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Immunocytochemical localization of myotonin protein kinase on muscle from patients with congenital myotonic dystrophy

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Summary. Using a polyclonal anti myotonin-protein kinase (M-PK) antibody against synthetic M-PK peptides corresponding to part of the amino acid sequence, and the immunohistochemical analysis of indirect immunoperoxidase, we have investigated localization of M-PK on muscle from patients with congenital myotonic dystrophy. In congenital myotonic dystrophy (MD) patients, one month and 3 months old, M-PK was weakly expressed at sarcolemma of muscle fibers. In congenital MD patients from 2 to 9 years of age, M-PK was clearly expressed at sarcolemma of muscle fibers. M-PK of immature muscle is weakly expressed at sarcolemma. With aging, M-PK is clearly expressed at sarcolemma of muscle from MD patient and normal control.

Key words: Immunochemistry, Myotomin dystrophy, Myotonin protein kinase

Introduction

Myotonic dystrophy (MD) is an autosomal-dominant inherited multisystemic disorder characterized by myotonia, muscular weakness, cataracts, gonadal atrophy, and cardiomyopathy (Harper, 1989). The molecular defect has been identified as an unstable trinucleotide (CTG) repeat, located in the 3' end of a transcript encoding a protein with putative serine/threonine protein kinase (myotonin-protein kinase, M-PK) (Brook et al., 1992; Fu et al., 1992; Mahadevan et al., 1992). We investigated localization of M-PK on muscle from patients with congenital MD by immunocytochemistry using antibody against synthetic M-PK peptide antigen.

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Materials and methods

We studied 5 patients with congenital MD, one male and 4 females, ranging in age from 1 month to 9 years. Their mothers were diagnosed as adult form MD. 5 congenital MD patients had characteristic clinical features, including marked hypotonia with facial diplegia, respiratory distress, and feeding difficulty during neonatal period. Patients' muscles and age-matched 5 controls' muscles were frozen in liquid nitrogen-cooled isopentane for Southern blot analysis, immunocytochemistry and histochemistry. Genomic DNAs from muscles were prepared by standard procedures.

Southern blot analysis

Seven micrograms of DNA were digested with EcoRI. Digested DNAs were separated by electrophoresis on 0.8% agarose gel, and transfered onto nylon membranes using vacuum transfer apparatus. After prehybridization, digested DNAs were hybridized to radiolabeled p5B1.4 for 24 hours. Autoradiography was performed for 2 to 5 days at -80 ºC.

Histochemical procedures

Immunocytochemical analysis of M-PK was performed in transverse cryostat sections. The immunoreaction was performed on 6 μm sections by indirect immunoperoxidase, using a polyclonal antibody. We used the original polyclonal anti M-PK antibody against synthetic M-PK peptides corresponding to part of the amino acid sequence of exon 8 within the serine/threonine protein kinase domain. This antibody was raised in rabbits and identifies 55 kDa protein in skeletal muscle, heart, and to a lesser extent in brain (a gift of Dr. C.T. Caskey). Histochemical stainings used were

hematoxylin and eosin, modified Gomori Trichrome, HADH-TH, and ATPase.

Results

Southern blot analysis showed an EcoRI polymorphism with alleles of 9.8kb and 8.6kb in normal muscle. An 8.6kb or 9.8kb plus expanding band was observed in muscle from congenital MD patients. Patient 1 (3 months, female) had alleles of 8.6kb/16.5 kb in muscle. Patient 2 (9 years, female) had alleles of 8.6kb/17.5kb. Patient 3 (2 years 5 months, female) had alleles of 8.6kb/17.5 kb. Patient 4 (2 years 5 months, female) had alleles of 8.6 kb/15.5 kb. Patient 5 (one month, male) had alleles of 9.8 kb/15.5 kb.

Histochemical and immunocytochemical study showed a variation in fiber size and type 1 fiber atrophy, from a slight to moderate degree, on muscle sections from all MD patients.

In patient 1 and 5, the most characteristic finding was the presence of small round fibers with large internal nuclei and a peripheral sarcoplasmic halo, indicating delay in muscle maturation (Fig. 1a).

Immunohistochemistry using anti M-PK antibody revealed a weak staining in the sarcolemma of muscle fibers from patients 1 and 5 (Fig. 1b). Immunostaining for M-PK expression in two-week-old normal muscle revealed similar M-PK expression to patient 1 and 5. In patients 2, 3, and 4, a characteristic finding was a type 1 fiber predominance and a decreased number of type 2A or 2B fibers. These findings were sometimes observed in muscle pathology of adult form MD. Immunohistochemistry revealed a clear staining in the sarcolemma of muscle fibers from patients with 2, 3, and 4 (Fig. 2a). The staining pattern of M-PK in adult normal muscle was similar to that of patients 2, 3, and 4 (Fig. 2b).

