Statistically Significant Differences in the Number of CD24 Positive Muscle Fibers and Satellite Cells between Sarcoglycanopathy and Age-Matched Becker Muscular Dystrophy Patients

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Object: The aim of this study was to reveal variations in the patterns of expression of the cell surface proteins in regenerating fibers and those in the number of satellite cells to gain an understanding of the pathological processes involved in sarcoglycanopathy. Methods: We have reported that there is a reduction of the beta-1 subunit of laminin, heparan sulfate proteoglycan (HSPG), and HCAM (CD44) in Japanese patients with sarcoglycanopathy. Here, we investigated immunohistochemically the expression of the neural cell adhesion molecule (NCAM), which is a marker for human regenerating muscle and satellite cell, and CD24, which appears to be expressed in the early stages of the regeneration process. Patients: We investigated six Japanese patients with sarcoglycanopathy, and compared to age-matched Becker muscular dystrophy. Results: We found that the incidences of muscle fibers with increased NCAM were not statistically different between the two groups. However, the incidences of muscle fibers with increased CD24 and those of NCAM positive satellite cells were very low in sarcoglycanopathy and were statistically different between sarcoglycanopathy and age-matched Becker muscular dystrophies. Conclusion: The poor expression of CD24 and the fewer satellite cells in sarcoglycanopathy without significant difference in the number of total regenerating fibers suggest that a different regeneration process is involved in sarcoglycanopathy compared to that in other types of muscular dystrophy.

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Key words: immunohistochemistry, neural cell adhesion molecule (NCAM), regeneration, cell surface proteins

Introduction

Sarcoglycanopathy is a genetically heterogeneous disease including LGMD2C, linked to the gamma-sarcoglycan gene on chromosome 13q (1); LGMD2D, linked to the alpha-sarcoglycan gene on chromosome 17q (2); LGMD2E, linked to the beta-sarcoglycan gene on chromosome 4q (3, 4); and LGMD2F, linked to the delta-sarcoglycan gene on chromosome 5q (5). The physiological role of each sarcoglycan protein and the pathophysiology of sarcoglycanopathy are poorly understood. We have reported that there is reduction of the beta-1 subunit of laminin, heparan sulfate proteoglycan (HSPG), and HCAM in Japanese patients with sarcoglycanopathy (6-8). The expression of the CD24 molecule, a glycoprotein expressed on the surface of most B lymphocytes and differentiating neuroblasts, was reported to be associated with a subpopulation of regenerative fibers in diseased muscles (9). Neural cell adhesion molecule (NCAM) was expressed in regenerating fibers, satellite cells, and denervated muscle fibers (10). The aim of the study was to reveal variations in the patterns of expression of the cell surface proteins in regenerating fibers and those in the number of satellite cells to gain an understanding of the pathological processes involved in sarcoglycanopathy.

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**Materials and Methods**

**Patients**

We have already reported the details of the 6 patients with sarcoglycanopathy (6, 8, 11, 12); and the clinical data for these 6 patients are presented in Table 1. As age-matched disease controls, 4 patients with Becker muscular dystrophy (BMD) were examined. Immunohistochemical analysis

Frozen biopsied muscles from the patients were cut and 8 μm sections were picked up on aminosilane-coated slides. Immunohistochemical analysis of regenerating fibers was performed using monoclonal antibodies to CD24 (YLEM, Italy), 1:50 dilution, and NCAM (Leu19; Becton Dickinson Immunocytochemistry Systems, CA), 1:20 dilution. The following antibodies were purchased from Novocastra: alpha-sarcoglycan (1:100 dilution), beta-sarcoglycan (1:100 dilution), gamma-sarcoglycan (1:100 dilution), beta-dystroglycan (1:50 dilution), dystrophin N-terminus (1:50 dilution), dystrophin rod (1:50 dilution), and dystrophin C-terminus (1:50 dilution). Biotinylated anti-mouse immunoglobulin GL (IgG) was used as the secondary antibody, and the ABC method (13) was used for signal detection (ABC kit; Vector). The sections were counterstained with hematoxylin for 5 seconds. All immunohistochemical procedures were as reported previously (8).

