Neurobiology

Role of proteoglycans and glycosaminoglycans in Duchenne muscular dystrophy

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Abstract

Duchenne muscular dystrophy (DMD) is an inherited fatal X-linked myogenic disorder with a prevalence of 1 in 3500 male live births. It affects voluntary muscles, and heart and breathing muscles. DMD is characterized by continuous degeneration and regeneration cycles resulting in extensive fibrosis and a progressive reduction in muscle mass. Since the identification of a reduction in dystrophin protein as the cause of this disorder, numerous innovative and experimental therapies, focusing on increasing the levels of dystrophin, have been proposed, but the clinical improvement has been unsatisfactory. Dystrophin forms the dystrophin-associated glycoprotein complex and its proteins have been studied as a promising novel therapeutic target to treat DMD. Among these proteins, cell surface glycosaminoglycans (GAGs) are found almost ubiquitously on the surface and in the extracellular matrix (ECM) of mammalian cells. These macromolecules interact with numerous ligands, including ECM constituents, adhesion molecules and growth factors that play a crucial role in muscle development and maintenance. In this article, we have reviewed in vitro, in vivo and clinical studies focused on the functional role of GAGs in the pathophysiology of DMD with the final aim of summarizing the state of the art of GAG dysregulation within the ECM in DMD and discussing future therapeutic perspectives.

Key words: Duchenne muscular dystrophy, extracellular matrix, glycosaminoglycans, proteoglycans

Introduction

Duchenne muscular dystrophy

Muscular dystrophies are inherited degenerative disorders of muscles associated with mutations in genes that encode numerous cellular proteins. Duchenne muscular dystrophy (DMD) is one of the most common and severe muscular dystrophy in humans and is an inherited fatal X-linked myogenic disorder characterized by progressive muscle wasting that is invariably fatal in men. The estimated DMD prevalence is 1 in 3500 male live births (Perumal et al. 2013). In contrast, female heterozygotes with clinical evidence of muscle weakness range from 2.5% to 17% of the population (Moser and Emery 1974). This can be explained by the Lyon hypothesis (Venugopal and Pavlakis 2018). Currently, there is no effective treatment for preventing the progressive skeletal and cardiac weakness associated with severe disability and death that characterizes DMD (Inui et al. 1996; Wilton et al. 2015).

The dystrophin gene contains 79 exons distributed over 2.3 million base pairs; it is the largest gene in the human genome (Hoffman et al. 2011) and its mutation is responsible for DMD (Ogura et al. 2014). The size of the DMD gene contributes to its high rate of
mutations, which can disrupt the translational reading frame or create a premature stop codon; the most common of these mutations include deletions of gene segments, point mutations and duplications.

DMD diagnosis is based on genetic testing for dystrophin gene mutations performed by multiplex ligation-dependent probe amplification and increased concentration of creatine kinase in serum (Kerr et al. 2013). For patients without a family history of DMD, diagnosis is usually not made until 3 years of age (Mirsiki and Crawford 2014). Children with DMD often learn how to walk late and show progressive impairment being unable to get up from the ground, jump or climb stairs (Santos et al. 2014). These problems cause severe scoliosis and compromise respiratory and cardiac functions (Suk et al. 2014). Most patients die around the age of 20 because of respiratory failure and/or cardiac insufficiency (Geng et al. 2012). In addition, specific deficits in information processing, complex attention, immediate verbal memory span, verbal working memory, verbal comprehension, reduced vocabulary, visuoconstruction ability, verbal learning and encoding may also occur in DMD patients (Perumal et al. 2013). Indeed, DMD is associated with low level of dystrophin, however, the level of dystrophin necessary for maintaining physiological function is a topic of debate. Studies in mdx mice suggest that 20–30% of functional dystrophin levels are necessary to reduce DMD pathology (Neri et al. 2007). DMD diagnosis has often been performed based on muscle dystrophin expression lower than 3% (Hoffman et al. 1988), while Neri et al. (2007) suggest that 10% of the normal dystrophin protein may be regarded as the minimum level to confer clinical benefits. However, questions regarding the relationship between dystrophin expression and functional outcome remain unanswered.

The protein dystrophin is a component of the dystrophin-associated glycoprotein complex (DGC) present in the muscular fiber plasma membrane (Blake et al. 2002) (Figure 1). The DGC role is to stabilize muscle cells by connecting actin filaments, intermediate filaments and microtubules to transmembrane protein complexes (Price et al. 2007). Therefore, the lack of dystrophin protein results in DGC disruption, membrane instability and increased susceptibility to mechanical stress and muscular fiber necrosis (Rando 2001). Moreover, in DMD, the regenerative capacity of muscle cells is compromised because of muscle stem cell weakness and excessive accumulation of extracellular matrix (ECM) components (Price et al. 2007) which will be discussed in the following sections of this review.

At present, no cure for DMD is available. Comprehensive clinical care recommendations do not exist, despite available guidelines for the treatment of various aspects of DMD pathology. One of the current promising therapeutic strategies for the treatment of DMD is exon skipping. The aim of this gene therapy approach is to improve severe DMD phenotype and symptoms using specific antisense oligonucleotides. These short synthetic strands of nucleic acid modulate expression of specific genes by binding to complementary mRNA (Salmaninejad et al. 2018). Various antisense oligonucleotides targeted to skip exon 51 have been developed. For example, drisapersen (also known as PRO051) treatment results in restoration of functional dystrophin protein. (van Deutkom et al. 2007; Flanigan et al. 2014). More than 60% of DMD patients could potentially benefit from this approach. The antisense oligonucleotides designed to skip exon 51 could help up to 13% of the DMD population (Guglieri and Bushby 2010). Other common targets for the exon skipping approach could be exons 45, 53, 44 and 2 and, together with exon 51, would still only target less than 35% of the DMD population (Guglieri and Bushby 2010). Therefore, other approaches used to treat DMD have been developed. They include gene therapy using viral or human artificial chromosome vectors. Unfortunately, a major drawback of viral therapy in DMD is the limited capacity to accept foreign DNA; this capacity is insufficient for the large dystrophin protein (Wilton et al. 2015). Major obstacles for the abovementioned strategies to restore normal levels of dystrophin are the large size of the dystrophin gene, differences in dystrophin expression in various types of muscles and the rare occurrence of point mutations in the dystrophin gene.

Animal models have been of key importance to study DMD pathophysiology. Mdx mice have been extensively used as an animal model of DMD due to the presence of a stop codon mutation in the dystrophin mRNA which results in dystrophin deficiency (Bulfield et al. 1984; Sciniski et al. 1989; Cox et al. 1993). In mdx mice, a cytosine is replaced by a thymidine at the nucleotide position 3,185 resulting in a termination codon in place of a glutamine codon mimicking the human pathology (Sciniski et al. 1989). While at 3 months of age, DMD rats present necrosis in the limb and diaphragm muscles, at 7 months, they present fibrosis and tissue infiltration in muscles (Larcher et al. 2014). In addition, muscular atrophy is accompanied by decreased motor activity and reduced muscle strength. Moreover, the DMD rat is characterized by dilated cardiomyopathy and abnormal diastolic function (Larcher et al. 2014) which resemble the clinical pathology.

Given the limited clinical improvement observed with therapies focused on increasing the levels of dystrophin, new approaches, based on the modulation of other proteins of the DGC, may represent a novel therapeutic strategy. In this review, we analyze the key results from in vitro, in vivo and human studies of proteoglycans (PGs) and glycosaminoglycans (GAGs) in DMD.

