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Gonzalez JM, Pla D, Perez-Sala D, Andres V. A-type lamins and Hutchinson-Gilford progeria syndrome: pathogenesis and therapy. *Front Biosci (Schol Ed)*. 2011;3:1133-46

which has been published in final form at <https://doi.org/10.2741/216>

A-type lamins and Hutchinson-Gilford progeria syndrome: pathogenic mechanisms and therapeutic strategies

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ABSTRACT

Lamin A and lamin C (A-type lamins, both encoded by the *LMNA* gene) are major components of the mammalian nuclear lamina, a complex proteinaceous structure that acts as a scaffold for protein complexes that regulate nuclear structure and function. Abnormal accumulation of farnesylated-progerin, a mutant form of prelamin A, plays a key role in the pathogenesis of the Hutchinson-Gilford progeria syndrome (HGPS), a devastating disorder that causes the death of affected children at an average age of 13.5 years, predominantly from premature atherosclerosis and myocardial infarction or stroke. Remarkably, evidence is accumulating that progerin is also present in normal cells and appears to progressively accumulate during aging of non-HGPS cells. Therefore, understanding how this mutant form of lamin A provokes HGPS may shed significant insight into physiological aging. In this review, we discuss recent advances into the pathogenic mechanisms underlying HGPS, the main murine models of the disease, and the therapeutic strategies developed in cellular and animal models with the aim of reducing the accumulation of farnesylated-progerin, as well as their use in clinical trials of HGPS.

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MAIN TEXT

1. THE NUCLEAR LAMINA

The nuclear envelope in eukaryotic cells separates the nucleoplasm from the cytoplasm (**Figure 1**). This structure is composed of the outer and inner nuclear membranes (ONM and INM, respectively), the nuclear pore complexes (NPCs) and the nuclear lamina. The ONM is continuous with the endoplasmic reticulum, while the INM is connected to the nuclear lamina. The ONM and INM are separated by a luminal space of 30 to 50 nm and are joined at the NPCs, which control the transport of macromolecules between the nucleus and the cytoplasm (1, 2). The nuclear lamina is a filamentous protein layer that provides mechanical stability to the INM and has important functions in a variety of cellular processes, such as nuclear positioning (3, 4), chromatin structure and NPC organization (5-10), nuclear envelope breakdown and reassembly during mitosis (11), DNA replication (12, 13), DNA damage response, cell cycle progression, cell differentiation (14-16), cell polarization during cell migration (17) and transcriptional control (10, 18). The main components of the nuclear lamina are type-V intermediate filament proteins called lamins, which have a central α -helical rod flanked by two globular domains, a short N-terminal “head” and a long C-terminal “tail” (19, 20). Lamins form coiled-coil dimers that associate longitudinally to form head-to-tail polymers (21).

Lamins are classified as A-type or B-type based upon their primary sequence and their biological properties. For example, A-type lamins are basic and B-type lamins acidic, as revealed by isoelectric focusing and conventional two dimensional SDS-PAGE (22, 23). Human B-type lamins are encoded by the *LMNB1* gene (protein lamin B1) located at chromosome 5q23.3-q31.1 (24) and the *LMNB2* gene (proteins lamin B2 and lamin B3) located at chromosome 19p13.3 (25). B-type lamins are expressed throughout development, with lamins B1 and B2 expressed in most cells (26-28) and lamin B3 only in spermatocytes (29). A-type lamins are encoded by the *LMNA* gene, which has 12 exons and maps to chromosome 1q21.2-q21.3. The main products of *LMNA* are the proteins lamin A (664 aminoacid residues in the non-processed form) and lamin C (572 residues), but the gene also encodes lamin A Δ 10 and lamin C2 (30-32). The mRNA sequence of lamin A coincides with that of lamin C up to codon 566, after which lamin C, which results from an alternate splice, lacks part of exon 10 and all of

exons 11 and 12 (32). Lamin A, like lamin B but unlike lamin C, possesses a C-terminal CAAX motif, which directs farnesylation of the protein (33, 34); in contrast, lamin C displays a unique six amino-acid sequence, VSGSRR, at its C-terminal end (32). Lamin C2, a germline-specific product of *LMNA*, contains a specific amino-terminal hexapeptide GNAEGR (31); and lamin A Δ 10, which is expressed in tumor cell lines and several normal cell types, lacks exon 10 (30). A-type lamins (lamin A and lamin C) are expressed in a developmentally regulated manner (33, 34). In general, lamin A and C are mainly expressed in differentiated cells and not in highly proliferating tissues (35), and have therefore been suggested as early markers of cellular differentiation (36).

