Matrix Metalloproteinase Activity that Disrupts the Dystroglycan Complex: Its Role in the Molecular Pathogenesis of Muscular Dystrophies

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Abstract

The dystroglycan (DG) complex, composed of two subunits αDG and βDG, interacts with the sarcoglycan complex to form the dystrophin-glycoprotein complex. αDG is a cell surface peripheral membrane protein which binds to the components of the extracellular matrix, while βDG is a type I integral membrane protein which anchors αDG to the cell membrane via the N-terminal extracellular domain. Although defects of the DG gene have not been identified as the primary causes of hereditary diseases in humans, secondary but significant abnormalities of the DG complex have been revealed in a number of muscular dystrophies. In this article, we characterize the matrix metalloproteinase (MMP) activity that disrupts the DG complex by cleaving the extracellular domain of βDG and discuss if this MMP plays a role in the molecular pathogenesis of muscular dystrophies. We also address the therapeutic potential of the drugs that inhibit this MMP activity to decelerate muscle degeneration in these diseases.

Key Words: Dystroglycan; Matrix metalloproteinase; Extracellular matrix; Cardiomyopathic hamster; Sarcoglycanopathy.

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I. Introduction: Molecular organization of the dystroglycan (DG) complex

The DG complex is a cell membrane-spanning complex composed of two subunits αDG and βDG [13]. In muscle, the DG complex interacts with the sarcoglycan complex to form the dystrophin-glycoprotein complex. αDG is a cell surface peripheral membrane protein which binds to the components of the basement membrane, laminin, agrin and perlecan, while βDG is a type I integral membrane protein which anchors αDG to the cell membrane via the N-terminus of the extracellular domain and binds to the cytoskeletal protein dystrophin via the C-terminal cytoplasmic domain [8, 13, 20, 27, 29]. Thus, the DG complex provides a tight link between the extracellular matrix (ECM) and intracellular cytoskeleton via the cell membrane. At present, the role of the DG complex in the assembly and maintenance of the basement membrane remains controversial [7, 11, 15, 30].

These recent findings indicate that the DG complex needs to be disrupted efficiently when tissue remodeling takes place in various conditions and suggest that a specific device may exist for this purpose [31]. As such a device, we have recently identified a matrix metalloproteinase (MMP) activity that cleaves the extracellular domain of βDG [31]. In this paper, we characterize this MMP activity and discuss its role in the molecular pathogenesis of muscular dystrophies. We also address the therapeutic potential of MMP inhibitors to decelerate muscle degeneration in these diseases.

II. Characterization of the MMP activity that disrupts the DG complex

In skeletal muscle, βDG is detected as a single 43-kDa band (βDG_{full}) by immunoblot analysis using the monoclonal antibody 43DAG/8D5 against the C-terminal cytoplasmic tail of βDG [1, 31] (fig. 1). 43DAG/8D5 also detects a 30-kDa fragment of βDG (βDG_{30}) in several non-muscle tissues [31]. We clarified the tissue distribution of βDG_{30} by immunoblot analysis of various bovine tissues using 43DAG/8D5. βDG_{30} was detected in peripheral nerve, smooth muscle, lung and kidney, whereas it was obscure or undetectable in cardiac muscle, skeletal muscle, cerebrum and cerebellum [31].

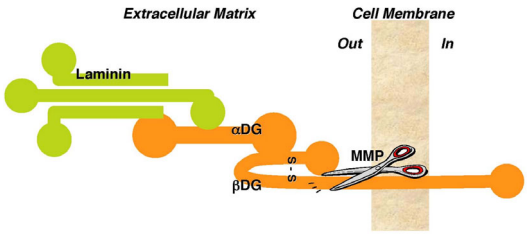

Fig. 1. Schematic model of the MMP activity (DG-MMP) that disrupts the DG complex by cleaving the extracellular domain of βDG.

