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Review

Beyond membrane channelopathies: alternative mechanisms underlying complex human disease

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Over the past fifteen years, our understanding of the molecular mechanisms underlying human disease has flourished in large part due to the discovery of gene mutations linked with membrane ion channels and transporters. In fact, ion channel defects ("channelopathies" — the focus of this review series) have been associated with a spectrum of serious human disease phenotypes including cystic fibrosis, cardiac arrhythmia, diabetes, skeletal muscle defects, and neurological disorders. However, we now know that human disease, particularly excitable cell disease, may be caused by defects in non-ion channel polypeptides including in cellular components residing well beneath the plasma membrane. For example, over the past few years, a new class of potentially fatal cardiac arrhythmias has been linked with cytoplasmic proteins that include sub-membrane adapters such as ankyrin-B (*ANK2*), ankyrin-G (*ANK3*), and alpha-1 syntrophin, membrane coat proteins including caveolin-3 (*CAV3*), signaling platforms including yotiao (*AKAP9*), and cardiac enzymes (*GPD1L*). The focus of this review is to detail the exciting role of lamins, yet another class of gene products that have provided elegant new insight into human disease.

Keywords: arrhythmia; channelopathy; heart disease; lamin; laminopathy; emerin; nesprin

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Introduction

In fact, ion channel defects ("channelopathies" — the focus of this review series) have been associated with a spectrum of serious human disease phenotypes including cystic fibrosis, cardiac arrhythmia, diabetes, skeletal muscle defects, and neurological disorders. However, we now know that human disease, particularly excitable cell disease, may be caused by defects in non-ion channel polypeptides including in cellular components residing well beneath the plasma membrane. For example, over the past few years, a new class of potentially fatal cardiac arrhythmias has been linked with cytoplasmic proteins that include sub-membrane adapters such as ankyrin-B (*ANK2*)[1–5], ankyrin-G (*ANK3*)[6–8], and alpha-1 syntrophin[9], membrane coat proteins including caveolin-3 (*CAV3*)[10], signaling platforms including yotiao (*AKAP9*)[11, 12] , and cardiac enzymes (*GPD1L*)[13]. The focus of this review is to detail the exciting role of lamins, yet another class of gene products that have provided elegant new insight into human disease.

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Lamins: critical intermediate filament components

Lamins are intermediate filament proteins and a major component of the nuclear lamina, a proteinaceous layer underlying the inner nuclear membrane, separating the nuclear envelope from the nuclear matrix. Lamins interact with proteins and chromatin, thus playing an important role in maintaining cell structure and cell regulation including apoptosis[14–17]. Lamin is involved in DNA repair and replication, transcriptional regulation, and maintaining the organization and structure of heterochromatin, nuclear lamina, inner nuclear membrane and nuclear pore complexes[14–19]. Further, lamin has been implicated to be involved in tumorigenesis and viral infections[18, 20, 21].

Lamins are divided into two groups originally based on isoelectric points observed by two-dimensional gel electrophoresis: A-type lamins (almost neutral isoelectric point) and B-type lamins (acidic isoelectric point)[22, 23]. A-type lamins are primarily located in differentiated cells while B-type lamins are located in all cells. Lamins have a conservative alpha helical central rod domain with an amino terminal globular head domain and carboxyl terminal globular tail domain[21]. The lamin tail domain contains an approximate 120-residue immunoglobulin fold, CAAX motif (except lamin C as described below) and a nuclear localization signal[24].