2. SYNTHESIS AND POST-TRANSLATIONAL PROCESSING OF LAMIN A AND C

Lamin A and lamin B contain a CAAX motif at their C-terminal end. CAAX boxes are consensus sequences for protein isoprenylation which occurs through the covalent attachment to the cysteine residue of isoprenoid moieties, either the 15 carbon isoprenoid farnesyl or the 20 carbon geranylgeranyl. The CAAX motifs of lamin A and lamin B —CSIM and CAIM, respectively— direct farnesylation of the protein. Isoprenylation is the first of a series of modifications which confer hydrophobicity to the C-terminal end of proteins, and is thought to facilitate their interactions with membranes (37, 38). Farnesylation of the cysteine at the CAAX motif occurs through the formation of a thioether linkage catalyzed by farnesyltransferase (FTase), an enzyme which uses farnesyldiphosphate as substrate. This step is required for subsequent processing of the protein by two enzymes that recognize the prenyl moiety: an endoprotease called Rce1 (39, 40) that cleaves the 3 terminal amino acids, and Icmt, a carboxyl methyltransferase (41, 42) that methylates the newly accessible cysteine. In lamin B, these modifications are thought to favor its interaction with the INM. Although isoprenylation is stable throughout the life-span of the protein, lamin B has been proposed to undergo a methylation-demethylation cycle during mitosis (43).

Uniquely among isoprenylated proteins, lamin A undergoes a second proteolytic cleavage after the three initial processing steps described above (**Figure 2**). This cleavage is carried out by the endoprotease FACE-1, a zinc metalloproteinase also known as Zmpste24 (zinc metalloproteinase STE24 homolog, *S. cerevisiae*), which recognizes farnesylated lamin A (44). Zmpste24 cleaves lamin A after Tyr 646,

removing the 15 C-terminal residues, including the farnesylated and carboxylmethylated C-terminal cysteine. Therefore mature lamin A is not an isoprenylated protein. The farnesylated precursor, non-cleaved form is known as prelamin A (**Figure 2A**). Since farnesylation is required for recognition by Zmpste24, prelamin A, which appears as a higher molecular weight form in SDS-PAGE, accumulates in conditions under which isoprenylation is inhibited (45). Although Zmpste24 has been detected in the endoplasmic reticulum, several lines of evidence situate this enzymatic activity in the nucleus (46). The current belief is that isoprenylation facilitates the initial assembly of lamin A in the nuclear lamina (47), while subsequent removal of the isoprenoid moiety allows its solubilization, for instance during mitosis (48). However, additional work is required to clarify these questions under normal and pathological conditions.

3. LAMINOPATHIES

LMNA mutations or defective posttranslational processing of prelamin A cause the majority of human diseases and clinical syndromes termed laminopathies, which can be divided into systemic disorders and diseases whose effects are restricted to specific tissues (49-52). Systemic diseases include: 1) Hutchinson-Gilford progeria syndrome (HGPS), in which patients exhibit premature aging, alopecia, loss of subcutaneous fat, premature atherosclerosis and associated myocardial infarction and stroke; 2) atypical Werner's syndrome, in which patients show premature aging, cataracts, sclerodermatous skin, premature atherosclerosis and hair graying; 3) Restrictive dermopathy (RD), a neonatal lethal form of HGPS characterized by intrauterine growth retardation, translucent and partly eroded skin, prominent superficial vasculature and epidermal hyperkeratosis, multiple joint contractures, facial deformities (small mouth, small pinched nose and micrognathia), skull defects, thin dysplastic clavicles, and pulmonary hypoplasia; and 4) Mandibuloacral dysplasia, in which patients exhibit mandibular and clavicular hypoplasia, acroosteolysis, delayed closure of the cranial suture, joint contractures and lipodystrophy, alopecia and insulin resistance (51, 52).