**Statistical analysis**

The incidences of NCAM-positive and CD24-positive fibers were obtained as a percentage of 500 randomly selected fibers exhibiting positive staining. The percentage of NCAM positive satellite cells per 500 myonuclei in non-necrotic muscle fibers, which were evaluated in the serial section stained with hematoxylin and eosin, was calculated for each case. The statistical significance of the differences between

**Results**

Dystrophin and beta-dystroglycan were expressed at near-normal level on the muscle cell membrane in all the patients with sarcoglycanopathy. However, alpha-sarcoglycan, beta-sarcoglycan, and gamma-sarcoglycan were deficient or markedly reduced on the muscle cell membrane in all the patients with sarcoglycanopathy.

The results of immunohistochemical analysis of CD24 and NCAM are shown in Fig. 1 and Fig. 2. The immunostaining of CD24 was the most intense on the membrane surface and moderately intense in the cytoplasm of muscle fibers in all patients. Most CD24 positive fibers showed basophilic appearance in the serial sections stained with hematoxylin and eosin. The immunostaining of NCAM varied from slight intensity to strong intensity in the sarcoplasm or on the muscle cell membrane in all patients. The mean diameter of CD24-positive fibers is smaller than that of NCAM-positive fibers. The results of detailed statistical analysis are shown in Table 1. We found that the incidences of muscle fibers with increased NCAM were not statistically different between the two groups. However, the incidences of muscle fibers with increased CD24 and those of satellite cells were statistically different between sarcoglycanopathy and BMD (Table 1). The number of CD24 positive fibers and satellite cells were low in patients with sarcoglycanopathy.

**Discussion**

In our previous immunohistochemical study on sarcoglycanopathy (6–8), we found poor expression of HSPG

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**Table 1. Clinical and Histochemical Data**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age of onset (years)</th>
<th>Age at biopsy (years)</th>
<th>Serum CK</th>
<th>SG gene</th>
<th>%NCAM positive fibers</th>
<th>%CD24 positive fibers</th>
<th>%Satellite cells/Myonuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGP1</td>
<td>M</td>
<td>5</td>
<td>30</td>
<td>235</td>
<td>alpha</td>
<td>25.5</td>
<td>0.6</td>
<td>6.7</td>
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<tr>
<td>2</td>
<td>M</td>
<td>10</td>
<td>41</td>
<td>220</td>
<td>unknown</td>
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<td>0.2</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>4</td>
<td>19</td>
<td>480</td>
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<td>16.0</td>
<td>1.0</td>
<td>15.5</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>9</td>
<td>46</td>
<td>240</td>
<td>unknown</td>
<td>12.3</td>
<td>0.4</td>
<td>18.1</td>
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<tr>
<td>5</td>
<td>F</td>
<td>5</td>
<td>31</td>
<td>680</td>
<td>alpha</td>
<td>38.2</td>
<td>1.5</td>
<td>6.7</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>6</td>
<td>13</td>
<td>3,550</td>
<td>gamma</td>
<td>35.1</td>
<td>2.1</td>
<td>20.0</td>
</tr>
</tbody>
</table>

BMD1    | M   | 4                   | 18                   | 800      | gamma   | 26.5                 | 2.8                  | 32.1                     |
| 2       | M   | 5                   | 21                   | 1,200    | unknown | 37.5                 | 8.6                  | 51.8                     |
| 3       | M   | 7                   | 42                   | 302      | unknown | 13.6                 | 2.2                  | 20.5                     |
| 4       | M   | 15                  | 52                   | 550      | unknown | 6.5                  | 1.4                  | 15.9                     |

CK: creatine kinase in U/l (normal: 24–195), SGP: sarcoglycanopathy, DMD: Duchenne muscular dystrophy, BMD: Becker muscular dystrophy, SG: sarcoglycan. %NCAM-positive and %CD24-positive fibers: percentage of 500 randomly selected fibers exhibiting positive staining. %satellite cells/myonuclei: percentage of satellite cells per 500 myonuclei in non-necrotic muscle fibers. "The differences of CD24-positive fibers are statistically significant between SGP and BMD (Mann-Whitney U test, p=0.03). "The differences of satellite cells are statistically significant between SGP and BMD (Mann-Whitney U test, p=0.03).
Figure 1. Immunohistochemistry for CD24 in patients 1 (A) and 5 (B) with sarcoglycanopathy, and in patients with BMD (C). Many CD24 positive muscle fibers are seen in the patients with BMD, but not in those with sarcoglycanopathy. ×150 (BMD: Becker muscular dystrophy).

