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Augmented Synthesis and Differential Localization of Heparan Sulfate Proteoglycans in Duchenne Muscular Dystrophy

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Abstract Muscular dystrophies are characterized by continuous cycles of degeneration and regeneration that result in extensive fibrosis and a progressive diminution of muscle mass. Cell surface heparan sulfate proteoglycans are found almost ubiquitously on the surface and in the extracellular matrix (ECM) of mammalian cells. These macromolecules interact with a great variety of ligands, including ECM constituents, adhesion molecules, and growth factors. In this study, we evaluated the expression and localization of three heparan sulfate proteoglycans in the biopsies of Duchenne muscular dystrophy (DMD) patients. Through SDS–PAGE analyses followed by specific identification of heparitinase-digested proteins with an anti-Δ-heparan sulfate specific monoclonal antibodies, we observed an increase of three forms of heparan sulfate proteoglycans, corresponding to perlecan, syndecan-3, and glypican-1. Immunohistochemistry analyses indicated a differential localization for these proteoglycans: glypican-1 and perlecan were found mainly associated to ECM structures, while syndecan-3 was associated to muscle fibers. These results suggest that the amount of specific heparan sulfate proteoglycans is augmented in skeletal muscle in DMD patients presenting a differential localization. J. Cell. Biochem. 85: 703–713, 2002.

Key words: Duchenne muscular dystrophy; extracellular matrix; heparan sulfate proteoglycans; syndecan; glypican; perlecan

Muscular dystrophies can arise from a number of clinically and genetically heterogeneous disorders whose molecular basis has only been elucidated in the last decade or so. These disorders are characterized by a progressive weakness and wasting of the musculature, due to continuous cycles of degeneration and regeneration of the skeletal muscle tissue. Duchenne muscular dystrophy (DMD) is a very severe pathology that appears before the fourth year and is fatal by the second decade of age [Betto et al., 1999]. The identification of dystrophin as the defective protein in DMD patients, inherited in an X-linked recessive manner [Koenig et al., 1987], was soon followed by the isolation of a number of dystrophin-associated proteins in skeletal muscle. These proteins were found to constitute a large oligomeric complex named the dystrophin-glycoprotein complex (DGC) that bridges across the sarcolemma and connects the extracellular matrix (ECM) with the actin cytoskeleton [Cohn and Campbell, 2000].

Several mutations, leading to a deficiency in laminin α2 in the ECM, have also been associated to muscular dystrophies [Allamand et al., 1997]. Laminin-2 is known to interact with α-dystroglycan, a component of the DGC, as well as the α7β1 integrin complex [Burkin

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and Kaufman, 1999]. Thus, skeletal muscle myofibers may require at least two separate but parallel attachment systems for their anchorage-dependent stability and survival, namely the basal lamina-DGC anchorage system and the α7β1 integrin-laminin-2 anchorage system. It also appears that both systems confer mechanophysical stability and ECM-survival signaling [Cohn and Campbell, 2000].

Another attractive group of molecules involved in the anchorage of plasma membrane to the ECM are heparan sulfate proteoglycans. Cell surface heparan sulfate proteoglycans are found almost ubiquitously on the surface and in the ECM of mammalian cells. These macromolecules interact with a great variety of ligands, including ECM constituents, adhesion molecules, and growth factors [Bernfield et al., 1992; Carey, 1997]. In skeletal muscle, heparan sulfate proteoglycans act as co-receptors for the asymmetric form of acetylcholinesterase at the neuromuscular junction [Brandan et al., 1985; Peng et al., 1999]. Perlecan is an intrinsic heparan sulfate proteoglycan constituent of skeletal muscle basal lamina [Larrain et al., 1997a]. It has been reported to be present at the neuromuscular junction [Jacobson et al., 2001] and to be involved in binding α-dystroglycan [Peng et al., 1998] as well as several ECM components, such as laminin [Yamagata et al., 1993], collagen type IV [Mayer et al., 1997], and fibronectin [Brown et al., 1997]. Glypican-1 is a heparan sulfate proteoglycan, which colocalizes with laminin in adult rat skeletal muscle sections and can bind to immobilized laminin [Carey and Stahl, 1990; Campos et al., 1993], suggesting a possible role as a cell surface receptor for ECM components. Glypican-1 can be released by phosphatidylinositol specific phospholipase C (PI-PLC) from the plasma membrane of differentiated skeletal muscle cells [Campos et al., 1993] and incorporated into the ECM [Brandan et al., 1996]. Membrane bound heparan sulfate proteoglycans, such as those of the syndecan family, have been shown to act as co-receptors for ECM constituents [Bernfield et al., 1992; carey, 1997], modifying cytoskeletal organizations and the adhesive phenotype [Bernfield et al., 1999]. Syndecans have been shown to modulate the activity of FGF-2 during skeletal muscle differentiation [Rapraeger et al., 1991; Fuentealba et al., 1999], development [Perrimon and Bernfield, 2000; Olguin and Brandan, 2001], and also in adult species [Bernfield et al., 1999; Rapraeger, 2000] by either sequestering or presenting the growth factor to transducing receptors via its glycosaminoglycan chains.