Tissue-restricted laminopathies that affect striated muscles or adipose tissue distribution include: 1) Emery-Dreifuss muscular dystrophy (EDMD), in which patients progressively develop contractures and muscle weakness, wasting of skeletal muscle and cardiomyopathy with conduction disturbance; 2) dilated cardiomyopathy, in which

patients show ventricular dilatation, impaired systolic contractility, arrhythmias, and conduction defects; 3) limb-girdle muscular dystrophy 1B, in which patients slowly develop progressive shoulder and pelvic muscle weakness and wasting, with later development of contractures and cardiac disturbances; 4) Charcot-Marie-Tooth neuropathy type 2B1, characterized by lower-limb motor deficits, walking difficulty, secondary foot deformities and reduced or absent tendon reflexes in the second decade (51, 52); and 5) Dunningan-type familial partial lipodystrophy, characterized by altered adipose tissue distribution, with loss of adipose tissue in the trunk and limbs and concomitant accumulation in the neck and face, often in association with insulin-resistant diabetes, hypertriglyceridemia and increased susceptibility to atherosclerosis (51, 52). EDMD, limb-girdle muscular dystrophy 1B and dilated cardiomyopathy are sometimes caused by the same *LMNA* mutations and occur in the same families, and can therefore be considered variants of the same disease (53).

4. HGPS AND RD

In many HGPS patients, a single de novo silent base pair mutation in codon 608 of *LMNA* (GGC to GGT, G608G) activates a cryptic splice site in exon 11, causing in-frame deletion of a sequence encoding a 50 amino acid stretch that contains the Zmpste24 cleavage site (54, 55). Therefore the last processing step leading to mature lamin A protein cannot occur and mutant prelamin A remains farnesylated throughout its life-span (**Figure 2A**). This anomalous protein, known as progerin, displays an apparent molecular weight on SDS-PAGE intermediate between prelamin A and the mature protein (56) (**Figure 2B**). RD patients either carry the G608G *LMNA* mutation or exhibit homozygous loss of *FACE-1/ZMPSTE24*. The absence of Zmpste24 function in RD patients causes accumulation of farnesylated-prelamin A, lack of lamin A, and misshapen nuclei with numerous folds and blebs (57-59).

Several lines of evidence demonstrate the key role played by the accumulation of progerin in the pathogenesis of HGPS. Transfection of either progerin or a non-cleavable form of prelamin A, both of which are tightly associated with the nuclear membrane, induces nuclear abnormalities in cells (60, 61). Similarly, antisense oligonucleotides against exon 11 sequences downstream from the exon 11 splice donor site promote alternate splicing in both wild-type and HGPS fibroblasts, and cause increased synthesis of progerin and the same nuclear shape and gene-expression

perturbations observed in HGPS fibroblasts (62). During mitosis, this abnormal association of progerin appears to delay the onset and progression of cytokinesis, and may also impair the targeting of lamina components to the nuclei of daughter cells and alter entry into S-phase mediated by hyperphosphorylation of the retinoblastoma gene product (pRB) (48). Progerin may also promote DNA-damage (51, 63), alterations in DNA repair and genome instability (64, 65), and appears to interfere with nuclear architecture by various mechanisms. Cells expressing progerin contain low levels of wild-type lamin A, which accumulates at the nuclear periphery (66). The deleterious effect of progerin accumulation thus seems to dominate over the function of wild-type lamin A. Interestingly, progerin is also present in small amounts in normal cells due to sporadic use in healthy subjects of the same splice site that produces HGPS, and this has been proposed to play a role in physiological aging (61, 66-69). Very recently, Olive et al. found that progerin-positive cells reside in non-HGPS arteries and that vascular progerin accumulates in vivo with age (70). Moreover, accumulation of pre-lamin A, possibly due to age-dependent downregulation of *Zmpste24*/FACE-1, has been identified as a novel biomarker of vascular smooth muscle cell aging and atherosclerosis that acts to accelerate senescence (71). Thus, accumulation of prelamin A and progerin in the vessel wall may be a potential new element causing age-dependent vascular alterations.

5. MOUSE MODELS OF HGPS AND RD

The development of mouse models has greatly increased knowledge about the etiopathogenesis of laminopathies. Disruption of the gene encoding *Zmpste24* in mice causes defective lamin A processing, the accumulation of prelamin A at the nuclear envelope, and muscular and adipocyte alterations that resemble those associated with some laminopathies (44, 72). This mouse model shows nuclear envelope abnormalities (44), hyperactivation of p53-dependent signaling (73), cellular senescence (73), stem cell dysfunction (74, 75), and the development of a progeroid-like phenotype characterized by a marked shortening of life expectancy (76).

