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Nuclear Envelope Diseases and Chromatin Organization

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Functions of the nuclear envelope and lamina in development and disease

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Abstract

Recent findings that some 24 inherited diseases and anomalies are caused by defects in proteins of the NE (nuclear envelope) and lamina have resulted in a fundamental reassessment of the functions of the NE and underlying lamina. Instead of just regarding the NE and lamina as a molecular filtering device, regulating the transfer of macromolecules between the cytoplasm and nucleus, we now envisage the NE/lamina functioning as a key cellular 'hub' in integrating critical functions that include chromatin organization, transcriptional regulation, mechanical integrity of the cell and signalling pathways, as well as acting as a key component in the organization and function of the cytoskeleton.

Introduction

The NE (nuclear envelope) consists of the INM (inner nuclear membrane) and ONM (outer nuclear membrane) that are separated by the PNS (perinuclear space). These membranes are, however, connected, at the point they are traversed by the NPCs (nuclear pore complexes). The ONM is also contiguous with the cytoplasmic ER (endoplasmic reticulum), making the ER, INM and ONM one continuous membrane system with lumen of the ER extending into the PNS. The other component of the NE, and underlying the INM, is the nuclear lamina, a thin proteinaceous meshwork of some 10–20 nm. The principal components of the lamina are intermediate filament proteins, the nuclear lamins. Most adult mammalian somatic cells contain four major lamin proteins: A, B1, B2 and C [1,2]. A single gene, *LMNA*, encodes the A-type lamins, which arise through alternative splicing of a common pre-mRNA, whereas the B-type lamins are each encoded by a separate gene. The main function of the lamina is to maintain interphase nuclear shape, but it also has important roles in regulating DNA synthesis, RNA transcription, chromatin organization and the selective retention of INM proteins [3]. Transcriptional cofactors associate with the lamins, suggesting that the lamina and NE are important in transcriptional regulation [4]. The lamins are developmentally regulated, with all cells expressing at least one lamin B, whereas A-type lamins are absent from early embryonic development, embryonic stem cells and certain stem cell populations in adults [5,6].

Laminopathies affecting striated muscle

The linkage of some 24 diseases and anomalies to mutations in NE proteins and the lamina has stimulated a major reassessment of the functions of the NE, and particularly the lamina, as more than half of these diseases are caused by mutations in the *LMNA* gene [7]. Our approach to investigating the function of the NE/lamina has been to derive mouse lines carrying the same mutations causing the laminopathies, to provide us with a resource to understand the molecular pathology of these diseases.

The first diseases linked to mutations in the *LMNA* gene were those affecting heart and striated muscle function, specifically AD-EDMD (autosomal dominant Emery–Dreifuss muscular dystrophy) [8], DCM (dilated cardiomyopathy) [9] and LGMD1B (limb-girdle muscular dystrophy-1B) [10]. As

Key words: Emery–Dreifuss muscular dystrophy (EDMD), Hutchinson–Gilford progeria syndrome (HGPS), lamina, nuclear envelope.

Abbreviations used: AV, atrioventricular; DCM, dilated cardiomyopathy; EDMD, Emery–Dreifuss muscular dystrophy; AD-EDMD, autosomal dominant EDMD; ER, endoplasmic reticulum; HGPS, Hutchinson–Gilford progeria syndrome; INM, inner nuclear membrane; LGMD1B, limb-girdle muscular dystrophy-1B; NE, nuclear envelope; NPC, nuclear pore complex; ONM, outer nuclear membrane; PNS, perinuclear space; pRb, retinoblastoma protein; X-EDMD, X-linked EDMD.