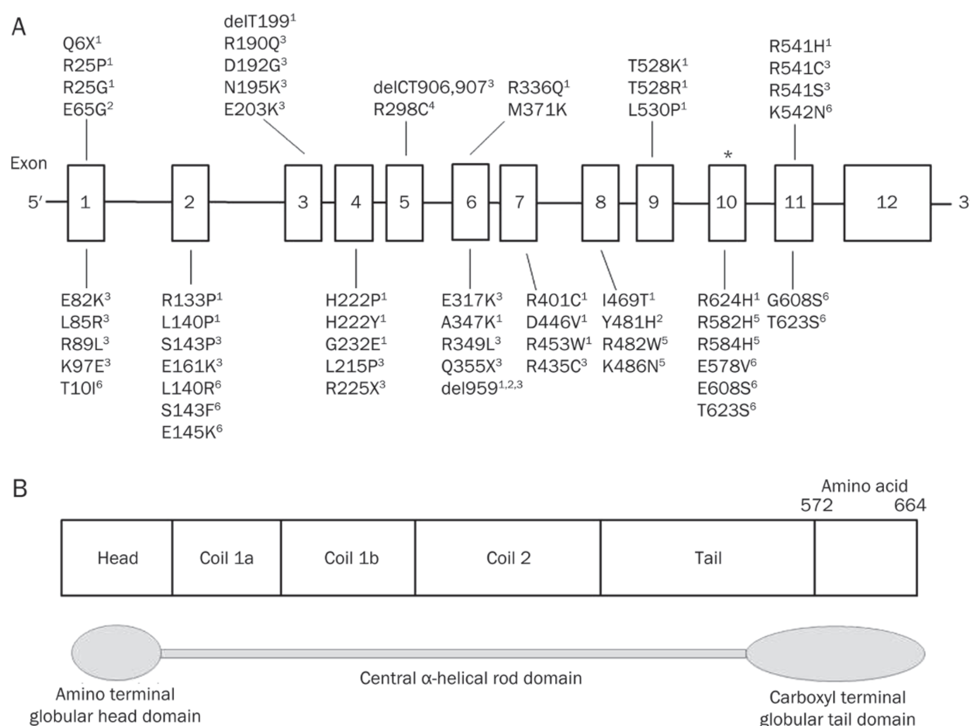

Figure 1. Schematic of (A) lamin gene (LMNA) and (B) lamin A/C protein. * indicates alternate splicing in exon 10 giving rise to the proteins lamin A (664 amino acids) and lamin C (572 amino acids). Shown are several mutations known to result in laminopathies with corresponding amino acid or nucleotide changes. 1=Emery-Dreifuss muscular dystrophy; 2=Limb girdle muscular dystrophy type 1B; 3=dilated cardiomyopathy; 4=Charcot-Marie Tooth type 2B1; 5=Familial partial lipodystrophy of the Dunnigan-type; 6=Hutchison-Gilford progeria syndrome.

In humans, the *LMNA* gene (Figure 1) codes for A-type lamins and is localized to chromosome 1q21.2[25]. *LMNA* consists of 12 exons and at exon 10 alternative splicing occurs giving rise to the proteins lamin A (664 amino acids) and lamin C (572 amino acids). The first 566 amino acids (exon 1–10) of lamin A and C (lamin A/C) are identical which code for an amino terminal globular head domain, central rod domain (coil 1a, 1b, and 2), and a portion of the carboxyl terminal globular tail domain[17, 26]. Mutations in the human *LMNA* gene encoding for lamin A/C results in several different clinical disorders referred to as "laminopathies"[27–35]. Interestingly, certain cases of laminopathies primarily affect the heart resulting in dilated cardiomyopathy with or without conduction system disease even though lamin is found in all differentiated cells in the human body[27–29]. *LMNA* also encodes the protein lamin C2 found in germ cells that is encoded by an alternative first exon[36].

B-type lamins in humans are encoded by the genes *LMNB1* and *LMNB2*[37]. *LMNB1* is localized to chromosome 5q23.3-q31.1 and encodes the protein lamin B1[25, 38]. A mutation in the *LMNB1* gene has been found to result in autosomal dominant leukodystrophy[39]. *LMNB2* is localized to chromosome 19p13.3 and encodes lamin B2 and lamin B3[37, 40]. A mutation in the *LMNB2* gene has been found to result in acquired partial lipodystrophy[41, 42]. Currently, these are the only two disorders discovered to be associated with mutations in the B-type lamins.

Laminopathies

Almost all lamin mutations discovered to-date resulting in human disease are located within the *LMNA* gene. These mutations result in several different clinical disorders with various phenotypes referred to as laminopathies; there are more than 10 clinical phenotypes that can be divided into four broad categories: myopathy, neuropathy, lipodystrophy and progeria, with overlap between groups. Well over 100 mutations have been discovered in the *LMNA* gene with the majority resulting in cardiac involvement. Over 90% of laminopathies are due to a nucleotide substitution[21, 43].

