Nuclear architecture remodelling in envelopathies

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Abstract
We performed ultrastructural studies on nuclear abnormalities in muscle from 8 patients with X-linked and autosomal dominant form of Emery-Dreifuss muscular dystrophy (EDMD) and one case with progeroid syndrome. The diagnosis was based on clinical and molecular findings. We detected various degrees of nuclear architecture remodelling ranging from misshapen shape, nuclear disintegration, nuclear chromatin condensation and decondensation, focal chromatin loss to complete nuclear fragmentation. The most interesting finding was the appearance of tubulofilamentous inclusions inside the nuclear matrix of X-linked EDMD patients. All these nuclear aberrations are considered to be structural indicators of nuclear dysfunction evoked by envelope protein deficiency.

Keywords: envelopathies, nuclear aberrations.

Introduction
For a long time, abnormalities of the muscle nuclei received little attention compared to the pathological changes of cytoplasmic cell components. During the last few years, knowledge about the nucleoskeleton, its proteins and structural abnormalities has emerged, and the molecular basis of this nuclear infrastructure, although still incomplete, is gradually being unravelled. The main structural elements forming the muscle nuclei include the internal nuclear matrix and nuclear membranous envelope that separates the nucleoplasm from the cytoplasm [18]. In recent years, mutations in nuclear envelope proteins have been shown to cause a surprisingly wide array of inherited diseases [15]. The mutated A/C lamin binding nuclear proteins (emerin, MAN1, LBR, Lap2) are linked to numerous human diseases collectively termed laminopathies [14,15,18,20]. They affect muscle, adipose, bone, nerve and skins cells ranging from muscular dystrophies to accelerated aging [2,3,6,11,19,22]. In this study we intend to characterize the major changes in nuclear architecture that accompany some mutations in the LMNA gene.

Material and methods
Muscle biopsies of four affected X-linked EDMD males were investigated. The diagnosis was based on clinical findings, DNA analysis and absence of emerin in immunostaining procedure. Muscle biopsies of four ADEDMD affected patients and one girl with progeroid syndrome and mutation in chromosome 1q21 and lamin A/C deficiency were analysed (Table I).

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Table I. Muscle biopsies of four ADEDMD affected patients and one girl with progeroid syndrome and mutation in chromosome 1q21 and lamin A/C deficiency

<table>
<thead>
<tr>
<th>Case</th>
<th>Disease</th>
<th>Age</th>
<th>Emerin activity</th>
<th>Emerin mutation</th>
<th>Lamin A/C activity</th>
<th>Lamin A/C mutation</th>
</tr>
</thead>
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<tr>
<td>DA</td>
<td>X-EDMD</td>
<td>42</td>
<td>–</td>
<td>C636T</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DA</td>
<td>X-EDMD</td>
<td>25</td>
<td>–</td>
<td>G421A</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>BB</td>
<td>X-EDMD</td>
<td>14</td>
<td>–</td>
<td>G421A</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>KM</td>
<td>X-EDMD</td>
<td>12</td>
<td>–</td>
<td>C.IV3de-10-27</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SD</td>
<td>ADEDMD</td>
<td>14</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>T743C</td>
</tr>
<tr>
<td>KM</td>
<td>ADEDMD</td>
<td>12</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>C1357T</td>
</tr>
<tr>
<td>KG</td>
<td>ADEDMD</td>
<td>41</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>C1357T</td>
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<tr>
<td>SM</td>
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<td>+</td>
<td>–</td>
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<td>G1072A</td>
</tr>
<tr>
<td>BN</td>
<td>H6PS</td>
<td>6</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>C428T</td>
</tr>
</tbody>
</table>

For electron microscopy, the muscle specimens were fixed in 3% glutaraldehyde in phosphate buffer and postfixed in 1% osmium tetroxide in the same buffer. Then they were dehydrated and embedded in spurr resin. Thin sections double stained with uranyl acetate and lead citrate were examined in a JEM 12000X/II electron microscope.

**Results**

Normal human muscles contain peripherally located nuclei with oval or round shape and smooth outline (Fig. 1). In addition, the organization of nuclear envelope-associated structure including pores, an extremely thin nuclear lamina and heterochromatin appear normal. In contrast, nuclei of laminopathic and emerinopathic patients frequently display irregular shape, folded outline, destruction of the nuclear envelope, abnormal composition of the nuclear lamina, remodelling of nuclear matrix and nuclear fragmentation.

Misshapen nuclei of irregular shape were observed in all investigated cases. More affected ADEDMD patients showed deep nuclear invagination (Fig. 2) and blebs projecting towards the cytoplasm (Fig. 3). Extensive nuclear deformation and segmentation

![Fig. 1. Nucleus with normal architecture. × 36.000](image-url)
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appeared in a child with progeroid syndrome and mutation in the LMNA gene (Fig. 4). In this last case, some nuclei contained thick nuclear lamina of 58-60 nm in diameter (norm 10-20 nm) adjacent to the INM (Fig. 5). In some nuclei, this unusual thick lamina penetrated into the nuclear matrix forming long narrow skeins (Fig. 6). Focal disruption and loss of the nuclear envelope and nucleoplasm extrusion into extranuclear space was the characteristic feature observed in EDMD patients (Fig. 7). More extensive nucleoplasm extrusion across a disrupted nuclear membrane was manifested by the presence of “naked” chromatin long tail in close contact with the nucleus (Fig. 8). Abnormal heterochromatin disruption and density were characteristic markers of X-linked as well as ADEDMD patients. In a number of nuclei, the heterochromatin appeared very dense and dark, completely filling the whole nucleus (Fig. 9). In some other nuclei, the heterochromatin reorganization was manifested by massive chromatin decondensation (Fig. 10), focal loss appearing as patches of varying shape and size (Fig. 11). A very interesting finding in