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with X-EDMD (X-linked Emery–Dreifuss muscular dystrophy), which is caused by mutations in the INM transmembrane protein emerin [11], AD-EDMD is characterized by progressive wasting of specific muscle groups, as well as the defining cardiac conduction defects that distinguish it from other muscular dystrophies. The conduction defects observed in DCM are similar to those in patients with EDMD (Emery–Dreifuss muscular dystrophy), suggesting that cardiomyopathy represents one extreme in a phenotypic continuum in which skeletal muscle involvement is not observed. Dilation of heart chambers, hypertrophy, arrhythmic conduction defects and cardiac arrest are all associated with *LMNA*-associated cardiomyopathy. At the other end of the spectrum, LGMD1B, which is also caused by mutations in *LMNA*, is associated with tendon contractures and fewer cardiac complications [10]. The heterogeneity in disease phenotypes, even among members of a single family carrying the same *LMNA* mutation, suggests that the pathological outcome may be strongly influenced by genetic or environmental modifiers [12,13]. To date, diseases affecting striated muscle comprise approx. 60% of the laminopathies. However, only approx. 50% of patients diagnosed with AD-EDMD or X-EDMD have *EMD* (emerin) or *LMNA* mutations, indicating that mutations in other genes, perhaps those encoding proteins that interact with the lamins [e.g. LAP2 (lamina-associated polypeptide 2) and nesprins 1 and 2], may account for the remaining 50% of patients [12,14,15].

We first established a mouse line in which the *Lmna* gene was disrupted so that no lamin A or C protein was expressed. Mice with no lamin A and C develop normally to birth, revealing that A-type lamins are not essential for embryonic development. However, postnatally, the *Lmna*-null mice show growth retardation starting at 2 weeks and, by 6–7 weeks, all are dead, with death being associated with extensive muscular dystrophy and cardiomyopathy [16,17]. Heterozygotes do not show any evidence of dystrophy; however, by 1 year, heterozygous mice have developed AV (atrioventricular) conduction defects with atrial and ventricular arrhythmias analogous to those in humans with *LMNA* mutations. $Lmna^{+/-}$ cardiomyocytes have impaired cell and sarcomere contractility, with the AV node cells having abnormal nuclei, which undergo apoptosis and are replaced by fibroblasts [18].

Another line of mice we derived carries an N195K mutation [19], originally identified in families with DCM [9]. The mouse line ($Lmna^{N195K/N195K}$), homozygous for the same mutation, develop cardiac conduction defects consistent with DCM with conduction system disease, with the mice dying at 3 months due to cardiac arrhythmia. The mice show minimal, if any, signs of muscular dystrophy. The transcription factor Hf1b/Sp4 and the gap junction proteins connexin 40 and 43 are misexpressed and/or mislocalized in the mutant hearts. Desmin staining revealed a loss of organization at sarcomeres and intercalated disks, suggesting that *LMNA* mutations may cause cardiomyopathy by disrupting the internal organization of the cardiomyocyte and/or altering the expression of transcription factors essential to normal cardiac development and function [19].

Progeria and lamin A

Probably the most intriguing disease that arises as a consequence of mutations in the *LMNA* gene is HGPS (Hutchinson–Gilford progeria syndrome). HGPS is a rare dominantly inherited disease in which patients show some aspects of premature aging, including growth retardation, loss of subcutaneous fat, hair loss, a reduction in bone density and weakened muscle function [20,21]. The average age of death in HGPS is 12–15 years, due to myocardial infarction or stroke caused by artherosclerosis. Artherosclerosis in the HGPS patients is not linked to abnormal systemic lipids levels [22], but may be associated with smooth muscle depletion in the sclerotic vessels and disruption to the microvasculature in various tissues [20,23]. Individuals with HGPS do not show any increase in tumour susceptibility, cataract formation or cognitive degeneration, features often associated with normal aging. This classifies HGPS as a segmental progeroid syndrome, as it may only partially reproduce some aspects of the normal aging processes [24,25]. However, the phenotypic effects of HGPS and the fact that individuals die from cardiovascular problems, despite having normal lipid levels, make this a particularly relevant disease to study in relation to some of the major health issues of the 21st Century.

Progeric mouse models

The most common mutation causing HGPS is a splicing defect in exon 11 of the *LMNA* gene, that arises due to a *de novo* single base substitution that introduces a cryptic donor splice site resulting in a 150 bp deletion and consequently a 50-amino-acid in-frame deletion in prelamin A, with lamin C being unaffected. The truncated prelamin A in HGPS is often called ‘Progerin’ or Δ50 [26,27].

