Dystrophin-Related Protein in Becker Muscular Dystrophy

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A benign Becker muscular dystrophy (BMD) patient with a marked decrease in dystrophin exhibited remarkable expression of dystrophin-related protein (DRP) on most of the muscle cell membrane. A phenotypic Duchenne muscular dystrophy patient with a truncated form of dystrophin exhibited no DRP expression on the muscle cell membrane except for the neuromuscular junction. Increased DRP expression might compensate for a lack of dystrophin in some BMD patients.

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Introduction

Dystrophin-related protein (DRP) (1) is a 13-kilobase transcript that maps to human chromosome 6 and exhibits entire length homology to dystrophin (2). Its localization in normal skeletal muscle is similar to that of acetylcholine receptor (3). On the other hand, DRP expression is increased on most of the muscle cell membrane in Duchenne muscular dystrophy (DMD) (4, 5). In Becker muscular dystrophy (BMD), some regenerating fibers show a positive immunoreaction to DRP on the muscle cell membrane (6). However, it remains to be determined whether or not DRP can functionally compensate for a dystrophin abnormality. Here we report a benign BMD patient and a phenotypic DMD patient exhibiting an unusual immunoreaction to anti-DRP antibody.

Patients and Methods

Patient 1 was a 42-year-old man who gradually developed progressive muscle weakness in childhood. He could not run at age 15 years and noticed difficulty in standing up from the floor at age 42 years. He had proximal dominant muscle weakness and hypertrophy of the calves. Serum CK activity was elevated to 302 U/L (normal: less than 55 U/L). He had no family history of neuro-muscular diseases. Patient 2 was a 13-year-old boy who had rapidly progressive muscle weakness and became wheelchair-bound at age 10 years. We reported the results of dystrophin tests for this patient previously (7). Biopsied specimens were obtained from the biceps brachii muscle of both patients.

Serial frozen sections of the specimens were stained by means of standard histologic and histochemical techniques. Immunohistochemical studies were performed according to the avidin-biotin complex (ABC) method (8). We used 3 types of monoclonal antibodies which were raised against the N-terminus, mid rod and C-terminus of dystrophin (purchased from Novocastra, England), and anti-DRP polyclonal antibody (provided by Dr. S. Ishiura, Tokyo University), which exhibits no cross-reactivity to the dystrophin molecule (3). Immunoblot analysis was performed by the method described by Hoffman et al (9). Blood samples were used for DNA studies performed by SRL (Tokyo, Japan) using the multiplex polymerase chain reaction (PCR) method. Oligonucleotide primer sequences that can be used to amplify a muscle specific promoter and 18 exons (exons 3, 4, 6, 8, 12, 13, 17, 19, 43, 44, 45, 47, 48, 49, 50, 51, 52 and 60) described by Chamberlain et al (10) and Beggs et al (11) were used.

Results

A standard histochemical study on the biopsied muscle specimens from the two patients showed similar findings, such as variation in fiber size, some necrotic fibers, occasional regenerating fibers, increased internal nuclei, and increases in fatty and fibrous connective tissues. Immunohistochemical studies for dystrophin in patient 1 disclosed almost the same pattern with the 3 types of antibodies, with 20% of the muscle fibers showing no immunoreaction and 70% showing a faint or
Fig. 1. Immunohistochemical analysis of biopsied muscle specimens from patient 1, a benign BMD patient (A, B), and patient 2, a phenotypic DMD patient with the C-terminal domain (C, D), using a monoclonal antibody against the C-terminal domain of dystrophin (A, C), and an anti-DRP antibody (B, D). The arrow indicates a neuro-muscular junction. Magnifications (A, B) x200 and (C, D) x350.

Patchy staining pattern (Fig. 1A). Immunohistochemical staining for DRP in patient 1 disclosed continuous marked expression of DRP on most of the muscle cell membrane (Fig. 1B). Immunohistochemical studies for dystrophin in patient 2 disclosed a patchy staining pattern with the antibodies against the C-terminus (Fig. 1C) and the mid rod domain of dystrophin, however, no immunoreaction was observed with the N-terminal antibody. Immunohistochemical staining for DRP in patient 2 showed no DRP expression on the muscle cell membrane except for the neuro-muscular junction (Fig. 1D). Immunoblot testing of patient 1 revealed an almost normal molecular weight and a markedly reduced amount of dystrophin. That of patient 2 revealed an abnormal molecular weight (340 kd) and a well-preserved amount of dystrophin with the C-terminal antibody; no immunoreaction was observed with the N-terminal antibody (7). Multiplex PCR analysis demonstrated the deletion of exons 3 and 4 in patient 1, and exons 3, 4, 6, 8, 12, 13, 17, and 19 in patient 2.

Discussion

Most muscle fibers in DMD patients show DRP expression on the cell membrane, and selective DRP expression on regenerating muscle fibers is the most common finding in BMD patients (6). However, we demonstrated marked continuous expression of DRP on the cell membrane not only in regenerating but also in normal appearing muscle fibers in patient 1. The patient was diagnosed as having BMD, and the clinical course seems to be benign (he can walk at age 42) in spite of the marked decrease in the amount of dystrophin. On the contrary, the muscle fibers in patient 2 showed no DRP expression on the muscle cell membrane except for the neuro-muscular junction. He had rapidly progressive muscle weakness and was clinically diagnosed as having DMD, however, he was later diagnosed as having BMD on the basis of dystrophin test results. As an explanation of the unusual severity in this patient, we previously reported that deletion of the N-terminal domain of dystrophin can cause a severe phenotype. The abnormality of DRP expression on the muscle cell membrane might have also
led to the clinical severity of this patient.

Recently, some investigators reported marked upregulation of DRP not only in patients with DMD or BMD but also with polymyositis or dermatomyositis (12, 13). We have not observed the upregulation of DRP in patients with polymyositis or dermatomyositis (data not shown). The differing results may be due to the different anti-DRP antibodies used in the studies.

The results of the present study suggest that an increased DRP expression on the muscle cell membrane may compensate for a lack of dystrophin in some BMD patients. A recent report concerning the association of DRP with dystrophin-associated proteins in mdx mouse muscle (14) may provide a basis for the upregulation of DRP as a therapeutic trial. Further studies on the present patients are necessary to elucidate the regulatory mechanism for DRP expression on the muscle cell membrane.

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