In a large French pedigree, Bonne *et al*, in 1999[30] discovered for the first time that a mutation (nonsense and missense) in the *LMNA* gene resulted in an inherited disorder, autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD). Since that time several mutations, mostly missense, have been discovered throughout the *LMNA* gene resulting in EDMD. EDMD is characterized by contractures of the elbows and Achilles, muscle wasting with humeroperoneal weakness and cardiomyopathy with conduction disease. Symptoms begin within the first few years of life with difficulty ambulating. Cardiac involvement usually occurs after the onset of skeletal myopathy between the first and fourth decades of life resulting in conduction system disease (atrioventricular block; atrial and ventricular arrhythmias), dilated cardiomyopathy and sudden cardiac death[43, 44]. Autosomal recessive EDMD is much less common with a few reported cases demonstrating an earlier phenotypic expression of skeletal myopathy, however, cardiac involvement has not been seen[45, 46].

Limb girdle muscular dystrophy type 1B (LGMD1B) results primarily from a missense mutation with an autosomal dominant inheritance; several missense mutations located throughout the *LMNA* gene resulting in LGMD1B have been identified. Affected individuals develop progressive limb

girdle weakness with or without calf hypertrophy and dilated cardiomyopathy with conduction system disease may occur[47]. Interestingly, a single nucleotide deletion at position 959 has been identified within exon 6 of the *LMNA* gene in one family resulting in different phenotypic expressions within the same family including LGMD1B-like symptoms, autosomal dominant EDMD-like symptoms and isolated dilated cardiomyopathy with conduction system disease[48].

Specific mutations in the *LMNA* gene can result in isolated cardiac involvement in which the affected individuals develop dilated cardiomyopathy with or without conduction system disease. Dilated cardiomyopathy is a disorder of the myocyte characterized by cardiac dilation and systolic dysfunction[49, 50]. Lamin mutations are likely the most common cause of idiopathic dilated cardiomyopathies. Approximately 30% of idiopathic dilated cardiomyopathies are inherited[51–53]. Several mutations have been discovered, mostly missense mutations, located throughout the *LMNA* gene[43]. An example of the natural history of this disease and evolution to the discovery of one of the *LMNA* genetic mutations is illustrated by the immigration of a young couple from Bavaria, Germany to Maryland, United States of America and then to central Ohio in 1830. Descendants of this couple in the 1960s presented to The Ohio State University Medical Center with high-grade atrioventricular (AV) block; careful family history revealed autosomal dominant inheritance after reconstructing an extensive nine generations pedigree. Following the family members closely for several decades, it was found that affected patients between 30 to 70 years of age also developed non-ischemic dilated cardiomyopathy; sudden cardiac death may also occur. Autopsy in several cases demonstrated severe fibrosis in the sinus node, AV node, atria and ventricles. Fibrosis was more severe in the atria compared to the ventricles fibrosis. More recently, ventricular fibrosis has been seen on cardiac magnetic resonance imaging. Family wide genotyping performed in family members revealed a 2-nucleotide pair deletion in the *LMNA* gene (cytosine in position 906 and thymine in position 907 at exon 5) resulting in a sequence of amino acid changes beginning at position 302 and eventually leading to the amino acid substitution of cysteine for serine at position 328 forming a premature stop codon with protein truncation[54].

Charcot-Marie Tooth (CMT) disorders are the most common group of inherited neuropathies affecting 10 to 40 per 100 000 individuals. One sub-type, CMT2B1, is a sensorimotor axonal neuropathy with an autosomal recessive inheritance that results from a missense mutation in the *LMNA* gene[34, 55]; ten Algerian families with CMT2B1 have demonstrated a missense mutation resulting in the substitution of the amino acid arginine for cysteine at position 298 (R298C)[34, 56]. Onset of symptoms ranges from early childhood to early adulthood with distal muscle weakness and wasting occurring in the distal extremities, more evident in the legs compared to the arms. A sensory deficit may occur in the feet and lower extremities[57]. There is one family from south France found to have an axonal neuropathy with cardiac involvement and an autosomal dominant inheritance. In this family, a missense mutation was found to result in the substitution of the amino acid glutamic acid for aspartic acid at position 33 (E33D) leading to CMT, cardiomyopathy with conduction system disease, muscular dystrophy and leuconychia[58].