We have previously shown that the expression of perlecan, syndecan-1, and syndecan-3 is downregulated during in vitro skeletal muscle terminal differentiation [Larrain et al., 1997b; Fuentealba et al., 1999], whereas the expression of glypican-1 is upregulated [Brandan et al., 1996]. This differential expression may be a reflection of differing functions and macromolecular specificity during the skeletal muscle differentiation process. Considering the multiple functions described for heparan sulfate proteoglycans in skeletal muscle physiology, the expression and localization of these macromolecules in skeletal muscle tissue, under different pathologies, is worth further study. Particularly relevant is the case of DMD, in which the anchorage of skeletal muscle to the ECM is known to be severely affected.

In this study, we evaluate the expression and localization of three heparan sulfate proteoglycans in the biopsies of DMD patients. We find that all three forms augmented with differential localization: glypican-1 and perlecan were found mainly associated to ECM structures, whereas syndecan-3 was associated to muscle fibers.

MATERIALS AND METHODS

Muscle Samples

Biopsies from individuals were obtained from quadriceps during diagnostic or surgical procedures, in accordance with university ethical rules. DMD Patient 1 (D1): 8-year-old boy first evaluated for delay in walking at 19 months of age and subsequently developing progressive muscle weakness. He had no relatives with myopathies. CPK was 20,000 IU/ml. Dystrophin was absent in his muscle biopsy. Patient 2 (D2): 4-year-old boy with muscle pains since the age of 2½, developing progressive muscle weakness shortly after that. Family history was negative for muscle disease. CPK was $>$ 18,000 IU/ml. EMG showed myopatic motor units with increased recruitment and muscle biopsy revealed muscle dystrophy. Immune histochemistry for dystrophin showed absence of the three-dystrophin domains.

Patient 3 (D3): 7-year-old boy who was brought to medical attention at the age of 4 due to frequent falls. He also had mild language delay and at examination displayed proximal muscle weakness. CPK was 25,000 IU/ml. He had no family history for muscle disease. Muscle pathology showed muscle dystrophy with no dystrophin staining. After sampling, all biopsies were frozen in deep-cooled isopentene and stored at −80°C until processing.

Quadriceps muscle samples were obtained as controls from patients of the same age, who underwent an orthopedic procedure. Informed consent was obtained from all patients. The Ethics Committee of the Hospital Clínico de la P. Universidad Católica de Chile approved the study.

Immunohistochemistry

Cryostat sections (8 μm) were obtained from frozen samples treated for immunohistochemistry, fixed with cold ethanol/acetic acid solution (1:1) for 1 min, rinsed with phosphate-buffered saline (PBS; pH 7.4), and blocked with an 8% BSA solution. The sections were incubated with indicated primary antibodies diluted in 1% BSA for 12 h at 4°C and then with either anti-rabbit-FITC (diluted 1:200) or anti-mouse-TRITC (diluted 1:1,000) for 3 h at room temperature. Sections were pre-treated with heparitinase prior to anti-Δ-heparan sulfate monoclonal antibodies incubation. For nuclear staining, sections were incubated in 1 μg/ml Hoechst 33258 in PBS for 5 min. After rinsing, the sections were mounted and viewed under a Nikon Eclipse microscope equipped for epifluorescence. Controls were done where the primary antibodies were not present.

The following primary antibodies were used: anti-mouse-laminin (1/100; Sigma Chemical, St. Louis, MO); anti-human-fibronectin (1/400; Sigma Chemical, St. Louis, MO); monoclonal anti-human-dystrophyn (1/50; clone MANDRA1; Sigma Chemical, St. Louis, MO); anti-rabbit-α-dystroglycan (1/150; Upstate Biotech, Sellex, Inc.); anti-Δ-heparan sulfate, mAb3G10 (1/500; Seikagaku, Tokyo, Japan) [Steinfeld et al., 1996], anti-rat-glypican-1 (1/100; kind gift of D.J. Carey, PhD, Sigfried and Janet Weis Center for Research, Danville, PA) [Brandan et al., 1996]; anti-mouse-perlecan (1/1000; kind gift of Dr. J. Hassell, Shriners Hospital for Children, Tampa, FL) [Larrain et al., 1997a]; anti-rat-syndecan-3 (1/25; kind gift of D.J. Carey, PhD, Sigfried and Janet Weis Center for Research, Danville, PA) [Fuentealba et al., 1999].

