Original article

Folia Neuropathologica

Nuclear architecture remodelling in cardiomyocytes with lamin A deficiency

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Folia Neuropathol 2008; 46 (3): 196-203

Abstract

We analysed the architecture of cardiomyocyte nuclei lacking lamin A activity in three patients with dilated cardiomyopathy. The diagnosis was established on the basis of clinical and electrophysiological examinations, chest radiography and electrocardiography.

An ultrastructural study of affected cardiomyocytes showed dramatic alterations in nuclear distribution and organization affecting nuclear shape, lamina structure, chromatin and nuclear interior organization. The most specific hallmark of nuclei with lamin A deficiency was the reorganization of the nuclear interior, the appearance of a various number of mitochondria within the nuclear matrix, and focal or total lack of nuclear membrane.

Key words: *lamin A deficiency, nuclear remodelling, appearance of mitochondria within nuclear interior.*

Introduction

The nucleoskeleton in cardiomyocytes is composed of many interacting structural proteins that provide a framework for DNA replication and a variety of other nuclear functions. The nuclear envelope separates the chromatin from the cytoplasm and consists of two membranes [29]. The outer membrane, which is continuous with the endoplasmic reticulum, connects to the inner nuclear membrane by nuclear pore complexes [7,13,23]. The nuclear lamina is located between the inner nuclear membrane and peripheral chromatin [5,22]. The nuclear lamina is a dense fibrillar network composed of A and B type lamins [15]. Lamins interact directly with chromatin and several internal proteins including lamin-associated proteins (LAMP2), emerin and the lamin B receptor (LBR). Lamin A/C are the major products of the LMNA gene located at chromosome 1q21. During the past few years, mutations in lamin A/C have caused a wide range of human disorders. These disorders, known as laminopathies [4,5,26], primarily affect cardiac and skeletal muscle, peripheral nerve, adipose tissue and several tissues in a generalized way consistent with premature aging syndromes [6,26,27]. The aim of our study is to reveal, by ultrastructural analysis, the characteristic nuclear changes in lamin A/C deficient cardiomyocytes.

Material and Methods

An endomyocardial biopsy was taken from the right ventricle of three affected patients with diagnosis

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of dilated cardiomyopathy. The diagnosis was established on the basis of clinical history, physical examination, chest radiography and electrocardiography. For indirect immunofluorescence examinations cryostat sections were stained using monoclonal antisera against four epitopes of lamin A/C (AC2, AC3, A4, AC5) as was previously reported [7]. For electron microscopy specimens were fixed in 1% 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 with a JEMII electron microscope.

Results

Indirect immunofluorescence analysis of endomyocardial biopsy revealed that all cardiomyocyte nuclei were immunostained by antibodies directed against lamin A/C2, A/C3 and A/C5 (Fig. 1A). However, antibody A4 was directed against lamin A only, indicating lack of A activity (Fig. 1B). The ultrastructural study of affected cardiomyocytes showed dramatic alterations in nuclear distribution and organization which affected the shape of the nuclei, nuclear lamina structure and chromatin organization. Extensive nuclear deformations appeared in affected nuclei while in control cardiomyocytes the nuclei were mostly regular in size and shape and appeared generally round or ovoid with a smooth nuclear outline (Fig. 2). Affected nuclei were highly elongated, irregular and misshapen (Fig. 3) and some nuclei had a cauliflower appearance. This type of nuclear shape remodelling was never observed in the normal cardiomyocytes. Two kinds of nuclear chromatin abnormality were found in investigated cardiomyocytes. Various degrees of chromatin aggregation were observed in two of the investigated cases. Heterochromatin masses were randomly distributed within the nuclear matrix, forming dense whirlpools and clumps (Fig. 4). In the third case, extensive remodelling of heterochromatin distribution was the most intriguing finding. Focal breakage or leakage of chromatin formed an area of various size which was situated centrally (Fig. 5A) or peripherally (Fig. 5B). In some nuclei, the entire territory of the nuclear matrix was denuded (Fig. 6). In addition, in the same nuclei disrupted heterochromatin territories extended beyond the nuclear lamina (Fig. 7), which might indicate a change in the organization and anchoring of chromatin to the nuclear lamina. In one of these investigated cases, very thin nuclear lamina exhibited focal loss of its continuity, forming empty spaces devoid of nuclear membrane as well as of peripherally located chromatin (Fig. 8).