Role of glycosaminoglycans in the extracellular matrix

The ECM is an organized complex macromolecule network of proteins such as collagen and elastin that are linked together through polymers called GAGs (Timaru et al. 2017). The ECM components are fibronectin, cell surface GAGs including chondroitin sulfate (CS), keratan sulfate (KS), heparin, heparan sulfate (HS), hyaluronic acid (HA), PGs, thrombospondins, tenasin, vitronectin and collagens. All these components are regulated by biochemical mediators such as interleukins (Zgheib et al. 2014), arachidonic acid and derivatives (e.g., prostaglandins and leukotrienes) (Olczyk et al. 2014), interferons, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor (TGF) and epidermal growth factor (Olczyk et al. 2014). The most important component of the ECM is HS. HS insures the storage of several growth factors and cytokines, necessary for the continuous and identical renewal of cells and for tissue regeneration. HS also provides a mechanical protection of the matrix signaling proteins against proteolytic degradation (Timaru et al. 2017). Among other functions, the ECM works as a supportive frame for cells and represents an environment for nutrient transport, product metabolism and signal conduction. In addition, the ECM regulates the physiological processes of differentiation, proliferation, migration and cell adhesion (Nita et al. 2014) and plays a key role in muscle fibrosis (Fadic et al. 2006). Endopeptidases, also called matrix metalloproteinases, regulate the physiological balance between synthesis and disintegration of the ECM components and can degrade all of the ECM structural
elements including collagen, gelatins, pro-α-defensin, Fas ligand, tumor necrosis factor-α and E-cadherin (Visse and Nagase 2003).

GAGs are long unbranched polysaccharides consisting of a repeating disaccharide unit; the repeating unit consists of an amino sugar (N-acetylglucosamine or N-acetylgalactosamine) along with an uronic sugar [glucuronic acid (GlcA) or iduronic acid (IdoA)] or galactose (Constantopoulos and Dekaban 1975) (Table 1). GAGs vary based on the type of hexosamine, hexose or hexuronic acid unit they contain. They also vary in the geometry of the glycosidic linkage.

PGs are heavily glycosylated proteins whose basic unit consists of a “core protein” with one or more covalently attached GAG chains (Timpl 1989). PGs include decorin, biglycan, versican, testican, perlecan, bikunin, neurocan, aggrecan, brevican, fribromodulin and lumican. In the present review, we will focus on CS proteoglycans (CS-PGs), decorin, biglycan, HS proteoglycans (HS-PGs) and HA, as these molecules may be promising novel pharmacological therapies for DMD treatment.

**Aim and searching criteria**

We reviewed in vitro, in vivo and clinical studies focused on the functional role of GAGs in muscle in the context of DMD pathophysiology and included articles published between 1976 and 2018. We searched Pubmed/Medline, using the keywords “Duchenne muscular dystrophy”, “proteoglycans”, “glycosaminoglycans” “chondroitin sulfate”, “decorin”, “biglycan”, “heparan sulfate” and “hyaluronic acid” alone or combined. This review aims to summarize the state of the art of PG and GAG dysregulation within the ECM in DMD outlining future therapeutic perspectives.

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**Fig 1.** Dystrophin is a large cytoskeletal protein found in the inner surface of muscle fibers. Dystrophin is associated with a large oligomeric complex of sarclemma proteins and glycoproteins named the dystrophin-associated glycoprotein complex (DGC). The DGC is a key link between the cytoskeleton and the extracellular matrix. The constituents of the DGC differ among different tissues. Panel (A) shows the main components of the DGC. At the DGC, biglycan interacts with the dystroglycans and contributes to synapse stability (B). Perlecan binds acetylcholine through dystroglycans at the DGC (C). Glypican is a heparan sulfate proteoglycan (PG) bound to the outer surface of the plasma membrane by a glycosyl-phosphatidylinositol anchor; it regulates Wnt and Hedgehog intracellular signaling routes (D). Hyaluronic acid is unique among glycosaminoglycans because it does not contain any sulfate and is not found covalently attached to proteins forming a PG. It forms noncovalent complexes with PGs (E).
Proteoglycans and glycosaminoglycans in Duchenne muscular dystrophy

Chondroitin sulfate proteoglycans

CS-PGs consist of a core protein and a CS side chain (Rhodes and Fawcett 2004). In connective tissues, CS molecules can be found covalently linked to decorin (Nikolovska et al. 2014). CS chains consist of repeated units of GlcA or its epimer IdoA and N-acetylgalactosamine (GalNAc) (Bartolini et al. 2013). CS-PGs can be categorized as large aggregating PGs, i.e., aggrecan and versican, and small leucine-rich protein (SLRP), i.e., biglycan, decorin, proline/arginine-rich end leucine-rich repeat protein (PRELP), keratocan, chondroadherin, proteoglycan-Lb and osteoglycin (Buchaille et al. 2000) which differ in their core protein structures (Jia et al. 2012) (Table I).

In the ECM, CS-PGs control the growth factor gradients and cell adhesion, growth, receptor binding and migration (Wegrowski and Fawcett 2004; Schaefer and Schaefer 2010; Theocharis et al. 2010). In addition, CS-PGs’ migration and interaction with ECM components are mediated by the charged GAG chains and proteins including growth factors, cytokines, chemokines, proteases and their inhibitors (ten Dam et al. 2007; Kiri-Safra et al. 2009). Rat neuron-glial antigen 2 (NG2) is the rat homolog of the human melanoma associated CS-PG (MCSP). NG2/MCSP is an integral membrane CS-PG that interacts with ECM components as well as cell surface molecules. The full-length form of NG2/MCSP (400–600 kDa) consists of a short cytoplasmic tail, a transmembrane portion and a large ectodomain with covalently attached CS chains. NG2/MCSP can bind actin filaments, triggering cytoskeletal rearrangements leading to cell spreading and motility, various molecules of the plasma membrane, basal lamina and ECM components (Petrini et al. 2003). NG2/MCSP can also interact with several ECM molecules such as laminin-1, tenascin-C, collagens II, V and VI and participate in signaling events with integrins, membrane receptors for growth factors, such as platelet-derived growth factor-AA (PDGF-AA) and basic FGF (Iida et al. 1995; Burg et al. 1996; Tillet et al. 1997; Goretzki et al. 1999). The CS-PGs in vitro, in vivo and clinical studies are described in the following paragraphs and summarized in Table III.

Decorin

Decorin is a secreted 36 kDa PG that belongs to the SLRP family. It consists of a protein core containing leucine repeats with a GAG chain of CS at the N-terminal end. Decorin is produced by stromal cells, including dermal fibroblasts, chondrocytes, chorionic villus mesenchymal cells and decidual cells of the endometrium during pregnancy (Lala and Nandi 2016). It is located in the ECM of all collagen-containing tissues including skin cartilage and bone (Zhang et al. 2007). Decorin binds type I, II, III and VI collagens to regulate the formation and functioning of connective tissue (Scott and Orford 1981; Krusius and Ruoslahti 1986). Therefore, it may also influence the manner in which collagen fibrils are formed in the ECM (Vogel et al. 1984). In the following sections, we will review in vivo and clinical studies assessing alterations in muscle decorin expression associated with DMD pathology.