To compare the biochemical properties of βDG_{30} with βDG_{full}, we performed extraction analysis of the crude membranes of bovine peripheral nerve and rat RT4 schwannoma cells, which express both βDG_{full} and βDG_{30} [31]. Although βDG_{full} and βDG_{30} were both extracted by 2% Triton X-100, they were not extracted by pH 11 or by 10 mM EDTA [31]. Because βDG is a type I integral membrane protein having a single transmembrane domain, these results indicate that βDG_{30} retains this transmembrane domain. Also because βDG_{30} is recognized by 43DAG/8D5 directed against the C-terminus of the cytoplasmic domain of βDG, these results indicate that the predicted cleavage site exists in the extracellular domain of βDG and βDG_{30} is its C-terminal fragment.

To test the hypothesis that MMP may be responsible for the processing of βDG_{full} into βDG_{30}, we cultured RT4 cells in the presence or absence of N-biphenyl-sulfonyl-phenylalanine hydroxamic acid (BPHA), which is a highly specific hydroxamate MMP inhibitor [19], harvested the living cells and performed immunoblot analysis using 43DAG/8D5. βDG_{30} decreased with increasing concentrations of BPHA [31]. We also tested the effects of 1,10-phenanthroline, which is a transition metal ion chelator and well established as a MMP inhibitor. Because RT4 cells did not grow well in the presence of 1,10-phenanthroline, we instead incubated the total homogenates of harvested RT4 cells in the presence of 1,10-phenanthroline at 37°C for varying time periods. 1,10-Phenanthroline inhibited the proteolysis of βDG in a concentration-dependent manner (Fig. 2). These results indicate that βDG_{30} is the processing fragment by MMP and that this MMP is active for the living RT4 cells. We tentatively refer to this MMP activity as DG-MMP in this article.

We next tested the effects of another newly developed hydroxamate derivative, (2R)-3-(1H-Indol-3-yl)-2-[4-(2-phenyl)-2H-tetrazol-5-yl] benzenesulfonylamino] propionoc acid (MMI-166), which has a narrower range of inhibitory activity than BPHA [18]. When RT4 cells were cultured in the presence of BPHA or MMI-166, BPHA, but not MMI-166, inhibited the proteolysis of βDG in a concentration-dependent manner (Fig. 3). In addition, BPHA, but not MMI-166, inhibited the

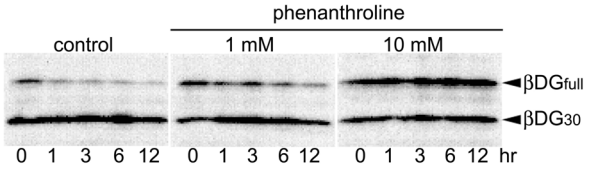

Fig. 2. 1,10-Phenanthroline inhibits the processing of βDG by DG-MMP. After 3 days of culture, RT4 cells were harvested, homogenized and then incubated in the presence or absence of 1,10-phenanthroline at 37°C for various time periods. The homogenates were analyzed by immunoblotting using 43DAG/8D5. 1,10-Phenanthroline inhibited the proteolysis of βDG in a concentration-dependent manner.

proteolysis of βDG when added to the total homogenates of harvested RT4 cells (not shown). Based on the inhibitory profiles of these reagents [18, 19], we presume that DG-MMP is distinct from MMP-1, MMP-2/MMP-14 or MMP-9. To clarify if DG-MMP is present in RT4 cells themselves or secreted into the culture medium, RT4 cells were harvested, homogenized and then incubated at 37°C for various times. βDG_{full} decreased and βDG_{30} increased with time, indicating that DG-MMP was present in RT4 cells themselves, not secreted into the culture medium [31]. We are currently investigating the possibility that DG-MMP might be a membrane-type MMP.

