physiological (such as morphogenesis, development, cell migration, proliferation and adhesion) as well as pathological processes, such as cancer, neurodegeneration, inflammation and muscular dystrophy [11].

Although dystrophin has recently been proposed as a new target of MMP-2 during ischemic injury [12] and a decrease in γ-sarcoglycan levels has been shown to correlate with an increase of MMP-2 activity, in an animal model of right ventricular failure [13], dystroglycan remains the only ascertained and direct DGC target of MMP-2 and MMP-9. In vitro, the two gelatinases disrupt the recombinant β-dystroglycan ectodomain by two distinct molecular mechanisms. MMP-9 induces a first cleavage leaving an intact C-terminal region of about 30 amino acids and an N-terminal region that is further processed [14], whereas MMP-2 produces multiple early cleavages on the entire protein [15] (see Fig. 1). MMP-2 has lately also been found to exert a significant proteolytic activity on native and recombinant α-dystroglycan in vitro [16].

Dystroglycan degradation is driven by gelatinases in physiological conditions

The ectodomain of β-dystroglycan represents the Achille's heel of the DGC. The possibility of a proteolytic breakdown, likely to take place at the ectodomain of β-dystroglycan, was first postulated after observing the electrophoretic behavior of a 30 kDa β-dystroglycan fragment in carcinoma cell lines [17].

The first direct evidence of an enzymatic activity driven by an MMP on dystroglycan comes from a study of Yamada and colleagues who found a truncated form of β-dystroglycan, devoid of part of its ectodomain and therefore unable to maintain its link with α-dystroglycan, in healthy tissues, such as peripheral nerve, kidney, lung and smooth muscle. In the same study the authors indicated the metalloproteinases MMP-2/MMP-14 and MMP-9, as the main players involved in the production of this 30 kDa β-dystroglycan fragment [18].
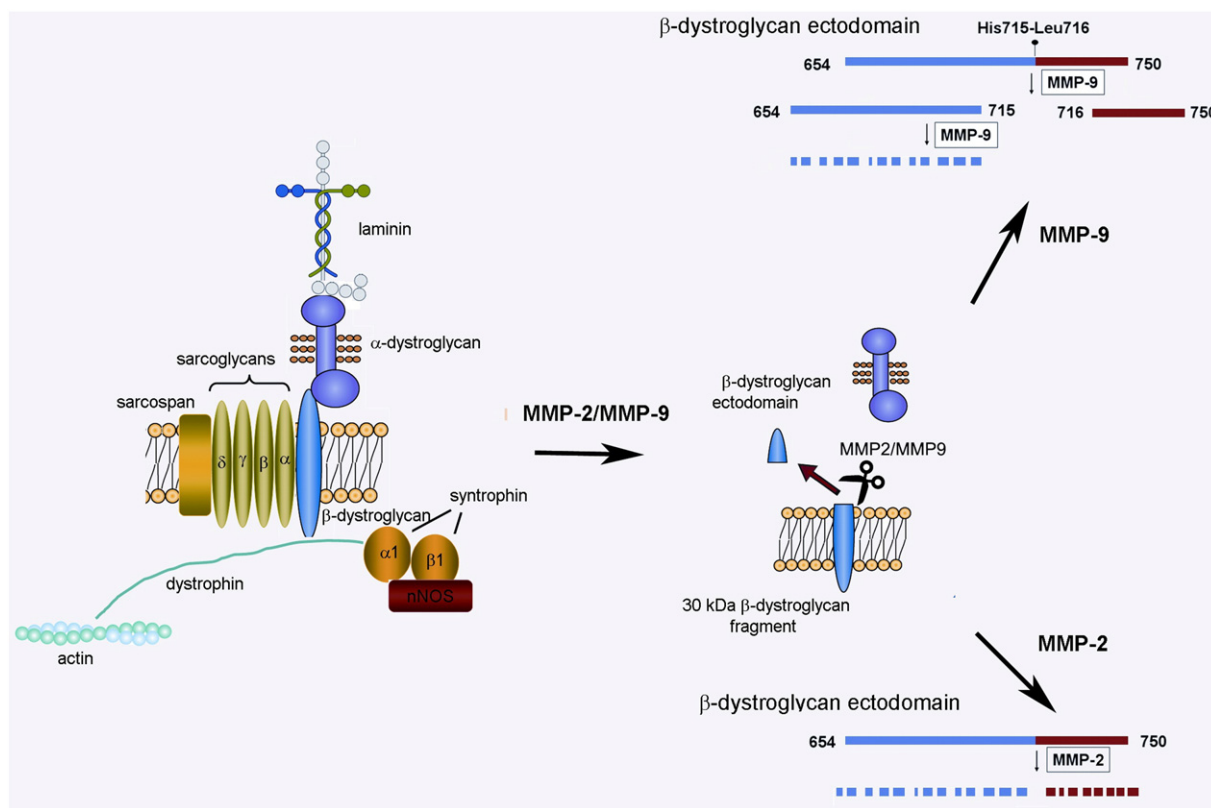
Fig. 1. *Scheme of the DGC and β-dystroglycan degradation driven by gelatinases.* In vitro, MMP-9 catalyzes a first cleavage within the β-dystroglycan ectodomain producing an intact C-terminal region of about 30 amino acids and an N-terminal region that is further processed [14], while MMP-2 induces multiple cleavages on the entire ectodomain [15].