Studies with the lamin C-only mouse, which produces lamin C but not lamin A or prelamin A, show that lamin A is dispensable in mice (77). Moreover, the presence of a single *LMNA(LCO)* allele eliminated the nuclear shape abnormalities and progeria-like disease phenotypes of *Zmpste24*^{-/-} mice (77). Analogous reversion of the nuclear

abnormalities and pathological manifestations of the disease occurred with the absence of an allele of lamin A/C in the *Lmna*^{+/-} *Zmpste24*^{-/-} mice (73). Similarly, heterozygous mice carrying a gene-targeted HGPS allele (*Lmna*^{HG/+}) show several progeria-related phenotypes, including bone alterations, reduction in subcutaneous fat and premature death (78). Knock-in mice expressing nonfarnesylated progerin (*Lmna*^{nHG/+}) have a milder phenotype and live longer than *Lmna*^{HG/+} mice, and the nuclei of *Lmna*^{nHG/+} embryonic fibroblasts are less misshapen (79). Mice expressing geranylgeranylated progerin (*Lmna*^{ggHG/+}) exhibit milder bone disease and survive longer than *Lmna*^{HG/+} mice, but also show a progeroid phenotype (80). Transgenic mice that carry a human bacterial artificial chromosome that contains the *LMNA* G608G mutation show progressive loss of vascular smooth muscle cells in large arteries—a common feature of HGPS—without the external phenotype seen in human progeria (81). Surprisingly, although the substitution of proline for leucine at residue 530 in lamin A causes autosomal-dominant EDMD in humans, homozygous *Lmna*^{L530P/L530P} mice show phenotypes markedly reminiscent of symptoms observed in progeria patients, including severe growth retardation and pathologies in bone, muscle and skin (82); nevertheless, these mice show no obvious defects in large vessels.

Other authors have developed mouse models expressing progerin in specific tissues. Using the keratin 14 promoter, Wang et al (83) generated a transgenic mouse line that expresses progerin in the epidermis. Although the skin keratinocytes of these mice show abnormalities in nuclear morphology, both hair growth and wound healing are normal. In contrast, Sagelius et al (84) reported abnormalities in the skin and teeth of transgenic mice carrying the HGPS-causing *Lmna*^{G608G} mutant under the control of the tetracycline-regulated (tet-off) keratin 5 promoter.

Recently, Davis et al (85) suggested that lamin C synthesis is dispensable in mice and that the failure to convert prelamin A to mature lamin A causes cardiomyopathy (at least in the absence of lamin C), but not progeria. These conclusions are based on the observation that knock-in mice harboring a mutant *Lmna* allele (*Lmnan*PLAO) that yields exclusively nonfarnesylated prelamin A (and no lamin C) have no evidence of progeria but died from cardiomyopathy, and that this phenotype could not be ascribed to an absence of lamin C because mice expressing an otherwise identical knock-in allele yielding only wild-type prelamin A (and no lamin C) appeared normal.

6. STUDIES WITH CELLS FROM HGPS AND RD PATIENTS

As a consequence of accumulation of progerin or prelamin A, the nuclei of cells from HGPS and RD patients are lobed and show nuclear lamina thickening, loss of peripheral heterochromatin and clustering of nuclear pores (59, 66, 86, 87). HGPS and RD cells also accumulate double-strand breaks that produce genome instability (64, 88), and fibroblasts from these patients exhibit alterations in DNA repair pathways and in the recruitment of repair factors (64, 88, 89). Accumulation of DNA damage causes cellular senescence (90, 91), a feature of HGPS cells with progerin accumulation (92). Cellular senescence is also increased by treatment with inhibitors of the human immunodeficiency virus protease that also inhibit Zmpste24, resulting in accumulation of farnesylated prelamin A (93). DNA damage induces p53 activation, a characteristic of cells from RD and HGPS patients (94). Moreover, preventing progerin accumulation associated to physiological aging reverts overexpression of p53 target genes in aged individuals (95). The activation of p53 might explain the absence of cancer in HGPS patients (96).

