Folia Neuropathologica

Nuclear architecture remodelling in envelopathies

Anna Fidziańska, Zofia Glinka

Neuromuscular Unit, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Folia Neuropathol 2007; 45 (2): 47-55

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.

Key words: *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).

Communicating author:

Anna Fidziańska, Neuromuscular Unit, Medical Centre, Polish Academy of Science, Pawiński 5, 02-106 Warsaw, Poland, tel./fax: +48 22 658 45 01, Email: neurmyol@cmdik.pan.pl

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

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

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

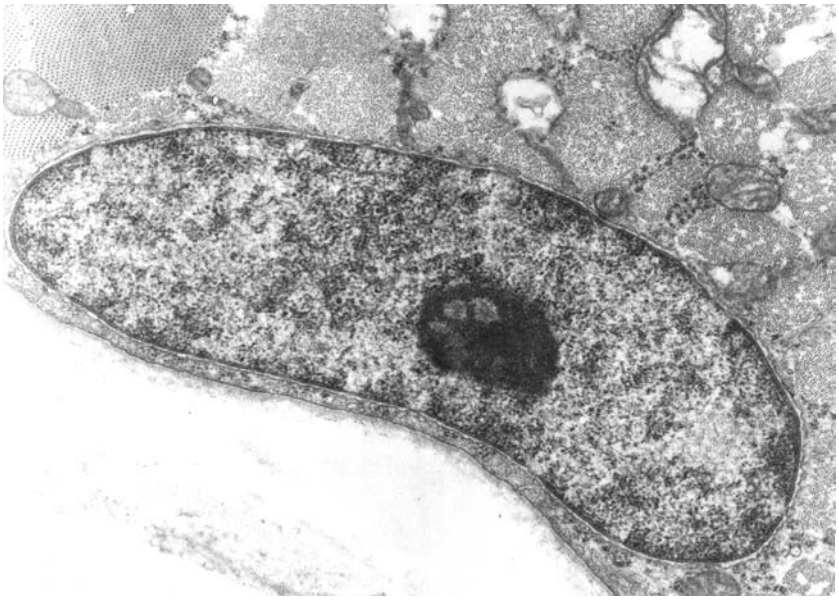

Fig. 1. Nucleus with normal architecture. × 36.000

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

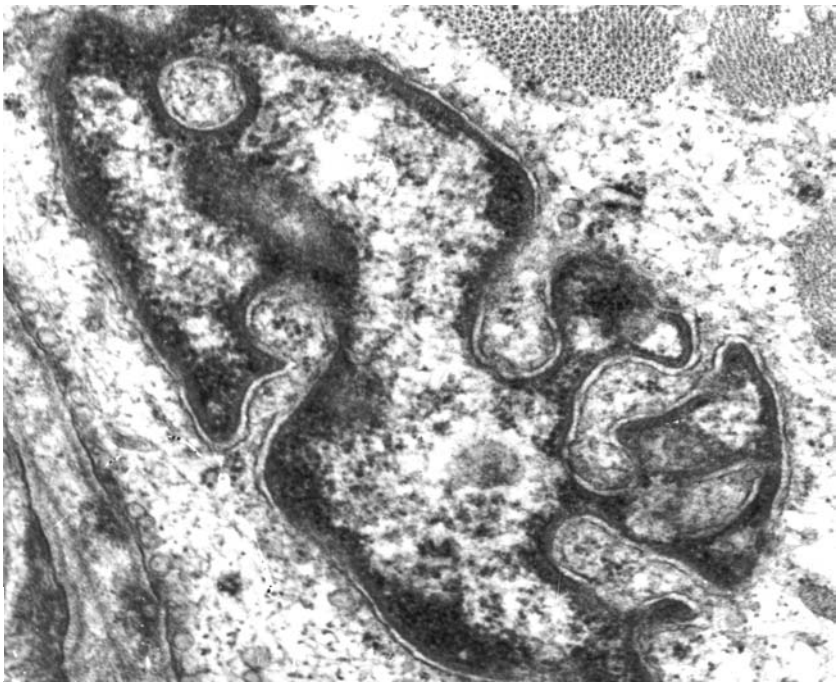

Fig. 2. Misshapen nucleus with numerous deep invaginations. × 40 000

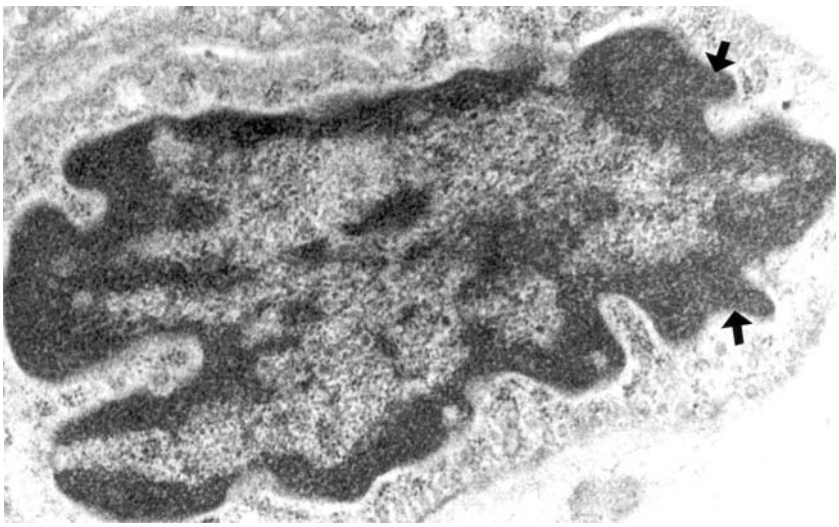

Fig. 3. Nucleus with blebs projecting towards the cytoplasm (arrowheads) × 60 000