Familial partial lipodystrophy of the Dunnigan-type (FPLD) has an autosomal dominance inheritance. FPLD most commonly occurs from a missense mutation in exon 8 of the *LMNA* gene resulting in the substitution of the amino acid arginine for tryptophan at position 482 (R482W) that encodes primarily the carboxyl terminal globular tail domain[59]. FPLD primarily affects adipocyte cells with progressive loss of fat from the extremities and trunk with accumulation of fat in the face and neck[60]. Further, affected individuals develop metabolic abnormalities including insulin resistance and glucose intolerance. Hypertriglyceridaemia may also occur. Onset of symptoms usually occurs at puberty[59, 61].

Hutchison-Gilford progeria syndrome (HGPS) is a multisystem disorder characterized by premature aging. Majority of affected individuals with HGPS results from a *de novo* heterozygous single base substitution of cytosine for thymine at position 1824. This substitution results in an abnormal splice donor site in exon 11 of the *LMNA* gene that produces a lamin A protein lacking 50 amino acids from the carboxyl terminal globular tail domain[35]. Affected individuals demonstrate skeletal abnormalities, micrognathia, mid-face hypoplasia, alopecia, loss of subcutaneous fat and pre-mature atherosclerosis. Most affected individuals die between the first and second decades of life from cardiovascular complications[43]. HGPS has also been found to have an autosomal recessive inheritance in one consanguineous family where a missense mutation results in the amino acid substitution of lysine for asparagine at position 542 (K542N)[62].

Mechanisms underlying laminopathies

Researchers have strived to elucidate why certain laminopathies result in specific tissue phenotypes even though lamin A/C essentially is found in all differentiated cells within the human body. In addition, different mutations have been shown to result in the same clinical phenotype. Moreover, the same single mutation can result in various phenotypic expressions[63]. Several hypotheses have been postulated to explain these observations including: structural, gene expression, cell proliferation and protein-protein interaction; however, a specific laminopathy may not be exclusive to one hypothesis.

Structural hypothesis

A mutation in the *LMNA* gene producing abnormal lamin weakens the nuclear envelope and develops abnormal nuclear-cytoplasmic interactions, thus decreasing the structural integrity of the cell. These changes make the cell susceptible to mechanical stress potentially leading to cell death, especially striated muscle or cardiomyocytes that are exposed to mechanical stress[64]. Embryonic fibroblasts obtained from *Lmna* knockout mice demonstrate the inability of the nuclear envelope to withstand physical force easily rupturing as compared to controls[65]. Skeletal muscle biopsies obtained from

patients with autosomal EDMD and cardiac biopsies from patients with dilated cardiomyopathies have shown physical damage to the cells including ruptured nuclear envelopes and localization of chromatin into the cytoplasm[66, 67]. In addition, fibroblasts from patients with HGP have shown to have an abnormal nuclear envelope shape, clustering of nuclear pores and loss of peripheral heterochromatin that worsen as the cells age[68]. Fibroblast from FPLD patients also revealed abnormal nuclei structure and when exposed to heat stress had an increase in cell death compared to controls[69].

Gene expression hypothesis

Lamin plays an important role in DNA repair and replication as well as transcriptional regulation, thus abnormal lamin will affect these functions[18, 19, 70]. The disruption of the normal organization of lamin in mammalian cells has been shown to inhibit RNA polymerase II-dependent transcription[71]. The gene expression hypothesis may particularly provide some insight in adipocyte disorders like FPLD. Peroxisome proliferator activator receptor gamma (PPARγ) and sterol regulatory element binding protein-1 (SREBP1) are two of several genes that regulate adipogenesis. SREBP1 binds to pre-lamin A and also activates PPARγ. Pre-lamin A in fibroblasts from patients with FPLD has been shown to accumulate at the nuclear envelope sequestering SREBP1, thus decreasing PPARγ activation and in turn inhibiting adipogenesis[72–75]. These findings may partially explain the progressive loss of fat in the extremities and trunk of individuals with FPLD. Further, deficient SREBP1 has been associated with type 2 diabetes mellitus, also seen in individuals with FPLD[76].