Table I. Members of the glycosaminoglycan family which vary based on the type of hexosamine, hexose or hexuronic acid unit they contain

<table>
<thead>
<tr>
<th>GAG</th>
<th>Hexuronic acid/hexose</th>
<th>Hexosamine</th>
</tr>
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<tbody>
<tr>
<td>CS</td>
<td>GlcUA or GlcUA(2 S)</td>
<td>GalNAc or GalNAc(4 S) or GalNAc(6 S) or GalNAc(4 S, 6 S)</td>
</tr>
<tr>
<td>Dermatan</td>
<td>GlcUA or IdUA or IdUA(2 S)</td>
<td>GalNAc or GalNAc(4 S) or GalNAc(6 S) or GalNAc(4 S, 6 S)</td>
</tr>
<tr>
<td>KS</td>
<td>Gal or Gal(6 S)</td>
<td>GalNAc or GlcNAc(6 S)</td>
</tr>
<tr>
<td>HS</td>
<td>GlcUA or IdUA or IdUA(2 S)</td>
<td>GlcNAc or GlcNS or GlcNAc(6 s) or GlcNS(6 S)</td>
</tr>
<tr>
<td>HA</td>
<td>GlcUA</td>
<td>GlcNAc</td>
</tr>
</tbody>
</table>

GlcUA, β-D-glucuronic acid; GlcUA(2 S), 2-O-sulfo-β-D-glucuronic acid; IdUA, α-L-iduronic acid; IdUA(2 S), 2-O-sulfo-α-L-iduronic acid; Gal, β-D-galactose; Gal(6 S), 6-O-sulfo-β-D-galactose; GalNAc, β-D-N-acetylgalactosamine; GalNAc(4 S), β-D-N-acetylgalactosamine-4-O-sulfate; GalNAc(6 S), β-D-N-acetylgalactosamine-6-O-sulfate; GalNAc(4 S, 6 S), β-D-N-acetylgalactosamine-4-O, 6-O-sulfate; GlcNAc, α-D-N-acetylgalactosamine; GlcNS, α-D-N-sulfoglucosamine; GlcNS(6 S), α-D-N-sulfoglucosamine-6-O-sulfate; GAG, glycosaminoglycan; CS, chondroitin sulfate; KS, keratan sulfate; HS, heparan sulfate; HA, hyaluronic acid; GlcA, glucuronic acid; IdoA, iduronic acid.

Table II. Classification of proteoglycans based on their size (large and small) and the nature of their glycosaminoglycan chains. A further classification takes into consideration members of the small leucine-rich proteoglycan family which includes decorin, biglycan, fibromodulin and lumican

<table>
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<tr>
<th>GAGs</th>
<th>Small PGs</th>
<th>Large PGs</th>
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<tr>
<td>CS/Dermatan sulfate</td>
<td>Decorin (36 kDa)</td>
<td>Versican (260–370 kDa)</td>
</tr>
<tr>
<td>HS/CS</td>
<td>Testican (44 kDa)</td>
<td>Perlecan (400–470 kDa)</td>
</tr>
<tr>
<td>CS</td>
<td>Bikunin (25 kDa)</td>
<td>Neurocan (136 kDa)</td>
</tr>
<tr>
<td>KS</td>
<td>Fibromodulin (42 kDa)</td>
<td>N/A</td>
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<tr>
<td></td>
<td>Lumican (38 kDa)</td>
<td>Brevican (145 kDa)</td>
</tr>
</tbody>
</table>

N/A, not applicable; GAG, glycosaminoglycans; PG, proteoglycan; HS, heparan sulfate; CS, chondroitin sulfate; KS, keratan sulfate.

Biglycan

Biglycan is an ECM-localized protein that belongs to the family of the SLRPs that includes decorin, fibromodulin, lumican, keratocan, PRELP, osteoadherin, epiphycan and osteoglycin (Iozzo 1998). Biglycan has a structure similar to decorin; biglycan and decorin can be distinguished from each other by the presence of one (decorin) or two (biglycan) CS side chains at their N-terminal ends (Krusius and Ruoslahti 1986). The composition of their GAG chains has been reported as varying according to the tissue (Cheng et al. 1994). In addition, biglycan is ubiquitously expressed and interacts through its core protein or GAG chains with numerous components of the ECM, e.g., type I, II, III and VI collagen and elastin (Nastase et al. 2012). In muscle cells, biglycan consists of a “PG” form and a “core” polypeptide that lacks the GAG chains (Mercado et al. 2006). In human and murine species, biglycan is primarily expressed in immature and regenerating muscles involved in muscle development (Amenta et al. 2011). For example, the expression of biglycan is high in newborn mice and its expression decreases during skeletal
### Summary of chondroitin sulfate proteoglycans in vitro, in vivo and clinical studies

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<tr>
<td>Mikami et al. (2012)</td>
<td>Mouse C2C12 myoblast cells</td>
<td>Quantification of CS by quantitative reverse transcription-polymerase chain reaction</td>
<td>CS chains were reduced at the stage of extensive syncytial myotube formation. Hyaluronidase-1 is involved in myogenesis and can catabolize endogenous CS to compensate its temporal decline.</td>
</tr>
<tr>
<td>Watanabe, et al. (1986)</td>
<td>Dystrophic (dy/dy) and phenotypically normal C57BL/6J-dy mice</td>
<td>CS analysis</td>
<td>While CS levels decreased from 3 to 18 weeks in healthy control muscle, CS increased from 20% to 30% in dystrophic muscle.</td>
</tr>
<tr>
<td>Mikami et al. (2012)</td>
<td>Mdx mice (C57BL/10 genetic background) and wild-type C57BL/10 mice</td>
<td>Histology and immunohistochemistry to investigate CS distribution in skeletal muscles. Injection of a bacterial CS-degrading enzyme solution into the mouse left tibialis anterior muscles</td>
<td>CS chains were reduced at the stage of extensive syncytial myotube formation. Intramuscular administration of the bacterial CS-degrading enzyme was effective for skeletal muscle regeneration and improvement of mdx pathology.</td>
</tr>
<tr>
<td>Geng et al. (2012)</td>
<td>Mdx mice transfected with ADSCs</td>
<td>Calculation of the rates of myogenic differentiation and adipogenesis. Western blotting for detection of myostatin and decorin expression in skeletal muscles</td>
<td>Decorin-induced myostatin inhibition improved the myogenic differentiation of ADSCs.</td>
</tr>
<tr>
<td>Mercado et al. (2006)</td>
<td>Biglycan-null mice and wild-type C3H mice</td>
<td>Histology and immunohistochemistry to identify nitric oxide synthase in skeletal muscles.</td>
<td>Biglycan is important for the maintenance of muscle cell integrity and plays a direct role in regulating the expression and sarcolemmal localization of the intracellular signaling proteins dystrobrevin-1 and -2, α- and β1-syntrophin and nitric oxide synthase.</td>
</tr>
<tr>
<td>Casar et al. (2004)</td>
<td>Biglycan-deficient mice and wild-type C57BL/6 mice</td>
<td>PG sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis to determine biglycan expression. Western blot analysis during barium chloride-induced skeletal muscle regeneration to determine biglycan expression</td>
<td>A transient upregulation of biglycan was associated with newly formed myotubes. Specifically, biglycan expression was initially high but decreased during skeletal muscle differentiation and maturation. Skeletal muscle maintains its regenerative capacity in the absence of biglycan; delayed fiber growth regeneration and decreased expression of embryonic myosin were observed despite the normal expression of myogenic differentiation antigen and myogenin.</td>
</tr>
<tr>
<td>Amenta et al. (2011)</td>
<td>Biglycan-null mice and wild-type C3H mice</td>
<td>Histological analysis to determine biglycan and utrophin expression. Quantitative reverse transcription-polymerase chain reaction and western blot analysis of utrophin expression. Eccentric contraction force measures to determine biglycan role in immature muscles</td>
<td>Recombinant human biglycan increased utrophin expression. Recombinant human biglycan treatment ameliorated muscle function by reducing the susceptibility to eccentric contraction-induced injury in absence of side effects.</td>
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<tr>
<th>Authors, year</th>
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<tr>
<td>Negroni et al. (2014)</td>
<td>Controlled clinical study</td>
<td>10 DMD patients and 11 healthy controls</td>
<td>Hematoxylin and eosin staining for muscle fibrosis quantification. CS disaccharide analysis by high-performance liquid chromatography in skeletal muscles biopsies. Reverse transcription-polymerase chain reaction and real-time reverse transcription-polymerase chain reaction analysis for assessment of enzymatic regulation.</td>
<td>CS deposition was higher in DMD muscle biopsies than in healthy controls. In DMD muscle biopsies, there was a reorganization of the sulfating pattern of CS disaccharides; specifically, there was an enriched proportion of MonoS units and a specific increase in 4-O-sulfation of N-acetyl-galactosamine residues, consistent with the upregulation of chondroitin 4-sulfotransferase-1.</td>
</tr>
<tr>
<td>Petriti et al. (2003)</td>
<td>Controlled clinical study</td>
<td>Muscle and skin biopsies from 10 DMD patients and 28 healthy controls</td>
<td>Immunofluorescence examination using human MCSP antibodies.</td>
<td>In postnatal muscles from healthy subjects from the first month of life to the age of 5 years, the NG2/MCSP was distinctly expressed in the sarcolemma of all myofibers.</td>
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Continued
Casar et al. 2004). Biglycan binds muscle-specific kinase, a receptor tyrosine kinase, important in neuromuscular synapse stability (Amenta et al. 2012). Biglycan is also a binding partner for α-dystroglycan, and interacts with the dystrophin-associated protein complex (DAPC) and the ECM, regulating the expression of dystrobrevins and syntrophins, particularly in immature muscle (Brenman et al. 1996; Rafii et al. 2006). Biglycan is upregulated during muscle regeneration. The mechanisms by which biglycan can influence muscle development signaling mechanisms and affect DAPC complex organization is not known yet. Biglycan also plays a key role in skeletal muscle development and regeneration. It is upregulated during muscle fiber regeneration (Casar et al. 2004). In addition, mice overexpressing biglycan demonstrate aberrant eyelid muscle development (Hayashi et al. 2005). However, it