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

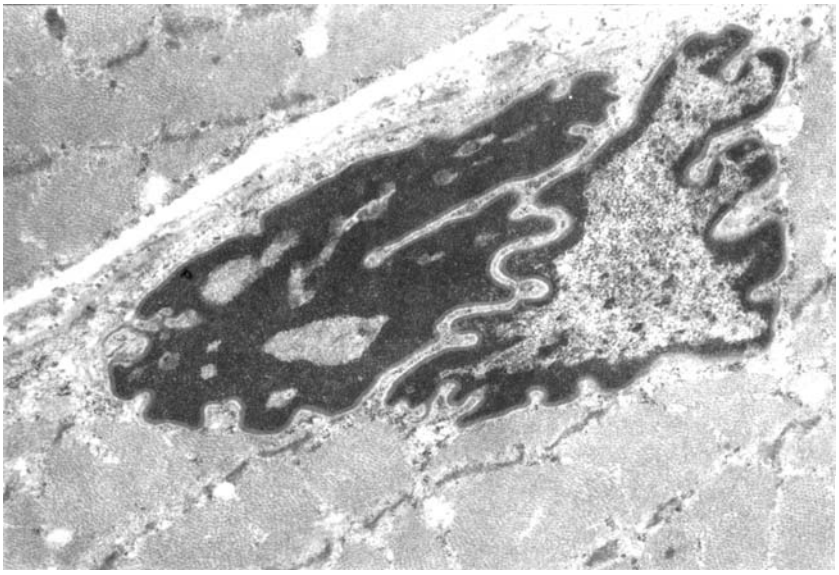

Fig. 4. Extensive nuclear deformation and segmentation. × 24 000

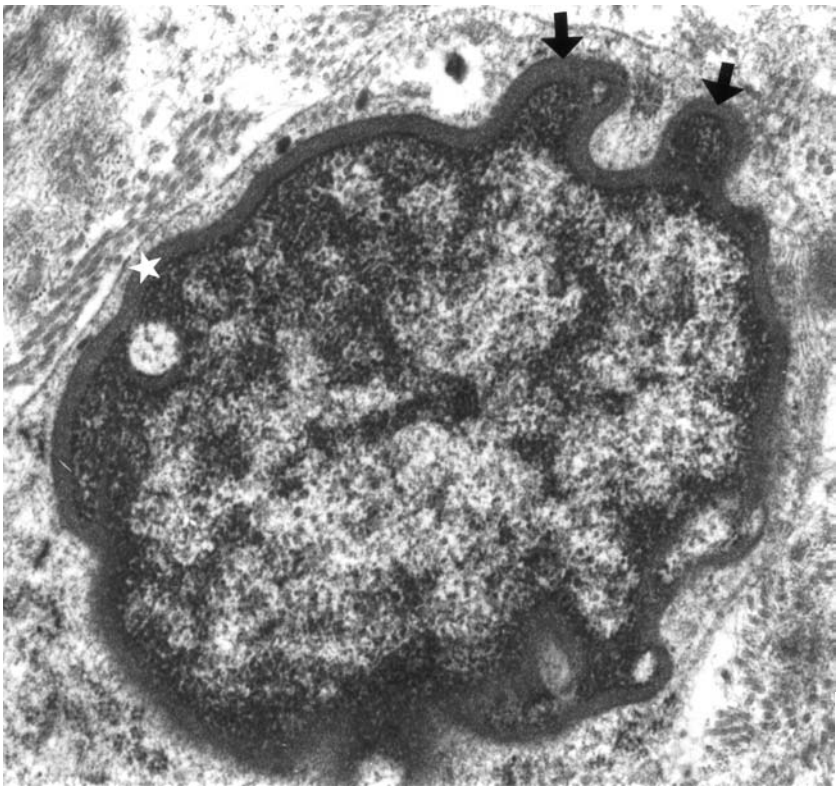

Fig. 5. Nucleus with blebs (arrowheads) and lamina thickness (asterisk). × 30 000

