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Review

LAMINOPATHIES: THE MOLECULAR BACKGROUND OF THE DISEASE AND THE PROSPECTS FOR ITS TREATMENT

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Abstract: Laminopathies are rare human degenerative disorders with a wide spectrum of clinical phenotypes, associated with defects in the main protein components of the nuclear envelope, mostly in the lamins. They include systemic disorders and tissue-restricted diseases. Scientists have been trying to explain the pathogenesis of laminopathies and find an efficient method for treatment for many years. In this review, we discuss the current state of knowledge about laminopathies, the molecular mechanisms behind the development of particular phenotypes, and the prospects for stem cell and/or gene therapy treatments.

Key words: Laminopathies, Nuclear lamina, Lamin, Emerin, Gene therapy

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Abbreviations used: ADLD – adult-onset autosomal dominant leukodystrophy; APL – acquired partial lipodystrophy; CMD1A – dilated cardiomyopathy 1A with conduction defect; CMT2B1 Charcot-Marie-Tooth disease type 2B1; DMD – Duchenne muscular dystrophy; EDMD – autosomal dominant Emery-Dreifuss muscular dystrophy; ERK – extracellular signal-regulated kinase; GL – generalized lipodystrophy; FPLD – Dunnigan familial partial lipodystrophy; HGPS – Hutchinson Gilford progeria syndrome; INM – inner nuclear membrane; JNK – c-Jun NH(2)-terminal kinase; LAP – lamina-associated polypeptide; LBR - p58 protein, lamin B receptor, 3 beta-hydroxysterol D14-reductase; LGMDB1 – limb-girdle muscular dystrophy type 1B; LINC – linker of the nucleoskeleton and cytoskeleton; *LMNA* – gene encoding A/C type lamins; MAD – mandibuloacral dysplasia; NE – nuclear envelope; PPARγ – peroxisome proliferator-activated receptor γ; pRB – retinoblastoma protein; ZMPSTE24/FACE1 – zinc metalloproteinase STE 24 homology

INTRODUCTION

The term laminopathies (envelopathies) defines a group of human disorders that are associated with mutations in the genes coding for nuclear lamina proteins (*LMNA*, *LMNB1* and *LMNB2*), proteins associated with their post-translational processing (e.g. *ZMPSTE24*), or proteins interacting with the lamins: emerin, LAP2, LBR (p58; 3 beta-hydroxysterol D14-reductase), MAN1, nesprin-1 and nuclear pore complex proteins [1]. The known mutations in the lamin genes give rise to at least 16 more or less distinct phenotypes with different extents of manifestation of the common phenotypic features. To date, more than 340 unique mutations have been reported from more than 1,000 patients (http://www.umd.be) [2]. The emerging data suggests that mutations in the nuclear lamina genes could also be involved in common diseases, particularly cancer [3].

Lamins are intermediate filament (IF) proteins (type V). They are the main component of the nuclear lamina, which is part of the nuclear envelope (NE). In addition to the peripheral lamina, lamins also form nucleoplasmic structures [4, 5]. Based on differences in their biochemical properties and expression patterns, lamins have been divided into two groups, the A and B types. Lamins have a conserved α-helical central rod domain and variable head and tail domains. The tail domain contains a ~120-residue immunoglobulin fold, a nuclear localization signal (NLS), and a CAAX motif (except lamin C) [6]. In humans, three genes (*LMNA*, *LMNB1* and *LMNB2*) encode nuclear lamins. The *LMNA* gene primary transcript is alternatively spliced to produce lamin A, C, C2 and Δ10. The two main protein products, lamin A and C, are present in the cells of many tissues, but not in undifferentiated cells [7]. They have an identical 566 aa length from the N-terminus, and differ in that pre-lamin A has additional 98 aa (exons 11 and 12 are specific for lamin A) on the C terminus with a CaaX motif (C-cysteine, a-aliphatic amino acid, X-unrestricted residue) that is necessary for the recruitment of the protein to INM. The maturation of lamins requires sequential enzymatic modifications of this motif: farnesylation, proteolytic cleavage and carboxy-methylation. In the first stage, farnesyl transferase adds a farnesyl group to the C-terminus cysteine residue. Next, either Ras-converting enzyme (Rce1/FACE2) or zinc metalloproteases related to Ste24p (Zmpste24/FACE1) cleave the last three amino acids (AAX) and then the methyltransferase methylates the farnesylated terminal cysteine. Finally, the last 15-amino acid fragment (containing the farnesylated and methylated cysteine residue) is cleaved off by Zmpste24, generating mature lamin A. B-type lamins remain carboxyfarnesylated and methylated (Fig. 3 IA) [8].

Initially, lamins were only considered to be structural proteins forming the nuclear lamina, which maintains nuclear shape and the spacing of nuclear pore complexes. Over the years, numerous reports have suggested that lamins take part in a wide range of processes. Currently, it is clear that lamins are involved directly or indirectly in the organization of chromatin, DNA replication, the

regulation of transcription factors, epigenetics, DNA repair, transcription, cell cycle regulation, cell development and differentiation, nuclear migration, and apoptosis. Recent studies have provided evidence in support of lamin function in virus infection, tumorogenesis and mitosis, and in linking the nucleoplasm to all the major cytoskeletal networks [9, 10]. Mutations in the nuclear lamina genes can cause a wide range of heritable human diseases with variable clinical phenotypes, i.e. laminopthies.

CLASSIFICATION OF LAMINOPATHIES

Most laminopathy symptoms develop during childhood or adolescence. Some laminopathies affect specific tissue types, while others act upon multiple types of tissue creating overlapping or systemic phenotypes. Based on the affected tissue, we can classify laminopathies into several categories (Fig. 1).

The most common group are laminopathies of the muscular dystrophy type, characterised by muscle wasting: autosomal dominant Emery-Dreifuss muscular dystrophy (AD-EDMD; OMIM 181350) [11]; autosomal recessive Emery-Dreifuss muscular dystrophy (AR-EDMD; OMIM 604929) [12]; limb-girdle muscular dystrophy type 1B (LGMD1B; OMIM 159001) [13]; dilated cardiomyopathy 1A with conduction defect (DCM-CD/CMD1A; OMIM 115200) [14]; X-linked Emery-Dreifuss muscular dystrophy (XL-EDMD; OMIM 31300) [15]; and heart-hand syndrome, Slovenian Type (HHS; OMIM 610140) [16].

The second group are lipodystrophies, which are characterized by an abnormal distribution of adipose tissue, which may be associated with metabolic disorders like diabetes and insulin resistance: Dunnigan familial partial lipodystrophy (FPLD2; OMIM 151660) [17-18]; acquired partial lipodystrophy (APL, also called Barraquer-Simons syndrome; OMIM 608709) [19]; and type A insulin resistance syndrome [20].

Systemic laminopathies covers disease types in which the associated symptoms are diverse and affect a variety of tissue types, frequently bearing features of premature aging: Hutchinson-Gilford progeria syndrome (HGPS; OMIM 176670) [21]; atypical Werner syndrome (AWS; OMIM 277700) [22]; restrictive dermopathy (RD; OMIM 275210) [23]; mandibuloacral dysplasia type A with lipodystrophy (MADA; OMIM 248370) [24-25]; and atypical progeroid syndrome [26-27]. Neuropathies include: Charcot-Marie-Tooth disease type 2B1 (CMT2B1; OMIM 605588) [28]; and adult-onset autosomal dominant leukodystrophy (ADLD; OMIM 169500) [29].

The last group of laminopathies is referred to as the overlapping laminopathies and syndromes, and it covers some heterogeneous clinical cases suggesting an overlapping continuum with different types of laminopathies. For example, patients with clinical features of lipodystrophy carrying the typical R482W FPLD mutations, but also exhibiting variable combinations of cardiac and skeletal

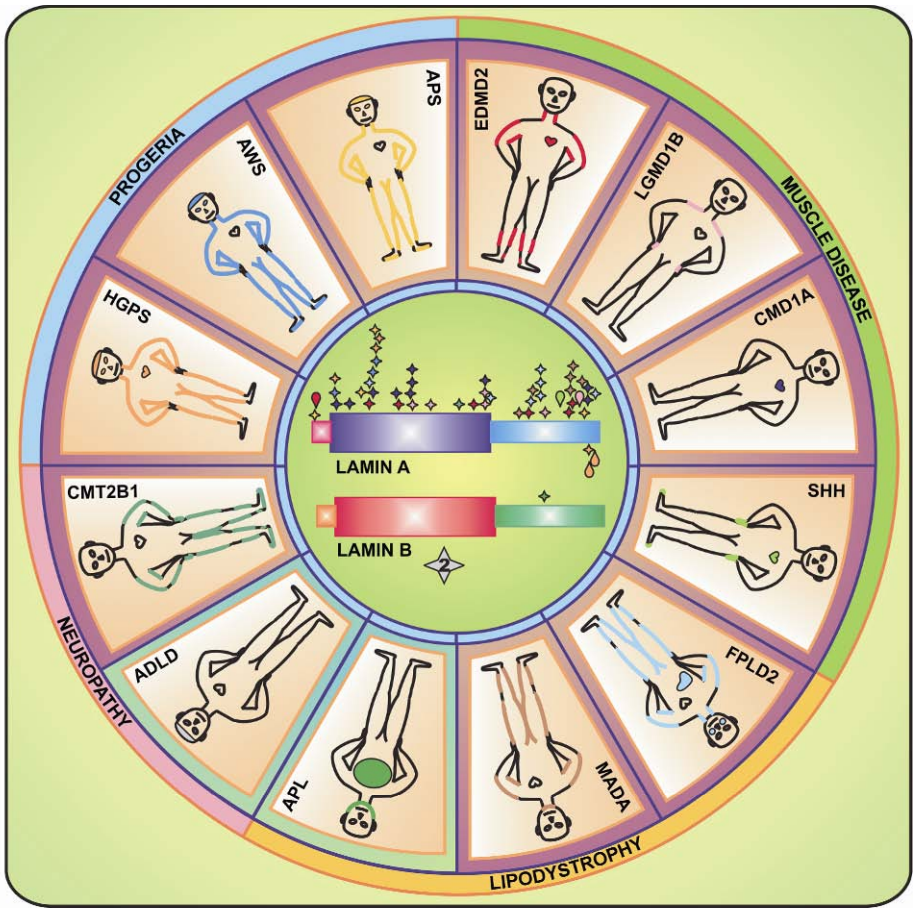

Fig. 1. A classification of laminopathies with a schematic representation of the distribution of the affected tissues and localization of typical mutations for each type. The green highlighting indicates diseases caused by mutations in genes encoding the B-type lamins, and the violet highlighting marks diseases caused by mutations in the *LMNA* gene. On the periphery, the various types of laminopathies are indicated: green – muscle diseases, orange – lipodystrophies, pink – neuropathies, and light blue – progeroid syndromes. Substitutions are represented by small stars, deletions by drops, and duplications by the large star. The colors of the mutations correspond with the colors used to show the parts of the human body affected by a particular disease: e.g. HGPS is orange.
Abbreviations: ADLD – adult-onset autosomal dominant leukodystrophy; APL – acquired partial lipodystrophy; CMD1A – dilated cardiomyopathy 1A with conduction defect; CMT2B1 Charcot-Marie-Tooth disease type 2B1; FPLD – Dunnigan familial partial lipodystrophy; HGPS – Hutchinson Gilford progeria syndrome; LGMDB1 – limb-girdle muscular dystrophy type 1B; MAD – mandibuloacral dysplasia

muscular alterations [30], multinodular goitre, extrapyramidal syndrome and primary hyperaldosteronism [31]. One report describes a patient with features of myopathy and peripheral neuropathy [32]. An E33D mutation is clinically

characterized by the combination of axonal neuropathy with myopathic features, cardiac disease, conduction disturbances and arrhythmia, and leuconychia [33].

Laminopathies have three modes of inheritance: autosomal dominant (AD-EDMD, CMD1A, LGMD1B, FPLD2, HGPS, AWS, RD); autosomal recessive (AR-EDMD, CMT2B1, MAD, HGPS); and X-linked (XL-EDMD). Patients with classical laminopathy have mutations in the *LMNA* gene and emerin gene (*EMD*). More recently, two disorders have been linked to mutations in the *LMNB2* and *LMNB1* genes. The first and to date only reported mutations for the *LMNAB2* gene were described in 2006 by Hegele *et al.* [19] for APL patients. However, acquired partial lipodystrophy is a complex phenotype, it is probably additionally connected with mutations in genes other than *LMNB2*, and it might require the presence of environmental factors or acquired disorders to be expressed [34]. The first human disease clearly attributed to a mutation in the B-type lamin gene was adult-onset autosomal dominant leukodystrophy (ADLD) [29]. Mutations in the genes encoding B-type lamins are probably not frequently connected to diseases (contrary to *LMNA* mutations) because of the functional importance of the B-type lamins, which are essential for cell function [35]. *LMNB1*-deficient mice are small during embryonic development and die soon after birth with defects in their lungs and bones [36]. Mice lacking lamin B2 are normal in size at birth, but die shortly thereafter, as they have severe brain abnormalities resembling lissencephaly, with abnormal layering of neurons in the cerebral cortex and cerebellum [37].

