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Lamin A/C assembly defects in Emery–Dreifuss muscular dystrophy can be regulated by culture medium composition

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Abstract

Emery-Dreifuss muscular dystrophy results from mutations in either emerin or lamin A/C and is caused by loss of some unknown function of emerin–lamin A/C complexes. This function must be of special importance in the skeletal and cardiac muscles that are affected by the disease. Some lamin A/C mutant proteins form 'nuclear foci' in the nucleoplasm when overexpressed by transient transfection and similar aggregates have been seen in cultured skin fibroblasts from patients with Emery–Dreifuss muscular dystrophy, suggesting that mis-assembly of the A-type lamina may be involved in the pathogenesis. Whereas an earlier study of cultured skin fibroblasts compared several different missense mutations in lamin A/C, we have chosen to study one particular Emery-Dreifuss mutation (R249Q) in greater detail. We found that the proportion of fibroblast nuclei containing abnormal lamin A/C aggregates can vary from 0.5 to 23.6% depending on the culture conditions. In particular, switching from a 'slow growth' medium to 'rapid growth' media increased both the number and size of nuclear aggregates. Similar results were obtained with fibroblasts from a second unrelated patient with the same mutation. In contrast to these aggregates of endogenous lamin A/C, 'nuclear foci' formed after transfection of mouse embryo fibroblasts by mutant lamin A/C were not affected by culture conditions. Faulty assembly of the nuclear lamina by mutated lamin A/C molecules could be partly responsible for the disease phenotype, though this has not been proven. The present study suggests that inappropriate lamin A/C assembly may be preventable by manipulation of cell growth conditions.

Keywords: Lamin A; Lamin C; Nuclear lamina; Skin fibroblast; R249Q; Cell culture

1. Introduction

The nuclear lamina is a dynamic, fibrous structure located beneath the inner nuclear membrane and consists of the intermediate filament lamin proteins together with associated integral membrane proteins. Higher mammals have three different lamin genes, LMNA, LMNB1 and LMNB2 that encode several different splice variants. There are two principle products encoded by the LMNA gene: lamins A and C. All lamins have a short head domain followed by a series of heptad repeats that form a 52 nm long rod in the homotypic dimer. They differ principally in their large globular tail region. Lamin C is for practical purposes a shorter form of lamin A with six unique amino-acids at its C-terminus, though it is never prenylated like lamin A [1]. Emerin is a 254 amino acid, type II integral membrane protein that is anchored to the inner nuclear membrane by its hydrophobic tail [2,3] and interacts directly with lamins A and C [4]. The emerin–lamin A/C interaction is of particular interest because mutations in either protein cause different variants of Emery-Dreifuss muscular dystrophy (EDMD) [5,6]; (reviewed in [7]). The interaction occurs between the globular tail region common to both lamins A and C [8] and a central region (amino-acids 70-164) of the emerin molecule [9]. It has been suggested that the loss of some specific function of lamin A/C–emerin complexes is responsible for the common pathological features of the two forms of EDMD [10]. Mislow et al. [11] have shown that both emerin and A-type lamins interact with syne proteins or nesprins [12,13]. Cytoplasmic actin

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also interacts with nesprins in the outer nuclear membrane [14] suggesting a possible analogy with the actin–dystrophin interaction at the sarcolemma that is important in the Duchenne form of muscular dystrophy caused by dystrophin mutations (reviewed in [15]).

Structural abnormalities at the nuclear rim, including disrupted chromatin attachment in electron micrographs, have been observed in muscle from EDMD patients with emerin deficiency or lamin A/C mutations [16–18] and in lamin A/C knockout mice [19]. More dramatic nuclear changes have been observed in cell cultures derived from EDMD patients, including formation of blebs, 'honeycomb' structures and nucleoplasmic 'aggregates' of lamina proteins [20–23]. Transfection of normal or lamin A/C knockout cells with mutant lamins can also result in the formation of large nuclear foci, depending on the particular lamin A/C mutation [10,24,25]. These nuclear changes, dramatic though they often seem, do not appear to be directly correlated with EDMD pathogenesis, particularly since they are observed in cells from skin, a tissue that is not affected in the disease. Defective assembly of emerin–lamin A/C complexes in the nuclear lamina, however, may underlie both EDMD pathogenesis and some of the nuclear abnormalities observed in cell cultures. In the present study, we show that it is indeed possible to ameliorate or exacerbate nuclear abnormalities in EDMD skin fibroblasts by changes in the culture conditions, so that at least some consequences of lamin A/C mutations are not fixed and unalterable.

