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Lamins A and C are differentially dysfunctional in autosomal dominant Emery-Dreifuss muscular dystrophy

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Abstract

Mutations in the *LMNA* gene, which encodes nuclear lamins A and C by alternative splicing, can give rise to Emery–Dreifuss muscular dystrophy. The mechanism by which lamins A and C separately contribute to this molecular phenotype is unknown. To address this question we examined ten *LMNA* mutations exogenously expressed as lamins A and C in COS-7 cells. Eight of the mutations when expressed in lamin A, exhibited a range of nuclear mislocalisation patterns. However, two mutations (T150P and delQ355) almost completely relocated exogenous lamin A from the nuclear envelope to the cytoplasm, disrupted nuclear envelope reassembly following cell division and altered the protein composition of the mid-body. In contrast, exogenously expressed DsRed2-tagged mutant lamin C constructs were only inserted into the nuclear lamina if co-expressed with any EGFP-tagged lamin A construct, except with one carrying the T150P mutation. The T150P, R527P and L530P mutations reduced the ability of lamin A, but not lamin C from binding to emerin. These data identify specific functional roles for the emerin–lamin C- and emerin–lamin A-containing protein complexes and is the first report to suggest that the A-type lamin mutations may be differentially dysfunctional for the same *LMNA* mutation.

Keywords: Nuclear lamins; Emerin; EDMD

Introduction

Emery-Dreifuss muscular dystrophy (EDMD) is characterised by slowly progressive muscle wasting and weakness, early contractures of the elbows, Achilles tendons and spine, and cardiomyopathy associated with cardiac conduction defects (Emery, 1989). Initially described as an X-linked disorder (X-EDMD), autosomal dominant and recessive forms are also now recognised (ADEDMD and AREDMD, respectively);

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the dominant form being the most prevalent of the three types (Bonne et al., 2003). X-EDMD arises as a consequence of mutations in the *STA* gene (also known as the *EMD* gene), located at Xq28, which encodes for the ubiquitously expressed inner nuclear membrane protein emerin (Bione et al., 1994) whereas AD/AREDMD arises from mutations in the *LMNA* gene located at chromosome 1q11–23 (Bonne et al., 1999). The *LMNA* gene is alternatively spliced to encode for two nuclear intermediate filament proteins, lamins A (74 kDa) and C (66 kDa). The connection between the muscular dystrophy phenotype and mutations in nuclear-associated proteins was unexpected, given that the majority of muscular dystrophies so far characterised arise from defects in either cytoskeletal or plasma membrane proteins the function of which is to maintain muscle cell integrity.

Mutations in the *LMNA* gene are also associated with dilated cardiomyopathy with conduction system disease (DCM-CD), limb-girdle muscular dystrophy 1B (LGMD1B) and Charcot-Marie-Tooth disease type 2B1 (CMT2), a peripheral neuropathy with muscle wasting and weakness (Mounkes et al., 2003). However, other mutations in the *LMNA* gene result in non-muscle tissue-specific disorders, including familial partial lipodystrophy of the Dunnigan type (FPLD), which affects adipose tissue, mandibuloacral dysplasia (MAD), which mainly affects the bony skeleton, and the premature ageing diseases Hutchison–Gilford progeria syndrome (HGPS), Werner's syndrome and cardiocutaneous progeria (Eriksson et al., 2003; Mounkes et al., 2003a; De Sandre-Giovannoli et al., 2003; Cao and Hegele, 2003; Chen et al., 2003). These disorders are collectively known as the "the laminopathies". Muscle, fat and bone tissue are all derived from the mesenchymal lineage, suggesting that the laminopathies may arise from defects in the differentiation, maintenance, repair or regulation of mesenchymal cells (Cohen et al., 2001). Since the normal ageing process is associated with an increased inability to repair damaged tissues, progeria arising from *LMNA* mutations may be an example of a laminopathy exhibiting an extreme phenotype. Although the laminopathies differ from one another pathophysiologically, they share abnormalities observed in nuclear architecture and mislocalisation of nuclear antigens. Two molecular mechanisms have been proposed to explain the phenotypic consequences of mutations in the *LMNA* gene, namely an increase in nuclear fragility in affected cell types (Manilal et al., 1999) or an alteration in nuclear organisation (Cohen et al., 2001); these two models are not necessarily mutually exclusive.

The nuclear lamina is a fibrous structure underlying the inner nuclear membrane, mainly composed of A- and B-type lamins. It is purported to provide both support to the nuclear envelope and anchorage sites for the chromatin (Goldman et al., 2002). The inner nuclear membrane and nuclear lamina interact through multi-subunit protein complexes. For example, emerin binds directly to A- and B-type lamins, as well as transcriptional regulators (e.g. Btf and E2F), heterochromatin-binding proteins (e.g. BAF, HP1) and other inner nuclear membrane proteins (Zastrow et al., 2004). Defects in emerin and the A-type lamins in these complexes disrupt directly nuclear architecture, which clinically results in the laminopathies. Although some lamin A- (Eriksson et al., 2003; De Sandre-Giovannoli et al., 2003; C.A. Brown, unpublished data) and one lamin C-specific mutation (Fatkin et al., 1999) have been identified, the majority of mutations occur in the common regions and affect both lamins A and C. Whilst it is known that lamin C is dependent on the presence of lamin A to be correctly incorporated into the nuclear lamina (Horton et al., 1992; Pugh et al., 1997; Raharjo et al., 2001), no published studies have addressed the issue of whether mutant forms of lamins A and C contribute differentially to the cellular phenotype. In the present paper we have analysed nine missense and 1 deletion *LMNA* mutations which are associated with an ADEDMD phenotype, to ascertain whether lamins A and C contribute differentially to the observed cellular phenotype. Our results show that mutant lamins A and C are both dysfunctional and that their inter-dependency identifies an additional molecular mechanism which may contribute to the disease phenotype.

Materials and methods

Cell culture and antibodies

COS-7 (green monkey kidney fibroblasts) and C2C12 cells (mouse myoblasts) were cultured as described in Fairley et al. (1999). The affinity-purified rabbit polyclonal anti-emerin antibody AP8 (Ellis et al., 1998) was used as described in Fairley et al. (1999), and a rabbit polyclonal anti-lamin C antibody AB3702 (Chemicon) was used at 1:50 for immunofluorescence. A mouse monoclonal anti-FLAG antibody M2 (Stratagene) was used at 1:250 for immunoblotting FLAG-tagged-lamin C in the in vitro transcription–translation reactions and 1:10 for immunofluorescence experiments. The mouse monoclonal antibody R27 against mature lamin A/C (Höger et al., 1991) was used at 1:500 on immunoblots of cell lysates. A universal anti-actin rabbit polyclonal antibody (Sigma) was used at 1:1000 to monitor loading onto gels. Detection of immunoblotted proteins was performed by enhanced chemiluminescence (ECL).