Nuclear damages and oxidative stress: new perspectives for laminopathies

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Abstract

Mutations in genes encoding nuclear envelope proteins, particularly *LMNA* encoding the A-type lamins, cause a broad range of diverse diseases, referred to as laminopathies. The astonishing variety of diseased phenotypes suggests that different mechanisms could be involved in the pathogenesis of laminopathies. In this review we will focus mainly on two of these pathogenic mechanisms: the nuclear damages affecting the chromatin organization, and the oxidative stress causing un-repairable DNA damages. Alteration in the nuclear profile and in chromatin organization, which are particularly impressive in systemic laminopathies whose cells undergo premature senescence, are mainly due to accumulation of unprocessed prelamin A. The toxic effect of these molecular species, which interfere with chromatin-associated proteins, transcription factors, and signaling pathways, could be reduced by drugs which reduce their farnesylation and/or stability. In particular, inhibitors of farnesyl transferase (FTIs), have been proved to be active in rescuing the altered cellular phenotype, and statins, also in association with other drugs, have been included into pilot clinical trials. The identification of a mechanism that accounts for accumulation of un-repairable DNA damage due to reactive oxygen species (ROS) generation in laminopathic cells, similar to that found in other muscular dystrophies (MDs) caused by altered expression of extracellular matrix (ECM) components, suggests that anti-oxidant therapeutic strategies might prove beneficial to laminopathic patients.

Laminopathies present a wide range of diseased phenotypes

Mutations affecting the *EMD* gene that encodes the inner nuclear membrane-associated protein emerin cause the X-linked form of Emery-Dreifuss muscular dystrophy (EDMD1).[1] An indistinguishable disease phenotype (EDMD2) is caused by mutations in the *LMNA* gene encoding lamin A/C.[2] The finding that the altered expression of two different proteins located at the nuclear envelope causes a similar diseased phenotype affecting the contractile tissues suggested the existence of a common pathogenic mechanism.[3] Mutations in *LMNA* cause several tissue-specific diseases: the autosomal dominant form of EDMD,[2] the autosomal recessive form of EDMD,[4] the limb-girdle muscular dystrophy type 1B (LGMD 1B),[5] the dilated cardiomyopathy and conduction-system disease (CMD-CD),[6] the Dunningan-type familial partial lipodystrophy (FPLD2),[7] and the Charcot-Marie Tooth disorder type 2 (CMT 2B1).[8] Each disease selectively strikes one or more specific tissues, including skeletal and cardiac muscle, tendons, adipose tissue, and peripheral neurons.

A further group of laminopathies has been then identified, characterized by a systemic involvement of almost all the tissues, which undergo premature senescence. The progeric laminopathies include the Hutchinson-Gilford progeria, syndrome (HGPS)[9,10] atypical progeroid syndrome,[11] mandibuloacral dysplasia (MADA),[12] and restrictive dermopathy (RD).[13]

Typical nuclear abnormalities consisting of altered nuclear envelope/lamina structure and focal loss of heterochromatin are observed in fibroblasts, myoblasts and muscle tissue from EDMD1 patients.[14,15] Characteristic non-uniform distribution of both lamin A/C and emerin have been reported in skin fibroblasts from EDMD2 patients.[16,17] Ultrastructural alterations include nuclear lamina thickening, nuclear pore clustering,[16] as well as focal absence of heterochromatin and loss of contact between heterochromatin and the nuclear lamina.[16,18] No accumulation of abnormal prelamin A has been found in either biopsies or cultured cells from EDMD2 patients.[19] Fibroblasts from FPLD2 patients present characteristic nuclear alterations, due to the accumulation of abnormal amounts of prelamin A.[20,21] The dysmorphic FPLD2 nuclei present intranuclear prelamin A aggregates, an enlarged and irregular nuclear profile, and a reduced amount of peripheral heterochromatin.[22]

In dermal fibroblasts from HGPS, a-WS and MADA patients, typical nuclear alterations have been observed, mainly consisting in local

or total loss of peripheral heterochromatin, associated with blebs and invaginations of the nuclear lamina.[22-24] In HGPS cells, the worsening of chromatin alterations have been reported to increase with the age of the patient, as well as with the increasing amount of progerin.[23,25,26] The cellular phenotype of RD, determined by accumulation of farnesylated prelamin A, causes severe nuclear envelope and chromatin abnormalities.[27]