Another group of laminopathies consists of diseases caused by mutations in the genes encoding the integral membrane proteins of NE. The best characterized so far are autosomal dominant Pelger-Huet anomaly (OMIM 21540) and autosomal recessive hydrops-ectopic calcification-moth-eaten (HEM)/Greenberg skeletal dysplasia (OMIM 215140). Both are probably caused by different mutations in the LBR protein gene [26, 38]. This protein is evolutionarilyy conserved, interacts with chromatin, and simultaneously displays 3 beta-hydroxysterol D14-reductase activity. Autosomal dominant Pelger-Huet anomaly would be then the classical "haplo-insufficiency" rather only associated with the structural functions of the LBR protein. A mutation of both alleles results in the second phenotype, probably due to the total elimination of the enzymatic activity of the LBR (3 beta-hydroxysterol D14-reductase activity) protein. Mutation in the *LEMD3* gene encoding the other member of the INM, LEM domain-containing protein 3/MAN1, causes Buschke-Ollendorff syndrome (BOS; OMIM 166700), manifested by a wide phenotypic variation, multiple subcutaneous nevi or nodules, and hyperostosis of the cortical bones [39]. Mutations in the *LAP2* gene located in the C-terminal domain of the LAP2α protein (a region which interacts with lamin A/C) has been proposed to cause dilated cardiomyopathy (DCM) [40].

Laminopathies are very rare diseases. The exact occurrence rate of each laminopathy is difficult to assess from the available data sources. The laminopathy with the highest frequency in human populations is EMD, which affects 3 in 1,000,000 people (Orphanet Report Series, 2010). The other described

disorders, such LGMD1B, RD, MAD and Barraquer-Simons syndrome occur with less frequency, in 1 person per 1,000,000. HGPS is estimated to be present with a frequency of 1 in 8,000,000, and FPLD in 1 in 10,000,000.

Identifying laminopathy can be quite difficult when only routine medical tests are used, but genetic tests might prove useful. However, even using these tests, there is sometimes little chance to correctly classify the disease, due to the overlapping syndromes. Moreover, there is currently no cure for laminopathies, and treatment is mainly symptomatic and supportive. Patients with muscular dystrophies are treated with physical therapy and corrective orthopedic surgery. For cardiac problems, pacemakers are used. Treatments for lipodystrophies are based on controlling the effects of the lipodystrophy, e.g. vascular disease prevention or control of glycemia, via the use of existing drugs.

However, the recent progress in uncovering the molecular mechanisms of laminopathies and lamin functions has opened up the possibility for the development of targeted treatment. Treatments designed to modulate the key steps in laminopathy pathogenesis, such as gene-based therapies, stem cell therapies and novel drug treatments, might provide hope for suffering patients. Moreover, successful stem and/or gene therapy treatment for laminopathies may be easily adapted for the treatment of other similar hereditary diseases and vice versa, e.g. muscular dystrophy of the EDMD type and DMD type would certainly respond to the same treatment strategy. Similarly, all leukodystrophies, regardless of their genetic background, may also require the same treatment strategy based on the transplantation of healthy or genetically corrected (autotransplantation) HSC cells.

THE MOLECULAR MECHANISMS UNDERLYING LAMINOPATHIES

Despite the large number of identified mutations, it is difficult to create a clear correlation between phenotype and genotype in laminopathies. It is still unclear how specific mutations result in a particular tissue-specific laminopathy phenotype [41]. Many different mutations can give rise to the same clinical conditions. On the other hand, the same single mutation can result in different phenotypes, probably depending on the genetic background of a particular patient [42]. Laminopathies mainly arise from substitution (91%) or deletion (5%), or rarely from duplication, insertion and “in-del” (4%) in the *LMNA* gene. At the protein level, 67% of the alterations cause missense substitutions, 4% nonsense substitution, 4% frameshift mutations. 13% of the identified mutations are silent mutations (http://www.interfil.org/index.php). Mutations causing EDMD are distributed more or less evenly through the entire gene. The DCM phenotype is caused by frameshift mutations affecting almost half of the protein and a number of missense mutations. The latter are distributed mostly through the central rod domain of the lamin A/C protein. Mutations caused by LGMD1B are distributed on the C-terminal side of the central rod domain and in the IgG fold. In FPLD, nearly 90% of mutations affect exon 8 of the *LMNA*

gene, and they are mainly localized in the IgG fold of the lamin A/C tail domain. Interestingly, different missense mutations of the same residue can cause different diseases (e.g. R527H and R527C result in MAD, but R527P, T528K and L530P result in EDMD). Also, mutations localized in the same region of the protein for example lying within the eighth β-sheet of the globular region with Ig-like structure, caused different diseases. The mutation that causes EDMD most probably destroys the structure of the entire β-sheet. It has recently been shown that the mutations R527P and L530P, which are predicted to disrupt the Ig-fold, reduced F-actin binding, contrary to lipodystrofic R482Q mutation [43]. Different mutations that lead to FPLD result in the loss of the positive charge on the surface of the domain, but do not affect structural integrity [44].

The important aim of current research in this field is the understanding of the molecular mechanisms underlying laminopathies. Deeper knowledge of such pathogenic mechanisms seems to be necessary for the planning of proper treatment.

Muscular dystrophy

Muscular dystrophies are a heterogeneous group of neuromuscular disorders. Numerous muscular dystrophies are caused by defects in genes encoding sarcolemmal proteins, interacting proteins and enzymes [45]. Others are caused by defects in nuclear membrane proteins such as lamins and emerin. Emerin is a 254-aa integral, inner nuclear membrane protein with a single transmembrane domain. It is a member of the LEM domain protein family (LAP2, Emerin, MAN1) [46-47] due to the presence of a common structural motif (LEM domain) required for binding to the chromatin protein BAF (barrier to autointegration factor), an essential protein with roles in higher-order chromatin structure, nuclear assembly, and gene regulation [48].

Emery-Dreifuss muscular dystrophy (EDMD) was the first laminopathy to be described [49]. AD-EDMD and AREDMD (respectively, autosomal dominant EDMD and autosomal recessive EDMD) are caused by mutations in the *LMNA* gene [11]. The X-linked EDMD (OMIM 31300) is caused by mutations in the emerin gene (*EMD*) localized on chromosome Xq28 [15]. It is known that lamin A is responsible for emerin targeting to the INM [50]. Thus, any mutations in lamin A impairing its ability to polymerize itself into the filament network inside the nucleus or its import and nuclear retention may result in the disease phenotype. EDMDs, both autosomal and X-linked, can be defined by slow progressive muscle wasting, life-threatening cardiac conduction defects, and contractures at the elbows, ankles and neck [51].

The lamin A and emerin genes have been found to be mutated in about 35-40% of cases of EDMD, suggesting the existence of additional major genes genetically elucidated with this disease. Recently, a whole-genome scan was used to identify linkage to the Xq26.3 locus containing the *FHL1* gene. FHL1 (four and a half LIM protein 1) proteins play a role in the maintenance of structural integrity and the regulation of cell signaling. A correlation has been

shown between the FHL1 expression level and myotube size [52]. Recently, 4 mutations in 2 other nuclear envelope proteins, nesprin-1 and -2, were found in EDMD patients [53].

Soon after the mutations in *LMNA* gene were shown to cause EDMD, the next mutations in this gene were shown to cause other dominantly inherited diseases affecting muscle, including dilated cardiomyopathy 1A (DCM-CD; OMIM 115200) [14] and limb girdle muscular dystrophy type 1B (LGMD1B; OMIM 159001) [13]. DCM is a heart muscle disease characterised by ventricular dilation and impaired systolic function [54]. The *LMNA* gene has been found mutated in about 33% of cases with an atrioventricular block [55]. LGMD1B appears during the first twenty years of life and is characterized by weakening of the shoulder and pelvic girdle musculature and proximal musculature, and later on also the distal musculature. In the case of LGMD1B, dilated cardiomyopathy can also develop [13]. LGMD1B is now classified as a variant of EDMD [56]. Investigations undertaken in recent years found new muscular disorders implied to be caused by *LMNA* mutation: congenital-type muscular dystrophy and "heart-hand" syndrome [16, 57].

Several hypotheses have been proposed to explain the molecular mechanisms underlying muscular dystrophies caused by nuclear protein mutation. Many groups suggest a model in which laminopathies arises in the muscle cells because of the unique features of the tissue: prolonged exposure to mechanical stress and the unique transcription pattern of the cells. Such cells and their organelles are exposed to prolonged physical stress and are in constant danger of physical destruction. There is no doubt that muscle cells are characterized by a unique protein pattern, also in case of nuclear lamina proteins. They do not express lamin B1 at all, and only moderate to normal amounts of lamin B2. Thus, with the loss of *LMNA* gene products or the impaired function of those products, only lamin B2 remains to support the interactions between the integral proteins of INM, the nuclear lamina network and chromatin. The resulting karyoskeleton structure and its link to the cytoskeleton is more susceptible to destruction. The level of susceptibility depends on the degree of impairment of lamin A/C and/or the emerin properties. There is a growing body of evidence that lamins interact with the LINC complex and are involved in mechanotransduction, by translating mechanical forces and deformations into biochemical signals, e.g. by activating diverse signalling pathways. Consequently, defects in the lamins can be implicated in the development of various diseases [58]. Interestingly, homozygous mutations in the *SYNE-1* gene encoding nesprin-1, which is one of the LINC complex components, can cause either a cerebellar ataxia or a syndrome with joint contractures and myopathy [59-60]. It is worth noting that emerin forms structural complexes with nuclear myosin I, αII-spectrin and actin [61], probably providing a structural junction to the nuclear lamina analogous to the plasma membrane-associated actin cortical network.

To date, some of the perturbations observed in the various signaling pathways, including MAPK, pRb, MyoD, Wnt-β catenin and TGFβ (transforming growth factor β), have been connected to laminopathic muscular dystrophies [62-63].

The mammalian family of mitogen-activated protein kinases (MAPKs) are serine-threonine kinases that mediate the intracellular signaling associated with a variety of cellular activities. The family comprises extracellular signal-regulated kinase (ERK), p38 and c-Jun NH(2)-terminal kinase (JNK). These activated kinases phosphorylate different substrates including cardiocspecific transcription factors such as GATA4 and MEF2 (myocyte-enhancing factor 2) [64]. ERK1/2 interacts with A-type lamins at the nuclear periphery and regulates activator protein 1 (AP-1) activity (Fig. 2 IA) [65]. Abnormal activation of ERK and JNK was observed in the heart tissue of emerin-deficient mice [66] and in animals the carrying *LMNA* missense mutation, H222P, and in cardiomyocytes and HeLa and COS-7 cells expressing the H222P mutant [67]. What is more, siRNA-mediated supression of lamin A or emerin in C2C12 skeletal myoblasts and HeLa cells reduces ERK activation [68]. Altered ERK function was noted for fibroblasts from EDMD and DCM patients (Fig. 2 IB) [69]. In light of these experiments, ERK inhibition could be treated as a therapeutic option to prevent or delay heart failure in such patients. The initial tests on animal models seem to be reasonably promising. Systemic treatment of lamin A-H222P mutant mice with an inhibitor of ERK activation, PD98059 (an inhibitor of the extracellular signal-regulated kinase, MEK, which activates ERK), inhibited ERK phosphorylation, blocked the activation of downstream genes in the heart, and delayed the development of left ventricular dilatation [70]. Either treatment with SP600125 (an anthrapyrazolone inhibitor), an inhibitor of JNK signaling, which inhibited JNK phosphorylation with no detectable effect on ERK, significantly delayed the development of left ventricular dilatation and prevented decreases in cardiac ejection fraction and fibrosis [71].

The activation of myogenesis is controlled by a series of complex transcriptional regulatory networks. One of the pathways is the Wnt/β-catenin signaling pathway, which has a vital role in myogenesis. This pathway is activated by Wnt through the transmembrane receptor Frizzled (Fzd) and its co-receptor LRP6 (lipoprotein receptor-related protein 6). In the absence of Wnt, cytoplasmic β-catenin is constantly phosphorylated by the protein complex composed of Axin, APC (adenomatous polyposis coli gene product), CK1 (casein kinase 1) and GSK3β (glycogen synthase kinase 3). This leads to β-catenin ubiquitination and proteasomal degradation [72]. Wnt binding to Fzd inhibits the phosphorylation of β-catenin. Hypophosphorylated β-catenin is released from the complex, accumulates in the cytoplasm and is translocated into the nucleus. Via the adenomatous polyposis coli-like (APC-like) domain, emerin binds nuclear β-catenin and regulates its concentration in a lamin A-dependent manner [73].