The expression of progerin affects the cell cycle through its abnormal association with membranes during mitosis, delaying the onset and progression of cytokinesis and impairing the targeting of nuclear envelope/lamina components to daughter cell nuclei in early G1-phase (48). Additionally, progerin delays the transition to S-phase by inhibiting the hyperphosphorylation of pRb by cyclin D1/cdk4 (48). Progerin accumulation also causes abnormal chromosome segregation and binucleation (61). Di Masi et al (97) found that the accumulation of unprocessed prelamin A in fibroblasts from mandibuloacral dysplasia patients induces DNA damage and reduces DNA repair after irradiation, resulting in alterations in the checkpoint response in the G1-to-S transition.

A-type lamins interact with peripheral chromatin (19), serving as a platform for genome organization (20). Accumulation of progerin or prelamin A in HGPS patients leads to chromatin disorganization (86), and prelamin A accumulation also alters histone methylation and epigenetic control (98, 99). It is also noteworthy that lamin A and C interact with several transcription factors and regulatory proteins, including pRb and MyoD (100-105), c-Fos (106, 107), SREBP1 (108, 109), MOK2 (110, 111), MEL-18 (112), and TonEBP (113). Lamin A and C also modulate the activity of several signaling molecules (reviewed in (10), including Wnt/ β -catenin (74, 114, 115), ERK1/2

(107, 116-118), Notch (75, 119), and transforming growth factor β (TGF- β) (16, 120), and progerin expression in mesenchymal stem cells causes defective signaling via Notch, a pathway that regulates stem cell differentiation (10). Although evidence is accumulating that these interactions play important roles in the regulation of signal transduction pathways and gene transcription in health and disease (reviewed in (10, 121), more work is needed to firmly establish whether the expression of lamin A mutants can provoke laminopathies at least in part through alterations in gene expression.

7. GENE THERAPY STRATEGIES FOR THE TREATMENT OF PROGERIA

Potential new approaches to reduce the pathological consequences of progerin accumulation include the use of a morpholino antisense oligonucleotide directed against the aberrant alternative splice donor site in *LMNA* exon 11 of HGPS patients. Transfection of this antisense oligonucleotide in HGPS fibroblasts reduces the levels of progerin mRNA and protein, reverses nuclear shape abnormalities and corrects alterations in gene expression associated with the disease (66). Similarly, reduced progerin expression and improved cellular phenotypes are achieved with a small-hairpin RNA against progerin, confirming that progerin downregulation can regress nuclear abnormalities (77). It will be of interest to test the effect of these approaches in mouse models of progeria. An important limitation of these gene therapy strategies is the small size of mRNA regions—the splice donor site or the exon 11-12 junction—that can be targeted by these approaches. As an alternative, the use of antisense strategies against the full transcript of prelamin A has been suggested (122), based on the observation that mature lamin A and prelamin A are apparently dispensable in mice, and also on experiments showing that RNA interference-mediated reduction of prelamin A transcript and protein improves nuclear morphology in *Zmpste24*-null fibroblasts (77).

8. FARNESYLTRANSFERASE INHIBITORS AS THERAPEUTIC AGENTS FOR THE TREATMENT OF PROGERIA SYNDROMES

Studies from several groups have demonstrated that reducing the expression of lamin A ameliorates the symptoms associated with progerin accumulation. For example disruption of one *Lmna* allele in *Zmpste24*-null mice protects against several disease

phenotypes, including retarded growth (73, 77). Moreover, using a tetracycline-regulated mouse model of progeria, Sagelius et al. demonstrated that the damage caused by progerin expression is not irreversible, at least in skin and teeth (123).

Initial interest in the clinical potential of inhibiting FTase was aimed at suppressing the transforming activity of Ras oncogenes, farnesylated proteins that require this posttranslational modification for function (124). Given the implication of isoprenylated progerin in the nuclear abnormalities of progeroid syndromes, the hypothesis was raised that pharmacological inhibition of progerin farnesylation might also be beneficial in this setting. Available FTase inhibitors (FTIs) fall into distinct classes. CAAX peptides were initially developed as competitive inhibitors of FTase and were later followed by CAAX peptidomimetics. Also, competitive inhibitors of farnesyl diphosphate have been developed, and other small compounds have been identified through screening (125). Amelioration of the nuclear morphology by FTIs has been reported in various cellular models of progeroid laminopathies, including fibroblasts from HGPS or RD patients (60, 126, 127). The beneficial effect was associated with efficient blocking of protein farnesylation, as assessed by the accumulation of nonfarnesylated-prelamin A and the reduction in the incorporation of radioactive isoprenoids on progerin (128), although progerin levels were not reduced. Treatment of control human fibroblasts with the peptidomimetic inhibitor FTI-277 was recently shown to cluster heterochromatin-associated proteins and lamin-associated polypeptide 2 α (LAP2 α) in the nuclear interior, suggesting that chromatin is an immediate target of this FTI (129). Interestingly, the effects of FTI-277 on chromatin are abolished upon the inhibition of prelamin A accumulation by treatment with 5-azadeoxycytidine. Moreover, FTI administration reversed the gene expression defects observed in the lamin A-pRb signaling network in fibroblasts from HGPS patients (130) and completely restored nucleolar antigen localization in treated progeria cells (131).