A mouse mutant with progeria was created by the fortuitous introduction of a splicing defect, resulting in the deletion of exon 9 of *Lmna* ($Lmna^{\Delta 9/\Delta 9}$), with the consequent in-frame removal of 40 amino acids from the C-terminal globular domain. This mutation produces a truncated lamin A protein that remains farnesylated. Mice homozygous for the $Lmna^{\Delta 9/\Delta 9}$ mutation develop to term and are seemingly normal at birth. However, postnatal development is associated with multiple pathologies resembling HGPS. Loss of subcutaneous fat, decreased bone density, osteoporosis, abnormal dentition, thin hyperkeratotic skin, growth retardation and death by 4 weeks post-partum are some of the most striking features of these mice [28]. Fibroblasts cultured from various tissues of postnatal $Lmna^{\Delta 9/\Delta 9}$ mice have misshapen nuclei, and undergo a rapid onset of senescence and death, a characteristic similar to that described for fibroblasts isolated from HGPS patients [28,29]. In the $Lmna^{\Delta 9/\Delta 9}$ fibroblasts, this premature loss of proliferative capacity appears to be largely due to the cells’ inability to produce a functional extracellular matrix, an observation consistent with some gene array studies performed on human progeric cells [30].

A second mouse line has also been engineered to only produce progerin ($Lmna^{HG}$) [31]. Heterozygous fibroblasts from these mice express large amounts of progerin and have

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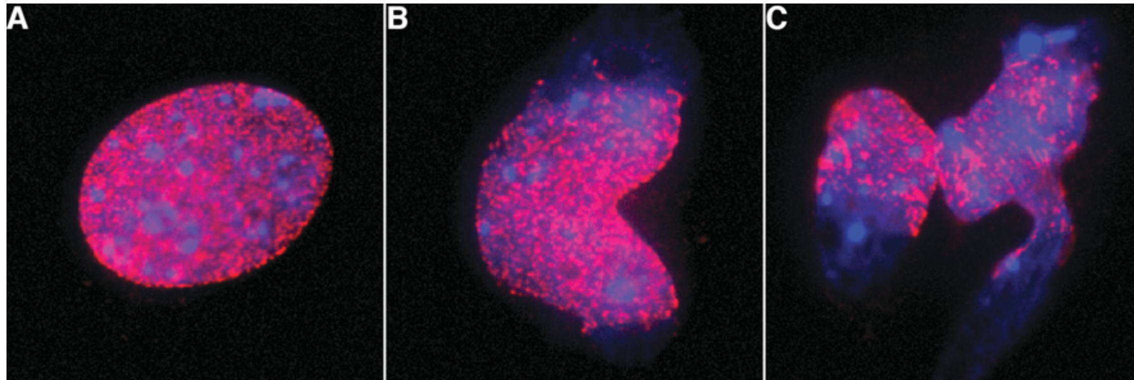
Figure 1 | Different lamin mutations have different effects on nuclear morphologies and on the distribution of NE components
(**A**) WT nucleus showing a normal oval morphology and even distribution of NPCs. DNA is stained blue with DAPI (4′,6-diamidino-2-phenylindole), and NPCs are stained red. (**B**) A nucleus null for lamin A and C expression. Note the change in nuclear morphology arising from loss of NPCs from one pole (owing to separation of the INM from ONM). (**C**) A nucleus from an N195K homozygous mouse. Note the extreme distortion in nuclear morphology and clumping of the NPCs.



misshapen nuclei. *Lmna*$^{HG/+}$ mice are normal at birth, but their post-weaning growth rate is retarded. The *Lmna*$^{HG/+}$ mice develop many phenotypes common to HGPS, including osteoporosis, alopecia, micrognathia, reduced subcutaneous fat and osteolysis of the clavicle. These phenotypes are progressive, with the mice dying by 6–7 months of age. Homozygous mice (*Lmna*$^{HG/HG}$) have severe osteoporosis, spontaneous bone fractures and die before weaning. In none of these mouse lines (*Lmna*$^{HG/+}$, *Lmna*$^{HG/HG}$ or *Lmna*$^{\Delta 9/\Delta 9}$) have the arterial lesions, thought to be a characteristic of HGPS, been found [32] and the cause of death remains to be established (S. Young, C.L. Stewart and L. Hernandez, unpublished work).

