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Non-Glycanated Biglycan and LTBP4: Leveraging the extracellular matrix for Duchenne Muscular Dystrophy therapeutics.

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Abstract

The extracellular matrix (ECM) plays key roles in normal and diseased skeletal and cardiac muscle. In healthy muscle the ECM is essential for transmitting contractile force, maintaining myofiber integrity and orchestrating cellular signaling. Duchenne Muscular Dystrophy (DMD) is caused by loss of dystrophin, a cytosolic protein that anchors a transmembrane complex and serves as a vital link between the actin cytoskeleton and the basal lamina. Loss of dystrophin leads to membrane fragility and impaired signaling, resulting in myofiber death and cycles of inflammation and regeneration. Fibrosis is also a cardinal feature of DMD. In this review, we will focus on two cases where understanding the normal function and regulation of ECM in muscle has led to the discovery of candidate therapeutics for DMD. Biglycan is a small leucine rich repeat ECM protein present as two glycoforms in muscle that have dramatically different functions. One widely expressed form is biglycan proteoglycan (PG) that bears two chondroitin sulfate GAG chains (typically chondroitin sulfate) and two N-linked carbohydrates. The second glycoform, referred to as ‘NG’ (non-glycanated) biglycan, lacks the GAG side chains. NG, but not PG biglycan recruits utrophin, an autosomal paralog of dystrophin, and an NOS-containing signaling complex to the muscle cell membrane. Recombinant NG biglycan can be systemically delivered to dystrophic mice where it upregulates utrophin at the membrane and improves muscle health and function. An optimized version of NG biglycan, ‘TVN-102’, is under development as a candidate therapeutic for DMD. A second matrix-embedded protein being evaluated for therapeutic potential is latent TGF β binding protein 4 (LTBP4). Identified in a genomic screen for modifiers of muscular dystrophy, LTBP4 binds both TGF β and myostatin. Genetic studies identified the hinge region of LTBP4 as linked to TGF β release and contributing to the “hyper-TGF β ” signaling state that promotes fibrosis in muscular dystrophy. This hinge region can be stabilized by antibodies directed towards this domain. Stabilizing the hinge region of LTBP4 is expected to reduce latent TGF β release and thus reduce fibrosis.

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The Muscular Dystrophies and the ECM

The muscular dystrophies are a group of >40 hereditary diseases characterized by progressive degeneration and/or loss of muscle tissue. Duchenne Muscular Dystrophy (DMD) is the most common and well characterized form of muscular dystrophy and arises due to loss of function of dystrophin [1]. Dystrophin is an extended intracellular protein that tightly adheres to the inner surface of the plasma membrane, which is known as the sarcolemma in cardiac, skeletal and smooth muscle. As an X-chromosome gene, mutations that disrupt dystrophin leads to DMD in males. DMD disorder manifests in early life as a loss of walking by about age 13 and with accompanying cardiomyopathy usually in the second decade. Dystrophin participates in a mechanically strong link between the cytoskeleton of muscle, the plasma membrane and the extracellular matrix that surrounds each myofiber [2]. Dystrophin binds to a complex of transmembrane proteins including dystroglycan and the sarcoglycans as well as targeting key signaling molecules including nNOS to the sarcolemma [3] (See Figure 1). Mutations that internally truncate dystrophin, leaving intact some dystrophin protein production, lead to the milder Becker Muscular Dystrophy (BMD). Mutations in the sarcoglycan genes also produce muscular dystrophy and cardiomyopathy [4, 5].

Boys with DMD exhibit muscle weakness by age four [6]. With supportive care providing respiratory support and cardiac management, those with DMD are living into the third decade and beyond [7, 8]. Disease progression at the cellular level has been well characterized over the thirty years since the discovery of dystrophin [9]. Early on there is disruption of membrane integrity and faulty signaling that leads to the death of the muscle cell and the activation inflammatory pathways. Starting in prenatal life the original cohort of muscle cells is replaced by regenerating myofibers that are derived from satellite cells, an adult stem cell population resident in muscle. However, the repeated cycles of muscle death, inflammation and fibrotic expansion result in reduced capacity for regeneration and functional muscle cells are eventually replaced by fibrous elements and fat. At the end-stage, little contractile tissue remains and the skeletal muscle is profoundly weakened. The heart also develops fibrotic replacement, leading to reduced function [10]. Death is usually caused by failure of the diaphragm and/or heart muscle.