SDS–PAGE Analysis of Heparan Sulfate Proteoglycans

Appropriate samples were analyzed by SDS–PAGE, using a 3–15% acrylamide gradient [Larrain et al., 1997b]. To determine the core proteins of the isolated heparan sulfate proteoglycans, Triton X-100 extracts [Olguin and Brandan, 2001] were incubated with heparitinase (Seikagaku, Tokyo, Japan), separated by SDS gel electrophoresis, transferred to nitrocellulose membranes, stained with anti-Δ-heparan sulfate, mAb3G10 (Seikagaku, Tokyo, Japan), and then revealed by ECL [Steinfeld et al., 1996; Olguin and Brandan, 2001]. Molecular weight standards were obtained from BIO-RAD, Hercule, CA.

Protein Determination

Protein was determined using the BCA protein assay kit (PIERCE, Rockford, IL) with BSA as standard [Riquelme et al., 2001].

RESULTS

Expression of Skeletal Muscle Extracellular Matrix Constituents Is Enhanced in Duchenne Skeletal Muscle Dystrophy

To evaluate the synthesis and localization of ECM constituents in DMD cases, biopsies from the quadriceps of DMD and control patients were submitted to immunohistochemistry. Figure 1A–D shows, as expected, the absence of dystrophin staining in DMD patients as compared to controls; the presence of Hoechst-stained nuclei can be seen in blue. Similarly, Figure 1E–H also shows that α-dystroglycan is very reduced in DMD patients in comparison with controls. On the other hand, both the amount and distribution of fibronectin, shown in Figure 1I–L, seemed to be affected as reported previously [Rampoldi et al., 1986]. The observed increase in the deposition of this ECM protein likely occurred in degenerating fibers (Fig. 1K) as well in the patient (D2) with more advanced muscular damages (Fig. 1L). The synthesis of laminin is also shown in Figure 1M–O, where a disorganized laminin distribution can be seen in DMD cases. These results indicate that the expression of two ECM proteins is