To know if the processing of βDG by DG-MMP affects the integrity of the DG complex, we performed sucrose density gradient sedimentation analysis of RT4 cell membranes. βDG_{full}, but not βDG_{30}, co-sedimented with αDG (Fig. 4). We isolated the DG complex from the RT4 cell membranes by laminin affinity chromatography. βDG_{full} co-isolated with αDG, which bound to laminin-Sepharose directly as a laminin-binding protein [31]. However, βDG_{30} did not co-isolate with αDG [31]. We isolated the DG complex from the RT4 cell membranes by wheat germ agglutinin (WGA) affinity chromatography. αDG and βDG_{full} were completely absorbed by WGA-Sepharose and recovered in the eluates [31]. However, βDG_{30} was not absorbed and undetectable in the eluates [31]. All together, these results indicate that βDG_{full}, but not βDG_{30}, is complexed with αDG and thus that the MMP cleavage of βDG into βDG_{30} disintegrates the DG complex. This is consistent with the report that the αDG-binding site exists in the N-terminus of the extracellular domain of βDG [27], since βDG_{30} is the C-terminal fragment of the cleavage. Because αDG and βDG are responsible for the binding to the ECM and cell membrane respectively, DG-MMP disrupts the link between the ECM and cell membrane via the DG complex.

III. Implications of DG-MMP in the pathogenesis of diseases

1. Cancer invasion/metastasis and infectious diseases

The aforementioned findings will have important implications in an array of pathological phenomena. For instance, it has been shown recently that certain carcinoma cell lines express β-DG_{30} abundantly [17]. Taken together with our results, carcinoma cells are presumed to employ DG-MMP to disrupt the dystroglycan complex. This will enable carcinoma cells to metastasize and invade other tissues. Interestingly in this respect, BPHA has been developed as a drug to inhibit cancer spread and metastasis [18, 19].

Processing of βDG by DG-MMP may also play a role in the molecular pathogenesis of viral and bacterial infections. It has been shown recently that pathogens such as arena viruses (several strains of lymphocytic choriomeningitis virus and Lassa fever virus) and *Mycobacterium leprae* bind to the cell surface αDG as an initial step of host cell infection [5, 23, 26]. Therefore, DG-MMP might be a natural defense mechanism against these pathogens, in analogy to matrilysin (MMP-7), which has been shown to play a defensive role against microorganisms in mucosal epithelial cells [16].

2. Muscular dystrophies

Another situation where DG-MMP is implicated is the molecular pathogenesis of hereditary neuromuscular diseases. Over the last 10 years, primary genetic defects have been identified in a number of these diseases. However, the precise molecular pathways by which the primary defects lead to muscle cell degeneration eventually in these diseases have not necessarily been clarified. Studies to elucidate the biological functions and dysfunctions of the proteins which work in close concert with the causative proteins *in vivo* will be useful in this context. As such, a research on DG processing by DG-MMP could provide us precious clues concerning the molecular pathogenesis of muscular dystrophies caused by the primary defects of the components of the dystrophin-glycoprotein complex and its related proteins, because abnormalities of the DG complex are well known in theses diseases.

For instance, defective glycosylation of αDG has been demonstrated in several forms of congenital muscular dystrophies, including Fukuyama-type congenital muscular dystrophy, MDC1C/LGMD2I, muscle-eye-brain disease, Walker-Warburg syndrome and $Large^{myd}$ mice, which are the model animals of congenital muscular dystrophy [2, 3, 9, 10, 14, 21, 32]. These diseases are all caused by the primary defects of the genes encoding glycosyltransferases, which are presumed to disturb glycosylation of αDG. Because glycosylation of αDG is crucial for the binding of laminin [6, 20], its defect is expected to perturb this binding and result in the disruption of the ECM-cell membrane linkage via the DG complex. This scenario is supported by the finding that the antibody against the carbohydrate residues of αDG involved in the binding of laminin induced a dystrophic phenotype in cultured muscle cells [4].