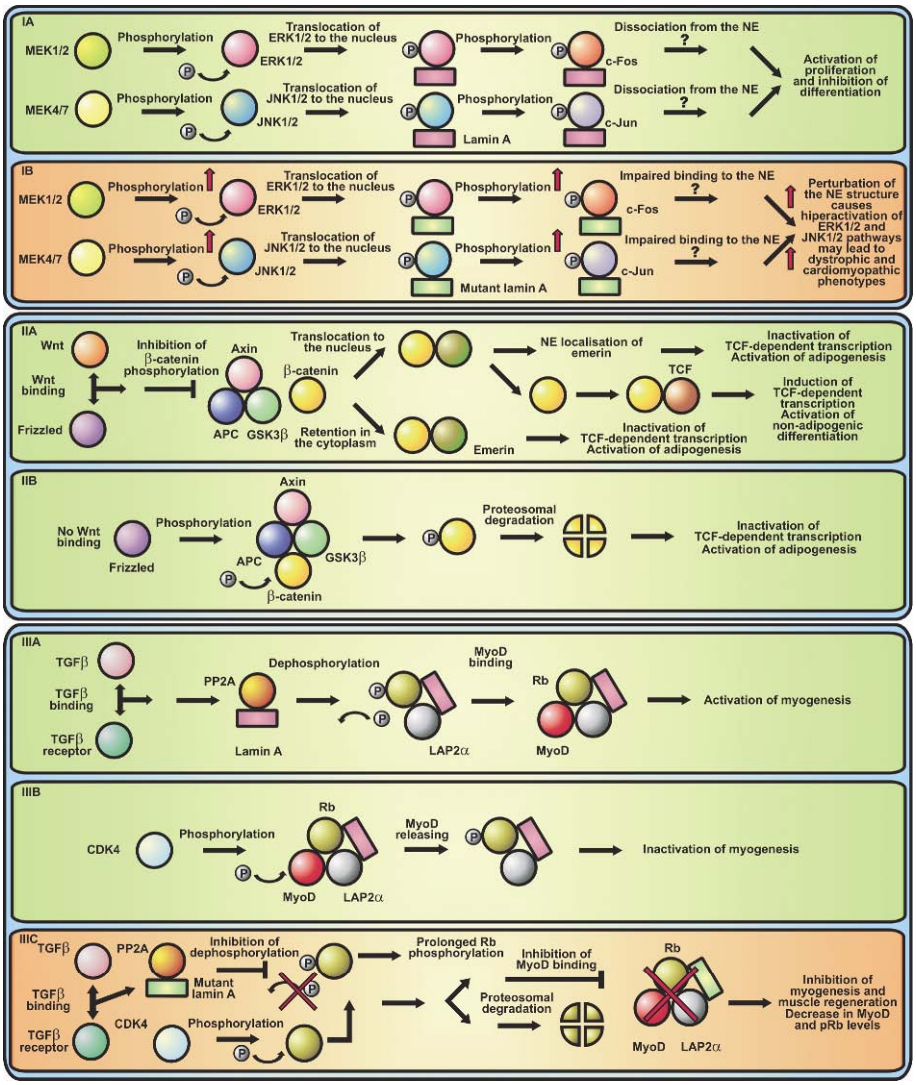

Fig. 2. The molecular background of muscular dystrophy type laminopathies. IA – The MAPK kinases activate ERK1/2 and JNK. Active, phosphorylated ERK1/2 and JNK kinases are translocated to the nucleus where they interact with A-type lamins at the nuclear periphery and phosphorylate c-Fos and c-Jun, causing their release from the NE, thus allowing the transcriptional activation of responsive genes. IB – Lamin A mutations, probably because of impaired binding of mutated lamin A/C with ERK and JNK kinases, cause the abnormal activation of the two kinases. IIA – Wnt/β-catenin signaling is activated by ligands of the Frizzled receptor, which triggers a signal cascade resulting in the displacement of the multifunctional kinase GSK-3β from the APC/Axin/GSK-3β complex. Free GSK-3β is not able to phosphorylate β-catenin. Hypophosphorylated β-catenin accumulates in the cytoplasm and is translocated to the nucleus, where it can be immobilized in NE by emerin binding or can translocate to the nucleoplasm, where it activates its target genes via TCF/LEF transcription factors. Mutations in emerin could change β-catenin cellular

localization and thus influence its activity. IIB – In the absence of Wnt, cytoplasmic β-catenin is constantly phosphorylated by GSK3β in a protein complex, which leads to β-catenin ubiquitination and proteasomal degradation. IIIA – Lamins could modulate TGF-β-dependent signaling through interaction with protein phosphatase 2A (PP2A). PP2A dephosphorylates pRB, and dephosphorylated Rb is able to bind MyoD. The MyoD-Rb-lamin A-LAP2α complex induces the expression of genes associated with myogenesis. IIIB – Cdk4 phosphorylates MyoD, releasing it from the lamina-Lap2α-pRb complex, leading to the inactivation of myogenesis. This creates an opportunity for pRb to take part in the regulation of cell cycle progression and proliferation. IIIC – In the absence of wild-type lamin A/C, PP2 is unable to dephosphorylate pRb in the lamin A-LAP2α complex. Phosphorylated pRb does not bind MyoD, and this leads to the inactivation of myogenesis and a decrease in the level of MyoD and pRb.

Nuclear β-catenin activates its target genes via TCF/LEF (T-cell factor/lymphoid enhancer factor) transcription factors [74]. Thus, a lack of emerin or emerin function might inhibit TCF-dependent transcription and play a crucial role in the regulation of the Wnt-β-catenin pathway (Fig. 2 IIA, B). β-catenin is also engaged in myoblast fusion by binding with the cell adhesion complexes containing cadherins [75]. Reducing the β-catenin level increases the myogenic differentiation of myogenic satellite cells. β-catenin promotes self-renewal of satellite cells and contributes to the maintenance of this stem-cell pool in adult skeletal muscle [76]. Early activation of the Wnt pathway leads to premature differentiation and significantly weakens muscle regeneration.

There is a hypothesis that lamin A/C protein is involved in the regulation of transcription of certain genes by interacting with transcription factors (directly or indirectly). The classical mechanism of the involvement of lamin A/C proteins in gene activation is the regulation of the expression of genes regulated by E2F transcription factors mediated by Rb protein [77]. The main function of this family of transcription factors is in the regulation of the transition from G_1 to S phase. E2F proteins are normally bound by pRb, and this protein in turn is bound and recruited to the nucleus by the lamin A-LAP2α complex. The mechanism of activation of E2F factors involves specific phosphorylation of pRb on pocket C and the release of E2F. The lamin A-LAP2α complex binds hypophosphorylated pRb and associated E2F and thereby reduces E2F-dependent gene expression [78-79]. In the absence of lamin A/C or in the case of loss of function of mutated lamin A in muscle cells derived from *LMNA*$^{-/-}$ mice, the level of Rb is reduced [80]. A lack of LAP2α prevents nucleoplasmatic localization of A-type lamins in G_1, which affects pRb functions and enhances proliferation [81]. A-type lamins might also influence TGF-β (transforming growth factor cytokine superfamily β) signaling. TGF-β controls differentiation, proliferation and apoptosis [82]. Lamins could modulate TGF-β-dependent signaling through interaction with protein phosphatase 2A (PP2A) and Smads. In the absence of wild type lamin A/C, PP2 is unable to dephosphorylate pRb (Fig. 2 IIIA) [83]. Smads undergo altered phosphorylation kinetics in the presence of pathogenetic *LMNA*

mutations causing EDMD [67]. These observations make the lamin A-LAP2α complex a key regulator of the cell cycle, especially in muscle and muscle progenitor cells, through the regulation of pRb location, phosphorylation and proprieties. Rb protein is also involved in the regulation of myogenesis. Dephosphorylation of Rb due to the inactivation of the cdk4 kinase is a key event of this process. Dephosphorylated pRb takes part in the acetylation of MyoD (a member of a family of myogenic regulatory factors that activate muscle-specific genes) by releasing it from HDAC1 (class I histone deacetylase) (Fig. 2 IIIB) [84]. A lack of lamin A and emerin decreases the pRb and MyoD levels. In skeletal muscles from EDMD and LGMDB1 patients, a reduced number of MyoD-positive nuclei is observed (Fig. 2 IIIC) [85].

Recent studies have demonstrated that mutations in lamin A cause somatic stem cell dysfunction, which leads to the attractive hypothesis that the differential effect observed for diverse tissues is a result of their different regenerative potential [86]. Adult stem cells are rare cells that are tissue-specific residents, uniquely capable of reproducing themselves and generating the differentiated cell types to provide a source of tissue replenishment and maintenance. They are present in a wide variety of tissues [87-88]. The first evidence that mutations in lamin A can be connected with the function of stem cells was provided by experiments on mouse C2C12 myoblasts, which are capable of differentiation into myotubes. Two lamin mutants were overexpressed in these cells: R453W (one of the most frequent mutations in EDMD) and R482W (a site responsible for FPLD). R453W clones had a significantly lower capacity to differentiate than clones expressing the R482W mutant and a wild-type lamin A [88].

Currently, there is no efficient treatment for patients with laminopathic muscular dystrophies. Great care should be given to proper diagnosis of patients and then to regular follow-up by a cardiologist. Permanent pacemaker implantation or cardiac transplantation can be the desired therapy for valvular disease in EDMD cardiomyopathy [89]. Another option to delay heart failure is ERK inhibition [70-71]. A very attractive option for treating laminopathic patients might be gene therapy. However, this therapeutic area remains unexplored at this time.

Progeroid syndrome

Many premature aging diseases are caused by mutations in the lamin A gene, resulting in severe nuclear abnormalities. One of them is Hutchinson-Gilford progeria syndrome (HGPS; OMIM 176670) [21]. HGPS is a very rare disease affecting about 1 per 4 to 8 million live births, but it has been the focus of very intense research because it might provide a new insight into the processes of ageing. The average life span of sufferers is approximately 13 years, and they often die because of cardiovascular disease. Characteristic features of HGPS patients are the profound atrophy of subcutaneous fat set, loss of bone density and diminished muscle development.

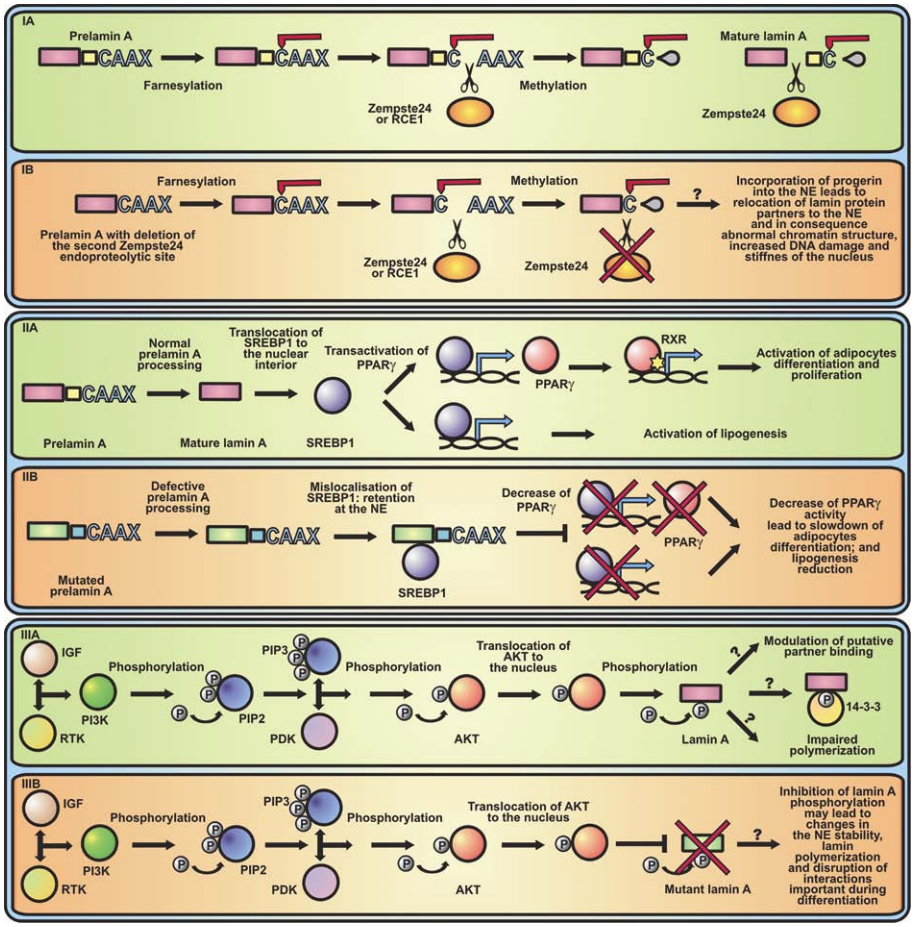

Fig. 3. The molecular background of laminopathies. IA – The maturation of lamins: farnesylation (red), proteolytic cleavage (AAX) and carboxy-methylation (grey) of terminal cysteine. In the last step of maturation, a 15-amino acid fragment (containing farnesylated and methylated cysteine residue) is cleaved off by Zmpste24, generating mature lamin A.
IB – Progerin contains an internal deletion of 50 amino acids (in pre-lamin A marked by a yellow square). The deleted region includes the second cleavage site cleaved by ZMPSTE24, which leads to retention of the toxic farnesyl-group and thus to the abnormal incorporation of mutant lamin into the NE, causing various mechanical defects, including abnormal chromatin structure and increased DNA damage. IIA – SREBP1 interacts with pre-lamin A, but not with the mature form of this protein. Following activation by retinoic acid, PPARγ forms heterodimers with RXR (retinoid X receptor) and modulates gene transcription. IIB – Mutations of lamin A cause the retention of SRBP1 at the nuclear envelope and decrease the SREBP1 level in the the nucleoplasm, which reduces the expression of PPARγ and other genes. IIIA – Lamin A is phosphorylated on the S404 residue by Akt/PKB kinase downstream of the phosphoinositide-3-kinase (PI3 kinase) signaling pathway. The phosphorylation of lamin A might modulate interaction with protein partners, impair lamin polymerization or promote association to the 14.3.3 protein. IIIB – Lamin mutations can inhibit S404 phosphorylation and lead to changes in NE stability.