Molecular mechanisms underlying the laminopathies

Mechanical stress hypothesis

A major question concerning the laminopathies is how do so many different diseases arise from mutations in the same protein that is expressed in the majority of adult cell types? Two theories have been proposed to account for the molecular basis of this spectrum of degenerative diseases. The first is that disruption of the lamina results in a physical weakening of the nucleus and its ability to withstand mechanical stresses. In mechanically stressed tissues, such as contracting striated muscle, or vascular smooth muscle, this weakening may result in increased cellular apoptosis and necrosis with the consequent loss of the cells and tissue. The second hypothesis is that disruption of the lamina and NE affects chromatin organization and gene expression. However, it should not be assumed that these hypotheses are mutually exclusive, as it is becoming increasingly apparent that the NE and lamina may have a central role in integrating cellular mechanics with signalling and transcriptional pathways.

Structural studies on the expression of mutant forms of the lamin A protein in cells have provided evidence to support the mechanical weakening hypothesis. The mutations causing the muscular dystrophies and DCM are distributed throughout the A-type lamins. Many of these mutations disrupt assembly of the lamins and their incorporation into the lamina [33,34]. These mutations result in abnormal nuclear morphologies, separation of the ONM from the INM and disruption in the localization of NE proteins, such as emerin and nesprin-3 ([17] and C.L. Stewart, unpublished work). A comparison of the nuclear mechanical properties between lamin A-deficient cells and those with a disrupted lamin B gene revealed that the A-type lamins are probably the principal contributors to the biophysical properties of the lamina, providing mechanical strength and stiffness to the nuclei, as loss of lamin A results in increased levels in apoptosis and necrosis following mechanically induced strain [35].

Although these observations are consistent with the notion that mutations in *LMNA* physically weaken the nucleus, a consensus is emerging that such a weakening does not provide a complete explanation. In EDMD patients, mutation or loss of emerin results in muscle wasting and cardiac conduction defects similar to those caused by *LMNA* mutations. In mice, *Emd*-deficient nuclei do not exhibit physical weakening. However, *Emd*-null fibroblasts, which have morphologically and physically normal nuclei [36], are more prone to apoptosis following mechanically induced strain and show reduced levels of *iex-1* (immediate early response gene-1) expression induced by mechanical strain, compared with normal cells [37]. In addition, *Lmna*$^{N195K/N195K}$ mice either do not show or have minimal dystrophy in their skeletal muscles. Yet *Lmna*$^{N195K/N195K}$ nuclei are severely morphologically abnormal and are just as weak as the *Lmna*$^{-/-}$ nuclei (J. Lammerding and C.L. Stewart, unpublished work) (Figure 1). Together, these findings suggest that it may be too simplistic to view physical weakening of the nuclei as

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the prime cause of striated muscle defects in the laminopathies.

Gene expression hypothesis

As the mechanical stress hypothesis does not provide a complete explanation for the muscle envelopathies, a second hypothesis, that the NE/lamina regulates gene expression, has been invoked. The basis for this hypothesis is that both A- and B-type lamins interact either directly or indirectly with many chromatin organization proteins, transcription factors and transcriptional regulatory factors. Furthermore, signalling pathways that culminate in transcription factor activity may also be regulated at the NE and lamina. The gene expression hypothesis should not, however, be seen to exclude effects that mechanical stress may have on cells. Indeed, much preliminary evidence from studying both mouse and human cells carrying laminopathy mutations indicate that gene expression, signalling pathways and mechanical integrity are all perturbed [1].

The lamina may regulate gene transcription by acting as a scaffold, which is necessary for the appropriate localization to the NE of factors important in muscle regeneration. Emerin was the first NE-associated protein to be identified that, when mutated, resulted in a disease, X-EDMD [11]. Emerin, besides interacting with A-type lamins, also binds to several transcription factors, transcriptional repressors and chromatin-organizing factors, such as BAF (barrier-to-autointegration factor), as well as the muscle-specific transcription factors Lmo7, Btf, GCL (germ cell-less) and the RNA splicing factor YT521-B [38,39]. Emerin may also regulate β-catenin accumulation in the nucleus, as there is an increase in the nuclear accumulation of β-catenin in fibroblasts from patients with *EMD* mutations [40]. Such effects on β-catenin localization or activity were not, however, observed in emerin-deficient mouse fibroblasts (C.L. Stewart and L. Hernandez, unpublished work).