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

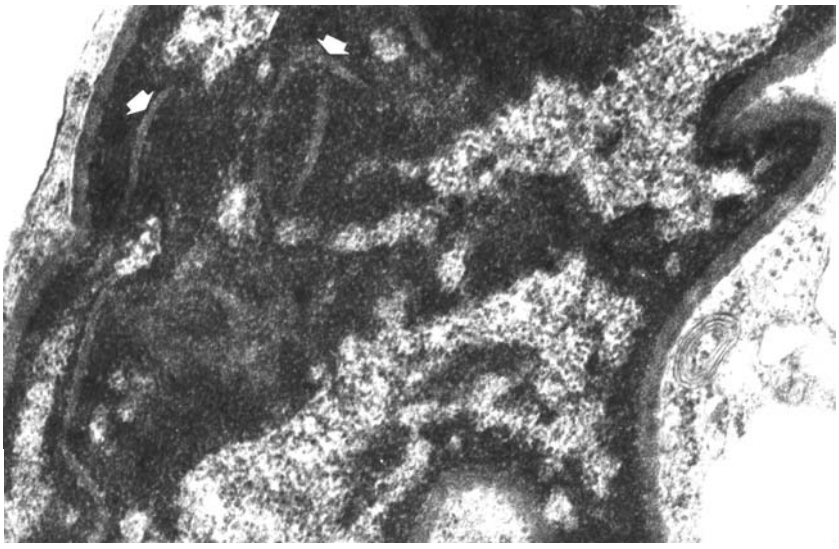

Fig. 6. Thick lamina penetrating inside nuclear matrix (arrowheads). × 60 000

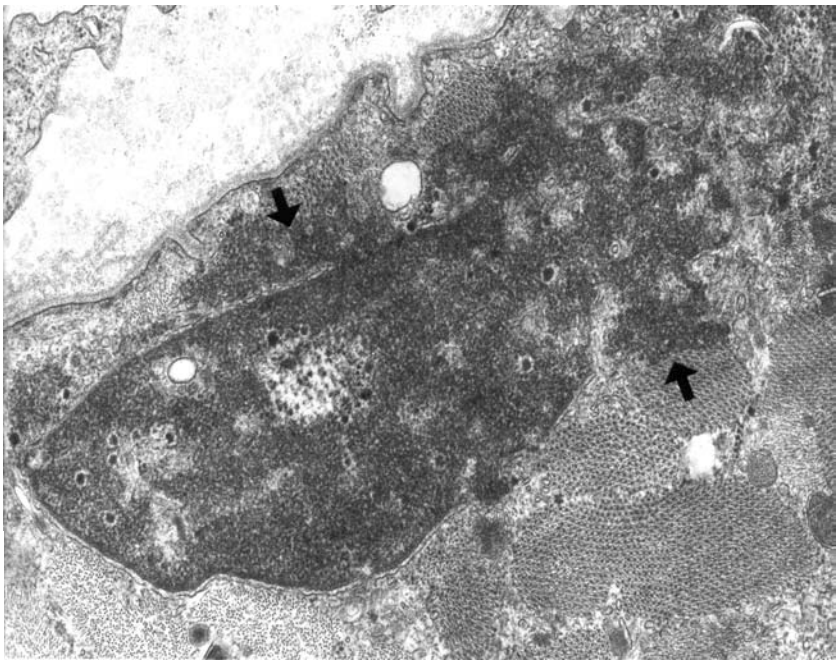

Fig. 7. Focal disruption of nuclear envelope with nucleoplasm extrusion (arrowheads) × 36 000