Many muscular dystrophies disrupt matrix-cell interactions. As noted above, the loss of dystrophin leads to mis-expression of its binding partner dystroglycan, a key link to laminin. Glycosylation of alpha-dystroglycan is necessary for its binding to laminin. Mutations in several glycosyltransferase genes required for this post-translational modification (*LARGE*, *POMT1*, *POMT2*, *POMGNT1*, *FKTN* and *FKRP*) result in hypoglycosylation, reduced laminin binding and disrupted dystroglycan stability [11]. Recently, matriglycan has been identified as a key carbohydrate structure that links dystroglycan to laminin [12, 13]. Mutations in these genes produce a constellation of muscular dystrophy, cardiomyopathy, and sometimes brain malformations. Like mutations in dystrophin and its associated proteins, mutations in these genes also produce excess cardiac fibrosis and cardiomyopathy [14]. Mutations in *LAMA2* or in *COL6A1*, *COL6A2*, or *COL6A3* cause the congenital muscular dystrophies MDC1A and UCMD, respectively [15]. Notably, all these disorders also produce fibrotic replacement of the muscles of respiration, especially in the diaphragm.

Impaired ventilation can indirectly affect heart function through poor gas exchange, thus providing more than one mechanism by which cardiac performance is adversely affected [16]. Thus understanding the role of cell-matrix interactions in DMD pathology, as well as how these mechanisms can be leveraged for creating therapies is a central question in these disorders.

DMD Therapies under development

Several avenues are being pursued for DMD treatment. Therapies to restore muscle cell integrity and function include exon skipping with antisense oligonucleotides or AAV-mediated gene therapy to replace mutated dystrophin with a truncated, Becker-like form [17]. In addition, the activation of a compensatory pathway based upon the upregulation of utrophin, a dystrophin homolog, is being targeted as a means to redress the primary pathology of DMD [18, 19] (and see below). Laminin 111 has also been shown to have benefit in ameliorating pathology in mdx mice [20]. Agents to combat fibrosis are also under development including those such as LTBP4 to target TGF β (see below) [21]. Myostatin ligand traps and anti-myostatin antibodies are being tested in settings where increasing muscle mass is useful [22, 23]. As in many chronic diseases, it seems likely that a combinatorial therapeutic approach will be required to provide optimal benefit to DMD patients. This review is focused on two therapeutic strategies in developed that each target the extracellular matrix in DMD. The first, NG Biglycan was developed and pursued based on biochemical and functional evaluation. The second, LTBP4, was identified through a genetic screen looking for modifiers of muscular dystrophy. NG Biglycan and LTBP4 have distinct actions, but both take an approach aimed at changing the matrix surrounding myofibers.

Biglycan in normal and diseased muscle

The rationale for biglycan therapy in DMD is based upon the ability of recombinant NG biglycan to activate a compensatory, utrophin-based pathway to preserve muscle health and function in the absence of dystrophin. In this section, we will discuss the studies that formed the basis for this approach and the data supporting its efficacy in the mdx mouse model of DMD.

Biglycan is a small leucine-rich repeat extracellular matrix protein expressed in nerve, bone, cartilage, skin and muscle [24–26]. In muscle biglycan is localized in close proximity to the sarcolemma as well as to the interstitial matrix [24, 27]. Notably, this dual localization is similar to that observed with collagen VI, which binds to biglycan [28]. In humans, mutations in biglycan lead to thoracic aortic aneurysm and dissection and spondyloepimetaphyseal dysplasias [29, 30].

The primary sequence of biglycan reveals a 37kD polypeptide core comprised of 12 leucine-rich repeats (LRR) flanked by amino- and carboxyl- terminal cysteine rich domains [31]. Biglycan has two sites for GAG addition and two for N-linked glycosylation. Importantly, as discussed below a functionally critical, endogenously expressed biglycan glycoform does not bear GAG chains - i.e. it is non-glycanated. We term this glycoform ‘NG biglycan’. Note

that NG biglycan is a glycoprotein as it bears two N-linked carbohydrates. Biglycan's role in the neuromuscular system was first indicated by the discovery that it binds alpha-dystroglycan and is selectively enriched in postsynaptic membranes fractions from *Torpedo* electric organ – a model for the neuromuscular junction [27]. Biglycan is also highly expressed in developing and damaged muscle where it is important for timely regeneration - suggesting that it may be particularly important in immature muscle [18, 32–34].