Most of the typical HGPS patients (approximately 90%) have a single-nucleotide substitution (1824 C > T) in the *LMNA* gene. This mutation activates a new splice site that results in alternative processing of pre-mRNA and the production of a mutant protein containing an internal deletion of 50 aa residues. The resulting protein is termed progerin or LAΔ50. Since the deleted region includes the second cleavage site in pre-lamin A cleaved by ZMPSTE24, this pre-lamin A protein is improperly processed and retains the farnesyl-group at its C terminus. Such a protein is abnormally incorporated mainly into the NE, which leads to various mechanical defects, including abnormal chromatin structure, expansion of the NE, and increased DNA damage (Fig. 3 IB) [90]. Progerin is also expressed sporadically in healthy individuals at low levels, and accumulates in old age [91]. It seems that progeroid syndrome is rather caused by the retention of farnesyl lipid than by the retention of the last 15 amino acids of the protein. In support of such a progerin toxicity model, the other premature aging laminopathy, restrictive dermopathy (RD), has farnesylated lamin A accumulating due to dominant mutations of the *LMNA* gene (primary laminopathy) or recessive mutations of the *ZMPSTE24/FACE1* gene (secondary laminopathy) [23], which encodes a zinc metalloproteinase necessary for the correct processing of pre-lamin A. Moreover, transfection with mutant cDNA or protein injection into normal cells induce the same changes as in patient cells proportionally to the amount of mutated protein present [92]. These observations lead to the hypothesis that permanent farnesylation of lamin A is toxic and can contribute to progeria disease.

The molecular mechanisms of the toxicity of progerin are as yet unclear, but progress has been made in the development of a potential treatment strategy since the discovery that farnesylation of pre-lamin A can be inhibited by farnesyl transferase inhibitors (FTIs; e.g. lonafarnib or tipifarnib), which are typically used in anti-tumor treatments. In cell cultures [93-94] and mice models [95-96], FITs yielded promising results, abnormalities in nuclear morphology were corrected and some, but not all of the progeria-like phenotypes were ameliorated. Altought FITs could be therapeutically useful, some data suggests that such an approach may be limited [97]. It has recently been shown that blocking farnesylation of pre-lamin A and progerin by FTIs causes an alternative modification via geranylgeranylation by geranylgeranyl transferase. This could explain the low efficiency of FTIs in progeroid mouse models. To solve this problem and efficiently inhibit any possible prenylation, a combination of statins and aminobisphosphonates was used to reduce farnesyldiphosphate production. Such treatment noticeably improves the phenotype and extends longevity [98].

Currently, clinical trials treating HGPS patients with lonafarnib are ongoing in the Children's Hospital in Boston (Phase II Trial) [99]. However, there are some questions remaining unanswered regarding the long-term consequences of the FTI strategy. First of all, such treatment inhibits modification of the other CaaX motif proteins (about 100 in the human genome), which includes all of the membrane-bound small GTP-ases (e.g. Ras), as well as some cell cycle

regulators (e.g. Abl). Secondly, after prolonged treatment with such inhibitors, a considerable level of non-farnesylated lamins would be accumulated (B-type lamins are normally constantly farnesylated). Besides, the lack of farnezylation of pre-lamin A may distort its nuclear import or retenction. Finally, we are not sure if non-farnesylated progerin is non-toxic. The latter findings have shown that in mice models expressing exclusively non-farnesylated pre-lamin A (CaaX motif mutated to SaaX), a range of cardiomyopathy developed, contrary to the situation in individuals expressing only the wild-type pre-lamin A, which appeared normal (at least in the absence of lamin C) [100]. Inhibition of pre-lamin A farnesylation prevented cellular senescence and oxidative stress [101]. Pre-lamin A accumulates in cells from HIV-infected patients, probably due to the anti-retroviral therapy with inhibitors of HIV protease, which also inhibits the activity of ZMPSTE24 [102]. This pre-lamin A accumulation can account for the side effect of such treatment, acquired lipodystrophy. Interestingly, in fibroblasts from Dunnigan-type familial partial lipodystrophy patients, pre-lamin A accumulation was also observed [101], supporting the observed connection between pre-lamin A and lipodystrophy.

The use of anti-sense oligonucleotides to selectively inhibit progerin synthesis is another avenue of current research into the development of accelerated aging treatment [103]. Experiments have shown that different antisense oligonucleotides (ASOs) can be used to increase or decrease 'HGPS splicing'. Treating *Zmpste24*$^{-/-}$ mouse fibroblast cells with a pre-lamin A-specific antisense oligonucleotide reduced the pre-lamin A levels and significantly reduced the frequency of misshapen nuclei [104]. Other ASO against exon 11 sequences downstream from the exon 11 splice donor site promote alternate splicing increasing the synthesis of progerin [105]. Also shRNA (short harpin RNA) was exploited to suppress progerin expression in fibroblasts, which leads to a partial correction of the rgw pathophysiological phenotype [103]. These studies suggest a new therapeutic strategy not only for treating progeria but also other laminopathies.

Despite many studies having clearly implicated farnesylated progerin in HGPS, the precise molecular mechanisms of the induced pathology are not yet understood. Some gene expression profiling experiments with fibroblasts from patients with progeria syndromes and transfected cell models have been studied in attempts to elucidate the issue. Recently, scientists demonstrated a link between progerin and the pRb signaling pathway for fibroblasts form HGPS children. What is more, this signaling network seems to be modulated by FTI treatment. There is evidence for a reduction of hyperphosphorylated pRb [90] and a decreased level of pRb expression [106], which may indicate an alteration in the pRb control of E2F1 activity. Therapeutic approaches to reestablish a proper lamin A-Rb signaling network may be beneficial in preventing the complications of physiological aging.

Some recent studies showed that somatic stem cells are misregulated (in terms of number and functional competence) in premature aging [107]. It was shown that

human mesenchymal stem cells (hMSCs) expressing GFP-LAΔ50 exhibit significant differentiation defects, and display activation of major downstream effectors of the Notch signalling pathway (HES1, HES5, HEY1, and TLE1), a major regulator of hMSCs, which give rise to many of the tissues affected in HGPS [107]. Notch proteins are cell surface receptors activated by ligands produced by neighboring cells. Activation of the Notch receptor leads to cleavage of the Notch intracellular domain (NICD), which translocates to the nucleoplasm and regulates downstream gene expression by acting as a co-activator of the transcription factor. Alterations observed in progerin-transducted hMSCs and human HGPS fibroblasts correlate with reduced levels of the transcriptional co-repressor NcoR and increased levels of SKIP. SKIP is a transcriptional co-activator of Notch target genes. In normal cells, it is associated with the karyoskeleton, unlike in the HGPS cell, where it is found in a soluble state in the nuclear interior [107-108]. These findings shed some light on the possible molecular mechanism of HGPS. SKIP and other Notch downstream effectors may be directly regulated by their physical association with the lamina, or their misregulation may be caused by changes in epigenetic modifications frequently found in HGPS. Further insight into the connection between stem cells and progeroid diseases comes from studies on the effects of ZMPST24 depletion in mice hair follicle stem cells, where an absence of the transcriptionally active form of β-catenin, the well-characterized regulator of the Wnt pathway, have been observed [109].

Next to the HGPS called progeria of childhood, the second best-known example of human accelerated aging syndrome with a similar phenotype is Werner Syndrome (WS, called progeria of the adult). Whereas most cases of WS have been caused by mutations in *WRN*, a gene encoding a RecQ family helicase, a subset of patients with atypical WS show heterozygous amino acid substitutions in the heptad repeat region of lamin A [22]. Other systemic laminopathy with some progeroid phenotype features are mandibuloacral dysplasia (MAD; OMIM 248370) [25] caused by a mutation in the *LMNA* gene (MAD type A) or *ZMPSTE24* gene (MAD type B) [24]. The disease is characterised by post-natal growth retardation, craniofacial anomalies, skeletal malformations, partial lipodystrophy, insulin resistance and mottled cutaneous pigmentation [25].

Lipodystrophy

Lipodystrophy is characterized by fat loss in some anatomical sites and fat accumulation in nonatrophic depots and unusual sites like the liver and muscle. Adipose tissues are composed of fat cells, called adipocytes. Adipocytes arise from mesenchymal precursor cells and are present in distinct anatomical locations. Lipodystrophies can be inherited or acquired, partial or generalized, and can also be a component of certain inherited multisystem syndromes (e.g. MAD). Inherited partial lipodystrophies are caused by mutations in several genes e.g. *LMNA* (FPLD2, MAD type A), *LMNB2* (APL), *ZMPSTE24* (MAD type B), and *PPARG* gene encoding PPAR γ (FPLD3) [110].

Familial partial lipodystrophy (FPLD), originally described in the 1970s, is caused by mutations in the *LMNA* gene and is characterized by a progressive, gradual subcutaneous adipose tissue loss from the extremities and gluteal region, usually commencing in the teenage years. Dunnigan type familial partial lipodystrophy (FPLD; OMIM 151660) is characterized by regional and progressive adipocyte degeneration often associated with insulin resistance, hyperlipidaemia and type 2 diabetes presenting in later life [17]. Concerning the metabolic complications, females affected with FPLD are more severely affected than males, but the reasons for this sex-dependent phenotype difference have not been elucidated. In addition, women with FPLD present hyperandrogenism and ovarian abnormalities. The above-mentioned MAD is sometimes classified as lipodystrophy, taking into the account the clinical phenotype of partial lipodystrophy and insulin resistance [25].

It has recently been shown how the adipogenic program of the cell can be associated with lamin and its protein partner emerin [111]. Emerin binds and regulates the nuclear fraction of the canonical Wnt-signalling effector, β-catenin, in a lamin A-dependent manner [73]. Nuclear β-catenin influences the expression and activation of PPARγ. These adipogenic factors regulate the differentiation program leading to the development of mature fat cells [112]. Canonical Wnt signaling represses adipogenesis by blocking the induction of PPARγ. Because the process of adipogenesis is affected by β-catenin and PPARγ signaling, it can be linked to the expression of A/C-type lamins and emerin. It is possible that a loss of these NE proteins can generate lipodystrophy phenotypes. Some experiments suggest a link between emerin mutation and the progressive replacement of skeletal muscle fibres and cardiomyocytes with fatty fibrotic tissue, observed in patients with X-linked EDMD [111].

PPARγ is itself activated by SREBP1 (sterol response element binding protein). SREBP1 is a transcription factor regulating lipogenesis in muscle satellite cells and contracting myotubes [113] and has been identified as a pre-lamin A binding protein (Fig. 3 IIA). It has been thought that FPLD arises due to the affected binding of transcription factor SREBP1-lamin A/C [114]. Thus FPLD would fit into the so-called “gene expression model”. In the case of such a complex, pre-lamin A would be the docking site for SREBP1 and responsible for its recruitment to the nucleus. Mutations in the lamin A/C gene affecting the SREBP1-binding domain (or entire protein) would cause the mis-localization of SREBP1 and the loss of its function, which would in turn affect the normal function of several transcription factors, e.g. PPARγ, changing the expression pattern of many genes associated with lipid metabolism (Fig. 3 IIB).

Unfortunately, to date there is no efficient treatment for lipodystrophic patients. Physicians attempt to manage the metabolic abnormalities with existing drugs for hypertension, diabetes and dyslipidaemia. However, different possibilities are emerging. Lately, the administration of a specific adipokine, such as leptin, in patients with generalized lipodystrophy (GL) was shown to yield very promising results. The long-term effect of treatment with R-metHuLeptin led to

significant improvements in glycemia, dyslipidemia, and hepatic steatosis [115]. Because patients with FPLD have variable serum leptin levels and similar metabolic abnormalities as in GL, the described therapy was also used in FPLD treatment. Patients demonstrated an improvement in insulin sensitivity, fasting glucose concentrations and triglyceride levels, but the changes were less significant than those seen in GL, probably due to the significant baseline differences between patients with FPLD and GL [116]. Some promising experiments have been performed with thiazolinidione (TZD), which acts as a synthetic co-activator of PPARγ and is used in the treatment of diabetes mellitus type 2 [117]. Other treatments for lipodystrophic patients might involve stimulating re-growth or replacing lost adipose tissue with normal white adipose tissue, or possibly gene therapy.

Neuropathy

Another class of laminopathies are neuropathies, like CMT2 and ADLD. Autosomal recessive Charcot-Marie-Tooth disorder type 2 (AR CMT2) is the motor and sensory neuropathy caused by a mutation in exon 5 of the lamin A/C gene [28]. It belongs to the wider group of autosomal dominant CMT1 and AD CMT2, which are caused by mutations in the NF-L gene (neurofilament protein) and microtubule motor protein from the kinesin family (KIF1Bβ). The disease is characterised by electrophysiological and neuropathic symptoms followed by muscle wasting. Adult-autosomal dominant leukodystrophy (ADLD) caused by an increased level of lamin B1 protein in the brain tissue, is a slowly progressive neurological disorder characterized by symmetrical widespread demyelination of the central nervous system [29].

TISSUE SELECTIVE PHENOTYPES

Any attempt to resolve the issue of the molecular mechanism of the laminopathies will always come to this principal question: why do at least some laminopathies have a tissue-selective phenotype, bearing in mind that A- and B-type lamins are expressed in nearly all differentiated cell types? Many theories have been suggested to explain this phenomenon. Classically, two distinct models are usually discussed: the structural model and the gene expression model [118]. The structural hypothesis assumes that alterations in the lamins result in weakened NE, an impaired nucleo-cytoskeleton link and cell damage, especially in muscle cells exposed to high mechanical stress. The lamins and the nucleoskeleton are interconnected with the cellular cytoskeleton via the LINC complex (linker of nucleoskeleton and cytoskeleton), and these connections probably aid in dispersing stressing forces. Additionally, in muscle cells, a proper connection between the karyoskeleton and cytoskeleton must be further extended into the extracellular matrix, since additional mechanical stress is generated during myotube contraction. Indeed, mutations in over 25 genes coding for proteins of the karyoskeleton, cytoskeleton, contractile apparatus and

mechanotransduction pathway give rise to the same dystrophy-like phenotype [58]. Moreover, one of the first histological features of the disease is centrally located nuclei. The lamin-LINC-cytoskeleton complex is also responsible for proper nucleus positioning within non-muscle cells. Recently, it was shown that failed muscle innervation caused by A-type lamin mutation leads to improper positioning of the synaptic nuclei at the neuromuscular junction in muscle fibers, which contributes to AD-EDMD [119]. Thus, impaired mechanotransduction may at least partially explain the typical muscular dystrophy phenotype, which seems to be similar to typical muscular dystrophies (e.g. DMD) and other muscular dystrophies associated with genes responsible for mechanotransduction from the contractile apparatus to the extracellular matrix.