Cell proliferation hypothesis

Stem cells fail to differentiate properly due to abnormal lamin within the cell. Individuals with HGPS have an increased production of progerin, a mutant form of the lamin A protein. Progerin accumulates near the nucleus altering the structure of the nuclear lamina. Studies have demonstrated that progerin interferes with the normal function of human mesenchymal stem cells (MSC) altering their ability to differentiate appropriately. MSC typically undergo differentiation to form several of the tissues affected in HGPS including bone (osteogenesis) and fat (adipogenesis); these effects are mediated by progerin activating downstream effectors of the Notch signaling pathway, a major regulator of human MSCs[77]. Further, studies have shown that the differentiation of mouse skeletal stem cells, satellite cells, is associated with the relocation of nucleoplasmic lamin A/C to the nuclear lamina and reorganization of the nucleoskeleton; C2C12 myoblasts transfected with a mutant lamin A, known to cause autosomal dominant EDMD, prevented the relocation of lamin and reorganization of the nucleoskeleton, resulting in the inhibition of myoblast differentiation[78].

Protein-protein interaction hypothesis

Altered lamin due to a *LMNA* gene mutation will develop an abnormal interaction with associated proteins resulting in disorganized cell structure and in-turn cell dysfunction[79–82]. Nikolova *et al*, demonstrated that in lamin A/C deficient mice the intermediate filament protein desmin, important in maintaining structural integrity of the cell, became disorganized and detached from the nuclear surface[79, 83]. In addition, the inner nuclear envelope proteins nesprin and emerin, both important in maintaining cell structure, mis-localized to the endoplasmic reticulum in SW-13 cells which lack lamin A and re-localized to the inner nuclear envelope in SW 13/20 cells that contain lamin A[80]. Cardiomyocytes of *LMNA* knockout mice demonstrated an altered nuclear envelope, disorganization of nesprin-1 and changes in the expression and distribution of nuclear and cytoskeletal actin[84]. Studies by Raharjo *et al*[82], showed that point mutations in lamin A/C resulting in the substitution of amino acids leucine for arginine at position 85 (L85R) and asparagine for lysine at position 195 (N195K), both known to cause dilated cardiomyopathy, altered the assembly of lamin A/C resulting in the partial mis-localization of emerin in HeLa cells; these findings were also seen in the point mutation resulting in the substitution of amino acid leucine for proline at position 530 (L530P), known to cause autosomal dominant EDMD[82]. Further, eliminating lamin A/C from the nuclear envelope of HeLa cells resulted in emerin mis-localization and the formation of aggregates within the endoplasmic reticulum[81].

Conclusions and perspectives

Lamins are intermediate filament proteins and are major components of the nuclear lamina playing an important role in cell regulation and structural integrity[14–17]. There are well over 100 mutations in the *LMNA* gene, encoding for the protein lamin A/C, that result in more than 10 clinical disorders collectively referred to as laminopathies[37, 43]. The challenge remains to determine why certain *LMNA* mutations result in tissue specific diseases, even though lamin A/C is found in all differentiated cells in the human body.

The laminopathy story is an elegant example of the importance of close collaboration that must exist between the physician-scientist and basic research-scientist in the study of heritable disorders. Careful physical examination of affected individuals and meticulous investigation of their family history allows the physician-scientist to understand the complexity of the disease, while the basic research-scientist helps in defining molecular mechanisms of that disease. Animal models, including *Lmna* knockout mice and mice carrying various *LMNA* missense mutations, have provided much insight into the mechanisms of laminopathies[85–87]. Knowledge gained from the clinic and bench will help to better understand the underlying mechanisms, and will result in therapeutic strategies to treat affected individuals and provide insight into molecular mechanisms of other human diseases.

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