2. Materials and methods

2.1. Cell culture

A skin biopsy was obtained from a 25-year-old male carrying a heterozygous LMNA mutation R249Q. The cell line G-9666 was established by the explant method using Eagle's MEM supplemented with ampicillin, streptomycin and 10% fetal bovine serum. In further passages, the line was maintained using the same culture medium. Phenotypically, the donor was characterized by slowly progressive proximal muscle weakness and wasting from early childhood on. In the beginning of the third decade, he developed cardiac conduction defects, including ventricular flutter, AV Block III and dilated cardiomyopathy, requiring a pacemaker implantation at age 25. The patient succumbed to a sudden cardiac heart death at age 26. A second skin biopsy was obtained from a 7-year-old male, G-13579, from an unrelated family. At age three, he showed a waddling gait and difficulty in climbing stairs. CK was slightly elevated. At age four a myopathic EMG was found. The disease progressed slowly up to age seven with contractures of the elbows and ankles. Weak reflexes and slight weakness of the biceps were also noted. No cardiac abnormalities occurred until age seven. G-13579 is a sporadic case in his family and was proven to be a new mutation by molecular genetic analysis of the LMNA gene in his relatives. Cell lines were established from skin biopsies with informed consent and Institutional Review Board Approval.

G-9666 skin fibroblasts were maintained in culture in DMEM (Gibco) with 20% decomplemented horse serum (Gibco), 2 mM L-glutamine and antibiotics (DMEM+ 20% HS), while G13579 fibroblasts were maintained in similar medium containing 20% fetal bovine serum, instead of horse serum. Cells were plated onto sterile glass coverslips in medium consisting of DMEM (with glutamine and antibiotics), skeletal muscle cell basal medium (SMBasal; C-23260; PromoCell, Heidelberg, Germany) or skeletal muscle cell growth medium (SMCGM; C-23060; PromoCell). Medium was supplemented with either decomplemented horse serum (HS; Gibco) or decomplemented fetal bovine serum (FBS; Gibco). At the end of the culture period, cells on coverslips were fixed in 50:50 acetone:methanol for 5 min and stored at −80 °C.

Mouse embryonic fibroblasts from the lamin A null mouse (MEFs lmna−/− [19]) were cultured in DMEM+ 20%HS prior to transfection with pSVK3 mammalian expression plasmid (Amersham Pharmacia) containing lamin A cDNA with pathogenic missense mutations, as described previously [10]. Transfected cells were plated onto coverslips and incubated for 48 h prior to fixation.

2.2. Immunohistochemistry

Coverslips of human skin fibroblasts were washed with casein buffer (0.1% casein in 154 mM NaCl, 10 mM Tris, pH 7.6) and incubated at 37 °C for 1 h with MANLAC1 mAb [26] diluted 1:3 in casein buffer. Cells were then washed four times in casein buffer and incubated with 5 μg/mL goat anti-mouse ALEXA 546 secondary antibody (Molecular Probes, Oregon, USA) diluted in PBS containing 1% HS, 1% FBS and 0.1% bovine serum albumin.

For double label experiments, coverslips were incubated with 1:100 rabbit anti-emerin polyclonal antiserum [10], washed with casein buffer, and then incubated with either 20 μg/mL anti-FLAG M2 mAb (Sigma, F3165) to label transfected mutant lamin A or MANLAC1 to label endogenous lamin A/C. After washing, these cells were further incubated with a mixture of goat anti-mouse ALEXA 546 and goat anti-rabbit ALEXA 488 (Molecular Probes), both at 5 μg/mL (in PBS, 1% HS, 1% FBS and 0.1% BSA). DAPI (diamidinophenylindole, 200 ng/mL; Sigma) was added for the final 5 min to counterstain the nuclei. Coverslips were mounted in Hydromount (Merck) and examined with a Nikon Eclipse E600 epifluorescence microscope (Nikon UK, Kingston, Surrey) with a 60× oil immersion objective (numerical aperture 1.40) and a BioRad MicroRadiance 2000