Biochemical and genetic studies showed that biglycan plays an important role in maintaining the integrity of the muscle membrane. NG (as well as PG) biglycan are components of the mature dystrophin-associated protein complex isolated by mild detergent extraction of muscle membrane preparations [34]. Structure-function studies with recombinant NG and PG glycoforms showed that biglycan binds via distinct sites on its polypeptide core to alpha- and gamma- sarcoglycan, which are critical transmembrane proteins of the Dystrophin/utrophin-associated protein complex; D/UPC [34]. The first 30 amino acids of the mature biglycan polypeptide are required for binding alpha-sarcoglycan while the gamma sarcoglycan binding site is distinct and is localized carboxyl-terminal to this region. NG biglycan is also sufficient to bind collagen VI [35]. Notably, alpha and gamma sarcoglycan are exclusively expressed in skeletal muscle and heart. Biglycan NG also binds to and modulates the activity of the receptor tyrosine kinase MuSK, the central organizer the neuromuscular junction [36]. Studies in null mice showed that biglycan is required for the expression of alpha- and gamma-sarcoglycan in immature muscle and also regulates the localization of the D/UPC associated intracellular signaling complex comprised of nNOS, syntrophin and dystrobrevin [37]. Importantly, the levels of membrane-associated utrophin are also reduced in immature biglycan null mice, showing that one role of endogenous biglycan is to regulate utrophin protein localization at the sarcolemma [18].

Non-glycanated biglycan selectively regulates the localization of utrophin and other D/UPC components at the muscle membrane

Since biglycan mutant mice lack both the NG and PG forms of the molecule, it was important to establish which glycoform is active in regulating D/UPC composition. Rescue experiments in biglycan null mice showed that recombinant NG, but not PG, restores the localization of dystrobrevin and syntrophin to the muscle membrane. Further, NG biglycan, but not PG, induces the localization of nNOS and utrophin to them muscle membrane [18, 37]. These studies are summarized in Fig. 2 and established three critical points: 1) Recombinant NG biglycan delivered to muscle could be detected up to three weeks following a single injection. 2) A single injection of NG biglycan restored the expression of D/UPC components to the muscle membrane for up to 3 weeks following a single injection. Thus, the injected recombinant NG biglycan is both stable and biologically active for prolonged periods. 3) Recombinant PG biglycan was inactive in all the in vivo and cell culture assays noted above.

The selective activity of recombinant NG biglycan in regulating D/UPC composition was a crucial discovery for developing this molecule as a DMD therapeutic for two reasons. First, manufacturing clinical grade biglycan PG would be extremely difficult. Obtaining the

homogeneity and consistency for an elaborately modified proteoglycan required to meet regulatory requirements, which includes GAG chain length and sulfation patterns, is beyond the capabilities of current biomanufacturing processes. Second, and more importantly, both endogenous and recombinant biglycan PG is well established as a proinflammatory molecule that activates the TLR2- and -4 receptors, triggering innate immunity. Several studies have shown that the GAG chains are required for this activity and that NG biglycan lacks the ability to activate TLR2/4 [38–41].

Utrophin and nNOS as therapeutic targets for DMD

One attractive target for DMD therapy is stimulating compensation by utrophin, an autosomal homolog of dystrophin [42]. Utrophin is highly expressed at the sarcolemma during development and regeneration, but in mature myofibers its localization is restricted to the neuromuscular and myotendinous junctions. The mdx mouse lacks dystrophin and it the most widely used animal model of DMD. This mouse undergoes a ‘crisis’ period where virtually all the myofibers die and are subsequently replaced by regeneration. After this recovery, the dystrophic phenotype stabilizes and is relatively mild compared to humans. Studies in these mice have shown that if utrophin is upregulated it can replace many functions of dystrophin. Notably, muscle death does not occur and ex vivo physiological measures show that the muscle function is restored to wild-type levels [19].

The enzyme nNOS is normally associated with the dystrophin complex at the sarcolemma. In DMD nNOS is no longer anchored at the membrane and becomes localized in the cytosol. Loss of nNOS from the sarcolemma is likely to compromise NO-regulated blood flow during muscle activity, leading to fatigue [43]. In addition, mislocalized nNOS can cause abnormal protein nitrosylation and dysregulated ROS, both of which are thought to contribute to muscle pathology. Finally, direct targeting of nNOS to the muscle membrane leads to upregulation of utrophin and amelioration of dystrophic pathology in mdx mice [44]. Thus, restoration of nNOS to the sarcolemma would also be expected to improve muscle health in DMD.