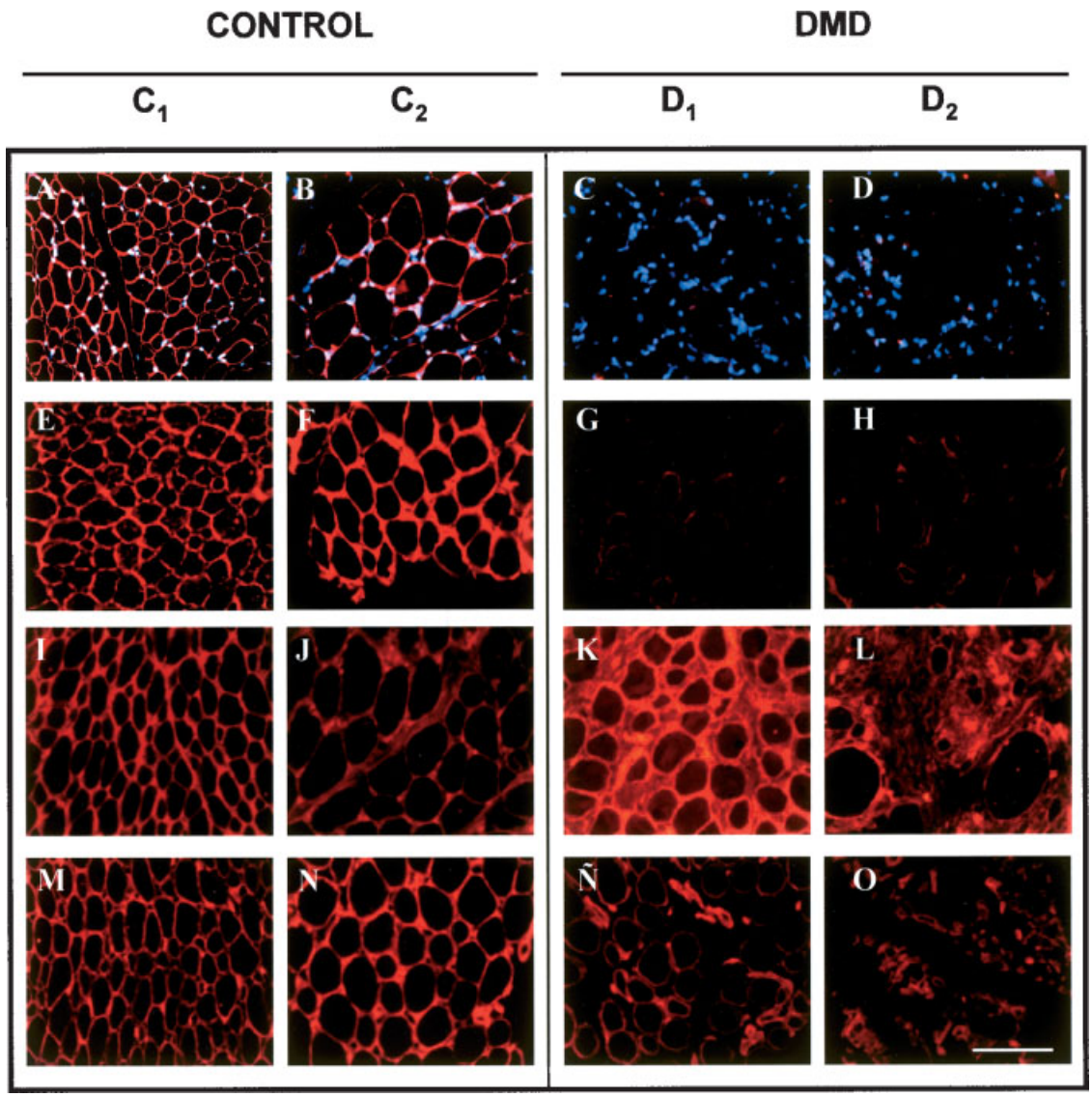

Fig. 1. Histological changes and expression of ECM proteins in DMD. Sample sections from quadriceps of DMD (D1 and D2) and control patients (C1 and C2) are shown. Serial cryosections were stained with anti-human dystrophin (**A–D**), anti-rabbit α-dystroglycan (**E–H**), anti-human fibronectin (**I–L**), and anti-mouse laminin (**M–O**). Nuclei were visualized by Hoechst 33258 nuclear staining (A–D; blue color). The bar (shown in O) corresponds to 100 μm.

altered in DMD patients as compared to control cases.

Synthesis of Skeletal Muscle Heparan Sulfate Proteoglycans Is Enhanced in Duchenne Skeletal Muscle Dystrophy

To identify and evaluate the expression of different heparan sulfate proteoglycans in DMD patients, biopsy sections were treated with heparitinase and stained with anti-Δ-heparan sulfate specific monoclonal antibodies [Steinfeld et al., 1996]. Figure 2A,B shows that heparan sulfate proteoglycans were found around individual fibers in control patients. In DMD patients (Fig. 2C,D), heparan sulfate proteoglycans located around degenerating fibers (Fig. 2C) or in ECM associated structures (Fig. 2D). No staining was observed in samples not treated with heparitinase (Fig. 2I–L), indicating that the staining was specific for heparan sulfate proteoglycans.

To determine the species of heparan sulfate proteoglycans involved, biopsies obtained from DMD and control patients were homogenized,