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<tr>
<td>Zanotti et al. (2013)</td>
<td>Controlled clinical study</td>
<td>5 DMD patients and 5 healthy controls</td>
<td>Investigation of the effect of decorin on the number and diameter of fibrotic nodules and the secretion of collagen I and fibronectin in skeletal muscle biopsies Study of the expression of collagen I and fibronectin transcripts in fibroblast monolayers of skeletal muscles</td>
<td>NG2/MCSP expression was reduced over the entire sarcolemma in skeletal muscle from subjects aged 7–8 years In DMD muscle biopsies, NG2/MCSP expression was upregulated and this upregulation was restricted to regenerating myofibers</td>
</tr>
<tr>
<td>Zanotti et al. (2005)</td>
<td>Controlled clinical study</td>
<td>9 DMD patients and 21 healthy controls</td>
<td>Competitive polymerase chain reaction and real-time polymerase chain reaction for the analysis of decorin Quantification of fibrosis by collagen VI immunostaining</td>
<td>Established an in vitro model to study muscle fibrosis consisting in cultured primary fibroblasts forming 3D nodules which grew on a substrate derived from human DMD muscle biopsies</td>
</tr>
<tr>
<td>Zanotti et al. (2007)</td>
<td>Controlled clinical study</td>
<td>7 DMD patients and 9 healthy controls</td>
<td>Competitive polymerase chain reaction and real-time polymerase chain reaction for the analysis of decorin and other ECM components Zymography to evaluate the inactive and active forms of matrix metalloproteinase-2 Western blot and immunocytochemistry for ECM components</td>
<td>Decorin mRNA was downregulated in DMD patients Decorin was mainly localized in muscle connective tissue and the content increased in relation to increased fibrosis Intensity of decorin bands on immunoblot did not differ from those of age-matched controls, although if the intensity of the bands was quantitated against vimentin and normalized against sarcomeric actin, the ratio of band intensities was significantly lower than in age-matched controls Biglycan mRNA was upregulated in skeletal muscle biopsies from DMD patients Biglycan expression increased proportionally to increased fibrosis in DMD dystrophic muscles</td>
</tr>
<tr>
<td>Haslett et al. (2002)</td>
<td>Controlled clinical study</td>
<td>12 DMD patients and 12 healthy controls</td>
<td>Microarray gene expression profiles of skeletal muscle biopsies Western blots and immunocytochemistry for decorin and biglycan expression in skeletal muscle biopsies</td>
<td>105 genes were significantly deregulated in DMD muscle biopsies Dystrophin was significantly downregulated in DMD patients Biglycan and decorin were increased in the ECM of DMD patient skeletal muscle biopsies Biglycan and decorin were augmented in the perimysium of muscle tissue</td>
</tr>
<tr>
<td>Fadic et al. (2006)</td>
<td>Case series</td>
<td>2 DMD patients and 2 patients without muscle diseases undergoing a surgical orthopedic procedure in the hip or femur (controls)</td>
<td>Western blotting and immunohistochemistry analysis for decorin and biglycan expression in skeletal muscle biopsies Gel filtration chromatography and sodium dodecyl sulfate polyacrylamide gel electrophoresis for decorin and biglycan expression in fibroblasts from explants obtained from DMD patient muscle</td>
<td>Decorin and biglycan were increased in the ECM of DMD patient skeletal muscle biopsies Incorporation of radioactive sulfate in fibroblasts showed an increased synthesis of both decorin and biglycan compared to controls</td>
</tr>
</tbody>
</table>

DMD, Duchenne muscular dystrophy; PG, proteoglycan; CS, chondroitin sulfate; TGF-β1, transforming growth factor beta 1; NG2, neuron-glial antigen; ECM, extracellular matrix; MCSP, melanoma-associated chondroitin sulfate proteoglycan; ADSC, adipose-derived stem cell.
remains unknown how extracellular biglycan affects processes that rely upon signaling inside the muscle cell and how biglycan influences the composition of the DAPC complex (Mercado et al. 2006). Biglycan controls the fate of skeletal stem cells by modulating the extracellular niche; absence of biglycan makes tendon stem cells unable to differentiate into mature tendons (Young and Fallon 2012). Biglycan regulates bone factors expression, e.g., TGF-β and BMP-2/4. In humans, a mutation in the extracellular domain of the Wnt-signaling molecule LRP-6 causes elevated cholesterol and osteopenia. Exogenous application of nonglycanated biglycan restored the Wnt signaling in mutant LRP-6 cells. Thus, nonglycanated biglycan may represent a valuable therapeutic strategy for pathologies affecting muscle such as DMD and caused by defective Wnt signaling. Taken together, these findings underline the importance of biglycan in modulating several key growth factor-mediated signaling pathways involved in the regulation of skeletal tissue architecture and function (Young and Fallon 2012).

In vitro studies
Mikami and coworkers tested the hypothesis that skeletal muscle differentiation and repair processes require temporal decline in CS levels (Mikami et al. 2012). Using differentiating C2C12 myoblast cultures, they found that CS chains are reduced at the stage of extensive syncytial myotube formation. In addition, they employed forced downregulation of CS (chondroitinase and hyaluronidase removal of CS) which resulted in enhanced myogenic differentiation in vitro. This effect was not observed when they employed forced downregulation of HA. Furthermore, they found that HYAL1, a catabolic enzyme for hyaluronan and CS, is involved in myogenesis and can catabolize endogenous CS indicating that a cell autonomous mechanism regulates the temporal decline in CS.

