

Perspective

From Immature Lamin to Premature Aging Molecular Pathways and Therapeutic Opportunities

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ABSTRACT

Accelerated aging or progeria has been a puzzling disease for many years. The recent findings involving the lamin A/FACE-1 (substrate/tearase) system in the etiology of Hutchinson-Gilford progeria syndrome and related pathologies have shed some light on the mechanisms underlying the development of these devastating conditions. Thus, genetic defects in the nuclear envelope protein prelamin A or in the FACE-1 metalloprotease (also called Zmpste24) involved in prelamin A proteolytic maturation, causes the accumulation of an abnormal form of this protein and the subsequent disruption of nuclear envelope integrity. Recently, we and others have observed how this disruption leads to alterations in chromatin organization, genomic instability, transcriptional changes, and activation of a p53-linked signaling pathway. By using genetic manipulation approaches in mouse, we have shown that lowering prelamin A levels results in a total recovery of Zmpste24-deficient mice from the accelerated aging process. Moreover, p53 nullizygosity allows a modest but significant improvement in the premature aging phenotype, and contributes to delaying the onset of the progeroid condition. On the basis of these results, we propose different potential therapeutic approaches that could be tested in Zmpste24-deficient mice. These strategies, some of which are based on existing drugs, might contribute to the development of effective treatments for these dramatic pathologies.

INTRODUCTION

Aging is a multifactorial process. It affects most biological functions of the organism and seems to be caused by the cooperation of diverse molecular events, including oxidative stress, telomere attrition and decline of DNA repair.¹ Alterations in the mechanisms that control these processes have been shown to compromise DNA integrity, thus accelerating the progression of cellular and organismal senescence.² In humans, disorders in which affected individuals develop various features resembling accelerated aging are known as segmental progeroid syndromes.² The two best-known examples of these diseases are Hutchinson-Gilford progeria syndrome (HGPS, ‘progeria of childhood’) and Werner syndrome (WS, ‘progeria of the adult’). Whereas most cases of WS have largely been known to be caused by mutations in WRN helicase,³ the mutation responsible for HGPS was identified only two years ago.^{4,5} Surprisingly, the gene affected by the HGPS mutation encodes lamins A and C, two structural proteins of the nuclear envelope that had been previously associated with a wide variety of genetic syndromes, including lipodystrophy, muscular dystrophy, dilated cardiomyopathy, and Charcot-Marie-Tooth neurodegenerative disorder.⁶ This LMNA mutation, consisting of a C to T transition at the third base of codon 608, does not change the encoded amino acid (G608G), but activates a cryptic splicing site (GTGGGC>GTGGGT), which results in the loss of a region coding for 50 amino acids of the lamin A precursor (prelamin A). One year before this finding, we had identified the protease involved in the maturation of prelamin A by using a genetic approach based on the generation and analysis of mice deficient in the metalloprotease FACE-1/Zmpste24.⁷ These mutant mice showed a marked accumulation of prelamin A in the nuclear envelope and lacked mature lamin A, displaying an accelerated aging phenotype with features shared by different laminopathies.^{7,8} Interestingly, the prelamin A cleavage site recognized by FACE-1/Zmpste24 to produce mature lamin A is contained within the 50 amino acids encoded by the mRNA region lost in the aberrant splicing that takes place in cells from HGPS patients. Thus, HGPS cells contain a truncated form of prelamin A called progerin.^{4,5} On the other hand, mutations in the lamin A/FACE-1 system have also been described in patients with progeroid diseases such as mandibuloacral dysplasia and restrictive dermopathy.^{9,10}

Very recently, we have utilized *Zmpste24*-deficient mice to explore the molecular events underlying accelerated aging processes.¹¹ These studies have revealed that severely affected progeroid mice show the activation of a p53-signaling pathway. Furthermore, we have also provided evidence that the observed phenotype can be totally rescued by *Lmna* heterozygosity, and partially reversed by *p53* nullizygosity, providing clues about potential therapeutic strategies to treat these devastating syndromes.