Another intriguing example is sarcoglycanopathy. Having the mutation of the δ-sarcoglycan gene, a genomic deletion including the first exon, cardiomyopathic hamsters are the model animals of sarcoglycanopathy LGMD2F [22, 25]. It is noteworthy that αDG has been shown to be dissociated from βDG and not recovered in the membrane fraction in the muscle of these animals [24, 28]. It has also been shown that αDG is reconstituted into the DG complex when dystrophic changes are corrected by the adenovirus transfer of the δ-sarcoglycan gene [12]. Furthermore, similar observations have been reported in other types of sarcoglycanopathies and their model animals. Based on these observations, we are currently analyzing the activity of DG-MMP in cardiomyopathic hamsters. Preliminary results indicate that DG-MMP is activated in the muscle of these animals (manuscript in preparation). We presume that activation of DG-MMP causes the disruption of the link between the ECM and

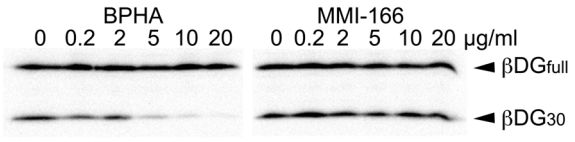

Fig. 3. BPHA, but not MMI-166, inhibits the processing of βDG by DG-MMP. Various concentrations of BPHA or MMI-166 were added to the culture medium of RT4 cells. After 3 days of culture, cells were harvested, homogenized and analyzed by immunoblotting using 43DAG/8D5. While BPHA inhibited the proteolysis of βDG in a concentration-dependent manner, MMI-166 did not.

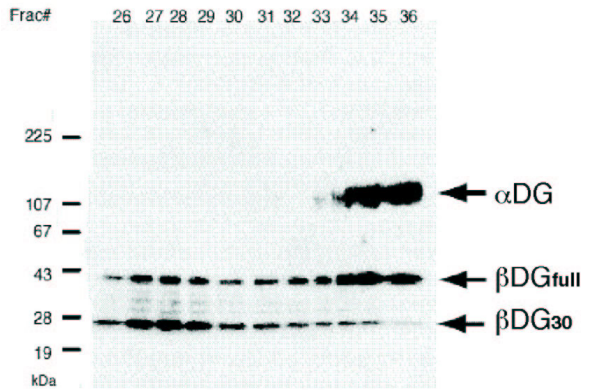

Fig. 4. βDG_{full}, but not βDG_{30}, co-sediments with αDG. Sucrose density gradient sedimentation of RT4 cell membranes was performed and analyzed by immunoblotting using 43DAG/8D5. βDG_{full}, but not βDG_{30}, co-sedimented with αDG.

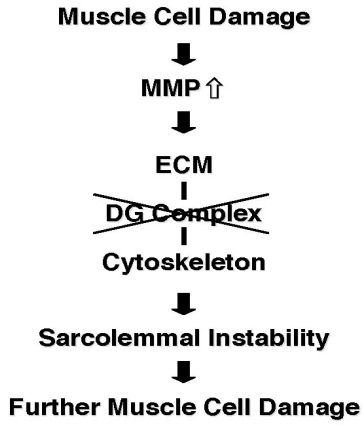

Fig. 5. Hypothetical scheme of the role of DG-MMP in the molecular pathogenesis of muscular dystrophies.
DG-MMP is hypothesized to be activated by the initial muscle cell damage in certain muscular dystrophies. This results in the disruption of the ECM-cell membrane linkage via the DG complex, which, in turn, destabilizes the sarcolemma and further augments muscle cell damage in a vicious cycle.

cell membrane via the DG complex in the muscle of cardiomyopathic hamsters and this may play an important role in the pathogenesis of muscle degeneration.

IV. Therapeutic implications of DG-MMP: do inhibitors of DG-MMP decelerate muscle degeneration in muscular dystrophies?

If DG-MMP turns out to be activated in certain muscular dystrophies, this will have significant implications for the molecular pathogenesis of muscle degeneration in these diseases, because the resulting disruption of the ECM-cell membrane linkage via the DG complex is expected to further augment muscle cell damage in a vicious cycle (Fig. 5). Moreover, it will raise the intriguing possibility of a novel pharmacological therapy for these diseases. The MMP inhibitors effective against DG-MMP have been developed as anti-cancer reagents and demonstrated to be not only effective but also safe without serious side effects when administered orally [19]. We are currently planning the oral administration of DG-MMP inhibitors to cardiomyopathic hamsters to see their effects on the progression of muscle degeneration.

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