In the nuclear membrane gaps, penetration of mitochondria into the nuclear interior was frequently visible (Fig. 6). Two repeated nuclear abnormalities were specific for lamin A deficiency. One was focal or total loss of nuclear membrane, sometimes leading to total nuclear destruction (Fig. 9). The second phenomenon frequently observed in lamin A deficient cardiomyocytes was the appearance of mitochondria within the nuclear interior. The penetration of mitochondria through broken nuclear membrane was seen in both types of nuclei, denuded (Fig. 10) and hyperchromatic. In other nuclei, mitochondria were

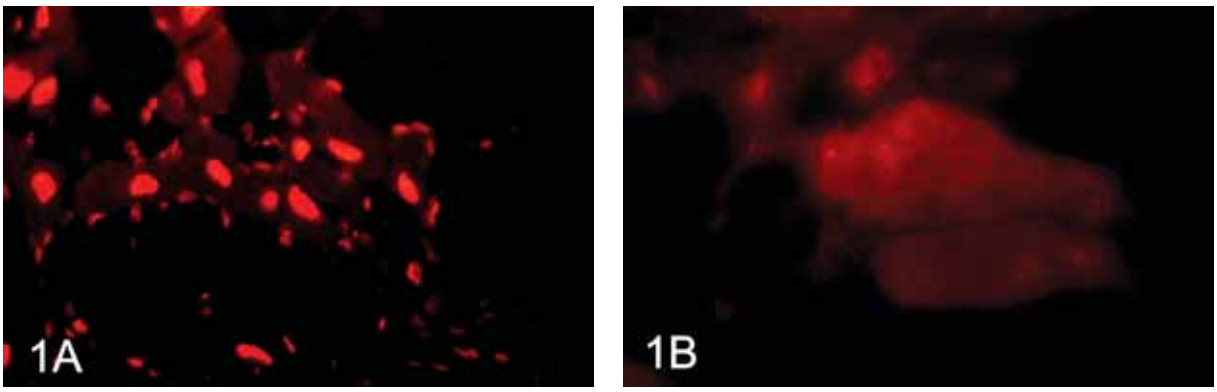


Fig. 1. Immunostaining of cardiomyocytes. A. Antibody AC2 shows intensive labelling of nuclei. B. Loss of lamin A activity in nuclei of affected cardiomyocytes. ×1050

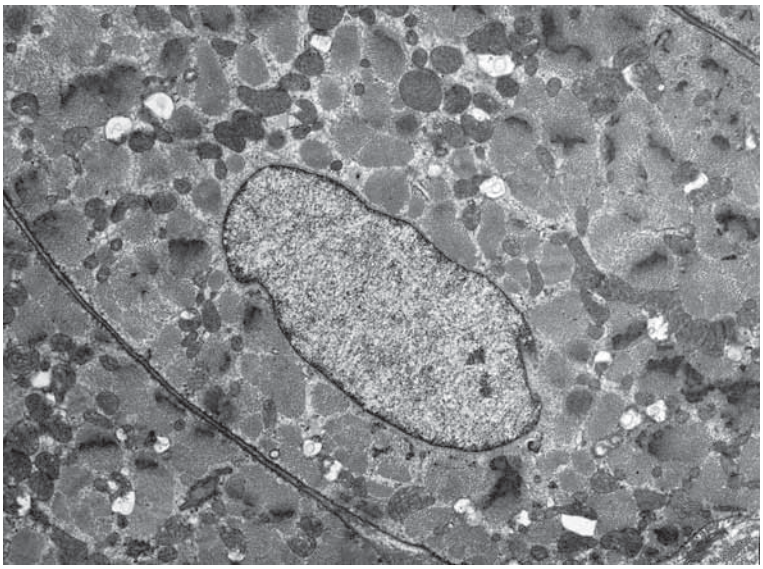


Fig. 2. Normal cardiomyocyte with nucleus of oval shape and smooth outline. ×15 000