The second plausible explanation for the tissue-specific phenotype of laminopathies is the gene expression model, which suggests that the defects in lamins lead to pathogenic and tissue-specific alterations in gene expression, based on the studies showing that lamins interact with many regulatory proteins and additionally could control chromatin organization and transcription [120]. Thus, we have a complex pattern of overlapping phenotypes, where impaired mechanotransduction mechanisms may coincide with modulatory functions of the nuclear lamina proteins in particular tissues.

Some new concepts have been published, based on the interaction of lamins with a wide spectrum of diverse proteins and on the mechanism by which lamin defects might alter these interactions to cause disease. Lamins not only interact with each other but also interact and modulate function or determine the location of key proteins of the nuclear membranes (emerin, nesprin, LBR, LAP, MAN1), LINC complex (SUN proteins), nuclear pore complex proteins (Nup153, Nup53), transcription factors (SREBP1, MOK2, pRb, c-Fos, Oct-1), nucleoplasmic proteins (BAF, LAP2α), proteins associated with signal transduction pathways (PKCα, PP1, PP2), DNA and chromatin (H2A, H2B histone dimers, heterochromatin protein-1) [for a review see 63, 121]. It should be considered that the formation of a definite complex must be directed by different signaling events. Based on what we already know, lamins are phosphorylated [122], farnesylated, carboxymethylated, proteolytically cleaved [8], and additionally lamin A is sumoylated at lysine 201 [123]. Perhaps knowing how to control and regulate these events one might be able to use them in designing a strategy for patient treatment. For instance, lamin A has been shown to be phosphorylated at its S404 residue by Akt/PKB kinase downstream of the phosphoinositide-3-kinase (PI3 kinase) signaling pathway, in response to insulin stimulation in myoblasts. In cells from an EDMD-2 patient carrying a mutation at Arg401, which lies at the Akt consensus, lamin phosphorylation was dramatically reduced [124]. The pathological mechanism of such an effect is unknown. We can speculate that lamin phosphorylation at Ser404, which lies in proximity to NLS, can regulate either lamin import into the nucleus, or the positioning of lamin A/C. Prediction programs indicate that phospho-S404 represents a fine 14.3.3 protein phosphomotif, which in turn suggests that lamin

phosphorylation at this particular site can be further modulated by its subsequent binding to a member of the 14.3.3 protein family [124] (Fig. 3 IIIA, B). Also, emerin is phosphorylated by tyrosine kinases: Her2, Scr and Abl. Her2 signalling is disrupted in emerin-deficient heart and skeletal muscles, which may suggest that emerin plays a role as one of the downstream effectors of Her2, but the mechanism remains unknown. Some connection has been made for nuclei located at the neuromuscular junction (NMJ) in skeletal muscle, which are exclusively regulated by neuregulin-stimulated Her2 signaling [125].

Misregulation of some signal cascades is observed in numerous laminopathies and may lead to an explanation of their pathogenesis and etiology. Several reports have shown that nuclear envelope proteins are involved in the modulation of Wnt-β-catenin, MAPK, TGF-β, PI 3-Kinase/Akt and Notch signal transduction pathways [63], but much more remains to be studied in order to completely comprehend the underlying disease mechanism. Understanding the interplay between lamins and their signaling partners may help to find a new therapeutic strategy involving inhibitory drugs.

ANIMAL MODELS FOR LAMINPATHIES

Investigations of the pathogenesis of laminopathies can be performed using biological material obtained from patient biopsies, cell cultures and recently generated genetically engineered mouse strains. Mouse models are very helpful for examining potential therapies. These include mice that are null for either *LMNA* [126], *Zmpste24* [127] or *EMD* [66, 128], and mice carrying *LMNA* missense mutations such as H222P [67, 129], N195K [130] or M371K [131]. It should be pointed out that the knockout of the lamin A/C gene in transgenic animals yields the EDMD phenotype, with rapidly progressive dilated cardiomyopathy (DCM) characterized by left ventricular (LV) dilation, and death before puberty [126, 132]. $LMNA^{-/-}$ mice are also characterized by impaired spermatogenesis [133], reduced thymus and spleen size, and severe defects in lymphocyte development [134], and on the cell level by decreased SREBP1 import and reduced PPARγ expression [132]. *Zmpste24*-deficient mice gain weight slowly, display progressive hair loss and suffer multiple spontaneous bone fractures [135]. By contrast, mice lacking emerin appear phenotypically normal [128].

PROSPECTS FOR TREATMENT

It is difficult to speculate at this moment about future prospects in cell and gene therapy for laminopathies due to the still insufficient knowledge of the molecular background of the diseases. Nevertheless, attempts at such treatment should be made at least on tissue-cultured patient cells and model organisms.

The general approach for gene therapy will depend on the type of mutation and the phenotype of the disease. Treatment of simple autosomal recessive disorders

would "only" require the expression of the wild-type allele in the diseased tissue. Autosomal dominant diseases, especially when the mutated protein has the property of "negative dominant mutant", may require a much more complex approach. Such a method would require at least the neutralisation of the mutant allele (protein). If the expression of the normal allele would prove not to be enough, the expression of the extra copy of the wild-type gene might be necessary. For laminopathies of the muscular dystrophy type, a similar approach to that for the development of gene therapy treatment for DMD should be the obvious method of choice. Unfortunately no preferred strategy has been developed for DMD. Model studies on mdx mice either use virus vectors of at least the third generation or attempt to use *ex vivo* gene therapy using muscle stem cells or transdifferentiated HSC CD133+ cells. Obviously, the treatment of dominant negative-type laminopathies presents considerably more difficulties.

Despite the technical difficulties in the development of a selective, specific and efficient vector for gene therapy, the main difficulty seems to be in developing a strategy to neutralise the mutated protein without affecting the expression of the normal allele or exogenous construct. The most promising method (the least dangerous and relatively fast to develop) would be to design small peptides or other molecules specifically interacting with mutated lamin protein only and make them inert for interactions with other proteins. It is also possible to imagine the design of small peptides interacting with mutated proteins and compensating for mutation helping to assume proper folding and interactions with other proteins. Alternatively, it is possible to modify the substrate specificity of exogenous proteases for specific processing of progerin protein only, which would result in the removal of farnesylated C-terminus. Some prospects can also give the use of specifically designed rybozymes, highly likely introduced by lentivirus vectors in order to incorporate the expression cassette into genomic DNA, to correct point (or small deletion) dominant mutations using the splicing apparatus. The most elegant and theoretically possible use of homologous recombination vectors in order to permanently correct the genomic DNA of the lamin locus seems to be (at least from the point of view of our current knowledge) impractical due to the low efficiency of such procedures even in tissue-cultured HGPS cells [103].

Some promise for efficient gene therapy was given by the discovery that human haematopoietic stem cells (HSC) or mesenchymal stem cells (MSC) can differentiate into different cell types [136-138]. Among others, they can differentiate into myoblasts and subsequently into myotubes. HSC cells have been widely used for the treatment of a large variety of human disorders [139-142]. Stem cells can be delivered to damaged tissue for regeneration or must be stimulated to differentiate into muscle satellite cells or mature cells *in vitro* and then delivered to patients. Such therapy could be based on three mechanisms: differentiation of the administered cells into the desired tissue; release of factors capable of paracrine signaling from the administered cells; and fusion of the administered cells with the existing constituents. The major drawback of the

ex vivo stem cell therapy with adult muscle stem cells in such an approach is the low migration potential of muscle satellite cells. Hence, due to the higher migration potential of MSC, there is an option for genetic manipulation of MSCs and adapting genetically-modified MSCs for clinical use. The proposed gene therapy for muscular dystrophy-type laminopathy may also be based on the isolation of HSC cells from the patient, transformation with the wild allele of the lamin gene and co-transfection with the gene(s) of transcription factors responsible for differentiation into "primary" myoblasts (e.g. Shh, Pax7, Myo-D, Sca-1) [143-144]. Such cells would be infused or microinjected back into patients. Infused cells should differentiate into primary myoblasts and as such should be directed very efficiently to the muscle cells, where they can repopulate and fuse with existing myotubes or form new ones. The advantage of this method is its universality: exchanging the therapeutic cDNA sequence in the DNA vector will allow for gene therapy treatment for other muscle genetic diseases as well as some inherited genetic metabolic diseases, especially those where the therapeutic gene product should be released into the peripheral blood (e.g. lysosomal storage diseases, factor IX deficiency, HGF deficiency, erythropoietin deficiency). Recently, stimulation of endogenous repair by injected cells, which might cause the regeneration of stem cell niches, was proposed for mesenchymal stem cells in cardiac repair [139].

The other question is how to deliver therapeutic nucleic acids to stem cells *in vitro* or alternatively directly to selected cells in human patients. At present, pseudotyped lentiviral vectors (LV) are used for both gene therapy strategies. LVs offer the advantages of a large packaging capacity, specific cell-type targeting through pseudotyping, low immunogenicity, stable integration in non-dividing and dividing cells, and long-term transgene expression [145]. Nowadays, hematopoietic cell transplantation corrected *ex vivo* using a lentiviral vector is used in some disease clinical trials [146].

CONCLUSIONS

In this review, we attempted to summarise the current state of knowledge on the subject of laminopathies. We suggested some models for the mutation-driven molecular mechanisms of the development of the various phenotypes. We also discussed possible prospects for future treatment addressing different aspects of the diseases, and suggested a possible systemic approach to patient treatment using the patients' own cells for gene therapy treatment.

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REFERENCES

1. Worman, H.J., Fong, L.G., Muchir, A. and Young, S.G. Laminopathies and the long strange trip from basic cell biology to therapy. **J. Clin. Invest.** 119 (2009) 1825-1836.
2. Worman, H.J., Fong, L.G., Muchir, A. and Young, S.G. The nuclear envelope from basic biology to therapy. **Biochem. Soc. Trans.** 38 (2010) 253-256.
3. Foster, C.R., Przyborski, S.A., Wilson, R.G. and Hutchison, C.J. Lamins as cancer biomarkers. **Biochem. Soc. Trans.** 38 (2010) 297-300.
4. Hetzer, M.W. The Nuclear Envelope. **Cold Spring Harb. Perspect Biol.** (2010) cshperspect.a000539.
5. Rzepecki, R., Smithe, C. and Hutchison, C.J. The role of mitotic vesicles in the nuclear assembly. **Cell. Mol. Biol. Lett.** 7 (2002) 299.
6. Herrmann, H., Bar, H., Kreplak, L., Strelkov, S.V. and Aebi, U. Intermediate filaments: from cell architecture to nanomechanics. **Nat. Rev. Mol. Cell Biol.** 8 (2007) 562-573.
7. Dechat, T., Pfleghaar, K., Sengupta, K., Shimi, T., Shumaker, D.K., Solimando, L. and Goldman, R.D. Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. **Genes Dev.** 22 (2008) 832-853.
8. Liu, Q., Kim, D.I., Syme, J., LuValle, P., Burke, B. and Roux, K.J. Dynamics of lamin-A processing following precursor accumulation. **PLoS One** 5 (2010) e10874.
9. Prokocimer, M., Davidovich, M., Nissim-Rafinia, M., Wiesel-Motiuk, N., Bar, D., Barkan, R., Meshorer, E. and Gruenbaum, Y. Nuclear lamins: key regulators of nuclear structure and activities. **J. Cell. Mol. Med.** 13 (2009) 1059-1085.
10. Zaremba-Czogalla, M., Dubińska-Magiera, M. and Rzepecki, R. [Nowe funkcje lamin - starzy znajomi w nowym świetle]. **Postępy Biologii Komórki** 37 (2010) 1-18.
11. Bonne, G., Di Barletta, M.R., Varnous, S., Becane, H.M., Hammouda, H.E., Merlini, L., Muntoni, F., Greenberg, C.R., Gary, F., Urtizberea, J.A., Duboc, D., Fardeau, M., Toniolo, D. and Schwartz, K. Mutations in the gene encoding laminA/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. **Nature Genet.** 21 (1999) 285-288.
12. Di Barletta, M., Ricci, E., Galluzzi, G., Tonali, P., Mora, M., Morandi, L., Romorini, A., Voit, T., Orstavik, K.H. and Merlini, L. Different mutations in the *LMNA* gene cause autosomal dominant and autosomal recessive Emery-Dreifuss muscular dystrophy. **Am. J. Hum. Genet.** 66 (2000) 1407-1412.

