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Electron microscopy in myofibrillar myopathies reveals clues to the mutated gene

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Abstract

We studied the ultrastructural characteristics in patients with myofibrillar myopathy (MFM) and differentiated between MFM-subtypes using electron microscopic (EM) findings. The ultrastructural findings in 19 patients with different genetically proven MFMs (9 desmin, 5 αB-crystallin, 3 ZASP, 2 myotilin) were analyzed. In one ZASPopathy, we additionally performed an immunoEM study, using antibodies against desmin, αB-crystallin, ZASP and myotilin. The ultrastructural findings in desminopathies and αB-crystallinopathies were very similar and consisted of electrondense granulofilamentous accumulations and sandwich formations. They differed in the obvious presence of early apoptotic nuclear changes in αB-crystallinopathies. ZASPopathies were characterized by filamentous bundles (labeled with the myotilin antibody on immunoEM), and floccular accumulations of thin filamentous material. Tubulofilamentous inclusions in sarcoplasm and myonuclei in combination with filamentous bundles were characteristic for myotilinopathies. We conclude that MFMs ultrastructural findings can direct diagnostic efforts towards the causal gene mutated, and that EM should be included in the diagnostic workup of MFMs.
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Keywords: MFM; EM; Ultrastructural; Genetic analysis; Diagnosis

1. Introduction

Myofibrillar myopathies (MFMs) are rare inherited or sporadic progressive neuromuscular disorders with considerable clinical and genetic heterogeneity. To date, mutations in five genes are known to cause MFM. These genes encode desmin [1], αB-crystallin [2], myotilin [3], Z-band alternatively spliced PDZ motif containing protein (ZASP) [4], and filamin C [5]. However, at present a genetic abnormality cannot be identified in more than half of MFM patients.

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The diagnosis of MFM is based on histological features observed by optical microscopy: the presence of single or multiple non-hyaline amorphous inclusions and small granules, and spherical, lobulated or serpentine hyaline structures, and/or cytoplasmic bodies, and both rimmed and non-rimmed vacuoles [6–10]. However, differentiating between the genetically different subtypes of MFMs is not possible using these histological results. Also, desmin and myotilin-positive aggregates are a characteristic but non-selective immunohistochemical finding, since they are present in all MFM subtypes. Although clinical observations can provide clues, in general the overlap in clinical phenotypes associated with the known genes prevents an accurate prediction of the causative gene.

At the ultrastructural level, MFMs are characterized by myofibrillar disorganization and focal accumulations of compacted degraded myofibrillar elements. In the present study, we analyze the ultrastructural characteristics of different genetically proven MFMs, and describe the morphological differences between the subgroups. Our results suggest that the distinct ultrastructural features in the MFM subgroups can be used to guide genetic analysis in patients with MFM.

2. Patients and methods

2.1. Patients

The study included 19 patients with genetically proven MFM and with muscle tissue available for ultrastructural analysis. Nine patients belonging to six families harboured a desmin mutation, five patients originating from one family had an αB-crystallin mutation, three unrelated patients had a ZASP mutation, and in two unrelated patients a mutation in myotilin was identified. We did not identify patients with a filamin C mutation in our cohort. For each patient the biopsied muscle, age at biopsy, mutated gene and mutation are indicated in Table 1. Open muscle biopsies and genetic analysis were performed after written informed consent according to the Declaration of Helsinki.

2.2. Optic microscopy

Specimens for light microscopy were immediately frozen and processed using standard histological and histochemical techniques according to previously described procedures [11]. For immunohistochemical studies we used monoclonal anti-mouse desmin (clone D33, Dako, Glostrup, Denmark), and monoclonal anti-mouse myotilin (clone RSO34, Novocastra, Newcastle upon Tyne, UK) antibodies.

2.3. Transmission electron microscopy

Small blocks of the muscle biopsies were fixed in 2.5% glutaraldehyde (pH 7.4), and postfixed in 2% osmium tetroxide. After dehydration the tissue blocks were embedded in resin (EMBed-812, Electron Microscopy Sciences, USA). Semithin sections of 0.5 μm stained with toluidine blue permitted a selection of the areas of interest. Within the regions of interest, ultrathin sections of 60 nm were cut using an ultramicrotome (Leica Ultracut UCT, Reichert, USA). Next, the sections were contrasted with lead citrate and uranyl acetate. For electron microscopy we used the Philips CM-120 microscope (voltage 80 kV) and for photodocumentation the Morada camera (Soft Imaging System, France). For each patient we investigated multiple longitudinal and transverse sections, except for patient 1 in whom only transverse sections were available for examination.

2.4. Immunoelectron microscopy

In patient 17 who had a ZASP mutation we also performed immunogold electron microscopy. Small blocks of muscle tissue (1 mm^3) were fixed in a solution of 2% paraformaldehyde and 0.1% glutaraldehyde with Hepes buffer (0.1 M pH 7.5), dehydrated with series of ethanol, and embedded in LR White (London Resin Company Ltd., Berkshire, UK). Ultrathin sections of 60–70 nm were preincubated in 50 mM glycine and after washing, were incubated in blocking buffer of 5% normal goat serum in PBS, 5% BSA, 0.1% fish gelatin for 1 h at room temperature, and after washing incubated in a wet chamber at 4 °C with the primary antibody: monoclonal anti-mouse desmin (clone D33, Dako, Glostrup, Denmark), polyclonal anti-rabbit αB-crystallin (Stressgen, Victoria, Canada), monoclonal anti-mouse myotilin (clone RSO34, Novocastra, Newcastle upon Tyne, UK), and anti-mouse ZASP (kindly provided by G. Faulkner, Trieste, Italy) were diluted in incubating buffer (PBS, 0.5% BSA, 0.1% fish gelatin) at 1:20. After overnight incubation with the primary antibody and subsequent washing, the grids were incubated with a secondary antibody coupled to colloidal gold (diluted 1/20, 10 nm particles) overnight at 4 °C in a wet chamber. Next, the grids were postfixed with 1% glutaraldehyde in PBS. Finally, the preparations were contrasted using conventional techniques. Ultrathin sections were examined using a Zeiss 900 (Oberkochen, Germany) electron microscope at an accelerating voltage of 80 kV.

3. Results

The optic microscopic findings in the patients belonging to the four MFM subgroups are summarized in Table 2. All patients showed marked pathology at the light microscopic level. Overall, the light microscopic observations were very similar amongst the four MFM subgroups.

The ultrastructural abnormalities in the biopsies of all patients typically had a focal character. Some fibers showed abnormalities, whereas adjacent fibers had a normal structure, and within an individual muscle fiber, regions containing normal sarcomeres alternated with areas of pronounced myofibrillar disorganization and the

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