NUCLEAR ENVELOPE ALTERATIONS LEAD TO TRANSCRIPTIONAL CHANGES: P53 SIGNALING ACTIVATION

Immature lamin A forms (prolamin A and progerin) assemble in the nuclear envelope, producing marked alterations in its architecture. We and others have associated such alterations with nuclear misshaping (blebbing and fragmentation) and chromatin conformational stress.^{7,12} Linked to progerin or prolamin A accumulation, Liu et al. have recently reported that the recruitment of the DNA repair factors 53BP and Rad51 to H2AX-tagged sites of DNA lesion is delayed in human HGPS cells as well as in mouse cells lacking FACE-1/*Zmpste24*.¹³ Furthermore, H2AX-53BP complexes take longer to dissociate in *Zmpste24*-null cells, and these cells show increased levels of genomic instability.¹³ In agreement with these observations, the transcriptional analysis we carried out in tissues from severely affected *Zmpste24*-deficient mice has revealed a clear upregulation of classical p53 target genes, including the growth arrest and DNA-damage inducible gene *Gadd45α*, the inhibitor of cyclin dependent kinases *p21/Waf1*, the nuclear protein *Pa26*, the anti-proliferative B-cell translocation gene *Big2*, the activating transcription factor *Atf3*, the DNA-damage inducible gene *RTP801*, and the regulator of G protein signaling *Rgs16*.¹¹ Moreover, other genes which are not clearly identified as direct p53 targets but have also been associated with the p53 signaling pathway, are upregulated in tissues from *Zmpste24*-deficient mice. These include the growth arrest inducible genes *Gadd45β* and *Gadd45γ*, which share multiple functional features with the p53-target *Gadd45α* in their response to DNA damage and other stresses.¹⁴ Interestingly, the overexpression of p53-responsive genes was progressive, becoming more frequent and intense as mice grew old, and rising dramatically during the last weeks of life.

In agreement with the observed upregulation of a tumor suppressor pathway in tissues from *Zmpste24*-deficient mice, adult fibroblasts from animals exhibiting progeroid features revealed a reduced proliferative capacity when compared with control cells. Additionally, cell cycle analysis demonstrated reduced levels of *Zmpste24*^{-/-} cells in S phase, along with increases in the percentages of cells in G₁ and G₂ phases. Taken together, these findings have prompted us to propose the existence of a structural cell cycle checkpoint involved in the detection of nuclear envelope and/or chromatin abnormalities. The chromatin conformational stress induced by the presence of progerin or prolamin A, and the genomic instability derived from the decline of repair mechanisms linked to the nuclear envelope abnormalities, would eventually lead to the activation of a tumor suppressor signaling pathway, conducting cells to senescence. Besides p53 signaling upregulation, we have also found important expression changes in genes involved in lipid metabolism. Thus, leptin receptor and steroyl CoA desaturase, both of which are transcriptionally regulated by circulating leptin,^{15,16} are upregulated in *Zmpste24*-deficient liver. Furthermore, fatty acid binding protein-1 and adipose differentiation related protein, which are involved in lipid transport and metabolism, are sharply downregulated in livers from *Zmpste24*^{-/-}

mice. These expression changes might be related to the lipodystrophic phenotype observed in mice deficient in FACE-1/*Zmpste24* metalloproteinase.

Since the phenotype of *Zmpste24*^{-/-} mice is similar to that of *Lmna*-deficient mice,¹⁷ we addressed the expression profile of *Lmna*^{-/-} livers to determine whether these similarities were based on common transcriptional patterns. Strikingly, out of the 949 genes upregulated in *Zmpste24*^{-/-} liver, 521 (54.9%) were also overexpressed under *Lmna* deficiency and only 9 (0.95%) were underexpressed. Additionally, out of the 1096 genes downregulated in *Zmpste24*^{-/-} liver, 422 (37.9%) were also underexpressed in *Lmna*^{-/-} liver and only 89 (8.1%) were overexpressed. Consistently, upregulation of p53 targets and changes in lipid metabolism were also observed in livers from *Lmna*-null mice. Thus, it seems that the absence of A-type lamins causes similar molecular alterations as prolamin A accumulation, indicating that this is the major cause of the progeroid phenotype observed in *Zmpste24*-deficient mice.

REDUCED PRELAMIN A AND P53 LEVELS RESCUE THE PROGEROID PHENOTYPE OF ZMPSTE24^{-/-} MICE

A powerful approach for demonstrating the causal involvement of a certain protein in a specific biological process is to target the protein and then observe the effects of such targeting in the studied process. The above described findings pointed at two potential molecular targets: prolamin A and p53. In the hope that downregulating prolamin A levels or eliminating p53 might alleviate the progeroid syndrome exhibited by *Zmpste24*-deficient mice, we initiated a series of breeding programs aimed at the generation of *Zmpste24*^{-/-} *Lmna*^{+/-} and *Zmpste24*^{-/-} *p53*^{-/-} mice. It is worthwhile mentioning that *Lmna* nullizygosity was not expected to provide any benefit in this regard, as *Lmna*^{-/-} mice develop a more severe laminopathy than *Zmpste24*^{-/-} mice.¹⁷ Remarkably, *Zmpste24*^{-/-} *Lmna*^{+/-} mice are virtually indistinguishable from controls. Whereas all *Zmpste24*-deficient mice are infertile, weigh considerably less than controls and live approximately 100 days, all *Zmpste24*^{-/-} *Lmna*^{+/-} animals are fertile, show a normal weight and are still alive one year after birth. Although a more extended analysis of the phenotype will be needed, it seems that *Lmna* heterozygosity leads to a total recovery of the progeroid phenotype caused by the *Zmpste24* deficiency.^{11,18} This observation is in perfect agreement with our previous proposal that prolamin A accumulation is the main cause of the accelerated aging observed in these mice, and suggests that prolamin A could be the only substrate targeted by the FACE-1/*Zmpste24* protease.