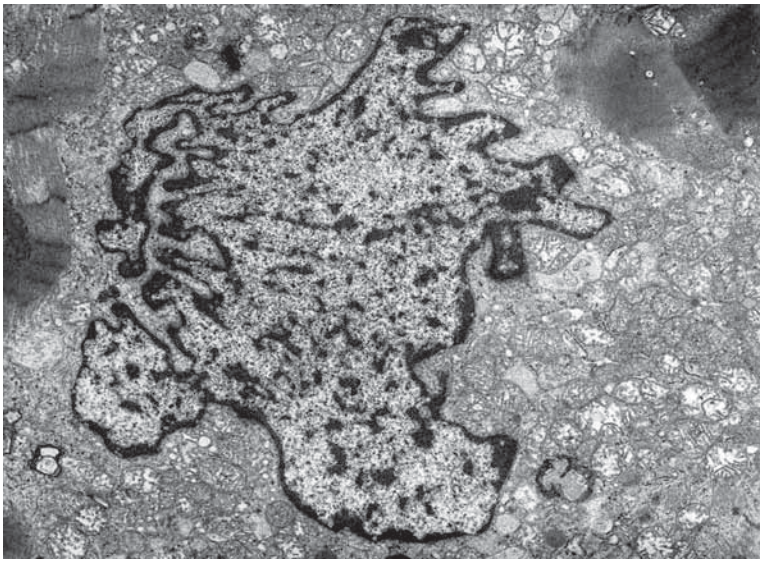


Fig. 3. Misshapen nucleus. ×15 000

loosely dispersed within the nuclear matrix. The most frequent finding in lamin A deficient nuclei was the appearance of mitochondrial clusters within the nuclear matrix and loss of nuclear envelope.

Discussion

The results of our study demonstrate that the lack of lamin A activity in cardiomyocyte nuclei resulted in a high frequency of dysmorphic nuclei. Structural modification of cardiomyocyte nuclei included altered nuclear shape, distorted nuclear envelope continuity, severe heterochromatin reorganization and nuclear interior remodelling. Nuclear deformation is a hallmark for most laminopathies and has been reported in affected cardiomyocytes [1,3,6,24,25,28], in skeletal muscles [9,10,19-21] and in fibroblasts [11,22,29]. Aberrant chromatin reorganization has also been observed in various conditions of lamin A/C misexpression [1,9,10]. Relocation of heterochromatin from the