Beneficial effects of FTI administration have also been observed in animal models. For example, FTIs reduce weight loss and bone and muscle alterations in *Zmpste24*-knockout mice (77), and improve survival in *Lmna*^{HG/+} mice (132). Importantly, a recent study supports the concept that the beneficial effects of FTIs are due to inhibition of progerin farnesylation, since ABT-100 ameliorates disease in the *Lmna*(HG/+) mouse model of HGPS but not in mice expressing a nonfarnesylated version of progerin (*Lmna*(nHG/+) (133). However, as judged from the accumulation of prelamin A, FTIs appear to be less effective at inhibiting lamin A farnesylation in

animal models than in cells, perhaps due to alternative prenylation by geranylgeranylation when FTase is inhibited (125).

Promising results obtained in cellular and animal models of cancer have led to several FTIs advancing to clinical trials, including the nonpeptidomimetic CAAX FTIs lonafarnib and tipifarnib. Clinical success of these compounds has been observed mainly with hematological tumors; however, their efficacy in the treatment of solid tumors has been somewhat limited. Nevertheless, clinical trials have shown that FTIs are fairly well tolerated, with adverse effects often limited to the gastrointestinal tract, and that they induce the accumulation of prelamin A in patients (125). Based on these findings, a first clinical trial was set up in 2007 to test the effect of FTIs, in particular lonafarnib, on the symptoms of children with progeria. This trial and the Triple Drug Trial now in progress (see below) have been possible in part thanks to the efforts of the Progeria Research Foundation (www.progeriaresearch.org), which has sponsored and spurred research in this area in recent years.

9. ADDITIONAL THERAPEUTIC STRATEGIES TO TREAT HGPS

Several studies demonstrate that FTI-277 reduces prelamin A farnesylation (134) and recovers the altered heterochromatin domains observed in HGPS cells (135). However, it has been recently suggested that drugs impairing prelamin A processing alter heterochromatin organization (129) and do not reduce the number of DNA double-strand breaks (94, 136). Moreover, although FTI treatment improves the phenotype of *Zmpste24*-deficient mice (137), it only reduces prelamin A processing by 5% (138), possibly due to alternative prenylation (geranyl-geranylation) of lamin precursors bypassing the effect of FTIs (125, 139-141). It will also be important to determine the effect of FTIs on other farnesylated proteins, such as B-type lamins (140). Possible negative effects of FTI treatment and the interest in using FTI in combination with other therapies suggest the need to identify alternative therapies for these devastating diseases. For these reasons, inhibitors of other steps in the maturation of lamin A, such as endoproteolytic processing or methylation, are also being tested for their ability to reverse the abnormal phenotype caused by progerin accumulation (56).

Lastly, other direct or indirect inhibitors of protein prenylation that are already in clinical use have shown beneficial effects in mouse models of progeria. Statins are well known inhibitors of the cholesterol biosynthetic pathway and are widely used in the

clinic to reduce hypercholesterolemia and associated disorders, such as atherosclerosis. Statins inhibit the synthesis of isoprenoid precursors involved in protein modification, thus reducing lamin A maturation (142-144). The aminobisphosphonates are effective therapeutic agents against disorders with increased bone resorption, such as that occurring in bone metastasis or in post-menopausal women (38). Bisphosphonates inhibit farnesylpyrophosphate synthase, thus reducing the synthesis of both geranylgeranyl and farnesyl groups (145, 146). Statins and aminobisphosphonates thus have potential in the treatment of progeria syndromes or of some of their symptoms, such as the vascular or bone defects. Indeed, combined administration of statins and aminobisphosphonates has recently been shown to efficiently inhibit the farnesylation and geranylgeranylation of progerin and pre-lamin A, and this effect is accompanied by an improvement in the phenotype of the *Zmpste24*-knockout mice (141). Based in these promising animal studies, the Triple Drug Trial is now in place to test the therapeutic effect of a combination of a statin (pravastatin), a bisphosphonate (zoledronic acid) and an FTI (lonafarnib, SCH 66336) in HGPS patients (ClinicalTrials.gov identifier: NCT00879034, www.progeriaresearch.org).