In vivo studies
In vivo studies using dystrophic mouse muscle biopsies have been conducted to identify and quantify CS expression in relation to age. For example, Watanabe et al. found that the muscle GAG content in healthy controls decreases slightly during the period from 3 to 10 weeks of age, and remains almost unaltered after 10 weeks (Watanabe et al. 1986). Moreover, Watanabe and colleagues (Watanabe et al. 1986) evaluated if CS is involved in muscular growth and maturation comparing healthy and dystrophic mouse skeletal muscles. While CS levels decreased from 3 to 18 weeks in healthy control muscle, CS increased from 20% to 30% in dystrophic muscle. Although, it is not clear whether the abnormality of age-related changes in GAG composition is involved in the maturational defect of the dystrophic muscle, this study supports the concept that differences in CS levels between healthy and dystrophic muscles may be related to the progressive muscular dysfunction that characterizes muscular dystrophy.

An interventional study tested the hypothesis that CS enzymatic removal would ameliorate dystrophic pathology in mdx muscles (Mikami et al. 2012). A cardiotoxin was injected into the left tibialis anterior muscles of mice to induce injury. Administration of a bacterial CS-degrading enzyme (ChABC) increased fusion index at day 7. Treatment with ChABC did not influence uninjured muscles but resulted in significant histological improvement in dystrophic muscles after 14 days. These findings suggest that a temporal decline in CS levels during skeletal muscle differentiation and repair processes is important in a therapeutic perspective for skeletal muscle injury and muscular dystrophy.

In mdx mice, the expression of decorin is increased (Caceres et al. 2000). Geng et al. (2012) tested the hypothesis that decorin-induced deactivation of myostatin may improve the myogenic differentiation of adipose-derived stem cells (ADSCs) (Geng et al. 2012). Indeed, myostatin is a negative regulator of myogenesis and decorin can bind myostatin and deactivate it (Geng et al. 2012). In this study, rat ADSCs were transfected with a lentivirus-containing green fluorescence protein and human decorin gene. The transfected ADSCs were induced by 5-azacytidine and injected into mdx mice. Decorin improved muscle mass and, in turn, dystrophin expression increased and myostatin expression decreased. Furthermore, serum creatine kinase and histological changes in centrally nucleated fiber decreased. These findings support the feasibility of combining decorin-mediated myostatin inhibition and ADSC transplantation as a new therapy for DMD.

Adult biglycan-null mice are mildly dystrophic displaying increased muscle fiber degeneration and regeneration, abnormal fiber size distribution and muscle membrane weakness (Mercado et al. 2006) suggesting a role for biglycan in DMD. In vivo studies using dystrophic mouse muscle biopsies have been conducted in order to quantify biglycan expression in DMD and identify biglycan-binding molecules involved in its pathogenesis. In mice, the lack of biglycan induces delayed muscle regeneration after muscle injury (Casar et al. 2004). Casar and colleagues (Casar et al. 2004) evaluated biglycan expression in skeletal muscle by western blot analysis during barium chloride-induced skeletal muscle regeneration in mice, showing that a transient upregulation of biglycan was associated with newly formed myotubes. Biglycan expression was initially increased but it decreased during skeletal muscle differentiation and maturation. In order to further evaluate the role of biglycan during the regenerative process, skeletal muscle regeneration was studied in biglycan-null mice. Indeed, skeletal muscle maintains its regenerative capacity in the absence of biglycan but delayed fiber growth regeneration and decreased expression of embryonic myosin was observed despite the normal expression of myogenic differentiation antigen (a skeletal muscle-specific transcription factor that is activated early during myogenesis and commits undifferentiated cells to the muscle lineage) and myogenin. These results suggest that transient upregulation of decorin during murine muscle regeneration may shed light on further roles of SLRPs in this process. Further research is necessary to confirm this finding.

Bowe and coworkers found that biglycan immunostaining was elevated in muscles from mdx mice (Bowe et al. 2000) and implicated biglycan in the pathogenesis of DMD. A further study showed that biglycan regulates utrophin expression in immature muscle and that recombinant human biglycan (rBGN) increases utrophin expression in cultured myotubes (Amenta et al. 2011). These results showed that systemically expressed rBGN upregulated utrophin at the sarcolemma and reduced muscle pathology in the mdx mouse model. RhBGN treatment also ameliorated muscle function by reducing the susceptibility to eccentric contraction-induced injury in absence of side effects. These findings suggest that rhBGN represents a new therapeutic molecule for treatment of DMD. Since biglycan is a binding partner of the DAPC with the ECM, regulating the expression of dystrobrevins and syntrophins in immature muscle, its role in muscular dystrophy in the above context has been investigated. Examination of DAPC elements revealed a selective reduction in the localization of α-dystrobrevin-1, and -2, α- and β1-syntrophin and neuronal nitric oxide synthase (nNOS) at the sarcolemma. Exogenous biglycan induced the relocalization of nNOS from the cytosol to the plasma membrane in cultured biglycan-null muscle.
cells. Finally, intramuscular injection of recombinant biglycan restored the expression of α-dystrobrevin-1, α-dystrobrevin-2 and β1-syntrophin to the sarcolemma of biglycan-null mice. Thus, biglycan is important for the maintenance of muscle cell integrity and plays a direct role in regulating the expression and sarcolemmal localization of the intracellular DAPC-signaling proteins dystrobrevin-1 and -2, α- and β1-syntrophin, and nNOS. Taken together, these findings further elucidate the role of biglycan in DMD and suggest that exogenous application of biglycan may be a novel therapeutic approach for this pathology.

Clinical studies
Clinical studies using muscle biopsies from muscular dystrophy patients have been performed with the final aim of identifying CS to understand its role in muscular dystrophy pathophysiology. A study investigated whether CS may represent a therapeutic target to improve the efficacy of gene therapy approaches for the treatment of DMD (Negroni et al. 2014). This study showed higher deposition of CS in skeletal muscle from 10 DMD patients, compared to healthy controls. In addition, high-performance liquid chromatography analysis revealed a reorganization of the sulfating pattern of CS disaccharide units in DMD patients’ muscle biopsies. Specifically, the authors observed an enriched proportion of MonoS units and a specific increase in 4-O-sulfation of GalNAc residues, consistent with the upregulation of the chondroitin 4-sulfotransferase 1 (CHST11/C4ST-1) enzyme. These results suggest that CS may be a novel therapeutic target to improve gene and cell therapy for DMD and other muscular dystrophies, suggesting a possible role for the enzyme CHST11/C4ST-1 which is implicated in the CS sulfation machinery (a part of the CS biosynthesis in the Golgi network involves the transfer of sulfate groups to specific carbon positions of the disaccharide units by sulfotransferase enzymes which is defined as CS sulfation) (Kluppel et al. 2012). A further study examined CS-PG expression in human post-natal and adult skeletal muscle biopsies from healthy subjects’ quadriceps femoris biopsy using immunofluorescence and western blotting (Petrini et al. 2003). The authors also analyzed the NG2/MCSP expression in merosin-deficient congenital muscular dystrophy. They found that NG2/MCSP is expressed at the sarcolemma of differentiated human skeletal muscle fibers. Specifically, in postnatal muscles from healthy subjects from the first month of life to the age of 5 years, the NG2/MCSP was distinctly expressed at the sarcolemma of all myofibers. In contrast, NG2/MCSP expression was reduced over the entire sarcolemma in the skeletal muscle from subjects aged 7 to 8 years. In DMD muscle biopsies, this PG expression was upregulated in regenerating and fully differentiated myofibers with the same degree of intensity. In DMD muscle biopsies, the staining pattern was correlated with the severity of dystrophin deficiency. These data suggest that NG2/MCSP is expressed in differentiated skeletal muscle fibers, creating new knowledge of this PG in muscular dystrophy. Indeed, in DMD, CS is associated with the cytoskeleton and its expression is regulated differently in human skeletal muscle fibers across lifetime.