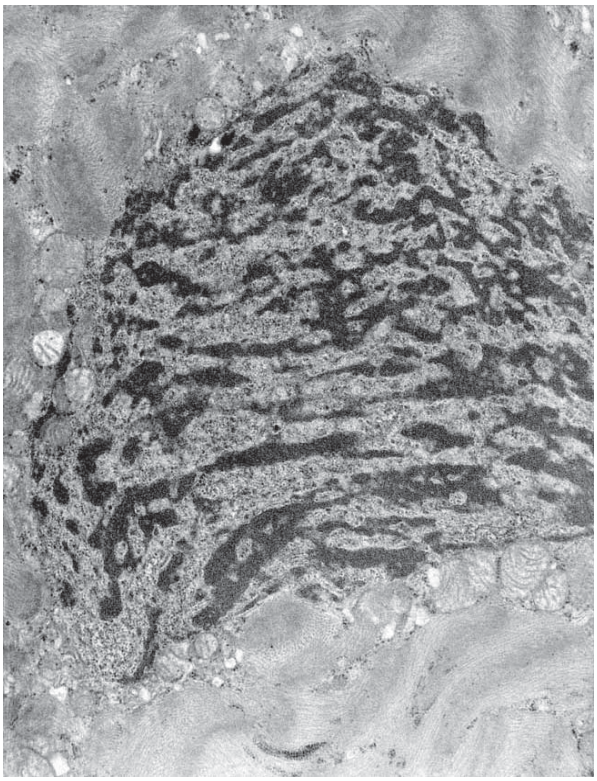
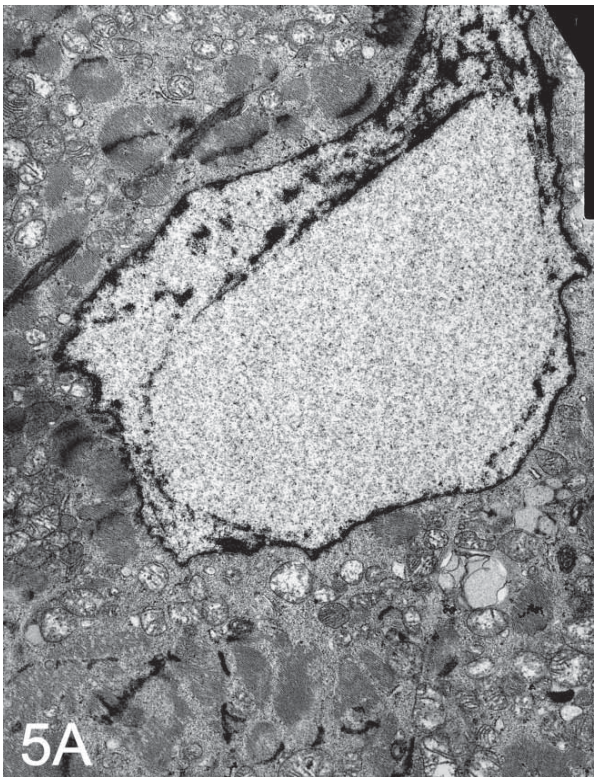


Fig. 4. Heterochromatin masses randomly distributed within nuclear matrix. ×15 000