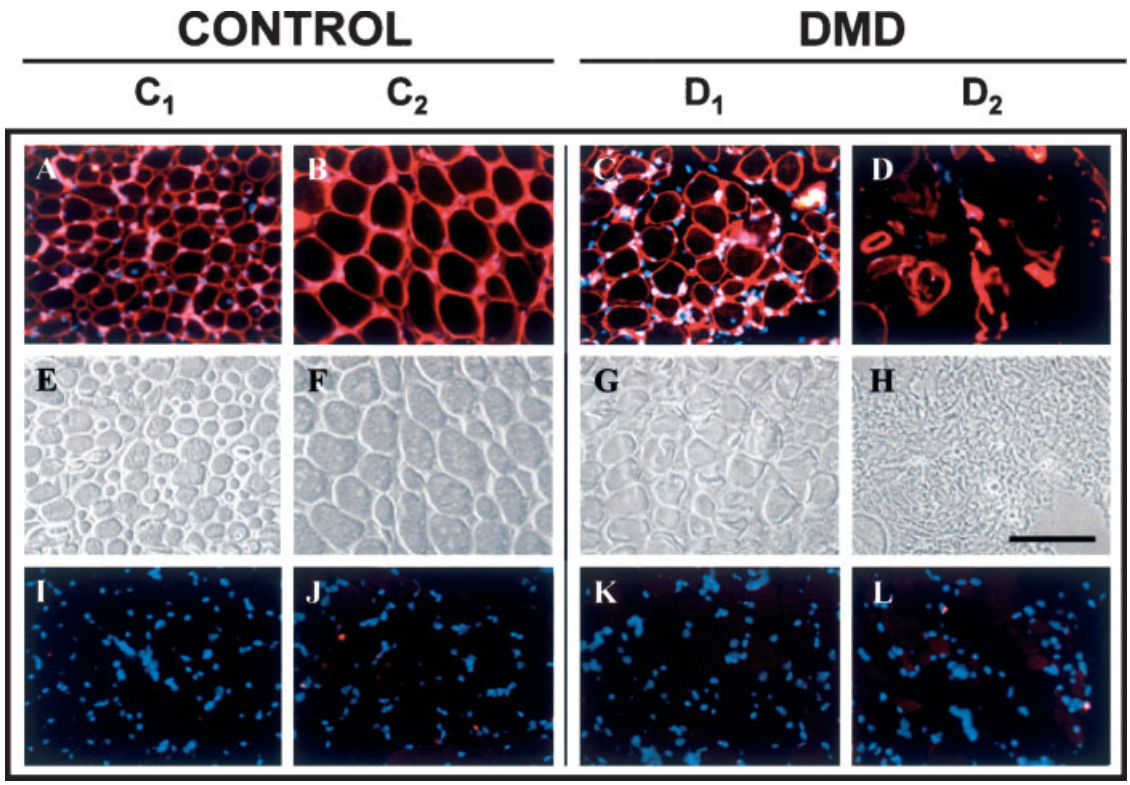

Fig. 2. Distribution of heparan sulfate proteoglycans in DMD. Sample sections from quadriceps of DMD (D1 and D2) and control patients (C1 and C2) are shown. Serial cryosections were either treated with heparitinase (**A–D**) or incubated with buffer as a control (**I–L**), and then stained with anti-Δ-heparan sulfate (A–D, I–L). Phase contrast micrographs corresponding to A–D samples are shown in **E–H**. Nuclei were visualized by Hoechst 33258 nuclear staining (A–D, I–L; blue color). The bar (shown in H) corresponds to 100 μm.

incubated with heparitinase, analyzed by SDS–PAGE, and revealed by incubation with anti-Δ-heparan sulfate specific monoclonal antibodies to reveal the core proteins of each heparan sulfate proteoglycan. This assay have been described and used with success to identified cores proteins of several heparan sulfate proteoglycans [Steinfeld et al., 1996; Fuentealba et al., 1999]. Figure 3A shows the protein profile of the biopsies analyzed after Ponceau S staining of equivalent amount of proteins. Figure 3B shows the presence of several bands in C3 and C4 of varying molecular weights:~310–250, 120, ~67–64 kDa, that likely correspond to perlecan, syndecan-3, and glypican-1. The amount of perlecan, syndecan-3, and glypican-1 was found to be increased in DMD patients (Fig. 3B; D2 and D3). A new band around 40 kDa, likely corresponding to syndecan-4, was also observed in one of the DMD patients (Fig. 3B; D3). As comparison an extract of differentiated mouse myotubes (C2C12) have been analyzed in the same manner (Fig. 3C), whereas previously identified heparan sulfate core proteins are indicated [Fuentealba et al., 1999].

Increased Expression of Perlecan, Syndecan-3, and Glypican-1 in Duchenne Skeletal Muscle Dystrophy

Specific antibodies were used to confirm the identities of the heparan sulfate proteoglycans and study their distribution. Figure 4A–D shows the localization of perlecan in control (Fig. 4A,B) and DMD patients (Fig. 4C,D). Perlecan has been described to be associated to the basal lamina in normal muscle tissue [Larrain et al., 1997a], as was observed in control biopsies (Fig. 4A,B). However, in DMD patients, an increased histochemical reaction was observed around degenerating fibers as well as around fiber packages (Fig. 4C). In a biopsy obtained from a patient with advanced