A study by Zanotti and coworkers tested the effect of decorin on: 1) the number and diameter of fibrotic nodules and the secretion of collagen I, and fibronectin in primary muscle-derived cell cultures obtained from the biobank “Cells, tissues and DNA from patients with neuromuscular diseases”; 2) the deposition of intracellular collagens and 3) the expression of collagen I and fibronectin transcripts in fibroblast monolayers (Zanotti et al. 2013). This study used muscle-derived cell cultures from quadriceps muscle biopsies from 5 DMD patients and 5 male controls to generate an in vitro model to study muscle fibrosis. This model consisted of cultured primary fibroblasts forming 3D nodules which grew on a substrate derived from human DMD muscle biopsies and healthy control biopsies. This model may be useful for drug screening in the context of muscular dystrophy as well as fibrotic diseases.

In order to investigate the role of decorin in muscle fibrosis, decorin expression was quantified in skeletal muscle biopsies from DMD subjects and patients with other forms of dystrophy compared with healthy controls using quantitative polymerase chain reaction (Zanotti et al. 2005). Although results showed that decorin mRNA was downregulated in patients with DMD, immunoblot analysis did not show a difference between dystrophic muscles and age-matched healthy controls. The reduction in decorin levels in DMD patients suggests the possibility of using decorin as a therapy to reinitiate its physiological levels in patients affected by DMD. Controversially, Fadic et al. (2006) showed an increase in decorin deposition in skeletal quadriceps biopsies and fibroblasts from DMD patients (Fadic et al. 2006). Western blot analysis showed an increase in decorin core protein and its glycated form in DMD skeletal muscles. This increase was localized in the perimysium and endomysium, as observed by immunohistochemical analysis. In fibroblasts isolated from DMD muscles, decorin levels were also increased. Further studies are necessary to assess if the increased decorin expression depends on a compensatory muscle mechanism or can be used as a marker of fibrosis in DMD. The study by Fadic and colleagues did not confirm results by Zanotti et al. (2007) who showed that mRNA expression of decorin was significantly downregulated in DMD patients. Discrepancies in the results from the studies described above can be explained by the methodological differences employed by Fadic et al. (2006) and Zanotti et al. (2007).

Zanotti et al. (2005) investigated the expression and localization of biglycan in nine patients with DMD compared with age-matched healthy controls. Biglycan mRNA was upregulated in skeletal muscle biopsies from DMD patients. In addition, biglycan expression increased proportionally to increased fibrosis in DMD dystrophic muscles, suggesting a putative role of biglycan in skeletal muscle fibrosis. Similarly, a further study (Fadic et al. 2006) investigated biglycan expression in skeletal muscle biopsies from DMD patients and fibroblast distribution. They found an increase in biglycan expression, and an increase in biglycan synthesis in cultured fibroblasts obtained from DMD samples. In addition, increased biglycan was localized in the fibrotic connective tissue, suggesting that biglycan plays a key role in the muscle response to dystrophic cell damage. Haslett at al. compared individual gene expression profiles of skeletal muscle biopsies from 12 DMD patients and 12 unaffected control patients using microarray analysis. In this study, biglycan and other components of the ECM were overexpressed in DMD skeletal muscle (Haslett et al. 2002).

In summary, biglycan has an important role in the pathogenesis of DMD although further experiments are required to determine if the increase in biglycan observed in this pathology can be ascribed to a compensatory response or indicates the beginning of fibrosis development. The nonglycanated form of biglycan may be therapeutically effective for the treatment of DMD probably because it is easily produced homogeneously in muscle cells and also because the nonglycanated form does not possess pro-inflammatory activity (Young and Fallon 2012).
Heparan sulfate proteoglycans

HS, a GAG found abundantly in the ECM of several tissues, is a highly sulfated polysaccharide composed of repeating disaccharide units, comprising 1α-L-iduronic acid (α-L-iduronic acid, IdoUA, or β-D-glucuronic acid, GlcUA) and α-D-glucosamine (GlcN) with varying patterns of 2-O-, 3-O- or 6-O-sulfation and N-sulfate or N-acetyl, respectively (Uniewicz et al. 2014). HS interacts with the heparin-binding region of fibronectin in ECM fibroblasts inducing intracellular signaling events leading to an increase in HS trafficking to the nucleus (Stewart and Sanderson 2014). Other functions of HS involve the regulation of angiogenesis, cell growth, migration and differentiation (Wegrowski et al. 2006). HS is a component of HS-PGs present in the cellular membrane (Uniewicz et al. 2014). HS-PGs are diverse and can be transmembrane (e.g., syndecans), bound by a glycosyl-phosphatidylinositol linkage to a plasma membrane lipid such as in the case of glypicans or secreted into basement membranes such as in the case of perlecan and agrin. (Perrimon and Bernfield 2000). These macromolecules interact with numerous ligands, including ECM constituents, adhesion molecules and growth factors (Alvarez et al. 2002). In skeletal muscles, HS-PGs are co-receptors of the asymmetric form of acetylcholinesterase at the neuromuscular junction (Peng et al. 1999).

Perlecan is also present in HS-PGs of the skeletal muscles basal lamina (Larrain et al. 1997). In the neuromuscular junction, perlecan binds α-dystroglycan and other ECM constituents such as laminin, collagen type IV and fibronectin (Alvarez et al. 2002). Perlecan is a large PG (400–500 kDa) containing five distinct structural domains to which long chains of HS and/or CS are attached (Ioizzo et al. 1994). It is upregulated by TGF and promotes muscle cell proliferation through its interaction with basic FGF-2 (Mongiat et al. 2001).

Lumican is a class 2 SLRP that contains primarily HS and 10 leucine-rich regions in their central domain. It interacts with collagen in interstitial collagenous matrices (Bowe et al. 2000) regulating collagenous matrix assembly. In addition, it modulates cell migration and proliferation during embryonic development and tissue repair (Coulson-Thomas et al. 2013). High levels of lumican occur in the human dermis, artery, aorta, lung, kidney, intervertebral disks and connective tissue.

Agrin is a multidomain HS-PG with different modules homologous to domains of other basement membrane proteins. Agrin enhances aggregation of acetylcholine receptors on muscle cells and it is necessary for the correct functioning of neuromuscular junctions (Eusebio et al. 2003). During development, agrin is expressed by the muscle fiber at the embryonal and neonatal stages. In adults, agrin is expressed at the neuromuscular junctions and only at a minor extent in extrajunctional regions (Hoch et al. 1993; Groffen et al. 1998). Agrin binds several cell membrane and ECM proteins (Ruegg and Bixby 1998), e.g., laminins and α-dystroglycan (Eusebio et al. 2003).

Glypican-1 is a HS-PG that is associated to laminin in adult skeletal muscles (Campos et al. 1993). Glypican represents 20% of total PGs associated with myotube membranes and is one of the principal cell surfaces and ECM HS-PGs (Brandan et al. 1996). Glypican-1 is released by phosphatidylinositol specific phospholipase C (PI-PLC) from the plasma membrane of differentiated skeletal muscle cells to be included into the ECM (Brandan et al. 1996).

Molecules of the syndecan family function as co-receptors for ECM constituents and regulate the cytoskeletal organization and the adhesive phenotype (Alvarez et al. 2002). During skeletal muscle cell differentiation and development, syndecans modulate the activity of FGF-2 by presenting FGF-2 to transducing receptors through their GAG chains (Alvarez et al. 2002). The GAG chains of syndecan-4 have similarity with the Hep II domain of fibronectin, a ligand involved in local adhesion assembly with syndecan-4 (Tumova et al. 2000). Syndecan-2 induces skeletal muscle fiber formation by a mechanism involving integrin a5b1 (Kusano et al. 2000). The synthesis of soluble HS-PGs declines in the last phases of differentiation and increases in the early phases (Alvarez et al. 2002). HS-PG levels in dystrophic muscles are increased, suggesting the engagement of HS-PGs in DMD (Pisconti et al. 2012). The HS-PGs in vivo and clinical studies are described in the following paragraphs and summarized in Table IV.