13. Muchir, A., Bonne, G., van der Kooi, A.J., van Meegen, M., Baas, F., Bolhuis, P.A., de Visser, M. and Schwartz, K. Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). **Hum. Mol. Genet.** 9 (2000) 1453-1459.
14. Fatkin, D., MacRae, C., Sasaki, T., Wolff, M.R., Porcu, M., Frenneaux, M., Atherton, J., Vidaillet, H.J., Spudich, S., De Girolami, U., Seidman, J.G., Seidman, C., Muntoni, F., Muehle, G., Johson, W. and McDonough, B. Missense mutations in the rod domain in the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. **New Eng. J. Med.** 341 (1999) 1715-1724.
15. Bione, S., Maestrini, E., Rivella, S., Mancini, M., Regis, S., Romeo, G. and Toniolo, D. Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. **Nat. Genet.** 8 (1994) 323-327.
16. Renou, L., Stora, S., Yaou, R.B., Volk, M., Sinkovec, M., Demay, L., Richard, P., Peterlin, B. and Bonne, G. Heart-hand syndrome of Slovenian type: a new kind of laminopathy. **J. Med. Genet.** 45 (2008) 666-671.
17. Cao, H. and Hegele, R.A. Nuclear lamin A/C R482Q mutation in Canadian kindreds with Dunnigan-type familial partial lipodystrophy. **Hum. Mol. Genet.** 9 (2000) 109-112.
18. Drac, H., Madej-Pilarczyk, A., Gospodarczyk-Szot, K., Gaweł, M., Kwieciński, H. and Hausmanowa-Petrusewicz, I. Familial partial lipodystrophy associated with the heterozygous *LMNA* mutation 1445G>A (Arg482Gln) in a Polish family. **Neurol. Neurochir. Pol.** 44 (2010) 291-296.
19. Hegele, R.A., Cao, H., Liu, D.M., Costain, G.A., Charlton-Menys, V., Rodger, N.W. and Durrington, P.N. Sequencing of the reannotated *LMNB*2 gene reveals novel mutations in patients with acquired partial lipodystrophy. **Am. J. Hum. Genet.** 79 (2006) 383-389.
20. Young, J., Morbois-Trabut, L., Couzinet, B., Lascols, O., Dion, E., Bereziat, V., Feve, B., Richard, I., Capeau, J., Chanson, P. and Vigouroux, C. Type A insulin resistance syndrome revealing a novel lamin A mutation. **Diabetes** 54 (2005) 1873-1878.
21. De Sandre-Giovannoli, A., Bernard, R., Cau, P., Navarro, C., Amiel, J., Boccaccio, I., Lyonnet, S., Stewart, C.L., Munnich, A., Le Merrer, M. and Levy, N. Lamin a truncation in Hutchinson-Gilford progeria. **Science** 300 (2003) 2055.
22. Chen, L., Lee, L., Kudlow, B., Dos Santos, H., Sletvold, O., Shafeghati, Y., Botha, E., Garg, A., Hanson, N., Martin, G.M., Mian, I.S., Kennedy, B.K. and Oshima, J. *LMNA* mutations in atypical Werner's syndrome. **Lancet** 362 (2003) 440-445.
23. Navarro, C.L., Cadinanos, J., De Sandre-Giovannoli, A., Bernard, R., Courrier, S., Boccaccio, I., Boyer, A., Kleijer, W.J., Wagner, A., Giuliano, F., Beemer, F.A., Freije, J.M., Cau, P., Hennekam, R.C., Lopez-Otin, C., Badens, C. and Levy, N. Loss of ZMPSTE24 (FACE-1) causes autosomal

recessive restrictive dermopathy and accumulation of Lamin A precursors. **Hum. Mol. Genet.** 14 (2005) 1503-1513.
24. Agarwal, A.K., Fryns, J.P., Auchus, R.J. and Garg, A. Zinc metalloproteinase, ZMPSTE24, is mutated in mandibuloacral dysplasia. **Hum. Mol .Genet.** 12 (2003) 1995-2001.
25. Novelli, G., Muchir, A., Sangiuolo, F., Helbling-Leclerc, A., D'Apice, M.R., Massart, C., Capon, F., Sbraccia, P., Federici, M., Lauro, R., Tudisco, C., Pallotta, R., Scarano, G., Dallapiccola, B., Merlini, L. and Bonne, G. Mandibuloacral dysplasia is caused by a mutation in *LMNA*-encoding lamin A/C. **Am. J. Hum. Genet.** 71 (2002) 426-431.
26. Hoffmann, K., Dreger, C.K., Olins, A.L., Olins, D.E., Shultz, L.D., Lucke, B., Karl, H., Kaps, R., Muller, D., Vaya, A., Aznar, J., Ware, R.E., Cruz, N.S., Lindner, T.H., Herrmann, H., Reis, A. and Sperling, K. Mutations in the gene encoding the lamin B receptor produce an altered nuclear morphology in granulocytes (Pelger-Huet anomaly). **Nat. Genet.** 31 (2002) 410-414.
27. Garg, A., Subramanyam, L., Agarwal, A.K., Simha, V., Levine, B., D'Apice, M.R., Novelli, G. and Crow, Y. Atypical progeroid syndrome due to heterozygous missense *LMNA* mutations. **J. Clin. Endocrinol. Metab.** 94 (2009) 4971-4983.
28. De Sandre-Giovannoli, A., Chaouch, M., Kozlov, S., Vallat, J.M., Tazir, M., Kassouri, N., Szepetowski, P., Hammadouche, T., Vandenberghe, A., Stewart, C.L., Grid, D. and 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. **Am. J. Hum. Genet.** 70 (2002) 726-736.
29. Padiath, Q.S., Saigoh, K., Schiffmann, R., Asahara, H., Yamada, T., Koeppen, A., Hogan, K., Ptacek, L.J. and Fu, Y.H. Lamin B1 duplications cause autosomal dominant leukodystrophy. **Nat. Genet**. 38 (2006) 1114-1123.
30. Vantyghem, M.C., Pigny, P., Maurage, C.A., Rouaix-Emery, N., Stojkovic, T., Cuisset, J.M., Millaire, A., Lascols, O., Vermersch, P., Wemeau, J.L., Capeau, J. and Vigouroux, C. Patients with familial partial lipodystrophy of the Dunnigan type due to a *LMNA* R482W mutation show muscular and cardiac abnormalities. **J. Clin. Endocrinol. Metab.** (2004) 5337-5346.
31. Vantyghem, M.C., Faivre-Defrance, F., Marcelli-Tourvieille, S., Fermon, C., Evrard, A., Bourdelle-Hego, M.F., Vigouroux, C., Defebvre, L., Delemer, B. and Wemeau, J.L. Familial partial lipodystrophy due to the *LMNA* R482W mutation with multinodular goitre, extrapyramidal syndrome and primary hyperaldosteronism. **Clin. Endocrinol (Oxf).** 67 (2007) 247-249.
32. Benedetti, S., Bertini, E., Iannaccone, S., Angelini, C., Trisciani, M., Toniolo, D., Sferrazza, B., Carrera, P., Comi, G., Ferrari, M., Quattrini, A. and Previtali, S.C. Dominant *LMNA* mutations can cause combined muscular dystrophy and peripheral neuropathy. **J. Neurol. Neurosurg. Psychiatry** 76 (2005) 1019-1021.

33. Goizet, C., Yaou, R., Demay, L., Richard, P., Bouillot, S., Rouanet, M., Hermosilla, E., Le Masson, G., Lagueny, A., Bonne, G. and Ferrer, X. A new mutation of the lamin A/C gene leading to autosomal dominant axonal neuropathy, muscular dystrophy, cardiac disease, and leuconychia. **J. Med. Genet.** 41 (2004) e29.
34. Hegele, R.A. and Oshima, J. Phenomics and lamins: from disease to therapy. **Exp. Cell Res.** 313 (2007) 2134-2143.
35. Harborth, J., Elbashir, S.M., Bechert, K., Tuschl, T. and Weber, K. Identification of essential genes in cultured mammalian cells using small interfering RNAs. **J. Cell Sci.** 114 (2001) 4557-4565.
36. Vergnes, L., Peterfy, M., Bergo, M.O., Young, S.G. and Reue, K. Lamin B1 is required for mouse development and nuclear integrity. **Proc. Natl. Acad. Sci. USA** 101 (2004) 10428-10433.
37. Coffinier, C., Chang, S.Y., Nobumori, C., Tu, Y., Farber, E.A., Toth, J.I., Fong, L.G. and Young, S.G. Abnormal development of the cerebral cortex and cerebellum in the setting of lamin B2 deficiency. **Proc. Natl. Acad. Sci. USA** 107 (2010) 5076-5081.
38. Waterham, H.R., Koster, J., Mooyer, P., van Noort, G., Kelley, R.I., Wilcox, W.R., Wanders, R.J.A., Hennekam, R.C.M. and Oosterwijk, J.C. Autosomal recessive HEM/Greenberg skeletal dysplasia is caused by 3beta-hydroxysterol delta14-reductase deficiency due to mutations in the lamin B receptor gene. **Am. J. Hum. Genet.** 72 (2003) 1013-1017.
39. Yuste-Chaves, M., Cañueto, J., Santos-Briz, A., Ciria, S., González-Sarmiento, R. and Unamuno, P. Buschke-Ollendorff Syndrome with Striking Phenotypic Variation Resulting from a Novel c.2203C>T Nonsense Mutation in LEMD3. **Pediatr. Dermatol.** (2010) DOI: 10.1111/j.1525-1470.2010
40. Taylor, M.R., Slavov, D., Gajewski, A., Vlcek, S., Ku, L., Fain, P.R., Carniel, E., Di Lenarda, A., Sinagra, G., Boucek, M.M., Cavanaugh, J., Graw, S.L., Ruegg, P., Feiger, J., Zhu, X., Ferguson, D.A., Bristow, M.R., Gotzmann, J., Foisner, R. and Mestroni, L. Thymopoietin (lamina-associated polypeptide 2) gene mutation associated with dilated cardiomyopathy. **Hum. Mutat.** 26 (2005) 566-574.
41. Landires, I., Pascale, J.M. and Motta, J. The position of the mutation within the *LMNA* gene determines the type and extent of tissue involvement in laminopathies. **Clin. Genet.** 71 (2007) 592-593.
42. Scharner, J., Gnocchi, V.F., Ellis, J.A. and Zammit, P.S. Genotype-phenotype correlations in laminopathies: how does fate translate? **BMC Dev. Biol.** 38 (2010) 257-262.
43. Simon, D.N., Zastrow, M.S. and Wilson, K.L. Direct actin binding to A- and B-type lamin tails and actin filament bundling by the lamin A tail. **Nucleus** 1 (2010) 264-272.
44. Magracheva, E., Kozlov, S., Stewart, C.L., Wlodawer, A. and Zdanov, A. Structure of the lamin A/C R482W mutant responsible for dominant familial

partial lipodystrophy (FPLD). **Acta Crystallogr. Sect. F: Struct. Biol. Cryst. Commun.** 65 (2009) 665-670.
45. Sewry, C. Muscular dystrophies: an update on pathology and diagnosis. **Acta Neuropathol.** 120 (2010) 343-358.
46. Rzepecki, R. The nuclear lamins and nuclear envelope. **Cel. Mol. Biol. Lett.** 7 (2002) 1019-1035.
47. Chmielewska, M., Dubińska-Magiera, M., Sopel, M., Rzepecka, D., Hutchison, C.J., Goldberg, M.W. and Rzepecki, R. Embryonic and adult isoforms of XLAP2 form microdomains associated with chromatin and the nuclear envelope. **Cell Tissue Res.** (2011), in press.
48. Margalit, A., Brachner, A., Gotzmann, J., Foisner, R. and Gruenbaum, Y. Barrier-to-autointegration factor--a BAFfling little protein. **Trends Cell Biol.** 17 (2007) 202-208.
49. Emery, A.E. and Dreifuss, F.E. Unusual type of benign X-linked muscular dystrophy. **J. Neurol. Neurosurg. Psychiatry** 29 (1966) 338-342.
50. Vaughan, O.A., Alvarez-Reyes, M., Bridger, J.M., Broers, J.L.V., Ramaekers, F.C.S., Wehnert, M., Morris, G.E., Whitfield, W.G.F. and Hutchison, C.J. Both emerin and lamin C depend on lamin A for localization at the nuclear envelope. **J. Cell Sci.** 114 (2001) 2577-2590.
51. Wheeler, M.A. and Ellis, J.A. Molecular signatures of Emery-Dreifuss muscular dystrophy. **Biochem. Soc. Trans.** 36 (2008) 1354-1358.
52. Gueneau, L., Bertrand, A.T., Jais, J.P., Salih, M.A., Stojkovic, T., Wehnert, M., Hoeltzenbein, M., Spuler, S., Saitoh, S., Verschueren, A., Tranchant, C., Beuvin, M., Lacene, E., Romero, N.B., Heath, S., Zelenika, D., Voit, T., Eymard, B., Ben Yaou, R. and Bonne, G. Mutations of the FHL1 gene cause Emery-Dreifuss muscular dystrophy. **Am. J. Hum. Genet.** 85 (2009) 338-353.
53. Zhang, Q., Bethmann, C., Worth, N.F., Davies, J.D., Wasner, C., Feuer, A., Ragnauth, C.D., Yi, Q., Mellad, J.A., Warren, D.T., Wheeler, M.A., Ellis, J.A., Skepper, J.N., Vorgerd, M., Schlotter-Weigel, B., Weissberg, P.L., Roberts, R.G., Wehnert, M. and Shanahan, C.M. Nesprin-1 and -2 are involved in the pathogenesis of Emery Dreifuss muscular dystrophy and are critical for nuclear envelope integrity. **Hum. Mol. Genet.** 16 (2007) 2816-2833.
54. Taylor, M.R.G., Fain, P.R., Sinagra, G., Robinson, M.L., Robertson, A.D., Carniel, E., Di Lenarda, A., Bohlmeyer, T.J., Ferguson, D.A., Brodsky, G.L., Boucek, M.M., Lascor, J., Moss, A.C., Li, W.-L.P., Stetler, G.L., Muntoni, F., Bristow, M.R., Mestroni, L. and Group, F.D.C.R.R. Natural history of dilated cardiomyopathy due to lamin A/C gene mutations. **J. Am. Coll. Cardiol.** 41 (2003) 771-780.
55. Arbustini, E., Pilotto, A., Repetto, A., Grasso, M., Negri, A., Diegoli, M., Campana, C., Scelsi, L., Baldini, E., Gavazzi, A. and Tavazzi, L. Autosomal dominant dilated cardiomyopathy with atrioventricular block: a lamin A/C defect-related disease. **J. Am. Coll. Cardiol.** 39 (2002) 981-990.
56. Muchir, A. and Worman, H.J. Emery-Dreifuss muscular dystrophy. **Curr. Neurol. Neurosci. Rep.** 7 (2007) 78-83.