Recombinant NG biglycan efficacy in a mouse model of DMD

Systemic recombinant NG biglycan treatment upregulates utrophin in mdx mice

The demonstration that biglycan can be delivered in vivo, coupled with its role in regulating several D/UPC components, most notably utrophin, motivated a study to test NG biglycan efficacy in mdx mice [18]. Utrophin levels were increased ~2.5 fold at the muscle sarcolemma of NG biglycan-treated mdx mice. A single systemic (IP) administration of biglycan significantly increased utrophin sarcolemmal expression for at least 2 weeks in mdx skeletal muscle. Further studies in vivo and in cell culture showed that NG biglycan acts postranscriptionally to recruit utrophin protein to the membrane. These studies established that NG biglycan effectively engaged its target – utrophin – in dystrophic muscle.

Recombinant NG biglycan administration ameliorates dystrophic pathology and improves muscle function in mdx mice

The finding that systemically NG biglycan upregulated utrophin at the sarcolemma motivated further studies to test the ability of this protein to ameliorate dystrophic pathology in mdx mice. Two hallmarks of muscle damage are centrally nucleated muscle cells, a measure of myofibers that have degenerated and regenerated, and mononuclear cell infiltration, which is triggered by damage to the myofiber. NG biglycan decreased the percentage of fibers exhibiting central nuclei in mdx muscle by >2-fold and reduced the percentage of muscle area that contained mononuclear cell infiltration. These results indicated that mice treated with NG biglycan have healthier skeletal muscle that is undergoing less degeneration and regeneration.

Finally, an effective treatment for DMD should improve muscle function. One of the primary causes of muscle pathology and dysfunction in DMD is the increased susceptibility of the myofiber to contraction-induced damage. This process can be modeled *ex vivo* by measuring the muscle force produced after each of several successive eccentric (lengthening) contractions (ECC) [45]. The DMD model, the *mdx* mouse, when dosed with NG biglycan for 3 months showed markedly reduced the degree of force drop following ECCs. NG biglycan was well tolerated in these 3-month dosing studies; no significant changes were observed in survival, body weight, organ weight or serum markers of liver and kidney function [18].

The favorable efficacy, pharmacodynamic, biodistribution, pharmacokinetic and safety profile of recombinant NG biglycan, combined with the ability to manufacture this glycoprotein at scale, has prompted the development of a humanized recombinant NG biglycan for clinical testing. A clinical candidate, TVN-102, is currently being developed by Tivrosan Pharmaceuticals. TVN-102 is a derivative of biglycan that has been modified to completely preclude the additional of GAG side chains, thus improving the yield and purity of the preparation.

TVN-102 could also be effective in indications in addition to DMD. Biglycan is important for the stability of the neuromuscular junction (NMJ) through its binding to and regulation of the receptor tyrosine kinase MuSK [36]. Since loss of NMJs is an early event in ALS and SMA that leads to muscle paralysis long before motor neurons degenerate, TVN-102 could prolong mobility in these patients. NMJ function is also compromised in MuSK-based myasthenia gravis and TVN-102 could stabilize MuSK in this autoimmune disease. Moreover, the nNOS sub complex of the DAPC, which is regulated by biglycan, is defective in several congenital muscular dystrophies including laminin 211-based MDC1A and the collagen VI-based Ullrich's CMD [46]. Finally, the recent discovery that biglycan deletions or point mutations lead to thoracic aortic aneurysms and dissections, while other point mutants cause spondyloepimetaphyseal dysplasia suggest that TVN-102 could be a replacement therapy for these disorders [29, 30]. Furthermore, this observation that biglycan loss of function mutations associate with increased TGF- β signaling underscores that reducing TGF- β signaling may be a consequence of biglycan replacement and that reducing TGF- β excess through other means can be an effective approach in muscular dystrophy.