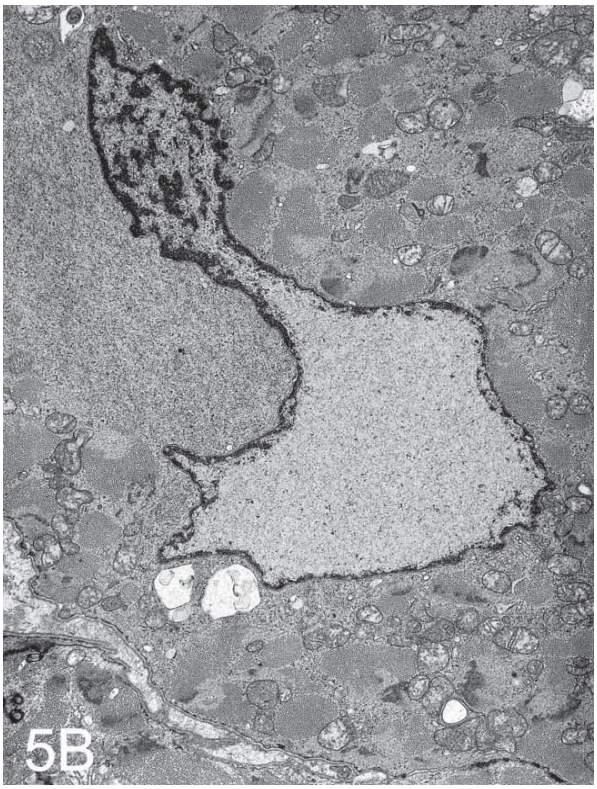


Fig. 5A-B. Areas of focal chromatin leakage. ×15 000

periphery to the interior of the nucleus was demonstrated in the nuclei of cardiomyocytes of lamin A/C deficient mice [18]. Defective nuclear shape and heterochromatin reorganization have been observed in cells from laminopathy patients independent of the site at which lamin mutations occur [12,18,29]. These morphological alterations are not surprising because nuclear lamins play an important role in nuclear assembly, organization and shape [14,15] and misshapen nuclei accompanied by changes in the nuclear membrane and chromatin have been reported in patients with Emery-Dreifuss muscular dystrophy [10,16,19-21], familial partial lipodystrophy [29] and progeroid syndromes [11,12]. The major surprising finding of our study was the evidence of mitochondrial accumulation within the nuclear interior. This phenomenon was found by us previously in cardiomyocyte nuclei with mutation D192G in the LMNA gene [7]. In the currently investigated cases with lamin A deficiency, an identical phenomenon was observed in the heterochromatic as well as in the denuded nuclei. This intriguing phenomenon occurring

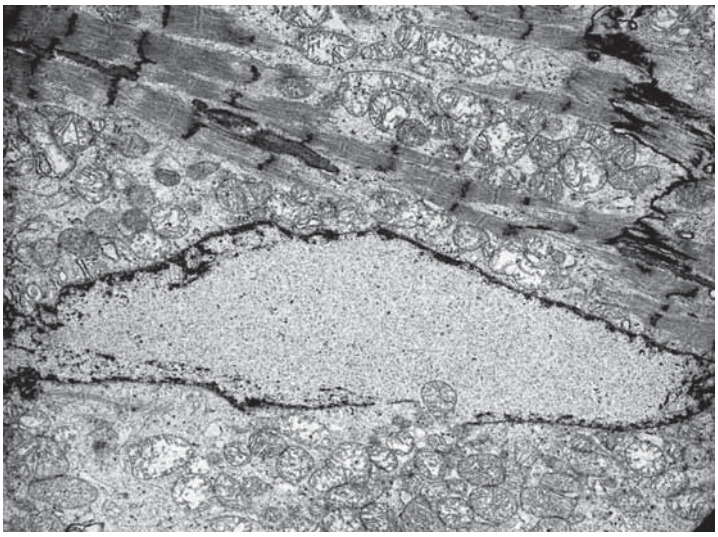


Fig. 6. Denuded nucleus with membrane gap. Note penetration of mitochondrion into nuclear interior. ×12 000