Discussion

The molecular basis of MD mutation has recently been identified as an unstable trinucleotide (CTG) repeat, located in the 3' end of a transcript encoding a protein with putative serine/threonine protein kinase (M-PK) (Brook et al., 1992; Fu et al., 1992; Mahadevan et al., 1992). Until now, little is known about the function and distribution of M-PK. Fu et al. (1993) first identified a 55 kDa protein product of the MD gene in muscle, brain and heart using specific antibodies against different

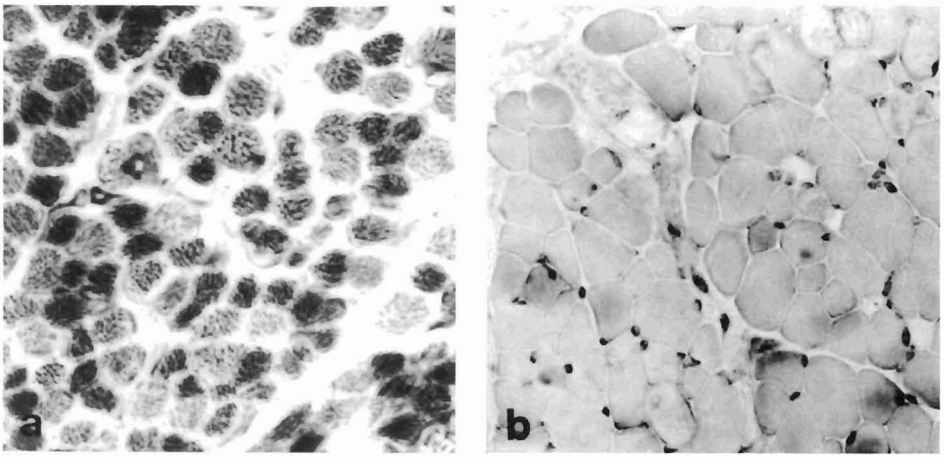

Fig. 1. a. Fibers with peripheral sarcoplasmic halo are seen in transverse section of patient 5. **b.** NADH-TR staining. In patient 1, M-PK immunolocalization is weakly observed at sarcolemma of muscle fibers. x 470

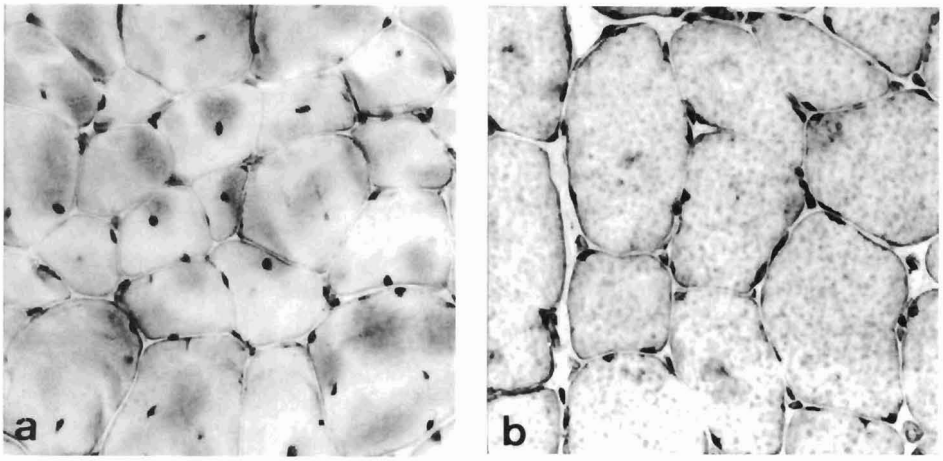

Fig. 2. a. In patient 2, M-PK staining is clearly observed at sarcolemma of muscle fibers. **b.** In normal adult muscle, M-PK is clearly shown at sarcolemma of muscle fibers. x 240

regions of the predicted amino acid sequence. They demonstrated decreased levels of M-PK and its mRNA in skeletal muscle of adult MD patients. Brewster et al. (1993) identified a 52 kDa M-PK as a major protein product of the MD gene. Koga et al. (1993) suggested the presence of tissue-specific isoforms of MD-PK using specific antibodies against synthetic MD-PK peptides. Etongué-Mayer et al. (1994) identified a 54kDa protein product of the MD gene using specific antibodies.

Strong et al. (1994) showed that 52kDa M-PK was not uniformly located in muscle cells but appeared to be present at myotendinous junctions and possibly associated with nuclei. Van der Ven et al. (1993) identified a 53kDa protein product of the MD gene and disclosed the distribution of its protein in human skeletal muscle.

M-PK was localized prominently at sites of neuromuscular and myotendinous junctions in both normal and MD muscle. Very low levels of MD-PK were present in the sarcolemma of predominantly type 1 fiber. Our data showed that immunohistochemical analysis for M-PK revealed weakly sarcolemmal staining on immature muscle observed in patient 1 and 5. With aging, immunostaining for M-PK showed clear sarcolemmal staining on muscle from patients 2, 3, and 4. We disclosed that M-PK was initially observed in the perinuclear area of myoblast in cultured normal human muscle.

Subsequently, M-PK was diffusely observed in cytoplasm of myotubes. Until those stages of cultured muscle, M-PK was not observed at sarcolemma of myotubes (Tachi et al., 1995).

Based on these observations, M-PK is initially localized in cytoplasm and perinuclear area of immature muscle cell, and migrates toward sarcolemma with maturity of muscle cell.

Congenital MD, which is the most severe form of the disease, has the largest size of CTG repeat. In our study, there was no correlation between pattern of expression of M-PK and expansion size of CTG repeat on muscle from congenital MD patients. It is well known that immature muscle is able to gain a certain degree of maturity in time in congenital MD patients. Further characterization and observation of the role of M-PK is needed.

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