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

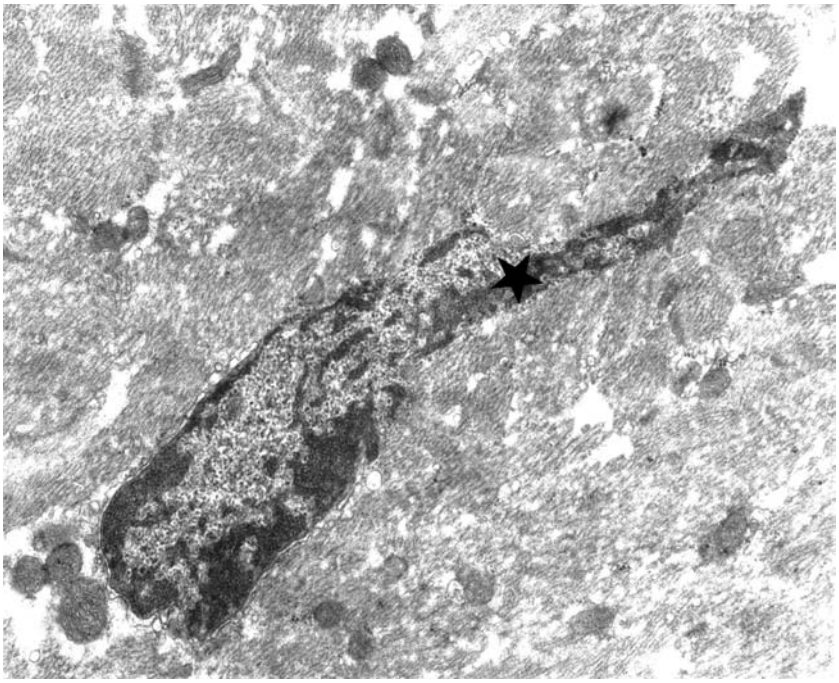

Fig. 8. A long "naked" chromatin tail in close contact with nucleus (asterisk). × 15 000