The matrix as a modifier of muscular dystrophy: LTBP4

Genetic modifiers are defined as genetic variants that can alter the course of a primary disease process. For muscular dystrophy, a mouse model was used to identify genetic modifiers and in so doing, highlighted the importance of the extracellular matrix and TGF- β as a direct modifiers of muscular dystrophy. Using a mouse model of sarcoglycanopathy, an intercross mouse breeding strategy was used to take an unbiased approach to identify genetic modifier regions [47]. One of the traits used in genome mapping was hydroxyproline content, a direct measure of collagen deposition into dystrophic muscle. A second trait used in these mapping studies was Evans blue dye uptake into muscle, which is a reflection of membrane leakage since normal muscle is impermeable to this dye. In contrast to normal muscle, dystrophic muscle has an increased content of dye uptake into the myofibers themselves. Genetic mapping identified the *Ltbp4* gene as a strong modifier of both fibrosis and membrane leakage, as two independent traits, and *Ltbp4* accounted for nearly 25-45% of the variance of these traits [48]. Notably, the deleterious form of *Ltbp4* is linked to excess TGF- β signaling [47].

Ltbp4 encodes the Latent TGF- β binding protein 4, an extracellular matrix protein that binds and sequesters latent TGF- β , contributing to its latency and inactive state. There are four members of the LTBP family, LTBP1-4. The LTBPs share a similar structure composed of multiple epidermal growth factor (EGF)-like repeats interspersed with domains containing eight cysteines, so called “8-cys repeats”. LTBP1 and LTBP3 were shown to bind avidly to TGF- β via the third 8-cysteine-containing repeat, which is found towards the carboxy-terminal region of the proteins [49]. LTBP4’s binding to TGF- β was not as strong as LTBP1 and 3, but was mediated through the orthologous third 8-cys repeat. More recently, the interaction between latent TGF β and LTBP4 was re-examined and robust binding was detected between the carboxy-terminus of LTBP4 and latent TGF- β 1, TGF- β 2 and TGF- β 3, the major canonical TGF- β forms [50]. The region within LTBP4 that mediated binding to TGF- β contains the third 8-cys repeat [49, 50]

Targeting the hyper-TGF β state of muscular dystrophy

The fibrotic signature of muscular dystrophy is characterized by elevated TGF- β , where unsupervised hierarchical clustering of gene expression changes identified a TGF- β -centric gene dysregulation profile in multiple forms of muscular dystrophy [51]. Inhibitors of latent TGF- β have been shown to be beneficial for fibrosis in mouse models of muscular dystrophy [52, 53]. Translating these findings into the clinical realm of DMD patient is partially challenged by limited efficacy of agents like losartan, an angiotensin receptor blocker, as most DMD patients have low systolic blood pressure and may not tolerate the doses of losartan necessary to achieve effect. Anti-TGF- β antibodies that target TGF- β 1-3, have been tested in mouse models of muscular dystrophy where some reduction of fibrosis was noted [54]. However, translating these findings into patients has been difficult owing to the side effect profile anticipated from broad TGF- β blockade.

An alternative approach was suggested from the modifier studies in mice with muscular dystrophy. Specifically, the *Ltbp4* polymorphism in mice responsible for regulating fibrosis and sarcolemmal leak was found to be a 36 bp polymorphism in the hinge region of the

LTBP4 protein. This polymorphism results in the insertion or deletion of 12 amino acids into the hinge region of LTBP4 protein [55]. In LTBP1, this same region is targeted by proteolysis by serine proteases that releases latent TGF- β [56, 57]. An antibody directed against the hinge region blocked its proteolysis by the serine proteases plasmin and elastase [58] (Figure 3). Moreover, an antibody directed against the amino-terminus of LTBP4 detected the cleavage product of LTBP4 in *mdx* muscle that had been engineered to express the human LTBP4 sequence [58]. Antibodies to LTBP4 are currently in development as a potential biological agent to treat DMD. Although the specific proteases responsible for in vivo LTBP4 processing have not been well studied, targeting these proteases may also be of benefit to reduce TGF- β mediated fibrosis.

LTBP4 also sequesters myostatin

In addition to LTBP4 binding TGF- β 1, 2, and 3, more recently it was shown that LTBP4 binds myostatin, a negative inhibitor of muscle mass [59]. Myostatin, also known as growth and differentiation factor 8, was originally described for its role in regulating muscle mass, as mice deleted for myostatin have nearly double normal muscle size [60]. As a TGF- β family member, myostatin has similar processing including its synthesis as a prodomain that is noncovalently linked to the active domain [61]. The prodomain of myostatin is being exploited for its ability to block function of the active protein [62, 63]. LTBP2 and 3 were found to regulate myostatin in skeletal muscle, emphasizing the importance of the extracellular pool of myostatin that resides in skeletal muscle [64]. For LTBP4, the N-terminus binds myostatin [59]. As it is the C-terminus of LTBP4 that binds TGF- β , this indicates that distinct domains on LTBP4 bind myostatin and TGF- β . Ligand traps aimed at myostatin, anti-myostatin antibodies, or anti-receptor antibodies are each viable a strategy for treating DMD and anti-myostatin antibodies have advanced to clinical trials [65]. LTBP4 has a theoretical advantage because it sequesters both TGF- β and myostatin.