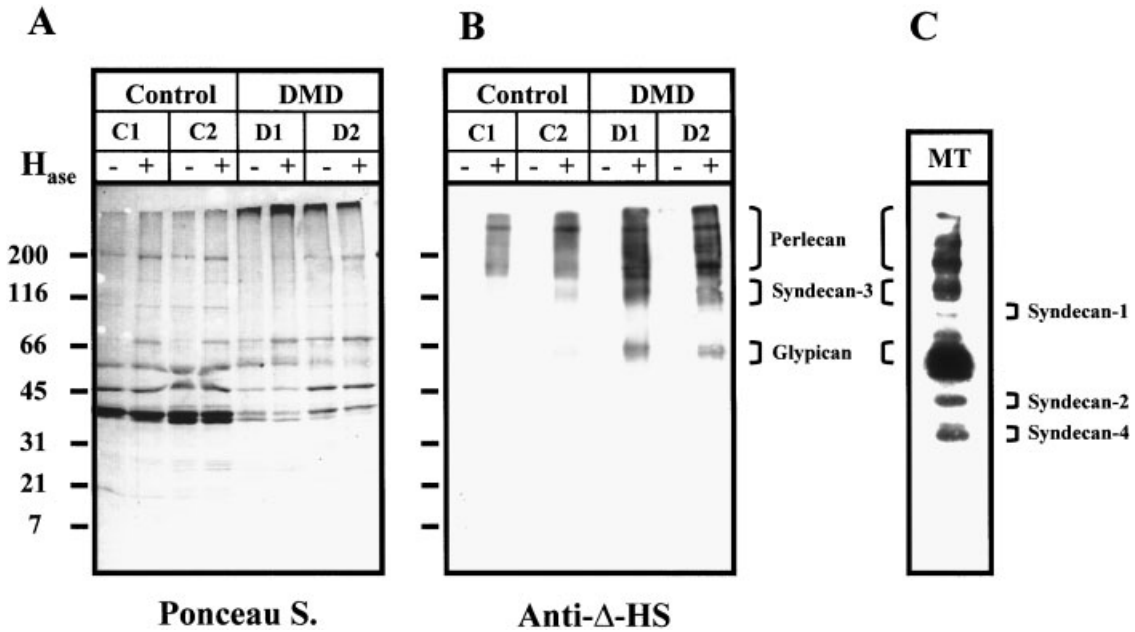

Fig. 3. Changes in the expression of heparan sulfate proteoglycans in DMD. Western blot analyses were carried out using soluble samples obtained from DMD biopsy extracts. C3 and C4 correspond to control biopsies and D2 and D3 to DMD patients. Fractions containing equivalent amounts of proteins were concentrated and incubated with heparitinase (Hase), separated by SDS gel electrophoresis, transferred to nitrocellulose membranes, stained with Ponceau S (**A**), and stained with anti-Δ-heparan sulfate specific monoclonal antibodies (**B**) to then reveal the core proteins of each heparan sulfate proteoglycan using ECL. It is possible to identify perlecan, syndecan-3, and glypican-1 based on the molecular weights of the core proteins. Molecular weight standards are shown on the left (kDa). **C**: Shows the identification, in parallel, of previously identified heparan sulfate core proteins obtained from mouse myotubes extract.

DMD (D2), the localization of perlecan was found in ECM-associated structures (Fig. 1L), resembling the localization of fibronectin.

The levels of syndecan-3 expression are known to be very low in normal mouse skeletal muscle [Fuentealba et al., 1999]. Here, low levels of syndecan-3 were obtained by immunohistochemical (Fig. 4I,J) and biochemical analyses in control biopsies (Fig. 3; C3 and C4). In contrast, an increased expression of syndecan-3 was observed in DMD patients (Fig. 4K,L), with positive reactions obtained in association with muscle fibers and following an intracellular pattern. The fibers could be identified by the absence of intracellular nuclei staining, as evidenced by the light blue color (Hoescht). This intracellular staining was not observed in samples were primary antibodies were not present (data not shown).

Finally, the localization of glypican-1 was evaluated. It has been described that glypican-1 is associated to the basal lamina of normal mouse skeletal muscle [Campos et al., 1993]. The same was found in normal human biopsies obtained from control individuals (Fig. 4P,Q), whereas an increased reaction for glypican-1 was obtained in DMD biopsies (Fig. 4R,S). Glypican-1 was found in association with degenerating fibers (Fig. 4R) and ECM structures (Fig. 4S). An important amount of glypican-1 co-localized with mono-nucleated cells (yellow stain). For aide, stained nuclei are shown in red (Fig. 4P–S).

These results show that the synthesis of the heparan sulfate proteoglycans perlecan, syndecan-3, and glypican-1 is increased in DMD patients and their localization altered. Perlecan and glypican-1 were found mainly associated to ECM structures, whereas syndecan-3 was associated with muscle fibers.

DISCUSSION

Several functional roles have been described for heparan sulfate proteoglycans. Among them are cell–ECM interactions and modulation of growth factor activities [Ruoslahti and Yamaguchi, 1991; Carey, 1997], functions which are relevant to skeletal muscle physiology. The attachment of skeletal muscle fibers to the ECM