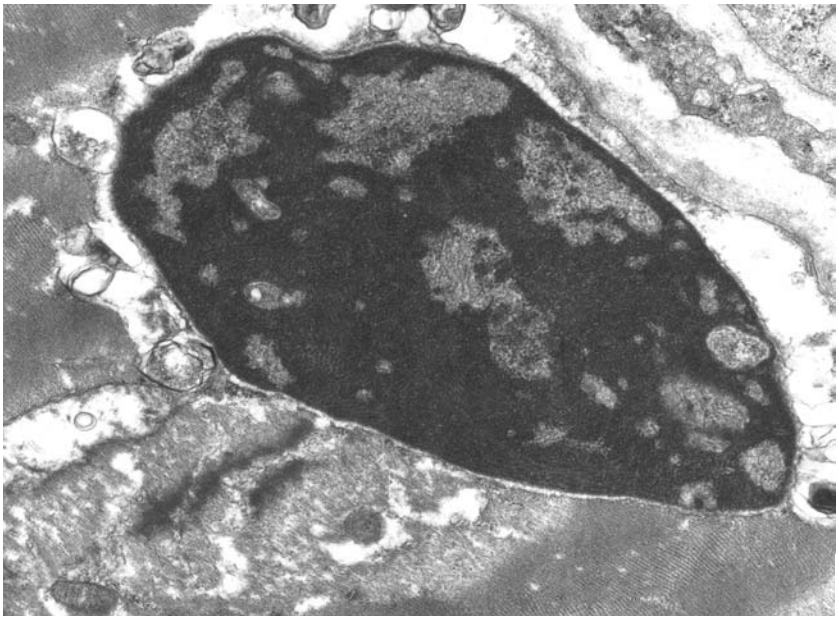

Fig. 9. Heterochromatin reorganization. × 22 000

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

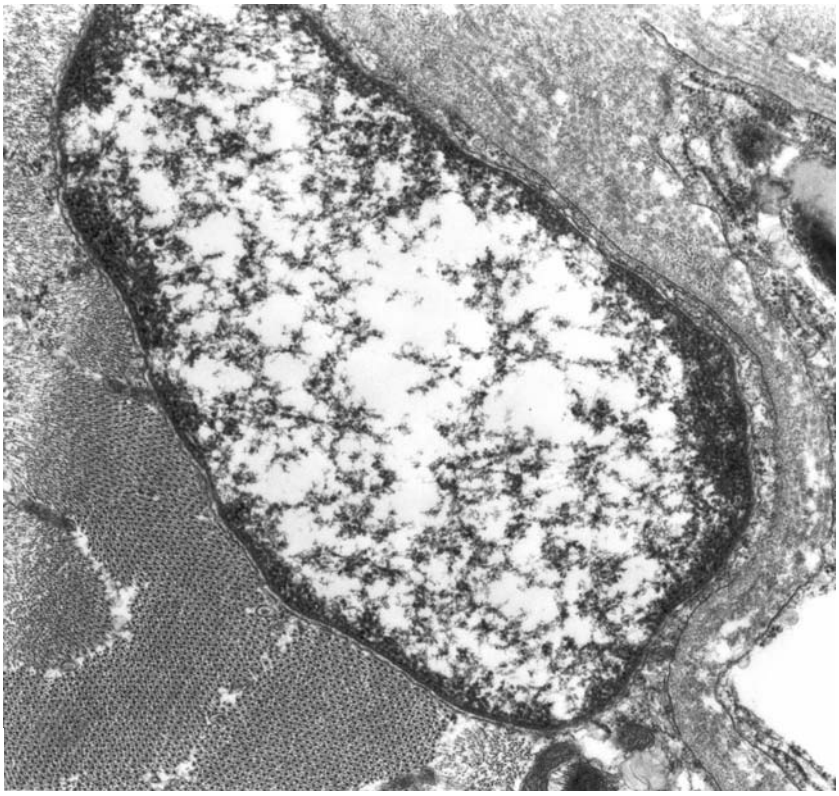

Fig. 10. Massive chromatin decondensation. × 22 000

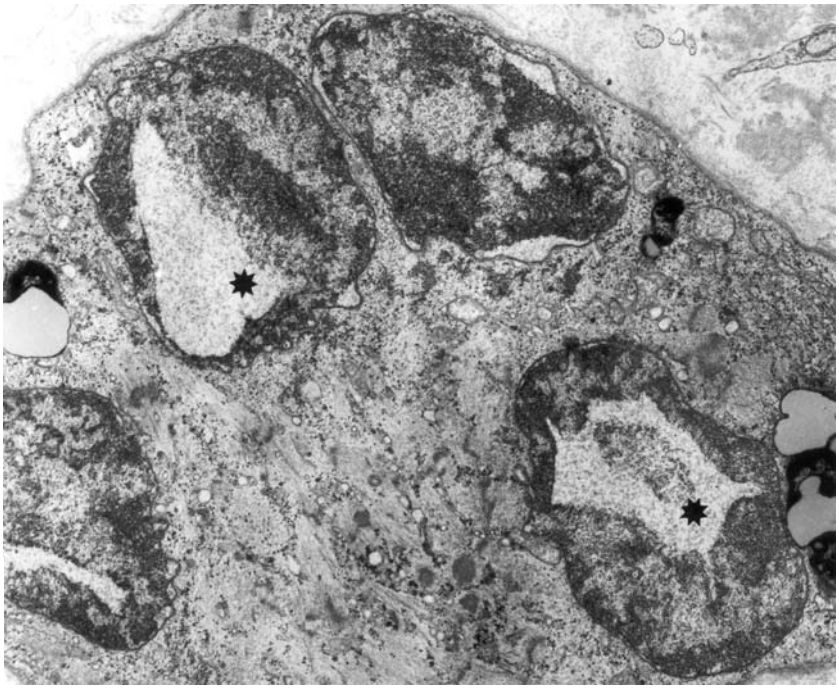

Fig. 11. Focal loss of heterochromatin with the appearance of empty patches (asterisk) × 20 000