Human LTBP4 polymorphisms modify DMD outcomes

Polymorphisms within the human *LTBP4* gene have been found to correlate with outcomes in DMD, underscoring the importance of this pathway in human disease. The human *LTBP4* gene has two major alleles, which differ at four different amino acid positions distributed across the LTBP4 protein. Particularly in populations derived from Europe, these two *LTBP4* alleles tend to have all four polymorphisms together, forming the two major alleles. One allele, when carried recessively, is linked to prolonged ambulation in DMD patients [66–68]. This allele, referred to as the “IAAM” allele for the amino acids isoleucine, alanine, alanine and methionine, was shown to have reduced SMAD signaling, consistent with a model where LTBP4 protein sequesters more latent TGF- β and effectively reducing the excess TGF- β signaling that characterizes DMD. The protective IAAM LTBP4 was also shown to bind more TGF- β [50], further providing support for this mechanism. The deleterious allele of *LTBP4* has been shown to associate with increased SMAD signaling and also associate with cardiomyopathy [50, 66, 69]. Together, these data underscore a model where the matrix embedded protein LTBP4 plays an active role in regulating latent TGF- β and myostatin availability.

Monitoring fibrosis as a biomarker for disease progression

Since muscular dystrophy is characterized by excessive muscle matrix deposition that progresses through the course of disease, matrix components have been explored as biomarkers of disease. Matrix protein can leak into serum or urine, where they can be monitored readily. Excess matrix deposition can also be visualized using modalities like magnetic resonance imaging (MRI). Biomarkers of fibrosis have significant potential for diagnosis, prognosis, and even as markers of therapeutic efficacy. Histopathological changes are readily evident in biopsy material, but biopsies are invasive and not suitable for repeated measures. For fibrotic skeletal muscle disorders like muscular dystrophy, MRI is advancing in the capacity to quantify fibrosis [70]. Not only can MRI detect fibrosis, but it can also detect fat fraction, which reflects lipid deposition and is another feature of dystrophic muscle [71–73]. Similar findings have been documented in mouse models of DMD [74], consistent with the utility of this method in preclinical models.

The increase of proteins like collagens, laminin, fibronectin, hyaluronan and TGF- β itself may result in proteins increased in the serum or urine. Larger matrix-embedded proteins may be represented in serum or urine proteins by smaller peptides that are more easily solubilized. For liver fibrosis and idiopathic pulmonary fibrosis, serum biomarkers have been shown to reflect the underlying disease process [75, 76]. However, in the case of liver biomarkers, many actually indicate the decline of liver function and are less useful for detecting fibrotic replacement in muscle.

Although biglycan or LTBP4 have not been evaluated as biomarkers, in DMD other matrix proteins have been. Matrix metalloproteinase-9 (MMP-9) was an early serum biomarker examined for its use in DMD, where it was shown to be elevated [77]. MMP-9 is increased due to the fibrotic process and is increased especially in older patients with a greater fibrotic burden. While MMP-9 is useful to detect fibrosis, its ability to indicate successful treatment was not suggested by a recent study [78]. Fibronectin has also been proposed as a serum biomarker in DMD [79], and the amino-terminal region of dystroglycan has also been promoted as a biomarker [80]. Broader proteomic profiling methods have been applied to both animal models and humans, which demonstrate an increase in muscle proteins in the serum of DMD patients, especially younger patients who retain significant muscle mass [81–83].

The extracellular matrix protein osteopontin has also been suggested as a biomarker as it is dramatically increased in dystrophic muscle [77, 84]. A single nucleotide polymorphism in *SPPI1*, the gene encoding osteopontin has been linked to severity in DMD, but evaluation of osteopontin as a modifier and as a biomarker has been complicated by its sex-dependent and steroid-responsive expression [84, 85]. While each of these matrix proteins has shown potential for monitoring disease progression, most of this work has been conducted in the setting of DMD, and therefore it is not known whether these are useful in other muscular dystrophies. It is expected that Biglycan therapy or anti-LTBP4 antibodies would promote changes in the matrix in DMD, and as such, these may prove useful for measuring the effect of these approaches.