57. Quijano-Roy, S., Mbieleu, B., Bönnemann, C.G., Jeannet, P.Y., Colomer, J., Clarke, N.F., Cuisset, J.M., Roper, H., De Meirleir, L., D'Amico, A., Ben Yaou, R., Nascimento, A., Barois, A., Demay, L., Bertini, E., Ferreiro, A., Sewry, C.A., Romero, N.B., Ryan, M., Muntoni, F., Guicheney, P., Richard, P., Bonne, G. and Estournet, B. De novo *LMNA* mutations cause a new form of congenital muscular dystrophy. **Ann. Neurol.** 64 (2008) 177-186.
58. Dahl, K.N., Booth-Gauthier, E.A. and Ladoux, B. In the middle of it all: mutual mechanical regulation between the nucleus and the cytoskeleton. **J. Biomech.** 43 (2010) 2-8.
59. Attali, R., Warwar, N., Israel, A., Gurt, I., McNally, E., Puckelwartz, M., Glick, B., Nevo, Y., Ben-Neriah, Z. and Melki, J. Mutation of SYNE-1, encoding an essential component of the nuclear lamina, is responsible for autosomal recessive arthrogryposis. **Hum. Mol. Genet.** 18 (2009) 3462-3469.
60. Gros-Louis, F., Dupré, N., Dion, P., Fox, M.A., Laurent, S., Verreault, S., Sanes, J.R., Bouchard, J.P. and Rouleau, G.A. Mutations in SYNE1 lead to a newly discovered form of autosomal recessive cerebellar ataxia. **Nat. Genet.** 39 (2007) 80-85.
61. Holaska, J.M. and Wilson, K.L. An emerin "proteome": purification of distinct emerincontaining complexes from HeLa cells suggests molecular basis for diverse rolesincluding gene regulation, mRNA splicing, signaling, mechanosensing, and nu clear architecture. **Biochemistry** 46 (2007) 8897-8908.
62. Andrés, V. and González, J.M. Role of A-type lamins in signaling, transcription, and chromatin organization. **J. Cell Biol.** 187 (2009) 945-957.
63. Marmiroli, S., Bertacchini, J., Beretti, F., Cenni, V., Guida, M., De Pol, A., Maraldi, N.M. and Lattanzi, G. A-type lamins and signaling: the PI 3-kinase/Akt pathway moves forward. **J. Cell. Physiol.** 220 (2009) 553-561.
64. Kim, E.K. and Choi, E.J. Pathological roles of MAPK signaling pathways in human diseases. **Biochim. Biophys. Acta** 1802 (2010) 396-405.
65. González, J.M., Navarro-Puche, A., Casar, B., Crespo, P. and Andrés, V. Fast regulation of AP-1 activity through interaction of lamin A/C, ERK1/2, and c-Fos at the nuclear envelope. **J. Cell Biol.** 183 (2008) 653-666.
66. Muchir, A., Pavlidis, P., Bonne, G., Hayashi, Y.K. and Worman, H.J. Activation of MAPK in hearts of EMD null mice: similarities between mouse models of X-linked and autosomal dominant Emery Dreifuss muscular dystrophy. **Hum. Mol. Genet.** 16 (2007) 1884-1895.
67. Muchir, A., Pavlidis, P., Decostre, V., Herron, A.J., Arimura, T., Bonne, G. and Worman, H.J. Activation of MAPK pathways links *LMNA* mutations to cardiomyopathy in Emery-Dreifuss muscular dystrophy. **J. Clin. Invest.** 117 (2007) 1282-1293.
68. Muchir, A., Wu, W. and Worman, H.J. Reduced expression of A-type lamins and emerin activates extracellular signal-regulated kinase in cultured cells. **Biochim. Biophys. Acta** 1792 (2009) 75-81.

69. Emerson, L.J., Holt, M.R., Wheeler, M.A., Wehnert, M., Parsons, M. and Ellis, J.A. Defects in cell spreading and ERK1/2 activation in fibroblasts with lamin A/C mutations. **Biochim. Biophys. Acta** 1792 (2009) 810-821.
70. Muchir, A., Shan, J., Bonne, G., Lehnart, S.E. and Worman, H.J. Inhibition of extracellular signal-regulated kinase signaling to prevent cardiomyopathy caused by mutation in the gene encoding A-type lamins. **Hum. Mol. Genet.** 18 (2009) 241-247.
71. Wu, W., Shan, J., Bonne, G., Worman, H.J. and Muchir, A. Pharmacological inhibition of c-Jun N-terminal kinase signaling prevents cardiomyopathy caused by mutation in *LMNA* gene. **Biochim. Biophys. Acta** 1802 (2010) 632-638.
72. MacDonald, B.T., Tamai, K. and He, X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. **Dev. Cell** 17 (2009) 9-26.
73. Markiewicz, E., Tilgner, k., Barker, N., van de Wetering, M., Clevers, H., Dorobek, M., Hausmanowa-Petrusewicz, I., Ramaekers, F.C., Broers, J.L., Blankesteijn, W.M., Salpingidou, G., Wilson, R.G., Ellis, J.A. and Hutchison, C.J. The inner nuclear membrane protein emerin regulates beta-catenin activity by restricting its accumulation in the nucleus. **EMBO J.** 25 (2006) 3275-3285.
74. Hoppler, S. and Kavanagh, C.L. Wnt signalling: variety at the core. **J. Cell Sci.** 120 (2007) 385-393.
75. Goichberg, P., Shtutman, M., Ben-Zeev, A. and Geiger, B. Recruitment of β-catenin to cadherin-mediated intercellular adhesions is involved in myogenic induction. **J. Cell Sci.** 114 (2001) 1309-1319.
76. Perez-Ruiz, A., Ono, Y., Gnocchi, V.F. and Zammit, P.S. β-catenin promotes self-renewal of skeletal-muscle satellite cells **J. Cell Sci**. 121 (2008) 1373-1382.
77. Hutchison, C.J. and Worman, H.J. A-type lamins: Guardians of the soma? **Nat. Cell Biol.** 6 (2004) 1062-1067.
78. Johnson, B.R. A-type lamins regulate retinoblastoma protein function by promoting subnuclear localization and preventing proteasomal degradation. **Proc. Natl. Acad. Sci. USA** 101 (2004) 9677-9682.
79. Markiewicz, E., Dechat, T., Foisner, R., Quinlan, R.A. and Hutchison, C.J. Lamin A/C binding protein LAP2 alpha is required for nuclear anchorage of retinoblastoma protein. **Mol. Biol. Cell.** 13 (2002) 4401-4413.
80. Frock, R.L., Kudlow, B.A., Evans, A.M., Jameson, S.A., Hauschka, S.D. and Kennedy, B.K. Lamin A/C and emerin are critical for skeletal muscle satellite cell differentiation. **Genes Dev.** 20 (2006) 486-500.
81. Naetar, N. and Foisner, R. Lamin complexes in the nuclear interior control progenitor cell proliferation and tissue homeostasis. **Cell Cycle** 8 (2009) 1488-1493.
82. Massagué, J. How cells read TGF-beta signals. **Nat. Rev. Mol. Cell Biol.** 1 (2000) 169-178.

83. Van Berlo, J.H., Voncken, J.W., Kubben, N., Broers, J.L., Duisters, R., van Leeuwen, R.E., Crijns, H.J., Ramaekers, F.C., Hutchison, C.J. and Pinto, Y.M. A-type lamins are essential for TGF-{beta}1 induced PP2A to dephosphorylate transcription factors. **Hum. Mol. Genet**. 14 (2005) 2839-2849.
84. Puri, P.L., Iezzi, S., Stiegler, P., Chen, T.T., Schiltz, R.L., Muscat, G.E., Giordano, A., Kedes, L., Wang, J.Y. and Sartorelli, V. Class I histone deacetylases sequentially interact with MyoD and pRb during skeletal myogenesis. **Mol. Cell.** 8 (2001) 885-897.
85. Park, Y.E., Hayashi, Y.K., Goto, K., Komaki, H., Hayashi, Y., Inuzuka, T., Noguchi, S., Nonaka, I. and Nishino, I. Nuclear changes in skeletal muscle extend to satellite cells in autosomal dominant Emery-Dreifuss muscular dystrophy/limb-girdle muscular dystrophy 1B. **Neuromuscul. Disord.** 19 (2008) 29-36.
86. Meshorer, E. and Gruenbaum, Y. Gone with the Wnt/Notch: stem cells in laminopathies, progeria, and aging. **J. Cell Biol.** 181 (2008) 9-13.
87. Jones, D.L. and Wagers, A.J. No place like home: anatomy and function of the stem cell niche. **Nat. Rev. Mol. Cell. Biol.** 9 (2008) 11-21.
88. Favreau, C., Higuet, D., Courvalin, J.C. and Buendia, B. Expression of a mutant lamin A that causes Emery-Dreifuss muscular dystrophy inhibits in vitro differentiation of C2C12 myoblasts. **Mol. Cell. Biol.** 24 (2004) 1481-1492.
89. Takai, H., Yamada, T., Tada, S. and Matsumaru, I. Successful surgical repair for Emery-Dreifuss muscular dystrophy valvular disease with long-term follow-up. **Interac. Cardiovasc. Thorac. Surg.** 10 (2010) 811-812.
90. Dechat, T., Shimi, T., Adam, S.A., Rusinol, A.E., Andres, D.A., Spielmann, H.P., Sinensky, M.S. and Goldman, R.D. Alterations in mitosis and cell cycle progression caused by a mutant lamin A known to accelerate human aging. **Proc. Natl. Acad. Sci. USA** 104 (2007) 4955-4960.
91. Rodriguez, S., Coppedè, F., Sagelius, H. and Eriksson, M. Increased expression of the Hutchinson-Gilford progeria syndrome truncated lamin A transcript during cell aging. **Eur. J. Hum. Genet.** 17 (2009) 928-937.
92. Goldman, R.D., Shumaker, D.K., Erdos, M.R., Eriksson, M., Goldman, A.E., Gordon, L.B., Gruenbaum, Y., Khuon, S., Mendez, M., Varga, R. and Collins, F.S. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. **Proc. Natl. Acad. Sci. USA**. 101 (2004) 8963-8968.
93. Mallampalli, M.P., Huyer, G., Bendale, P., Gelb, M.H. and Michaelis, S. Inhibiting farnesylation reverses the nuclear morphology defect in a HeLa cell model for Hutchinson-Gilford progeria syndrome. **Proc. Natl. Acad. Sci. USA** 102 (2005) 14416-14421.
94. Toth, J.I., Yang, S.H., Qiao, X., Beigneux, A.P., Gelb, M.H., Moulson, C.L., Miner, J.H., Young, S.G. and Fong, L.G. Blocking protein farnesyltransferase improves nuclear shape in fibroblasts from humans with progeroid syndromes. **Proc. Natl. Acad. Sci. USA** 102 (2005) 12873-12878.