Table IV. Summary of heparan sulfate proteoglycans in vivo and clinical studies

<table>
<thead>
<tr>
<th>Authors, year</th>
<th>In vivo model</th>
<th>Experimental outline</th>
<th>Outcome</th>
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<tbody>
<tr>
<td><strong>In vivo studies</strong></td>
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<tr>
<td>Alvarez et al. (2000)</td>
<td>Mdx (C57BL/10 genetic background) and wild-type C57BL</td>
<td>Mice were injected intraperitoneally with radiosulfate in order to quantify ECM components</td>
<td>Significant increase in the synthesis of HS-PGs in the leg muscle from mdx mice compared to age-matched controls. The increased HS-PG synthesis was revealed by direct quantification of radiosulfate incorporation by HS-PGs</td>
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<table>
<thead>
<tr>
<th>Authors, year</th>
<th>Design</th>
<th>No. of patients</th>
<th>Clinical study outline</th>
<th>Outcome</th>
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<tbody>
<tr>
<td><strong>Clinical studies</strong></td>
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<tr>
<td>Alvarez et al. (2002)</td>
<td>Controlled clinical study</td>
<td>3 patients with DMD and 3 healthy controls</td>
<td>Quadriceps femoris biopsies were analyzed using antibodies directed against ECM components; sodium doceyl sulfate polyacrylamide gel electrophoresis was used to analyze HS-PGs</td>
<td>Perlecan, syndecan-3 and glypican-1 increased in skeletal muscles from DMD patients and were distributed irregularly. Glypican-1 and perlecan were localized mainly connected to ECM components; syndecan-3 was mainly associated to muscle fibers</td>
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</tbody>
</table>

DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; HS-PG, heparan sulfate proteoglycan.
In vivo studies

Only one study investigated the synthesis of HS-PGs in mdx mice during skeletal muscle regeneration and showed a significant increase in the synthesis of HS-PGs in leg muscle from mdx mice compared to age-matched healthy controls (Caceres et al. 2000). Although further studies are necessary to understand the role of HS-PGs, the upregulation of HS-PGs may contribute to the regenerative capacity observed in mdx mice.

Clinical studies

HS-PGs have been quantified (Alvarez et al. 2002) in biopsies from DMD patients. An increase in three different HS-PGs (perlecan, syndecan-3 and glypican-1) was observed in skeletal muscles from DMD patients using sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis with anti-D-HS-specific monoclonal antibodies. However, a differential localization for these PGs was observed. Glypican-1 and perlecan were localized mainly connected to ECM components, while syndecan-3 was mainly associated to muscle fibers. These findings support results from previous studies showing that perlecan and glypican-1 are normally found in the basal lamina surrounding individual muscle fibers (Campos et al. 1993; Larrain et al. 1997). Syndecan-3 was mainly associated to the skeletal muscle fibers in DMD and was almost absent in normal muscle fibers. The differential localization of the augmented HS-PGs could be responsible for the accumulation of ECM material that characterizes DMD-associated fibrosis. These HS-PGs may be linked to the regeneration of muscle fibers that occurs in DMD patients at a mild stage of disease development (Alvarez et al. 2002). Furthermore, some of the HS-PGs whose levels are increased may be involved in the adhesion of the fibers to the ECM, in an attempt to overcome the absence of DGC that connects the ECM to the actin cytoskeleton.

Hyaluronic acid

HA is a polysaccharide synthesized as a large, negatively charged, unbranched polymer, consisting in the repetition of disaccharides of GlcA and N-acetylglucosamine: [β(1,4)-GlcUA-β(1,3)-GlcNAc]-n (Toole 2004). HA consists in 2,000–25,000 disaccharides with relative molecular masses of 106–107 and polymer lengths of 2–25 μm (Toole 2004). HA is a member of the GAGs family together with HS and CS but differs because it is synthesized as an unmodified polysaccharide by one of the HA syntheses (HASs) (Toole 2004). On the other hand, the other GAGs are synthesized as PGs and their assembly takes place in the rough endoplasmic reticulum and Golgi apparatus with a mechanism similar to glycoprotein assembly (Toole 2004). HA can be found associated with cells in a pericellular coat surrounding cells as well as mobile in solution. (Hunt et al. 2013). HA is ubiquitously expressed; however, it is principally localized in the skin (about 50% of total HA), skeleton (about 30% of total HA) and skeletal muscles (about 10% of total HA) (Reed et al. 1988). HA surrounds the myofibers (e.g., the endomysium, perimysium, and epimysium) and the mechanoreceptor spindle fibers (Hunt et al. 2013). HA can influence cell motion, migration, proliferation and differentiation from the mesenchymal lineage, including osteoblasts, chondrocytes, fibroblasts and adipocytes, as well as the skeletal muscle-forming myoblasts (Elson and Ingwall 1980; Yoshimura 1985; Kujawa, Carrino, et al. 1986; Kujawa, Pechak, et al. 1986; Huang et al. 2003; Allingham et al. 2006; Meran et al. 2007). In dystrophic muscles, HA is the major component in the extracellular space at the muscle cell surface. Defects in the structure of the basement membrane are also associated with dystrophic pathology (Fidzianska et al. 1987). HA participates in muscle repair upregulating myoblast migration and reversibly suppresses myoblast differentiation (Calve and Simon, 2012). The in vitro, in vivo and clinical studies of HA in DMD are described in the following paragraphs and summarized in Table V.

In vitro studies

In vitro studies have been performed with the final aim of identifying HA expression and understanding its role in muscular dystrophy pathophysiology. For instance, Hunt et al. (2013) studied HAS expression during skeletal muscle development, postnatal regeneration following injury, dystrophic pathology and in vitro myogenesis, hypothesizing that HAS2 is involved in syncytial muscle cell formation and muscle growth and repair. This evidence suggests, for the first time, that HA synthesis is fundamental for myogenic differentiation and that HAS2 contributes to myogenic differentiation, muscle cell HA synthesis, and maintenance of a pericellular matrix around myoblasts.

In vivo studies

In vivo studies on dystrophic mice muscle biopsies have been conducted to identify HA expression and distribution in dystrophic muscles. For instance, the use of a novel type of cell scaffold, based on HA, to deliver different types of myogenic progenitor cells in partially ablated murine muscles, has been described (Rossi et al. 2011). This study evidenced that the combination of isolated satellite cells with the photopolymerizable hydrogel promoted histological and functional reconstruction of a partially ablated skeletal muscle. These findings show, for the first time, that hydrogel HA-based technology can be used to repair damaged skeletal muscles. A further study showed differences in HA distribution in the ECM of skeletal muscles from normal and dystrophic mice at different ages (Watanabe et al. 1986). Physiologically, HA contents partially decreased with advancing age, while in dystrophic muscles HA distribution increased significantly, if compared to age-matched controls, confirming that DMD is characterized by an abnormal augmentation of the connective tissue. In summary, results of in vivo studies identify a putative role for HA in muscle injury and propose HA-based technologies as a potential therapy for the treatment of DMD.

Clinical studies

HA deposition within skeletal muscle biopsies from DMD patients has been described (Fidzianska et al. 1987). HA was localized as one of the principal constituents on the muscle cells surface and in the extracellular space together with a diffuse thickness of vascular and muscular basement membrane. These results suggest that dystrophic muscle displays a defective structure of the basement membrane where HA may play a role that requires further clarification.