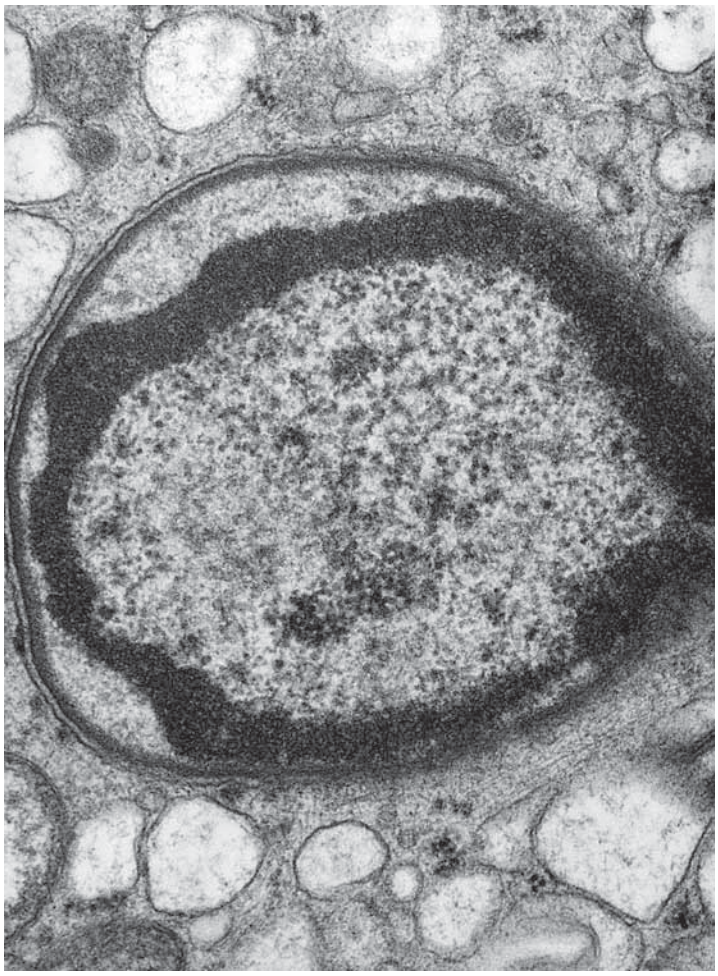


Fig. 7. Disrupted heterochromatin territory extending beyond nuclear lamin. ×90 000

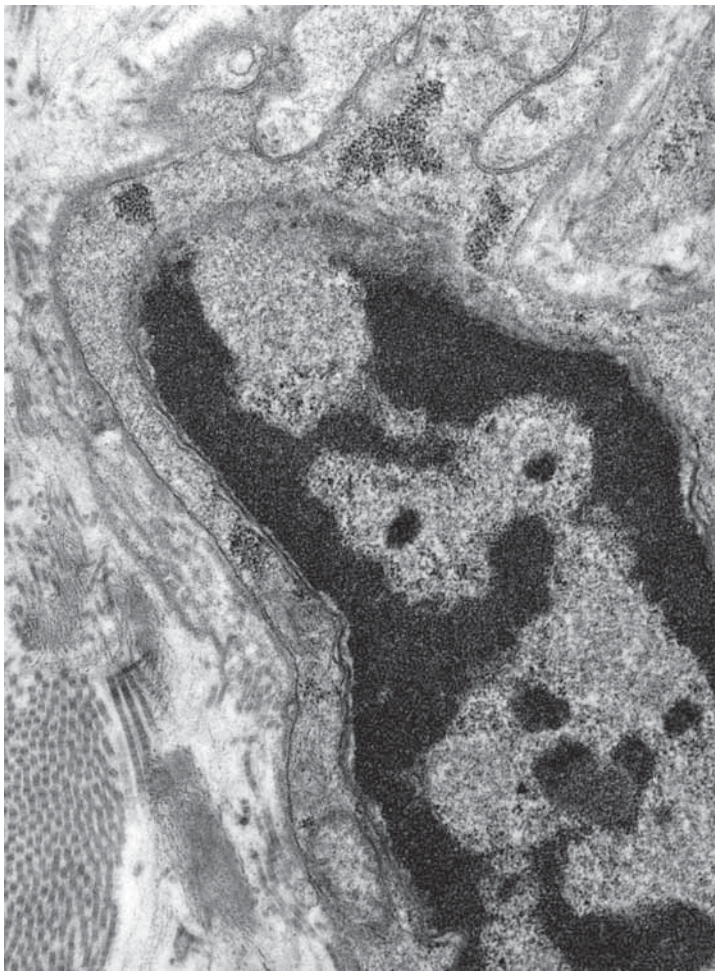


Fig. 8. Nuclear space devoid of nuclear membrane (arrow) and peripherally located chromatin. ×45 000