95. Capell, B.C., Olive, M., Erdos, M.R., Cao, K., Faddah, D.A., Tavarez, U.L., Conneely, K.N., Qu, X., San, H., Ganesh, S.K., Chen, X., Avallone, H., Kolodgie, F.D., Virmani, R., Nabel, E.G. and Collins, F.S. A farnesyltransferase inhibitor prevents both the onset and late progression of cardiovascular disease in a progeria mouse model. **Proc. Natl. Acad. Sci. USA** 105 (2008) 15902-15907.
96. Yang, S.H., Qiao, X., Fong, L.G. and Youn, S.G. Treatment with a farnesyltransferase inhibitor improves survival in mice with a Hutchinson-Gilford progeria syndrome mutation. **Biochim. Biophys. Acta** 1781 (2008) 36-39.
97. Yang, S.H., Andres, D.A., Spielmann, H.P., Young, S.G. and Fong, L.G. Progerin elicits disease phenotypes of progeria in mice whether or not it is farnesylated. **J. Clin. Invest.** 118 (2008) 3291-3300.
98. Varela, I., Pereira, S., Ugalde, A.P., Navarro, C.L., Suárez, M.F., Cau, P., Cadiñanos, J., Osorio, F.G., Cobo, J., de Carlos, F., Lèvy, N., Freije, J.M.P. and López-Otín, C. Combined treatment with statins and aminobisphosphonates extends longevity in a mouse model of human premature aging. **Nat. Med.** 14 (2008) 767-772.
99. Kieran, M.W., Gordon, L. and Kleinman, M. New approaches to progeria. **Pediatrics** 120 (2007) 834-841.
100. Davies, B.S., Barnes, R.H., Tu, Y., Ren, S., Andres, D.A., Spielmann, H.P., Lammerding, J., Wang, Y., Young, S.G. and Fong, L.G. An accumulation of non-farnesylated pre-lamin A causes cardiomyopathy but not progeria. **Hum. Mol. Genet.** 19 (2010) 2682-2694.
101. Caron, M., Auclair, M., Donadille, B., Béréziat, V., Guerci, B., Laville, M., Narbonne, H., Bodemer, C., Lascols, O., Capeau, J. and Vigouroux, C. Human lipodystrophies linked to mutations in A-type lamins and to HIV protease inhibitor therapy are both associated with pre-lamin A accumulation, oxidative stress and premature cellular senescence. **Cell Death Differ.** 14 (2007) 1759-1767.
102. Coffinier, C., Hudon, S.E., Farber, E.A., Chang, S.Y., Hrycyna, C.A., Young, S.G. and Fong, L.G. HIV protease inhibitors block the zinc metalloproteinase ZMPSTE24 and lead to an accumulation of pre-lamin A in cells. **Proc. Natl. Acad. Sci .USA** 104 (2007) 13432-13437.
103. Huang, S., Chen, L., Libina, N., Janes, J., Martin, G.M., Campisi, J. and Oshima, J. Correction of cellular phenotypes of Hutchinson-Gilford Progeria cells by RNA interference. **Hum. Genet.** (2005) 1-7.
104. Fong, L.G., Ng, J.K., Lammerding, J., Vickers, T.A., Meta, M., Cote, N., Gavino, B., Qiao, X., Chang, S.Y., Young, S.R., Yang, S.H., Stewart, C.L., Lee, R.T., Bennett, C.F., Bergo, M.O. and Young, S.G. Pre-lamin A and lamin A appear to be dispensable in the nuclear lamina. **J. Clin. Invest.** 116 (2006) 743-752.
105. Fong, L.G., Vickers, T.A., Farber, E.A., Choi, C., Yun, U.J., Hu, Y., Yang, S.H., Coffinier, C., Lee, R., Yin, L., Davies, B.S., Andres, D.A., Spielmann,

H.P., Bennett, C.F. and Young, S. Activating the synthesis of progerin, the mutant pre-lamin A in Hutchinson–Gilford progeria syndrome, with antisense oligonucleotides. **Hum. Mol. Genet.** 18 (2009) 2462-2471.
106. Marji, J., O'Donoghue, S.I., McClintock, D., Satagopam, V.P., Schneider, R., Ratner, D., Worman, H., Gordon, L.B. and Djabali, K. Defective lamin A-Rb signaling in Hutchinson-Gilford Progeria Syndrome and reversal by farnesyltransferase inhibition. **PLoS One** 5 (2010) e11132.
107. Scaffidi, P. and Misteli, T. Lamin A-dependent misregulation of adult stem cells associated with accelerated ageing. **Nat. Cell Biol.** 10 (2008) 452-459.
108. Scaffidi, P. and Misteli, T. Lamin A-dependent nuclear defects in human aging. **Science** 312 (2006) 1059-1063.
109. Espada, J., Varela, I., Flores, I., Ugalde, A.P., Cadinanos, J., Pendas, A.M., Stewar, C.L., Tryggvason, K., Blasco, M.A., Freije, J.M. and Lopez-Otin, C. Nuclear envelope defects cause stem cell dysfunction in premature-aging mice. **J. Cell Biol.** 181 (2008) 27-35.
110. Hegele, R.A., Joy, T.R., Al-Attar, S.A. and Rutt, B.K. Thematic review series: Adipocyte Biology. Lipodystrophies: windows on adipose biology and metabolism. **J. Lipid Res.** 48 (2007) 1433-1444.
111. Tilgner, K., Wojciechowicz, K., Jahoda, C., Hutchison, C. and Markiewicz, E. Dynamic complexes of A-type lamins and emerin influence adipogenic capacity of the cell via nucleocytoplasmic distribution of {beta}-catenin. **J. Cell Sci.** 122 (2009) 401-413.
112. Christodoulides, C., Lagathu, C., Sethi, J.K. and Vidal-Puig, A. Adipogenesis and WNT signalling. **Trends Endocrinol. Metab.** 20 (2009) 16-24.
113. Guillet-Deniau, I., Pichard, A.-L., Kone, A., Esnous, C., Nieruchalski, M., Girard, J. and Prip-Buus, C. Glucose induces de novo lipogenesis in rat muscle satellite cells through a sterol-regulatory-element-binding-protein-1c-dependent pathway. **J. Cell Sci.** 117 (2004) 1937-1944.
114. Lloyd, D.J., Trembath, R.C. and Shackleton, S. A novel interaction between lamin A and SREBP1: implications for partial lipodystrophy and other laminopathies. **Hum. Mol. Genet.** 11 (2002) 769-777.
115. Javor, E.D., Cochran, E.K., Musso, C., Young, J.R., Depaoli, A.M. and Gorden, P. Long-term efficacy of leptin replacement in patients with generalized lipodystrophy. **Diabetes** 54 (2005) 1994-2002.
116. Park, J.Y., Javor, E.D., Cochran, E.K., DePaoli, A.M. and Gorden, P. Long-term efficacy of leptin replacement in patients with Dunnigan-type familial partial lipodystrophy. **Metabolism** 56 (2007) 508-516.
117. Maraldi, N.M., Capanni, C., Lattanzi, G., Camozzi, D., Facchini, A. and Manzoli, F.A. SREBP1 interaction with pre-lamin A forms: a pathogenic mechanism for lipodystrophic laminopathies. **Adv. Enzyme Regul.** 48 (2008) 209-223.

118. Broers, J.L., Ramaekers, F.C., Bonne, G., Yaou, R.B. and Hutchison, C.J. Nuclear lamins: laminopathies and their role in premature ageing. **Physiol. Rev.** 86 (2006) 967-1008.
119. Méjat, A.V., Decostre, J., Li, L., Renou, A., Kesari, D., Hantaï, C.L., Stewart, X., Xiao, E., Hoffman, G., Bonne, G. and Misteli, T. Lamin A/C – mediated neuromuscular junction defects in Emery-Dreifuss muscular dystrophy. **J. Cell Biol.** 184 (2009) 31-44.
120. Bridger, J.M., Foeger, N., Kill, I.R. and Herrmann, H. The nuclear lamina. Both a structural framework and a platform for genome organization. **FEBS J.** 274 (2007) 1354-1361.
121. Wilson, K.L. and Foisner, R. Lamin-binding Proteins. **Cold Spring Harb Perspect. Biol.** 2 (2010) a000554.
122. Rzepecki, R. and Fisher, P.A. In vivo phosphorylation of Drosophila melanogaster nuclear lamins during both interphase and mitosis. **Cell. Mol. Biol. Lett.** 7 (2002) 859-876.
123. Zhang, Y.Q. and Sarge, K.D. Sumoylation regulates lamin A function and is lost in lamin A mutants associated with familial cardiomyopathies. **J. Cell Biol.** 182 (2008) 35-39.
124. Cenni, V., Bertacchini, J., Beretti, F., Lattanzi, G., Bavelloni, A., Riccio, M., Ruzzene, M., Marin, O., Arrigoni, G., Parnaik, V., Wehnert, M., Maraldi, N.M., de Pol, A., Cocco, L. and Marmiroli, S. Lamin A Ser404 is a nuclear target of Akt phosphorylation in C2C12 cells. **J. Proteome Res.** 7 (2008) 4727-4735.
125. Tifft, K.E., Bradbury, K.A. and Wilson, K.L. Tyrosine phosphorylation of nuclear-membrane protein emerin by Src, Abl and other kinases. **J. Cell Sci.** 122 (2009) 3780-3790.
126. Sullivan, T., Escalante-Alcalde, D., Bhatt, H., Anver, M., Bhat, N., Nagashima, K., Stewart, C.L. and Burke, B. Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. **J. Cell Biol.** 147 (1999) 913-920.
127. Leung, G.K., Schmidt, W.K., Bergo, M.O., Gavino, B., Wong, D.H., Tam, A., Ashby, M.N., Michaelis, S. and Young, S.G. Biochemical studies of Zmpste24-deficient mice. **J. Biol. Chem.** 276 (2001) 29051-29058.
128. Ozawa, R., Hayashi, Y.K., Ogawa, M., Kurokawa, R., Matsumoto, H., Noguchi, S., Nonaka, I. and Nishino, I. Emerin-lacking mice show minimal motor and cardiac dysfunctions with nuclear-associated vacuoles. **Am. J. Pathol.** 186 (2006) 907-917.
129. Arimura, T., Helbling-Leclerc, A., Massart, C., Varnous, S., Niel, F., Lacene, E., Fromes, Y., Toussaint, M., Mura, A.M., Keller, D.I., Amthor, H., Isnard, R., Malissen, M., Schwartz, K. and Bonne, G. Mouse model carrying H222P-*LMNA* mutation develops muscular dystrophy and dilated cardiomyopathy similar to human striated muscle laminopathies. **Hum. Mol. Genet.** 14 (2005) 155-169.

130. Mounkes, L.C., Kozlov, S.V., Rottman, J.N. and Stewart, C.L. Expression of an *LMNA*-N195K variant of A-type lamins results in cardiac conduction defects and death in mice. **Hum. Mol. Genet.** 14 (2006) 2167-2180.
131. Wang, Y., Herron, A.J. and Worman, H.J. Pathology and nuclear abnormalities in hearts of transgenic mice expressing M371K lamin A encoded by an *LMNA* mutation causing Emery-Dreifuss muscular dystrophy. **Hum. Mol. Genet.** 15 (2006) 2479-2489.
132. Nikolova, V., Leimena, C., McMahon, A.C., Tan, J.C., Chandar, S., Jogia, D., Kesteven, S.H., Michalicek, J., Otway, R., Verheyen, F., Rainer, S., Stewart, C.L., Martin, D., Feneley, M.P. and Fatkin, D. Defects in nuclear structure and function promote dilated cardiomyopathy in lamin A/C-deficient mice. **J. Clin. Invest.** 113 (2004) 357-369.
133. Alsheimer, M., Liebe, B., Sewell, L., Stewart, C.L., Scherthan, H. and Benavente, R. Disruption of spermatogenesis in mice lacking A-type lamins. **J. Cell Sci.** 117 (2004) 1173-1178.
134. Hale, J.S., Frock, R.L., Mamman, S.A., Fink, P.J. and Kennedy, B.K. Cell-extrinsic defective lymphocyte development in *Lmna*$^{-/-}$ mice. **PLoS One** 5 (2010) e10127.
135. Bergo, M.O. Zmpste24 deficiency in mice causes spontaneous bone fractures, muscle weakness, and a pre-lamin A processing defect. **Proc. Natl. Acad. Sci. USA** 99 (2002) 13049-13054.
136. Anjos-Afonso, F., Siapati, E.K. and Bonnet, D. In vivo contribution of murine mesenchymal stem cells into multiple cell-types under minimal damage conditions. **J. Cell Sci.** 117 (2004) 5655-5664.
137. Cousins, J.C., Woodward, K.J., Gross, J.G., Partridge, T.A. and Morgan, J.E. Regeneration of skeletal muscle from transplanted immortalised myoblasts is oligoclonal. **J. Cell Sci.** 117 (2004) 3259-3269.
138. D`Ippolito, G., Diabira, S., Howard, G.A., Menei, P., Roos, B.A. and Schiller, P.C. Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential. **J. Cell Sci.** 117 (2004) 2971-2981.
139. Mazhari, R. and Hare, J.M. Mechanisms of action of mesenchymal stem cells in cardiac repair: potential influences on the cardiac stem cell niche. **Nat. Clin. Pract. Cardiovasc. Med.** 4 (2007) S21-26.
140. Dwyer, R.M., Khan, S., Barry, F.P., O'Brien, T. and Kerin, M.J. Advances in mesenchymal stem cell-mediated gene therapy for cancer. **Stem Cell Res. Ther.** 1 (2010) 25.
141. Pistoia, V. and Raffaghello, L. Potential of mesenchymal stem cells for the therapy of autoimmune diseases. **Expert Rev. Clin. Immunol.** 6 (2010) 211-218.
142. Griffin, M., Greiser, U., Barry, F., O'Brien, T. and Ritter, T. Genetically modified mesenchymal stem cells and their clinical potential in acute cardiovascular disease. **Discov. Med.** 9 (2010) 219-223.

143. Epting, C.L., Lopez, J.E., Shen, X., Liu, L., Bristow, J. and Bernstein, H.S. Stem cell antigen-1 is necessary for cell-cycle withdrawal and myoblast differentiation in C2C12 cells. **J. Cell Sci.** 117 (2004) 6185-6195.
144. Rivier, F., Alkan, O., Flint, A.F., Muskiewicz, K., Allen, P.D., Leboulch, P. and Gussoni, E. Role of bone marrow cell trafficking in replenishing skeletal muscle SP and MP cell populations. **J. Cell Sci.** 117 (2004) 1979-1988.
145. Banasik, M.B. and McCray, P.B.J. Integrase-defective lentiviral vectors: progress and applications. **Gene Ther.** 17 (2010) 150-157.
146. Cartier, N., Hacein-Bey-Abina, S., Bartholomae, C.C., Veres, G., Schmidt, M., Kutschera, I., Vidaud, M., Abel, U., Dal-Cortivo, L., Caccavelli, L., Mahlaoui, N., Kiermer, V., Mittelstaedt, D., Bellesme, C., Lahlou, N., Lefrère, F., Blanche, S., Audit, M., Payen, E., Leboulch, P., l'Homme, B., Bougnères, P., Von Kalle, C., Fischer, A., Cavazzana-Calvo, M. and Aubourg, P. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. **Science** 326 (2009) 818-823.