10. CONCLUDING REMARKS

Recent years have witnessed important advances in the knowledge of the molecular basis of HGPS and other laminopathies. From various types of evidence it seems clear that an aberrant processing of prelamin A leading to the accumulation of progerin, a persistently farnesylated form of the protein, is critical for the pathogenesis of HGPS. This has made researchers to turn to FTIs in the search for a pathogenic treatment. These compounds have been shown to ameliorate HGPS manifestations in cellular and animal models of the disease, although with lesser efficacy in the latter. Importantly, the mechanisms leading to these potentially beneficial effects, as well as the limitations of FTI treatment, are not completely understood. On one hand, FTI could exert part of their beneficial effects through the inhibition of the farnesylation of targets other than progerin, or even through unrelated mechanisms of action. Moreover, inhibition of FTase could prove insufficient if alternative prenylation of progerin can occur. In addition, some evidence exists that even nonfarnesylated forms of progerin could be deleterious. In the light of these findings the search continues for alternative or

complementary treatments, like the combination of several drugs with the ability to block isoprenylation, which may provide some hope for these devastating diseases. Moreover, additional mechanistic insight into the pathogenesis of progeroid syndromes and the mechanisms of drug action are essential to develop novel therapeutic strategies against these devastating diseases. Since progerin is present in normal cells and appears to progressively accumulate during normal aging, research in this field may also improve our understanding of the mechanisms underlying physiological ageing.

ACKNOWLEDGMENTS AND NOTICE OF GRANT SUPPORT

We thank Simon Bartlett for English editing. V.A.'s lab is funded by the Spanish Ministry of Science and Innovation (MICINN) and the Fondo Europeo de Desarrollo Regional (FEDER) (grant SAF2007-62110), the Instituto de Salud Carlos III (ISCIII) (RECAVA, grant RD06/0014/0021), the Fundación Ramón Areces and Fina Biotech. D.P.-S. laboratory is supported by grants from the MICINN (SAF2009-11642) and ISCIII (Retics RIRAAF, RD07/0064/0007). J.M.G. is supported by the ISCIII and D.P. by the FPI programme of MICINN. The CNIC is supported by the MICINN and the Pro-CNIC Foundation.

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Running title:

HGPS: pathogenic mechanisms and therapeutic strategies

Supplementary key words:

A-type lamins, nuclear envelope, Hutchinson-Gilford progeria syndrome, Restrictive Dermopathy, laminopathies, farnesyl transferase inhibitors.

Non-standard abbreviations:

EDMD: Emery-Dreifuss muscular dystrophy; FTase: farnesyltransferase; FTI: farnesyltransferase inhibitor; HGPS: Hutchinson-Gilford progeria syndrome; INM: inner nuclear membrane; LAP2 α : lamin-associated polypeptide 2 α ; NPC: nuclear pore complex; ONM: outer nuclear membrane; pRb: Retinoblastoma gene product; RD: restrictive dermopathy; Zmpste24: zinc metallopeptidase STE24 homolog, *S. cerevisiae*

FIGURE LEGENDS

Figure 1. The mammalian nuclear envelope. A representation of the nucleus showing the location of lamins and other lamin-interacting proteins. BAF: Barrier to autointegration factor; LAP1/2: Lamin-associated polypeptide 1/2; SUN: Sad1p/UNC-84 domains; LBR: Lamin B receptor.