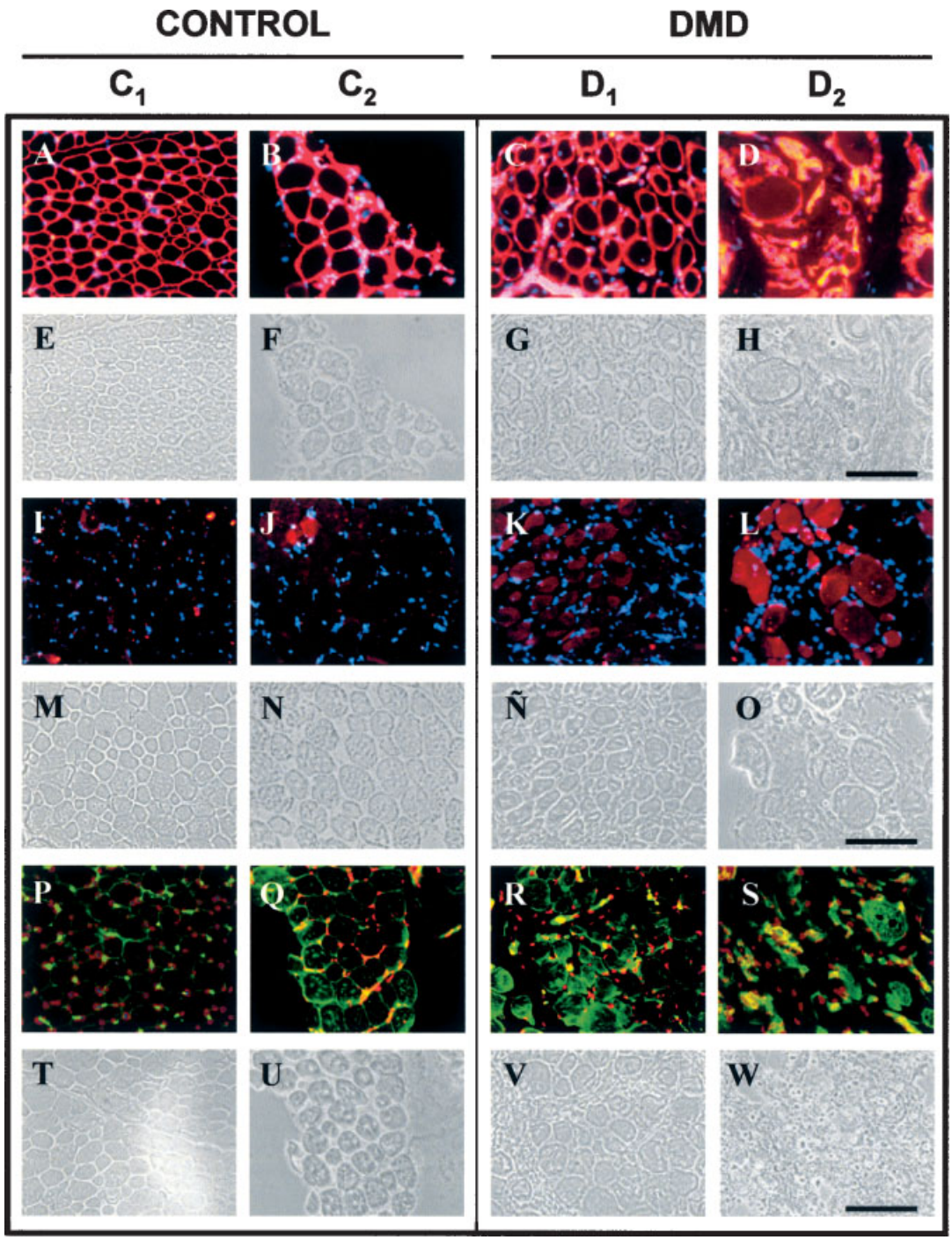

Fig. 4. Increased expression of perlecan, syndecan-3 and glypican-1 in DMD. Sample sections from quadriceps of DMD (D1 and D2) and control patients (C1 and C2) are shown. Serial cryosections were stained with anti-mouse perlecan (**A–D**), anti-rat syndecan-3 (**I–L**), and anti-rat glypican-1 (**P–S**). Corresponding phase contrast images are shown below each series of immuno-sections (**E–H**, **M–O**, and **T–W**, respectively). Nuclei were visualized by Hoechst 33258 nuclear staining (A–D and I–L, blue color; P–S, red pseudo color). The bars (shown in H, O, and W) correspond to 100 μm.

appears to be essential for normal muscle function and stability. Heparan sulfate proteoglycans interact with collagen type IV, laminin, and fibronectin to name a few ECM constituents [Bernfield et al., 1992]. Membrane bound proteoglycans, such as syndecan and glypican-1, are excellent candidates to act as receptors for ECM molecules [Bernfield et al., 1999]. The

glycosaminoglycan chains of syndecan-4 were found to have affinity for the Hep II domain of fibronectin, a ligand known to promote focal adhesion formation through syndecan-4 [Tumova et al., 2000]. There is also direct evidence that syndecan-2 participates selectively in the induction of stress fiber formation, in cooperation with integrin $\alpha5\beta1$ through specific binding of its heparan sulfate side chains to the fibronectin substrate [Kusano et al., 2000]. Recently, it has been shown that the globular domain of laminin is responsible for cell adhesion, with the suggestion that syndecan-2 and -4 mediate this activity [Utani et al., 2001]. On the other hand, glypican-1 interacts and co-localizes with laminin in normal skeletal muscle tissue [Carey and Stahl, 1990; Campos et al., 1993]. The heparan sulfate proteoglycan agrin [Denzer et al., 1995] is released by the nerve at the neuromuscular junction and induces differentiation of the nerve terminal, the synaptic basal lamina, and the postsynaptic membrane [McMahan, 1990]. Agrin binds to α-dystroglycan, one of the skeletal muscle laminin receptors, while the latter binds to other ECM molecules, including collagen, entactin, and perlecan [Peng et al., 1998]. On the other hand, the basal lamina protein acetylcholinesterase (AChE) interacts with heparan sulfate proteoglycans [Brandan et al., 1985; Deprez et al., 2000] localized at the neuromuscular junction, the anchorage enzyme which can be released specifically by heparinase treatment [Brandan et al., 1985], and also co-localizes with perlecan [Peng et al., 1998], which in turn can bind to α-dystroglycan.