Pathogenic mechanisms

The impressive variety of disease phenotypes of laminopathies rises the question of how mutation of a gene expressed in nearly every differentiated cell could give rise to many tissue-restricted pathologies. Besides their role in maintaining, in association with B-type lamins, the mechanical stability of the nuclear envelope (NE) throughout the phases of the cell cycle, A-type lamins and associated NE proteins represent scaffolds for molecular interaction with elements that regulate DNA synthesis and repair, higher-order chromatin organization, nuclear positioning, gene transcription, and cell differentiation.[28,29] Many of these functions involve lamin A interplay with signal transduction pathways, transcription factors and chromatin-associated proteins. On this basis, different pathogenic mechanisms of laminopathies have been proposed which include: nuclear envelope defects affecting nuclear stiffness,[29,30] nuclear envelope defects as determinants of altered nucleo-cytoplasmic

interplay,[31,32] altered cell cycle control,[33] telomere dysfunction,[34] altered cellular signalling,[35,36] altered stem cell functioning.[37,38]

In the present review we will focus mainly on two further pathogenic mechanisms: i) alterations of the nuclear morphology affecting chromatin rearrangement; ii) altered DNA repair due to oxidative stress.

Alterations of nuclear morphology affecting the chromatin arrangement

Alterations of nuclear morphology are present in many types of laminopathies, frequently associated with alterations of the chromatin arrangement. Interestingly, distinct altered patterns of chromatin distribution characterize different classes of laminopathies. Severe nuclear abnormalities have been reported in HGPS, a-WS, RD and MADA cells, including lobulation, blebbing, and loss of heterochromatin.[22,24-27,39] Aberrant nuclear morphology results in cellular senescence, down-regulation of transcription, and apoptosis.[20,40] Furthermore, there is increasing evidence of a role for lamins in the regulation of epigenetic marks in chromatin. Loss of heterochromatin in HGPS and RD cells is associated with down-regulation of trimethylation at Lys-9 of histone H3 (H3K9), and reduced association with heterochromatin protein 1α (HP1α).[23,27] Cells from older MADA patients also exhibit loss of peripheral heterochromatin, together with mislocalization of HP1β, trimethylated H3K9, and LBR.[24] In all these cases, the nuclear defects appear to be not related to a loss of mature wild-type lamin A, but to a dominant-negative effect of aberrant prelamin A accumulation.[41]

In laminopathies affecting the muscle, nuclear alterations, that include nuclear lamina thickening, nuclear pore clustering, and focal detachment of peripheral heterochromatin, appear to be related to a loss of mature wild-type lamin A and to defective lamin phosphorylation.[42,43] Alterations of the nuclear morphology occurring in muscular laminopathies also characterize sarcopenia, the age-related decline of muscle mass, strength and quality in elderly. It has been recently reported, by means of *in situ* immunocytochemistry, that the activity of pre-mRNA splicing and cleavage is impaired in the myonuclei of old muscles[44] and that similar alterations are detectable in cultured myoblasts from DM2 patients,[45] suggesting the possibility that these nuclear alterations might represent the basis of common pathogenic mechanisms in sarcopenia, myotonic dystrophy and laminopathies.[46]

Alterations in the nuclear arrangement have been reported in the tissues affected in different laminopathies and in cells cultured *in vitro* obtained from laminopathic patients or animal models of laminopathies. On the other hand, the specificity of nuclear alterations which characterize systemic laminopathies causing premature senescence with respect to those detected in muscular laminopathies, suggests that different mechanisms are involved in the pathogenesis of the two classes of laminopathies. In fact, the accumulation of farnesylated prelamin A occurring in systemic laminopathies results in defective interactions of the nuclear envelope with chromatin-associated proteins such as HP1, thus impairing the correct localization of heterochromatin domains at the nuclear periphery, and could have a repressive effect on gene transcription of chromosome domains or tissue-specific gene *loci*.[47,48] In muscular laminopathies, nuclear alterations are less evident[49] and mainly detectable with immunocytochemical methods, such as defects related to defective lamin A phosphorylation,[42] altered pRb-mediated activation of cellular differentiation,[50] excess accumulation of phosphorylated Smad2 and Smad3 in nuclei.[51] It is also conceivable that, as it has been reported in sarcopenia and myotonic dystrophy,[46] pre-mRNA splicing and cleavage is impaired in muscular laminopathies.