Further studies highlighted that MMP-2, rather than MMP-9, is constitutively expressed in injured neurons and is responsible for the production of the 30 kDa β-dystroglycan fragment. This leads to loss of pre- and post-synaptic connections preceding the synaptic remodeling induced by injury [19].

A basal enzymatic activity level of the two gelatinases producing low amounts of the 30 kDa β-dystroglycan fragment was also found in healthy neurons and hippocampus, but, under stimulation with glutamate or bicuculline, the MMP-9 driven cleavage of β-dystroglycan was significantly increased in post-synaptic elements and in the hippocampus, indicating that this process might be involved in learning and memory processes [20]. A consistent increase of the MMP-9 levels, induced by the transcription factor AP-1, with a consequent increase of the 30 kDa β-dystroglycan fragment, has also been observed in animals subjected to fear conditioning [21]. It was hypothesized that the MMP-9-driven cleavage of β-dystroglycan might reorganize the network of interactions that dystroglycan, expressed in the post-synaptic elements, establishes with other proteins, such as neurexin, expressed in the pre-synapse. The β-dystroglycan cleavage might influence also the cytoskeletal organization; for example, in Schwann cells, it was found that the 30 kDa β-dystroglycan displays a higher affinity for a short isoform of utrophin, UP71, than for the dystrophin isoform Dp116, whose interaction with full length β-dystroglycan is indeed stronger than that with its truncated form [22]. Accordingly, it has been revealed that in normal nerves MMP-2 and MMP-9 (that are differentially expressed during development) shed the N-terminal extracellular domain of β-dystroglycan making it not longer able to bind α-dystroglycan, thus modulating the dystroglycan complex composition and the size of the Schwann cell compartments [23]. Recently, a gelatinolytic activity targeting β-dystroglycan has been proposed to be involved in the remodeling of the postsynaptic domain of the neuromuscular junction after physical exercise [24].

Gelatinases are overexpressed in DGC-associated muscular dystrophies

Duchenne muscular dystrophy is a lethal X-linked disease due to a mutation in the dystrophin gene causing complete loss of protein function [25]. Muscular tissues of patients affected by Duchenne muscular dystrophy show sarcolemma instability, loss of Ca^{2+} homeostasis and reduced resistance to mechanical stress with consequent degeneration of muscular fibers followed by cycles of regeneration [26]. Complete regeneration of the muscular fibers is prevented by inflammatory cell invasion that triggers fibrosis, progressively reducing the amount of func-

tional muscular tissue [1]. Many proteases, including calpains, caspases and lysosomal proteases, increase their enzymatic activity in these conditions; although controversial, an increased proteasome activity has also been found [27].