Several heparan sulfate proteoglycans have been described in skeletal muscle tissue. As our earlier work has shown, skeletal muscle formation generates a concerted pattern of regulated expression of several heparan sulfate proteoglycans [Brandan and Larrain, 1998]. The synthesis of soluble heparan sulfate proteoglycans that accumulate in conditioned medium decreases during terminal differentiation [Brandan et al., 1991]. In contrast, the synthesis of cell and substratum-associated proteoglycans increases when skeletal muscle cells are induced to differentiate [Larrain et al., 1998]. These results are in accordance with a previous report on primary cultures of embryonic chicken skeletal muscle cells [Pacifici and Molinaro, 1980]. Specifically, the expression of syndecan-1 [Larrain et al., 1997b], syndecan-3 [Fuentealba et al., 1999], and perlecan [Larrain et al., 1997a] was found to decrease during skeletal muscle differentiation, whereas the synthesis of glypican-1, a lipid anchored heparan sulfate proteoglycan [Campos et al., 1993], was found to increase [Brandan et al., 1996]. This proteoglycan accounts for 20% of total proteoglycans associated with myotube membranes and is the main heparan sulfate proteoglycan associated with the cell surface and the ECM [Brandan et al., 1996]. Thus, a complex and vast array of interactions involving heparan sulfate proteoglycans occurs at the skeletal muscle fiber surface. These interactions might be critical for the correct assembly of the ECM and for creating signaling pathways throughout the fiber.

With regard to growth factor modulation, heparan sulfate proteoglycans have been involved in the control and triggering of skeletal muscle differentiation [Rapraeger et al., 1991; Larrain et al., 1998]. Specifically, syndecan-1 and -3 have been implicated in the modulation of FGF-2 activity [Larrain et al., 1998; Fuentealba et al., 1999]. On the other hand, decorin, a chondroitin/dermatan sulfate, has been found to modulate the activity of TGF-β during skeletal muscle differentiation [Riquelme et al., 2001]. Other proteoglycans, such as betaglycan [Lopez-Casillas et al., 1991] and biglycan [Kresse et al., 1994], also seem to be actively involved in the modulation of TGF-β activity [Brandan et al., unpublished communications]. Interestingly, it has been shown that biglycan associates with the DGC and is augmented in *mdx* mice, which are dystrophin negative [Bowe et al., 2000]. We also have evidence suggesting that chondroitin/dermatan sulfate forms are increased in DMD biopsies [Alvarez et al., unpublished communications].

To our knowledge, the present study is the first attempt to investigate the expression and localization of several heparan sulfate proteoglycans in DMD biopsies. We have previously shown that the total synthesis of proteoglycans is increased in *mdx* mice, in particular chondroitin/dermatan sulfate proteoglycans [Cáceres et al., 2000]. However, no attempts were made to specifically identify the forms that underwent this increase. An interesting and recent report evaluated the expression of several genes in biopsies of DMD patients, through expression profiles determined by microarray techniques [Chen et al., 2000]. Levels of versican transcripts, a large chondroitin sulfate

proteoglycan, glypican-1 and -3, and lumican were found to be higher in DMD than in control cases. Among the proteoglycans analyzed, only versican was evaluated by indirect immunohistochemistry, showing a diffuse increase at the endomysium.

In this work, we show that skeletal muscle biopsies obtained from DMD patients expressed elevated levels of heparan sulfate proteoglycans, specifically perlecan, syndecan-3, and glypican-1. Interestingly, a differential localization of these proteoglycans was observed in analyzed DMD samples, with glypican-1 and perlecan seemingly associated to the ECM, with increased material mainly focalized around skeletal muscle fibers. These observations are consistent with the view that perlecan and glypican-1 are normally found in the basal lamina surrounding individual muscle fibers [Larrain et al., 1997a; Campos et al., 1993]. In contrast, syndecan-3 seemed to be mainly associated to the skeletal muscle fibers in DMD cases and was almost absent from normal muscle fibers. It has been previously shown that syndecan-3 expression decreases during skeletal muscle differentiation, and that its expression occurs in myoblasts [Fuentealba et al., 1999] and during skeletal muscle formation [Olguin and Brandan, 2001] and recently shown to be a satellite cell marker together with syndecan-4 [Cornelison et al., 2001]. As mentioned, one of the functions of syndecan-3 is the modulation of FGF-2 during skeletal muscle differentiation [Fuentealba et al., 1999]. It is attractive to speculate that syndecan-3 expression occurs in muscle fibers under regeneration, with many embryonic events occurring once again [Weis et al., 2000]. The differential localization of the augmented heparan sulfate proteoglycans could account for cellular processes such as the accumulation of ECM material, characteristic of DMD-associated fibrosis [Rampoldi et al., 1986], and the induction of proteoglycan forms normally expressed during development and fiber formation. In the process of mice skeletal muscle regeneration, we have observed a transient increase in the same heparan sulfate proteoglycans [Casar et al., submitted]. This observation suggests that at least some of the heparan sulfate proteoglycans described in this paper are associated with the regeneration of muscle fibers, a characteristic observed in DMD, particularly in patients at a mild stage of disease development. It is tempting to speculate that some of the increased heparan sulfate proteoglycans observed here may be involved in the adhesion of the fibers to the ECM, in an attempt to overcome the absence of the DGC that connects the ECM to the actin cytoskeleton.

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