Altered DNA repair due to oxidative stress

Oxidative stress refers to a cell state where the production of reactive oxygen species (ROS), mainly produced by mitochondria and NADPH oxidases, is higher than its removal. In response to oxidative stress, the gene expression is modulated in order to activate various redox-transcription factors, such as Rb, p53, NF-kB and FoxO;[52] however, whilst oxidants in the cytoplasm are able to activate this redox signalling, oxidative stress in the nucleus blocks this process.[53]

Recent evidence points to a relationship between lamin mutation and altered ROS metabolism.[54] Accumulation of fanesylated prelamin A in progeric laminopathies induces excessive ROS and reduces the level of antioxidant enzymes.[55-57] Apart from a direct damaging effect to coding sequences, ROS can affect the lamin structure, thus promoting cellular senescence and susceptibility to ROS.[55] Downstream effects of ROS include persistent DNA damage,[58] and telomere shortening,[59] mechanisms that have been suggested to be involved into the pathogenesis of laminopathies. ROS accumulate at a higher rate in HGPS fibroblasts, as well as in normal-

ly aged fibroblasts;[56] this may contribute to increased levels of DNA damage and may trigger senescence in HGPS cells.[60] Moreover, oxidative stress appears involved in the pathogenesis of FPLD, whose fibroblasts undergo p16-dependent senescent arrest.[21] Impaired DNA repair, and genomic instability also characterize MEFs of Zmpste24-null mice, as well as bone marrow cells and HGPS or RD fibroblasts undergoing premature senescence.[61] Fibroblasts from HGPS and MADA patients show increased amounts of basal phosphorylated histone variant H2AX (γH2AX), a marker of DNA damage sites.[62,63] Gamma-H2AX colocalizes with XPA foci, an essential factor of nucleotide excision repair (NER) and not with double-strand breaks (DBSs), suggesting that the damage in HGPS cells may be different from that accrued by other genotoxic agents.[62] Furthermore, fibroblasts from HGPS patients show a marked delay in the recruitment of p53 binding protein 1 (53BP1) to sites of DNA repair.[61]

A dysfunctional lamina may influence ROS in several ways, because the lamina serves a a docking site for transcription factors and chromatin-associated proteins.[35] In HGPS fibroblasts and in *ZMPSTE24^{-/-}* MEFs the recruitment of the repair factor p53-binding protein (53BP1) to sites of DNA damage is impaired, as well as the presence of fragmented DNA after irradiation.[61] Expression of HGPS mutant lamin A in HeLa cells increases the levels of phosphorylated histone H2AX (γ-H2AX), a hallmark of double strand breaks, and this is correlated with defects in DNA repair *foci*.[64] Other DNA damage signalling pathways, including ATM and ATR kinases as well as Rad50 and Rad51, are affected in HGPS and RD fibroblasts.[65] These findings suggest that defects in the DNA repair machinery are due to expression of abnormal levels of farnesylated prelamin A in progeric laminopathies.

DNA damage-initiated genomic instability as well as p53-mediated cell senescence and apoptosis in response to DNA damage may contribute to aging.[66] In a recent study, it has been demonstrated that whilst the DNA damage induced by etoposide could be repaired in progeroid fibroblasts, many of double strand breaks (DSB) induced by ROS are unrepairable. Moreover, because the treatment with a ROS scavenger can reduce the amount of DSB, it has been suggested that the accumulation of un-repairable DNA damages could be mainly due to the ROS-generating environment present in progeroid fibroblasts.[67]

As far as the mechanism leading to DBS in laminopathic cells is concerned, it has been hypothesized that, in normal condition, the nuclear lamina could represent a nuclear shield against ROS, being a preferential docking site for ROS defusing enzymes, such as

CAT, GPX and GST, whose concentration in the nucleus are several fold higher than in the cytosol.[68] In the presence of mutant lamin A, the formation of the nuclear shield could be affected, resulting in a higher susceptibility to oxidative stress.