In such circumstances, the integrity of the entire DGC is severely compromised; besides loss of dystrophin, the levels of nNOS [28,29] and of one or both the dystroglycan subunits are dramatically reduced, when they are not completely lost [30].

In 1999, Kherif and colleagues observed that the expression and the activity of MMP-9 were increased in mice deficient of the dystrophin gene, called mdx mice, which represent an animal model of Duchenne muscular dystrophy [31]. This observation has been corroborated by many other studies that revealed an upregulation of gelatinases also in hearts of mdx mice [32–34].

MMP-2 and MMP-9 are overexpressed also in the brain of mdx mice and they co-localize with the vascular endothelial growth factor (VEGF), thus explaining the increased angiogenesis and vascular permeability observed in the brain of mdx mice [35].

Recently, many studies revealed that MMP-9 also increases in serum and muscular biopsies of patients affected by Duchenne muscular dystrophy and that the MMP-9 levels correlate with the severity of the disease [36].

Other members of the MMPs family display altered expression patterns and enzymatic activities in the dystrophic phenotype. For example, an increased activity of MMP-1, which limits fibrosis mainly by degrading collagen I and III, has been reported in dystrophic muscles, during remodeling of the extracellular matrix [34]. Altered levels and activities of MMP-1 and MMP-7, favoring a fibrotic phenotype, have also been found in fibroblasts from Duchenne muscular dystrophy biopsies [37], whereas overexpression of MMP-10 in dystrophic muscles plays a role in the muscle regeneration process [38]. Interestingly, alterations of the MMP levels are very often accompanied by alterations in the levels of TIMP-1 and TIMP-2, the endogenous inhibitors of MMPs [39,40]. In general, the increased expression of MMPs in mdx mice is probably due to macrophages, mast cells and fibroblasts that infiltrate the muscular tissue in pathological conditions and secrete different endopeptidases [39]. Indeed, it is well known that fibroblasts express high amounts of MMPs [41,42].

What is the exact role of gelatinases in the pathogenesis of DGC-related muscular dystrophies? This question still awaits a complete and exhaustive answer. A characteristic shared by many neuromuscular diseases is the presence of the aforementioned 30 kDa β-dystroglycan fragment that therefore represents an interesting biomarker (see Fig. 1). Indeed, this peptide was revealed in skeletal muscle of tissues affected by Duchenne

muscular dystrophy [43] and sarcoglycanopathy [43,44].

The quest for therapeutic targets: inhibiting the gelatinases activity by molecular genetics or pharmacological methods

Important clues regarding the role of gelatinases in the pathogenesis of DGC-related muscular dystrophies come from studies in which the corresponding genes have been up- or down-regulated in healthy and/or mdx mice or have been inhibited by molecular drugs.

In general, genetic ablation of MMP-9 in mdx mice improves the dystrophic phenotype, as indicated by a number of parameters [33,45,46]. For example, disruption of the MMP-9 gene reduces the serum levels of creatine kinase (a marker of muscular fibers damage), as well as the amount of macrophages that infiltrate muscular fibers, promotes muscle regeneration and ameliorates the fibers' stability increasing the amount of the two known targets of MMP-9, β-dystroglycan and collagen IV, this latter being essential for the stability of the basal membrane. Moreover, genetic depletion of MMP-9 increases nNOS while reducing the levels of caveolin-3 [45]. It was also observed that partial deletion of MMP-9 in 8-week old mdx mice i) improves the number of satellite cells in dystrophic muscles, ii) suppresses the pro-inflammatory M1 macrophages that contribute to muscular necrosis, and iii) increases the anti-inflammatory M2 macrophages that stimulate muscle regeneration by reducing the transcript levels of the inflammatory cytokines INF-γ and IL-6 while increasing the ones of the anti-inflammatory cytokine IL-4 [46].