Discussion and conclusions

The role of GAGs has been extensively studied in DMD. However, the main focus has been on in vitro, in vivo and ex vivo studies using human muscle biopsies. These studies have shown a deregulation of GAGs considering these molecules as hallmarks of DMD pathology. Studies performed using DMD mouse models have shown an increase in muscle CS (Watanabe et al. 1986), decorin (Zanotti et al. 2007), HS-PGs (Caceres et al. 2000; Alvarez et al. 2002) and HA (Watanabe et al. 1986) which could be linked to pathological processes in DMD pathology. Specifically, the increase in HS-PGs, which are involved in the adhesion of muscle fibers to
the ECM, may be a compensatory effect to overcome the absence of the DGC that connects the ECM to the actin cytoskeleton (Alvarez et al. 2002). Biglycan mRNA is also upregulated in mdx mouse muscle. Biglycan expression increases with progression of fibrosis in dystrophic mouse muscles suggesting that biglycan may play a role in DMD-associated fibrosis (Amenta et al. 2011). A preclinical interventional study using rhBGN showed a reduction in muscle pathology in the mdx mouse model of DMD and improved muscle function without side effects (Amenta et al. 2011). This study employed a nonglycanated form of biglycan that is more stable and does not display pro-inflammatory activity. Taken together, this evidence suggests that exogenous administration of biglycan warrants further investigation as a therapeutic approach for the treatment of DMD (Young and Fallon 2012).

Table V. Summary of hyaluronic acid in vitro, in vivo and clinical studies

<table>
<thead>
<tr>
<th>Author(s), year</th>
<th>In vitro model</th>
<th>Experimental outline</th>
<th>Outcome</th>
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<td><strong>In vitro studies</strong></td>
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<tr>
<td>Hunt et al. (2013)</td>
<td>C2C12 myoblast line</td>
<td>C2C12 myoblasts were induced to differentiate and fuse into multinucleate myotubes over a period of 72 hours in differentiation medium mRNA was extracted, and HAS1 and HAS2 transcript levels were examined by qPCR A hyaladherin G1 domain of aggrecan was used to investigate the presence and localization of HA in cultured C2C12 myoblasts</td>
<td>HA synthesis is fundamental for myogenic differentiation and HAS2 contributes to myogenic differentiation, muscle cell HA synthesis and maintenance of a pericellular matrix around myoblasts</td>
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<tr>
<td><strong>In vivo studies</strong></td>
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<tr>
<td>Rossi et al. (2011)</td>
<td>Wild-type C57BL/6J mice</td>
<td>A biodegradable, injectable, and photo-cross-linkable hydrogel, based on ester of HA linked to a synthetic photoinitiator was developed and injected in the partially ablated tibialis anterior of mice engrafted with freshly isolated satellite cells</td>
<td>The combination of satellite cells with the photopolymerisable hydrogel promoted histological and functional reconstruction of a partially ablated skeletal muscle in terms of improvement in muscle structure and the number of new myofibers</td>
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<tr>
<td>Watanabe et al. (1986)</td>
<td>Dystrophic (dy/dy) and phenotypically normal C57BL/6J-dy mice</td>
<td>Skeletal muscles of both hind legs were quickly removed and analysis of HA was conducted by using a specific extraction enzymatic kit and 2D electrophoresis on a cellulose acetate film</td>
<td>HA was the major GAG (over 60% of the total GAGs) in the skeletal muscle of the normal mice at various ages examined. In normal mice, the relative amount of HA increased slightly (from 70% to 80%) with age. In dystrophic muscle, the proportion of HA was ~65% between the third and the eighteenth week of age</td>
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<td><strong>Clinical studies</strong></td>
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<tr>
<td>Fidzianska et al. (1987)</td>
<td>Controlled clinical study</td>
<td>Quadriceps femoris muscles were analyzed for vascular basement membrane, muscular basement membrane and ECM organization with tannic acid and specific GAG-degrading enzymes</td>
<td>HA showed abnormal localization as one of the principal constituents on the muscle cell surface and in the extracellular space together with a diffuse thickness of the vascular basement membrane and muscular basement membrane in DMD muscles that was absent in control muscles The muscular basement membrane was enlarged and had irregular and wavy inner and outer contours The lamina densa of the vascular basement membrane lost its regular, ribbon-like appearance and was split into several interwoven strands, delineating clearer spaces where a delicate meshwork was present In dystrophic muscles additional structures were identified; these structures were absent in control muscles and included bundles of fine parallel arranged fibrils with electron-dense cross-bands corresponding to collagen fibrils found around the blood vessels and in the extracellular spaces</td>
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</table>

DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; GAG, glycosaminoglycan; HA, hyaluronic acid; HAS1, hyaluronic acid synthase 1; HAS2, hyaluronic acid synthase 2.
In summary, the role of ECM components dysregulated in DMD physiopathology is very relevant and has not been exhaustively investigated, especially in a therapeutic perspective. As a matter of fact, the muscle interstitial environment is strictly involved in myogenic stem cell chemotaxis, differentiation and turnover, and PGs may be properly administered as a therapy to restore muscle function, modulate the scar fibrotic process and support the tendon physiological function avoiding retraction and ankyloses (Fidzianska et al. 1987). However, studies investigating PGs as a therapy for DMD are still lacking.

Preclinical studies have focused on finding effective and translational strategies to engineer myogenic cells and biocompatible scaffolds to reconstruct skeletal muscle. This could have important implications for the treatment of muscular dystrophy. Delivery of satellite cells in combination with a photopolymerisable HA-based hydrogel promoted histological and functional reconstruction in a mouse model obtained by partial ablation of the tibialis anterior muscle (Rossi et al. 2011). In light of these findings, viscosupplementation with HA may have a place in the treatment of DMD.

In conclusion, a growing body of evidence is delineating that several GAGs are hallmarks of DMD. Administration of a ChABC, resulted in significant histological improvement in dystrophic muscles suggesting a therapeutic repair process in muscular dystrophy that involves a reduction in inflammatory cytokines (Mikami et al. 2012). Importantly, comprehensive strategies integrating diverse approaches and effects will most likely produce the highest curative value in clinical settings, particularly in light of the high genetic and clinical heterogeneity of dystrophic patients. In conclusion, identification and mechanistic understanding of GAGs are reshaping our knowledge of DMD and other muscular dystrophies and significantly priming the therapeutic quest for this yet incurable disease.

Conflict of interest statement

None declared.

Abbreviations

DMD, Duchenne muscular dystrophy; GAGs, glycosaminoglycans; ECM, extracellular matrix; DGC, dystrophin-associated glycoprotein complex; PGs, proteoglycans; CS, chondroitin sulfate; KS, keratan sulfate; HS, heparan sulfate; HA, hyaluronic acid; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; TGF, transforming growth factor; GlcA, glucuronic acid; IdoA, iduronic acid; GlcUA, β-D-glucuronic acid; GlcUA(2S), 2-O-sulfo-β-D-glucuronic acid; IdoUA(2S), 2-O-sulfido-l-iduronic acid; Gal, β-D-galactose; Gal(6S), 6-O-sulfo-β-D-galactose; GalNac, β-D-N-acetylgalactosamine; GalNAc(4S), β-D-N-acetylgalactosamine-4-O-sulfate; GalNAc(6S), β-D-N-acetylgalactosamine-6-O-sulfate; GalNAc(4S, 6S), β-D-N-acetylgalactosamine-4,6-O-sulfate; GlcN, α-N-acetylgalactosamine; GlcNAc, α-D-N-acetylgalactosamine; GalNAc, α-D-N-acetylgalactosamine-4-O-sulfate; GalNAc, α-D-N-acetylgalactosamine-6-O-sulfate; F abolished stem cell; ChABC, CS-degrading enzyme; rhBGN, recombinant human biglycan; nNOS, neuronal nitric oxide synthase; HAS, hyaluronic acid synthase; DAPC, dystrophin-associated protein complex.

References