The loss of or a reduction in the assembly of A-type lamins results in a redistribution of emerin from the nucleus to the ER [17,34], which in turn suggests that it is the reduction in emerin levels at the NE which contributes to the aetiology of AD-EDMD and X-EDMD. Loss of emerin from the NE also results in the centrosome detaching from the NE [41,42].

Emerin deficiency in mice, however, does not phenocopy human X-EDMD. *Emd*-deficient mice appear overtly normal and do not show any muscle or significant cardiac abnormalities, perhaps delineating a key difference that age and stress presents to human muscle [36,43]. Despite this lack of an overt pathological phenotype, evidence suggests that satellite-cell-mediated muscle regeneration depends, to some extent, on emerin [36].

Following injury or disease, muscle regeneration is initiated by the activation of muscle satellite cells with the muscle fibre with which they are associated. The satellite cells proliferate, then undergo pRb (retinoblastoma protein)-mediated exit from the cell cycle and fuse with the underlying myotubes [44]. Hypophosphorylated pRb complexes with the E2F family of transcription factors and, in these complexes, E2F is inactive and unable to transactivate cell-cycle-inhibitor genes, such as p21, resulting in continued cell proliferation. When pRb is hyperphosphorylated, its activity is inhibited and it no longer binds to E2F. Cell-cycle arrest genes are then induced, resulting in cells exiting the cycle.

Lamin A/C and perhaps emerin are part of the pRb–E2F complex required for cell-cycle exit [45,46]. Regenerating muscle of *Emd*-deficient mice shows up-regulation of the pRb–MyoD transcriptional pathway components and a delay in the induction of myogenic genes [36]. A similar up-regulation of pRb–MyoD pathway components is also observed in human EDMD muscle. Accordingly, disruption of lamin A/C and emerin localization may result in destabilization of pRb complexes, resulting in a compensatory up-regulation of pRb and MyoD target genes and defects in muscle regeneration [47]. These observations implicate inappropriate regulation of pRb in the pathogenesis of NE muscular dystrophies [36]. This may also explain why immortalized $Lmna^{-/-}$ myoblasts, but not primary $Lmna^{-/-}$ myoblasts, are impaired in their *in vitro* differentiation to myotubes [48], as the immortalized myoblasts may be defective in pRb regulation and/or expression.

In contrast with the above molecular findings that have largely been uncovered by studying the striated muscle laminopathies, our understanding of the molecular basis of HGPS is still limited. Studies on fibroblasts from progeric patients indicated that these cells suddenly lose the ability to proliferate [29]. This may be due to a failure in DNA-repair mechanisms and defective mitoses [49,50]. Such findings may provide an explanation for the observation that HGPS patients apparently lose smooth muscle cells in the aorta, as do a line of transgenic mice expressing progerin [51]. Whether this is due to accumulation of progerin and thickening of the lamina with increased passage is unclear, as different groups have reported contradictory results [50,52,53]. These results, however, do not address why progerin appears to primarily affect a different range of tissues, including the skin, vascular system and skeletal systems, whereas other tissues such as the nervous system, liver, skeletal muscle and kidneys are apparently unaffected, since virtually all adult tissues express A-type lamins [54]. Progeric fibroblasts are, like other *LMNA* mutations, more sensitive to the apoptotic effects of mechanical strain than normal fibroblasts [53]. However, what makes cells from different lineages carrying the same mutation in the *LMNA* gene respond differently to the same type of mechanical strain is not understood. Clearly, there is still much to learn about the molecular pathological basis of the laminopathies and the higher-order structure of the nucleus in relation to its function, with particular regard to whether the NE/lamina linkage of the cytoskeleton to chromatin has a central role in mechanosignalling.

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