Figure 2. Defective pre-lamin A processing in HGPS, and nuclear abnormalities induced by progerin accumulation. (A) In cells carrying a normal *LMNA* gene, prelamin A undergoes a series of post-translational modifications. First, the cysteine in the C-terminal CSIM moiety is farnesylated by the action of farnesyl transferase (FTase). Subsequently, the three terminal amino acids are cleaved by the endoprotease Rce1 and the newly accessible cysteine is methylated by the carboxyl methyltransferase ICMT. Finally, the fifteen C-terminal residues, including the isoprenylated and carbosymethylated C-terminal cysteine, are cleaved by the endoprotease Zmpste24/FACE-1. In many HGPS patients, a single de novo silent base pair mutation in codon 608 of *LMNA* (GGC to GGT, G608G) activates a cryptic splice site, causing the in-frame deletion of a sequence encoding a fifty amino acid stretch that contains the Zmpste24 cleavage site. Therefore the last processing step cannot occur and cells produce progerin, a mutant form of farnesylated pre-lamin A whose accumulation produces nuclear abnormalities. (B) Top: Confocal microscopy images showing lamin A/C distribution in the nuclei of human fibroblasts from a healthy subject (left) and a HGPS patient (right). The arrow marks a site of nuclear blebbing. Middle: Western-blot analysis of cell extracts from control subjects and HGPS patients showing the presence of lamin A/C and progerin. Bottom: Confocal microscopy images showing the subcellular distributions of HA-lamin A, HA-progerin and chromatin in transfected U2OS cells. The arrow marks a site of nuclear blebbing.

Figure 1

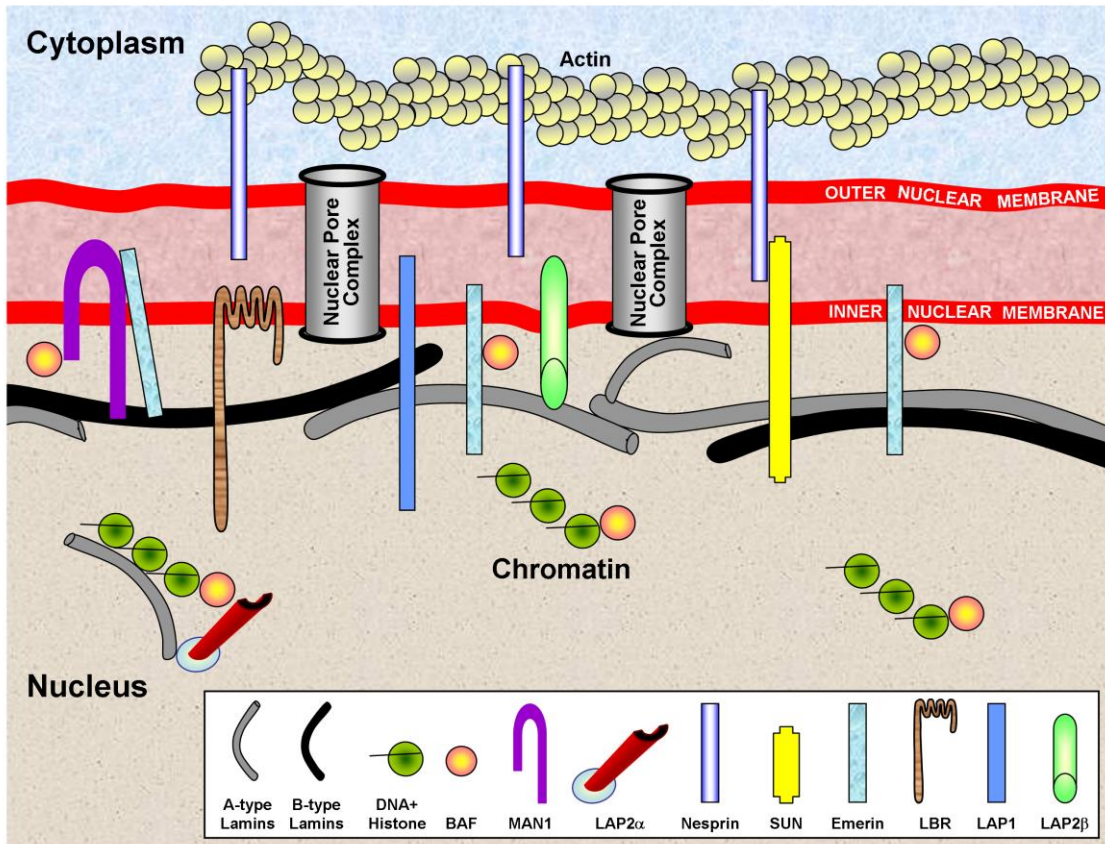


Figure 2