Conclusions

The ECM actively participates in the progression of muscular dystrophy, especially DMD. As such, ECM proteins are emerging as potential targets around which to develop therapy for treating this disorder. This review discusses biglycan and LTBP4 as two matrix proteins that can be targeted using biological approaches. As a chronic disorder, it is anticipated that repeated dosing of these biological agents would be needed, although the dosing and frequency needs further evaluation. Alongside these manipulations of the ECM in muscular dystrophy, the matrix and fibrosis in particular is viewed as a source of biomarkers, which may be useful to monitor disease progression and importantly, the effect of therapy.

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Highlights

Non-glycanated biglycan, which lacks GAG side chains, is endogenously expressed in muscle and selectively regulates the assembly of the utrophin-based protein complex.

A recombinant form of this non-glycanated biglycan (TVN-102) is under development for treatment of Duchenne Muscular Dystrophy.

Latent TGF β binding protein 4 (LTBP4) was identified in genomic screens for modifiers of muscular dystrophy. The hinge region of LTBP4 is linked to TGF β release.

Antibodies directed towards the LTBP4 hinge domain are being developed to limit fibrosis in Duchenne Muscular Dystrophy and other disorders.

Figure 1.

NG biglycan and the dystrophin/utrophin-associated protein complex (D/UPC) in skeletal muscle. The complex is anchored by the actin binding proteins utrophin or dystrophin (not shown) that in turn associate with three membrane-associated subcomplexes: the dystroglycans, sarcoglycan-sarcospan, and the signaling nNOS/ α -dystrobrevin/syntrophin (NODS) [2]. The α -dystroglycan binds laminin and agrin via a complex carbohydrate that includes matriglycan [86–89]. NG Biglycan binds to α -sarcoglycan and γ -sarcoglycan via distinct domains and also collagen VI, thus providing a second linkage of the D/UPC to the ECM [34, 89]. Proteins indicated by red stars are down-regulated in biglycan mutant mice and upregulated by systemic delivery of recombinant NG biglycan in mdx and/or biglycan mutant mice [18, 37]. Abbreviations: Cav3, caveolin 3; nNOS, neuronal nitric oxide synthase; SP, sarcospan. See text for details.

	Non-Glycanated 'NG'	Proteoglycan 'PG'
Upregulate utrophin, sarcoglycans, nNOS	Yes	No
Improve muscle health and function in mdx mice	Yes	n.d.
Stabilize synaptic specializations	Yes	No
Activate TLR2/4 Proinflammatory 'Danger' signal	No	Yes

Figure 2.

Distinct functions of biglycan glycoforms. Endogenous biglycan is expressed in forms bearing (PG) or lacking (NG) GAG side chains. Only NG-biglycan counters dystrophic pathology and stabilizes synaptic specializations. NG-biglycan is the basis for a recombinant protein, TVN-102, that is currently being developed as a therapeutic for DMD and other neuromuscular disorders. PG biglycan is a proinflammatory danger signal that requires GAG side chains for this activity [38, 39, 41]. Note that both forms have two N-linked carbohydrates. See text for details.

Figure 3.

Schematic of LTBP4 action in sequestering TGF- β and myostatin. LTBP4 is an ECM-associated protein that binds the latent forms of TGF- β (orange) and myostatin (blue) [59]. LTBP4 was identified as a modifier of muscular dystrophy in mice and humans with DMD [47, 66–68]. TGF- β family members like TGF- β 1, 2, and 3 and myostatin are produced as latent forms containing a prodomain linked to the active C-terminal domain (blue and orange ovals represent the active domains of myostatin and TGF- β , respectively). These latent forms are held inactive in the matrix until released, which then undergo further processing to release the active domains that subsequently engage cell surface receptors (purple). The

TGF- β receptor and Alk4/5 are the primary receptors, and once engaged by their ligands, these receptors promote SMAD activation regulating a cascade of gene expression changes (reviewed in [90]). The hinge region in LTBP4 undergoes proteolytic cleavage associated with release of latent TGF- β [47, 58]. Antibodies directed at LTBP4's hinge region are being developed by Solid Biosciences as a biological agent to treat DMD with the goal of limiting active TGF- β and myostatin.

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