Surprisingly, MMP-9 ablation in mdx mice reduces fibrosis by reducing the levels of collagen I and III [45], whereas overexepression of MMP-9 in healthy mice suppresses fibrosis by reducing the levels of collagen I and IV but not collagen III [47]. These contradictory results may be in part explained considering that in pathological conditions, MMP-9 deletion alters the levels of other MMPs, whose specificity is often overlapping, and/or of their endogenous inhibitors [33]. Deregulation of other cytokines and inflammatory cells that modulate the entire fibrotic process, makes this scenario even more complex [47]. Interestingly, in addition to reducing fibrosis, overexpression of an active form of MMP-9 in healthy mice stimulates growth and regeneration of the muscle fibers (also increasing their cross-sectional area), promotes the fusion of myoblasts into myotubes, and improves the contractile force in soleus muscle. Accordingly, an upregulation of the insulin growth factor and follistatin, both promoting skeletal muscle regeneration and growth [48,49], is observed [47].

Disruption of the MMP-2 gene in mdx mice leads to different results compared to the MMP-9 knockout. Indeed, ablation of MMP-2 significantly reduces muscle regeneration, as indicated by decreased centronuclear regenerating fibers. This is probably due to decreased levels of vascular endothelial growth factor-A (VEGF-A) that impair angiogenesis by reducing the mean size of the vessels. Nitric oxide (NO) is a vasodilator, produced by nNOS from arginine, which stimulates capillary proliferation [50]. Depletion of MMP-2 gene in mdx mice further reduces the levels of nNOS. Surprisingly, degradation of full-length β-dystroglycan producing the 30 kDa fragment occurs at 1 month of age but not at 3 months [51].

These studies suggest that gelatinase overexpression compensates for muscular damage. For example, they partially degrade the basal lamina favoring the migration and fusion of myogenic cells devoted to regeneration of the damaged fibers [31,52]. A sustained and prolonged activation of the two gelatinases, on the other hand, leads to an excessive protein degradation that exacerbates the dystrophic phenotype.

In accordance with the knockout studies, suramin, an antifibrotic drug, restores the diaphragm function, decreasing the activity of MMP-9 but not that of MMP-2, and increasing the β-dystroglycan levels [53]. Moreover, spironolactone, an aldosterone antagonist, and lisinopril, an angiotensin-converting enzyme (ACE) inhibitor, if co-administered before functional impairment reduce the damage of cardiac and skeletal muscle by decreasing the gelatinase activity [54]. Other molecules can be listed that ameliorate the dystrophic phenotype by suppressing the gelatinase activity. Among them, drugs that support the signal transduction pathway mediated by nitric oxide, which is otherwise attenuated in mdx mice, have beneficial effects on these animal models. For example, L-arginine, a target of nNOS enzyme, reduces the β-dystroglycan cleavage and reinforces its interaction with utrophin, by suppressing the MMP-2 and MMP-9 activity. A correct re-localization of nNOS is also observed in short-term treated mdx mice [55], although prolonged treatment with this drug stimulates fibrosis and is no longer effective on the gelatinase activity [56]. Similar results are obtained with Sildenafil, an inhibitor of phosphodiesterase 5, an enzyme that reduces the levels of cGMP, a second messenger involved in the NO induced signal transduction pathway, reinforcing NO-driven signaling [57]. Beneficial effects were obtained also with Batimastat, a broad-spectrum MMP inhibitor, which reduces fibrosis and the number of macrophages and increases the diaphragm force restoring the levels of nNOS and β-dystroglycan [40]. A mixture of free radical scavengers, composed of α-lipoic acid and L-carnitine, also reduces damages of dystrophy, suppressing the gelatinase activity and increasing the levels of β-dystroglycan [58].

All these data strongly support the view that MMPs may contribute to the progression of DGC-linked muscular dystrophy, mainly by degrading β-dystroglycan.