A general relationship between ROS, mitochondrial dysfunction, and the pathophysiology of muscular dystrophies (MDs) has been widely reported.[69,70] This relationship has been further supported by genetic[71] or pharmacological reduction of the open probability of the PTP which prevent myofiber injury in animal models of MDs,[72] as well as in patients with collagen VI myopathies.[73] In laminopathies, accumulation of farnesylated prelamina A also affects the expression of the mtDNA encoded subunit II of the cytochrome oxidase comple IV (COX 2), lowering the mitochondrial potential and generating ROS;[21] moreover, accumulation of farnesylated prelamin A in Zmpste24^{-/-} mice also causes an increased mitochondrial response to oxidative stress, further supporting a relationship between defective lamin A processing and mitochondrial dysfunction.[74]

A relevant source of ROS in mitochondria is represented by MAOs, located in the outer mitochondrial membrane, that catalyze the oxidative deamination of neurotransmitters and dietary amines, generating hydrogen peroxide. MAO have been studied at the level of nervous system,[75] leukemic cells,[76] and in cardiac diseases.[77] In skeletal muscle it has been recently reported that ROS related to MAO activity plays a pivotal role in loss of cell viability and contractile derangements in different animal models of MDs, causing oxidation of myfibrillar proteins.[78]

Molecular targets for a pharmacological treatment of laminopathies

Farnesylated lamin A exerts a dominant effect on the pathophysiology of HGPS[25] and MAD;[24] farnesyl transferase inhibitors (FTIs) can block prelamin A processing and reduce the percentage of cells with misshapen nuclei[79] and recover nuclear transcription activity.[23,41]

Signaling pathway required to maintain normal stem cell function have been reported to be perturbed in cells expressing high levels of unprocessed prelamin A. In particular, expression of progerin activates down-stream effectors of the Notch signalling pathway and alters the differentiation potential of stem cells.[80] Alterations of signalling pathways involved in regulation of stem cells, such as Wnt, have been reported in Zmpste24 null mice.[81] FTI treatment of HGPS cells did not result in a reduction in DNA double strand breaks and damage checkpoint signalling, suggesting that DNA damage accumulation and aberrant nuclear morphology are independent phenotypes due to accumulation of progerin.[65] Ideally, the therapy of progeroid syndromes needs to achieve both restoration of nuclear morphology and transcriptional ability, as well as improvement of DNA damage repair mechanisms. Amino-bisphosphonates (N-BPs), that are used to prevent osteoporosis and the risk of pathological fractures in bone malignancies, act as inhibitors of farnesyl-phyrophosphate synthase, thus reducing the synthesis of both geranyl-geranyl and farnesyl groups. Their use, in combination with statins, reduces nuclear defects and partly rescues the disease phenotype in Zmpste24 null mice.[82] Pharmacological inhibitors of ERK1/2, whose hyperactivation has been related to the pathogenesis of DCM in EDMD,[83] have been found to block the development of cardiomyopathy in Lmna[H222P/H222P] mice, before the appearance of clinical symptoms.[84] We have recently reported that activation of the m-TOR-dependent autophagic pathway using rapamycin can counteract progerin accumulation in HGPS cells, leading to the rescue of the chromatin phenotype of senescent cells.[85] The availability of a drug capable of triggering degradation of farnesylated prelamin A paves the way to the treatment of other laminopathies featuring accumulation of farnesylated prelamin forms different from progerin. The treatment of progeroid fibroblasts with the ROS scavenger N-acetyl cysteine (NAC) has been demonstrated to reduce the levels of un-repairable DSB and to improve their growth rate in culture. Because the ROS-generating environment is the primary cause of the accumulation of un-repairable DNA damage, the use of ROS scavengers in conjunction with FTIs might improve quality of life for progeric laminopathic patients.[67]

The recent demonstration that pargyline, an MAO inhibitor, is able to reduce ROS accumulation and exerts a beneficial effect on the dystrophic phenotype in mice models of collagen VI and Duchenne myopathies, adds evidence of the pivotal role of mitochondrial dysfunction in different muscular dystrophies[78] and suggest a therapeutic potential for MAO inhibitors also in laminopathies. Determining the molecular mechanisms by which the loss of A-lamins impacts on the different pathways regulating chromatin rearrangements, and DNA repair in the presence of increased ROS production, will be fundamental not only for understanding the pathogenic mechanisms but also for the development of further therapeutic strategies to treat these diseases.

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