The alternative genetic approach we carried out consisted of eliminating the p53 tumor suppressor. In this case, there was also an improvement in the phenotype, as assessed by the significant increase in weight and life expectancy of *Zmpste24*^{-/-} *p53*^{-/-} mice when compared with their *Zmpste24*^{-/-} littermates. Nevertheless, and despite these results support the involvement of p53 in the generation of the observed phenotype, the accelerated aging process was only delayed, as *Zmpste24*^{-/-} *p53*^{-/-} mice eventually lose fat, hair and motility, and die prematurely. This observation is in agreement with the situation of the tumor suppressor p53 at a lower level on the vertical cause-effect axis topped by prolamin A accumulation which results in the development of these progeroid syndromes (Fig. 1). However, the fact that the elimination of p53 results in only a partial recovery of the phenotype indicates that additional signaling pathways are probably involved in the generation of these pathologies.

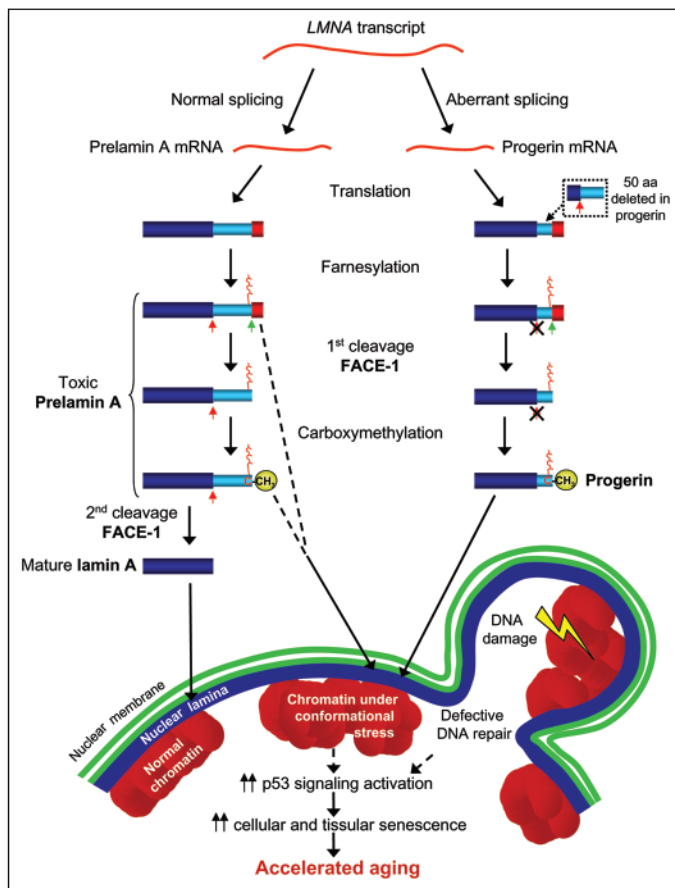


Figure 1. Molecular mechanisms underlying immature lamin A-induced progeria. Transcription from a wild-type *LMNA* locus yields normal preRNA that is correctly spliced producing prelamina A mRNA. In HGPS cells, a silent C to T transition introduces a cryptic splicing site, and the deletion of a 150 nt region from exon 11 yields the aberrant progerin mRNA. Prelamin A is a CaaX prenylated protein that after farnesylation undergoes three additional post-translational modifications: proteolytic removal of the C-terminal-aaX tripeptide (green arrow indicates the cleavage site), carboxymethylation of the newly exposed C-terminal cysteine and, finally, a second proteolytic step (red arrow indicates the cleavage site), producing mature lamin A. FACE-1/Zmpste24 protease is necessary for the second and most likely also for the first proteolytic step. Therefore, cells lacking FACE-1/Zmpste24 cannot complete lamin A maturation, accumulating toxic prelamin A. On the other hand, progerin lacks 50 amino acids including the cleavage site for the second proteolytic event, thus making impossible its correct processing to produce mature lamin A. Both farnesylated progerin and prelamin A assemble in the nuclear lamina, altering the stability of the nuclear envelope and causing conformational chromatin stress, as well as defective recruitment of DNA repair factors. Accumulated DNA damage and altered chromatin would activate a p53-signaling pathway, inducing cell and tissue senescence and eventually leading to accelerated aging.