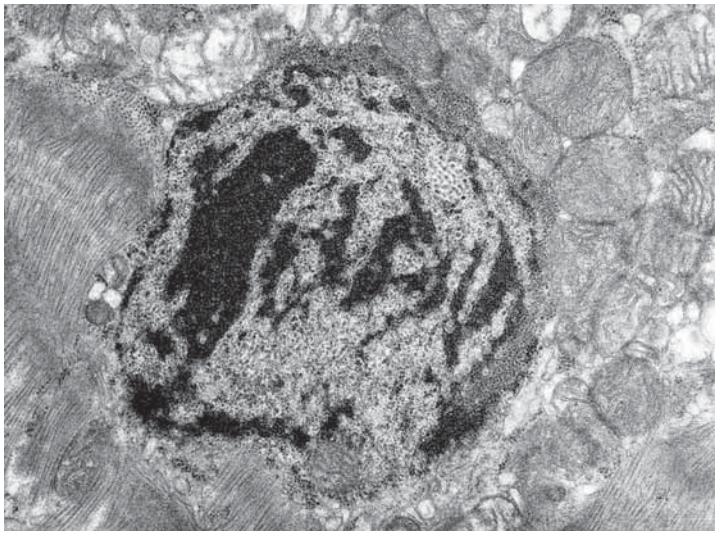


Fig. 9. Nucleus devoid of nuclear membrane. ×45 000

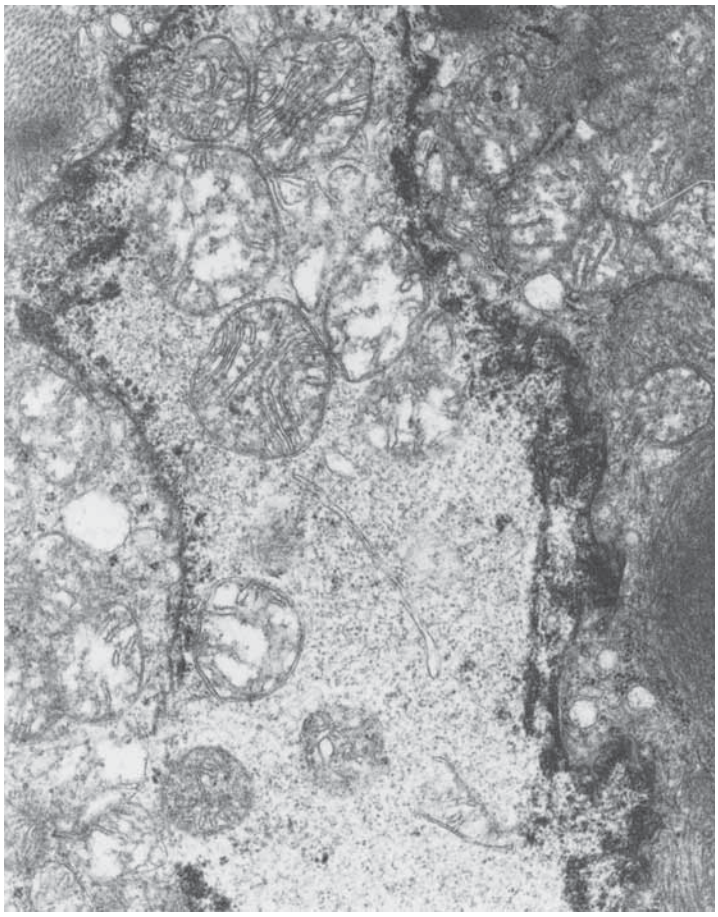


Fig. 10. Fragment of denuded nucleus with mitochondria located within nuclear matrix. ×45 000

only in cardiomyocyte nuclei was never observed in skeletal muscle or in fibroblasts.

The appearance of mitochondria in the nuclear matrix of cardiomyocytes as a result of heart pathology was supported in 2001 by Bakeeva and co-workers [2]. They found mitochondrial clusters in nucleoplasm of patients with hypertrophic and alcoholic cardiomyopathy. In our study, several serial sections of affected cardiomyocytes clearly demonstrated that the entry of mitochondria into the nuclear interior is closely related to the lack of lamin A and nuclear membrane destruction. Central position of single nuclei in cardiomyocytes with close proximity of mitochondria as well as continuous contractile function of myocytes may result in the insertion of sarcoplasmic organelles into the interior of membrane-lacking nuclei. Given all the data presented above, it can be suggested that the lack of lamin A exerts various effects on the architecture of cardiac and skeletal muscles. This variability could be a result of differences in cardiomyocyte structure and function.

Acknowledgements

The work was supported by the State Committee on Research grant No. 2P05B 106 29.

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