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

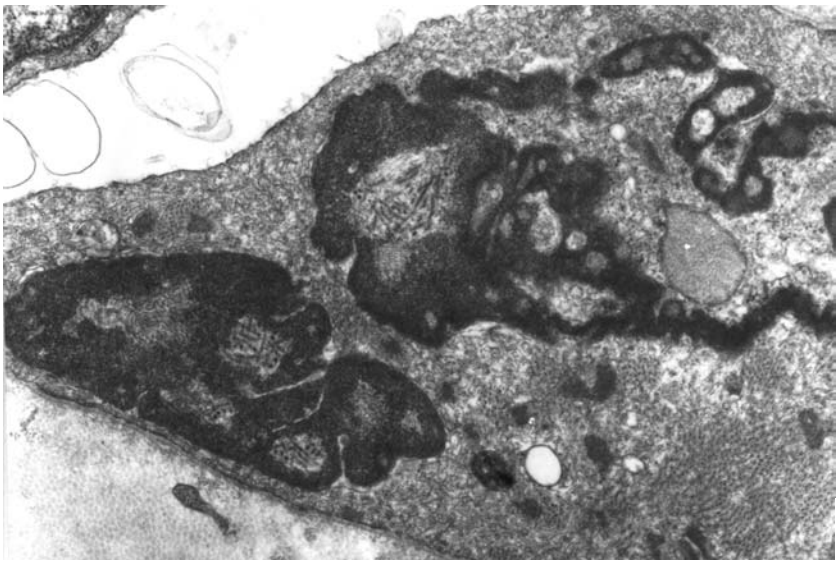

Fig. 12. Nuclear fragmentation. × 30 000

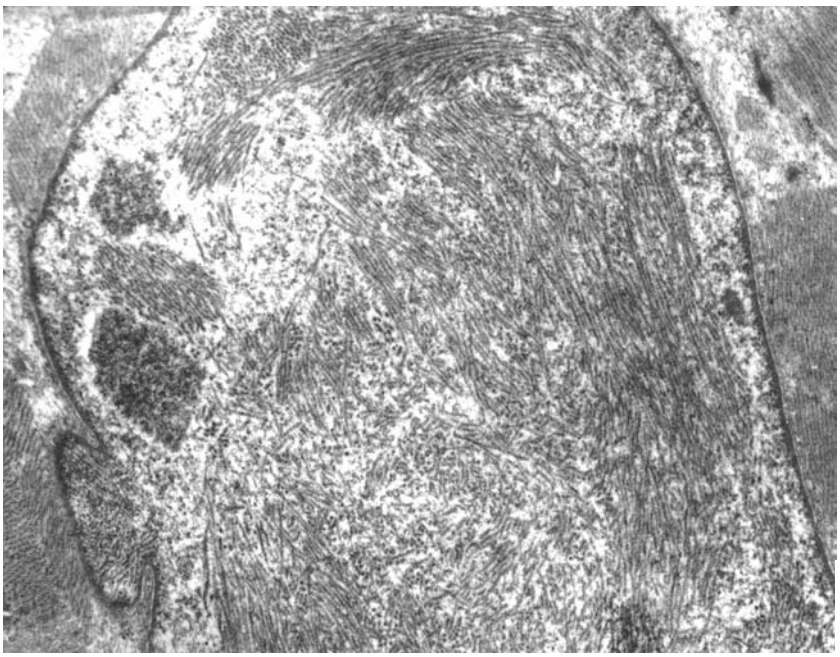

Fig. 13. Fragment of nucleus with tubulofilamentous intranuclear inclusions. × 36 000