Figure 2. Immunohistochemistry for NCAM in patients 3 (A) and 5 (B) with sarcoglycanopathy, and in patients with BMD (C). The number of NCAM positive satellite cells (arrowhead) is less in patients with sarcoglycanopathy. ×300 (BMD: Becker muscular dystrophy, NCAM: neural cell adhesion molecule).

and HCAM, and suspected the failure of signal transduction of growth factors, or the dysfunction of other heparan sulfate-binding proteins. In our previous study (8) we reported that NCAM was upregulated in a fair number of muscle fibers in muscular dystrophy including sarcoglycanopathy, however we did not count the NCAM-positive satellite cells separately. The present study was focused on the regeneration of muscle fibers, and disclosed the poor expression of CD24 and fewer satellite cells (small regenerating fibers with little sarcoplasm may be included) in sarcoglycanopathy compared to BMD. We consider that the difference is not due to a difference in the number of total regenerating fibers, but due to a difference in the regeneration process of muscle fibers, or to other unknown
fewer satellite cells are common to all patients with sarcoglycanopathy. It is important to determine if the poor expression of CD24 and the beta-1 subunit of laminin, HSPG, and HCAM. It would be important in the regeneration process in diseased muscle fibers and the fewer satellite cells are characteristic of sarcoglycanopathy, in addition to reduction of the number of CD24-positive fibers is always lower than that of NCAM-positive fibers (9), however, the details of the pathological difference between the fibers are largely unknown. It has been reported that very few muscle fibers express polysialylated NCAM and CD24 in childhood spinal muscular atrophies (14). Since the diameter of CD24-positive fibers seem to be smaller than that of NCAM-positive fibers, CD24 appears to be expressed in the early stages of the regeneration process. Taken together, the poor expression of CD24 and the fewer satellite cells in sarcoglycanopathy suggest that the time schedule of necrosis to regeneration is different between sarcoglycanopathy and BMD. One possible explanation for the difference is that the differentiation of satellite cells in sarcoglycanopathy is similar to that in BMD but the proliferation or recruitment of satellite cells in sarcoglycanopathy may be less active than that in BMD.

The CD24 is a glycosyl phosphatidylinositol (GPI)-linked glycoprotein expressed on hematopoietic cells, including B cells, T cells and granulocytes, and on non-hematopoietic cells, including neural cells. It has been reported that CD24 may play a role in cell adhesion, or as a signal transducing molecule (15–18). A cDNA that directs the expression of CD24 on the surfaces of transfected COS cells was cloned on the basis of its homology to a cDNA encoding the murine heat stable antigen (19). Figarella-Branger et al (9) reported that CD24 immunoreactivity was only observed in some unmystelined nerve fibers in normal adult muscles, and CD24 expression was always associated with a subpopulation of regenerative fibers in diseased muscles. The function of CD24 in muscle fibers has not been fully determined, but since the molecule is not expressed in developing human muscle fibers (9), CD24 expression may be important in the regeneration process in diseased muscle but not in the normal development of muscle fibers.

The present study disclosed that the poor expression of CD24 in muscle fibers and the fewer satellite cells are characteristic findings in sarcoglycanopathy, in addition to reduction of the beta-1 subunit of laminin, HSPG, and HCAM. It would be important to determine if the poor expression of CD24 and the fewer satellite cells are common to all patients with sarcoglycanopathy, in particular, in patients at early stages of the disease. Future experiments are necessary to determine the exact role of CD24 in muscle fibers, and to determine if the dysfunction of regeneration is relevant to the pathogenesis of sarcoglycanopathy.

References