Downregulation or pharmacological inhibition of gelatinases suppresses the MAPK kinase cascades

In various dystrophic models the susceptibility to contraction-induced injury and the sarcolemma integrity are not always clearly correlated with fiber degeneration and muscular dystrophy [59-61]. This suggests that some other factors are likely to contribute to muscle cell degeneration and its apoptosis. Detrimental signal transduction pathways have been indicated as a possible cause of muscular dystrophy. In mdx mice, an alteration of the mitogen-activated protein kinase (MAPK) signaling cascades, that regulate proliferation, differentiation and cell-survival, has been observed; dystrophic animals in fact display an increased phosphorylation of the extracellular signal regulated kinases 1 (ERK1) and 2 (ERK2) [55,62,63] and of the c-jun N-terminal kinases 1 (JNK1) and 2 (JNK2) [64–66], and a reduction in the phosphorylation of p38 [63]. Activation of the ERK1/2 pathway has been found also in limb–girdle muscular dystrophy, due to a deficiency in γ-sarcoglycan [67]. Moreover, the phosphatidylinositol 3-kinase (PI3K/Akt) signaling pathway, which regulates cell viability and protein synthesis [68], resulted altered in DMD and limb–girdle muscular dystrophy, showing an increased synthesis and phosphorylation of Akt to compensate muscular damages [69,70]. Indeed, an increase of the expression levels of Akt in mdx mice stimulates muscle regeneration and muscle hypertrophy, by elevating the levels of utrophin [71].

Interestingly, overexpression of MMP-9 in healthy mice leads to increased phosphorylation levels of Akt as well as of mTOR and FOXO1, two of its downstream phosphorylation targets (the PI3K/Akt/mTOR pathway promotes protein synthesis and hypertrophy, whereas FOXO1 stimulates protein degradation, by modulating the ubiquitin–proteasome pathway) rather than of ERK1/2 and JNK1/2, whereas p38 MAPK phosphorylation is reduced. Conversely, ablation of MMP-9 in mdx mice leads to reduced phosphorylation levels of Akt and p38 MAPK, but does not affect phosphorylation levels of ERK1/2 and JNK1/2 in the diaphragm of mdx mice [33]. In the hearts of mdx mice, depletion of MMP-9 reduces the phosphorylation of ERK1/2 and Akt, thus stimulating heart hypertrophy, but not the phosphorylation of JNK1/2 and p38 MAPK [47]. Many drugs that ameliorate the dystrophic phenotype by suppressing the gelatinase activity also affect the MAPK kinase signaling. For example, a mixture of α-lipoic acid and L-carnitine reduces the phosphorylation levels of p38 MAPK, ERK1/2 and JNK1/2 kinases, with a concomitant reduction of the creatine kinase levels [58]. Moreover, Batimastat reduces the levels of phosphorylated ERK1/2 and p38 MAPK, but does not affect the levels of phosphorylated JNK1/2 in mdx mice [41], although it should be noted that this drug may have a more complex effect, since it inhibits also other MMPs as well as ADAM proteases [72,73].

However, the reason why and the mechanism by which downregulation, or pharmacological inhibition of MMPs suppresses MAPK kinase cascades has yet to be clarified. There is some evidence that some transcription factors or cytokines, involved in inflammation and fibrosis, may represent a liaison between inhibition of MMPs and suppression of MAPK kinase cascades.

The nuclear factor (NF)-kB, a ubiquitous transcription factor, which activates genes involved in inflammation, immune and stress responses [74,75] and induces the expression of MMP-9, is overexpressed in mdx mice and in DMD patients [76–78]. MMP-9 expression is also induced by the transcription factor activator protein-1 (AP-1) [79,80]. Genetic ablation of MMP-9 in mdx mice reduces the activity of NF-kB and AP-1, suggesting that MMP-9 regulates its own expression by a positive feedback mechanism [45]. On the other hand, activation of MAPK signaling triggers the expression of many genes involved in inflammation and fibrosis, including AP-1 and NF-kB.

All these observations suggest that MAPK signaling, as well as the activities of NK-kB, AP-1 and gelatinases, might be reciprocally regulated. Indeed, the downregulation of MAPK signaling may produce beneficial effects both per se and by reducing the gelatinase levels, with possible important repercussions for the development of effective therapeutic protocols for muscular dystrophies.

Gelatinase;
Duchenne muscular dystrophy

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