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

1. Askanas V, Engel WK. Inclusion-body myositis and myopathies: different etiologies, possibly similar pathogenic mechanisms. Curr Opin Neurol 2002; 15: 525-531.
2. Bione S, Maestrini E, Rivella S, Mancini M, Regis S, Romeo G, Toniolo D. Identification of a noval X-linked gene responsible for Emery-Dreifuss muscular dystrophy. Nat Genet 1994; 8: 323-327.
3. Bonne G., Di Barletta M.R., Varnous S, Becane HM, Hammouda EH, Merlini L, Muntoni F, Greenberg CR, Gary F, Urtizberea JA, Duboc D, Fardeau M, Toniolo D, Schwartz K. Mutation in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. Nat Genet 1999; 21: 285-288.
4. Clements L., Manilal S, Love DR, Morris GE. Direct interaction between emerin and lamin A. Biochem Biophys Res Commun 2000; 267: 709-714.
5. Csoka AB, Cao H, Sammak PJ, Constantinescu D, Schatten GP, Hegele RA. Novel lamin A/C mutation in atypical progeroid syndromes. J Med Genet 2004; 41: 304-308.
6. De Sandre-Giovannoli A, Chaouch M, Kozlov S, Vallat JM, Tazir M, Kassouri N, Szepetowski P, Hammadouche T, Vandenberghe A, Stewart CL, Grid D, Levy N. Homozygous defects in LMNA encoding lamin A/C nuclear envelope proteins cause autosomal recessive axonal neuropathy in human (Charcot-Marie-Tooth disorder type 2) and mouse. Amer J Hum Genet 2002; 70: 726-736.
7. Fidziańska A, Toniolo D, Hausmanowa-Petrusewicz I. Ultrastructural abnormality of sarcolemmal nuclei in Emery-Dreifuss muscular dystrophy. J Neurol Sci 1998; 159: 88-93.
8. Fidziańska A, Hausmanowa-Petrusewicz I. Architectural abnormalities in muscle nuclei. Ultrastructural differences between X-linked and autosomal dominant forms of EDMD. J Neurol Sci 2003; 210: 47-51.
9. Fidziańska A., Rowińska-Marcińska K., Hausmanowa-Petrusewicz I. Coexistence of X-linked recessive Emery-Dreifuss muscular dystrophy with inclusion body myositis-like morphology. Acta Neuropathol (Berl) 2004; 107: 197-203.
10. Goldman RD, Goldman AE, Shumaker DK. Nuclear lamins: building blocks of nuclear structure and function. Novartis Found Symp 2005; 264: 3-16.
11. Goldman RD, Shumaker DK, Erdos MR, Eriksson M, Goldman AE, Gordon LB, Gruenbaum Y, Khuon S, Mendez M, Varga R, Collins FS. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. Proc Natl Acad Sci U S A 2004; 101: 8963-8968.
12. Gruenbaum Y, Goldman RD, Meyuhas R. The nuclear and its functions in the nucleus. Int Rev Cytol 2003; 226: 1-62.
13. Holaska JM., Wilson KL. Multiple roles for emerin: implications for Emery-Dreifuss muscular dystrophy. Anat Rec A Discov Mol Cell Evol Biol 2006; 288: 676-680.
14. Hutchinson CJ. Lamins: building blocks or regulators of gene expression. Nat Rev Mol Cell Biol 2002; 3: 848-858.
15. Maraldi NM, Squarzoni S, Sabatelli P, Capanni C, Mattioli E, Ognibene A, Lattanzi G. Laminopathies: involvement of structural nuclear proteins in the pathogenesis of an increasing number of human diseases. J Cell Physiol 2005; 203: 319-327.
16. Mattout A, Dechat T, Adam SA, Goldman RD, Gruenbaum Y. Nuclear lamins, diseases and again. Curr Opin Cell Biol 2006; 18: 335-341.
17. Moir RD, Spann TP, Goldman RD. The dynamic properties and possible functions of nuclear lamins. Int Rev Cytol 1995; 162B: 141-182.
18. Muchir A, Worman HJ. The nuclear envelope and human diseases. Physiology (Bethesda) 2004; 19: 309-314.
19. Sabatelli P, Lattanzi G, Ognibene A, Columbaro M, Capanni C, Merlini L, Maraldi NM, Squarzoni S. Nuclear alternations in autosomal-dominat Emery-Dreifuss muscular dystrophy. Muscle Nerve 2001; 24: 826-829.
20. Stuurman N, Heins S, Aebi V. Nuclear lamins: their structure, assembly, and interactions. J Struct Biol 1998; 122: 42-66.
21. Wilson KL, Zastrow MS, Lee KK. Lamins and disease: insights into nuclear infrastructure. Cell 2001; 104: 647-650.
22. van der Kooi AJ, Bonne G, Eymard B, Duboc D, Talim B, Van der Valk M, Reiss P, Demay L, Merlini L, Schwartz K, Busch HF, de Visser M. Lamin A/C mutations with lipodystrophy, cardiac abnormalities and muscular dystrophy. Neurology 2002; 59: 620-623.