POTENTIAL THERAPEUTIC STRATEGIES FOR IMMATURE LAMIN-INDUCED PROGERIA

Considering the above described mechanisms associated with the development of immature lamin-induced progeroid syndromes (Fig. 1), there are several approaches which could be of putative therapeutic relevance for patients with these diseases.

Lamin A mRNA interference. Small interfering RNAs (siRNAs) aimed at targeting *LMNA* mRNA have already been used to effec-

tively downregulate lamin A production.¹⁹ However, to date no widespread delivery mechanism has been proven to allow siRNAs to reach the several tissues affected in these progeroid syndromes. Therefore, substantial improvements in this field will be necessary before the in vivo downregulation of lamin A levels mediated by siRNAs can be an effective strategy for the treatment of progeria.

Inhibition of aberrant splicing of the *LMNA* transcript. Morpholino antisense oligonucleotides designed to target the cryptic splicing site activated by the C to T transition in HGPS have been shown to inhibit the production of mRNA encoding progerin in vitro.²⁰ This type of modified oligonucleotides has been successfully used for sustained delivery in animals and humans,²¹ thereby opening the possibility of using them in HGPS patients.

Inhibition of prelamin A/progerin farnesylation. Blocking of farnesyl transferase with several drugs, some of which have already been approved for treating cancer patients, has been recently shown to improve nuclear shape in cultured cells expressing progerin or prelamin A²²⁻²⁵ (and our unpublished results). Since a decrease in nuclear shape abnormalities has been shown to correlate with suppression of the progeroid phenotype in *Zmpste24*^{-/-} *Lmna*^{+/-} mice,^{11,18} it seems reasonable to expect that farnesyl transferase inhibitors will be effective for the treatment of HGPS patients. Therefore, preclinical testing of these compounds in animal models of progeria is the imperative next step previous to the design of clinical trials.

Inhibition of p53 signaling. Although total p53 inhibition could lead to an increase in tumour susceptibility,²⁶ partial p53 signaling inhibition might improve some symptoms. Thus, p53 heterozygosity is sufficient to extend the lifespan of *Zmpste24*^{-/-} mice to levels similar to those achieved by p53 nullizygosity (our unpublished results). However, strategies aimed at inhibiting the function of p53 or some of its downstream targets and pathways should be carefully evaluated before application to progeria patients.

The availability of *Zmpste24*-deficient mice will be essential to test the proposed approaches for treating the progeroid disorders caused by defects in the proteolytic processing of prelamin A. Nevertheless, the development of a mouse model more accurately reflecting the molecular alteration responsible for HGPS in humans will likely be necessary to further validate such therapeutic strategies. Moreover, it should be emphasized that mice seem to be more resistant than humans to mutations in the *Lmna*/*Zmpste24* system. Thus, whereas *Zmpste24*-null mice develop normally and only start to show accelerated aging seven weeks after birth, lack of FACE-1/Zmpste24 in humans leads to restrictive dermopathy (RD),¹⁰ a genodermatosis that causes premature delivery and neonatal death. However, if we consider the relative life expectancy of C57Bl/6 mice (3 years) and humans from western countries (70-80 years), mice deficient in *Zmpste24* (life expectancy: 3-4 months) reflect more accurately the phenotype of patients with HGPS than that of RD patients (12-15 years vs few days life expectancy, respectively). Furthermore, mice carrying the *Lmna* C to T transition characteristic of HGPS patients might not develop an accelerated aging phenotype, as this mutation is expected to generate only low levels of progerin, and mice with low levels of prelamin A seem to be completely normal.^{11,18} Despite these limitations, the development of these novel mouse models will likely help to better understand the differences between human and mouse nuclear lamina dynamics, and between progerin and prelamin A properties.

In summary, over the last three years we have witnessed an exponential growth in the knowledge of the etiology of several pathologies

affecting the lamin A/FACE-1 system, and more specifically those causing progeroid syndromes. The advances in this field allow us to be optimistic about the possibility that some of the proposed therapies will be useful in the treatment of these previously hopeless conditions. However, additional work will be needed to elucidate the details of the connections between immature lamin A, chromosomal instability, activation of tumor suppression pathways and development of progeria, as well as to address whether nuclear lamina alterations are among the multiple factors that contribute to normal aging.

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