![Fig. 2. Misshapen nucleus with numerous deep invaginations. × 40 000](image)

![Fig. 3. Nucleus with blebs projecting towards the cytoplasm (arrowheads) × 60 000](image)
some nuclei was the detachment of peripheral heterochromatin from the nuclear lamina, forming narrow or large splits (Fig. 11). This phenomenon was observed only in cases with mutation in the LMNA gene. Massive disappearance of the nuclear matrix with preserved very narrow peripherally located heterochromatin ring was a rare finding seen in lamin A deficiency. Various stages of nuclear fragmentation were observed in both forms of EDMD (Fig. 12). A surprising phenomenon seen in two cases with emerin deficiency as well as in one case with a deficiency of both proteins (emerin and lamin A) was

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**Fig. 4.** Extensive nuclear deformation and segmentation. × 24 000

**Fig. 5.** Nucleus with blebs (arrowheads) and lamina thickness (asterisk). × 30 000
the appearance of tubulofilamentous structures (TFs), analogues to paired helical structures seen in inclusion body myositis (IBM). TFs of 16-20 nm in diameter were located in the nuclear matrix of euchromatic nuclei (Fig. 13) as well as in disrupted nuclei.

**Discussion**

In this study, we present major changes in the nucleoskeleton architecture that accompany emerin and lamin A/C deficiency. The nucleoskeleton is composed of structural proteins that provide the framework for DNA replication, transformation, repair and a variety of other nuclear functions [10]. The nucleus is surrounded by an envelope composed of three parts: the nuclear membranes (inner, outer), the nuclear complex and the nuclear lamina [21,20,12]. The outer nuclear membrane is directly continuous with the endoplasmic reticulum. The pore membranes connect the inner and outer nuclear membranes at numerous points. The inner nuclear membrane is associated with the nuclear

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**Fig. 6.** Thick lamina penetrating inside nuclear matrix (arrowheads). × 60 000

**Fig. 7.** Focal disruption of nuclear envelope with nucleoplasm extrusion (arrowheads) × 36 000
lamina. The nuclear lamina is a layer located between the inner nuclear membrane and the peripheral chromatin. The main components of the nuclear lamina are intermediate filaments known as a lamins [10,14,16,20]. Nuclear envelope herniation, rupture and chromatin extrusion into the extranuclear space found in patients with X-linked EDMD [7] are likely symptoms of defective internal membrane assembly. Emerin, an integral part of INM, belongs to the LEM-domain family of proteins and interacts with lamin A/C in the nuclear envelope [4]. Lamins are divided into A types expressed in differentiated cells and B types found in all cells [17]. Lamin proteins have been shown to bind to chromatin and several inner nuclear proteins and have many different functions in the cell. Emerin

Fig. 8. A long "naked" chromatin tail in close contact with nucleus (asterisk). × 15 000

Fig. 9. Heterochromatin re-organization. × 22 000
and lamin A/C form a stable complex with other protein binding partners [13]. Growing evidence also indicates that emerin plays a role in both tissue-specific gene regulation and mechanical integrity of the nucleus [13]. Heterochromatin remodelling, focal loss of nuclear membrane and chromatin extrusion.

**Fig. 10.** Massive chromatin decondensation. × 22 000

**Fig. 11.** Focal loss of heterochromatin with the appearance of empty patches (asterisk) × 20 000
were described previously in X-EDMD patients [7]. More advanced abnormalities of the nuclear architecture have been observed in ADEDMD patients. Their nuclei were abnormally shaped with deep invaginations leading to formation of blebs, pseudoinclusions [8] and nuclear fragmentation. In the literature the most irregularly shaped nuclei were reported in the progeria syndrome with mutation in the LMNA gene. The extreme lobulation of the nuclear membrane somewhat resembles a cauliflower or a bunch of grapes [5]. The many other changes that occurred in the nuclei of ADEDMD patients include focal appearance of empty plaques seen in the nuclear matrix. The characteristic detachment of heterochromatin from INM found in ADEDMD patients highlighted the critical role of mutant lamin A not only in anchoring heterochromatin to the nuclear envelope, but also in maintaining heterochromatin architecture. The structural alterations observed by us and others in

![Fig. 12. Nuclear fragmentation. × 30 000](image1)

![Fig. 13. Fragment of nucleus with tubulofilamentous intranuclear inclusions. × 36 000](image2)
ADEDMD patients are not surprising because it has been shown that lamins play a major role in nuclear assembly, organization and shape [10]. Nuclear deformability with nuclear matrix reorganization was reported in fibroblasts of patients with familial partial lipodystrophy [22] and progeroid syndrome [11]. A surprising and inexplicable finding in three cases with X-EDMD [9] is the presence of TFs within the nuclear matrix. Their structure, size and location were identical to paired helical structures described in IBM [1]. Our ultrastructural study indicates that nuclear envelope disorganization and heterochromatin remodelling in muscle cells of patients with X-EDMD and ADEDMD are a hallmark of these diseases. The appearance of TFs within the nuclei of X-EDMD patients requires further investigation. The work was supported by the State Committee on Research grant no. 2P05B 106 29.

References