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REVIEW

Hutchinson–Gilford progeria syndrome through the lens of transcription

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Summary

Lamins are nuclear intermediate filaments. In addition to their structural roles, they are implicated in basic nuclear functions such as chromatin organization, DNA replication, transcription, DNA repair, and cell-cycle progression. Mutations in human *LMNA gene* cause several diseases termed laminopathies. One of the laminopathic diseases is Hutchinson–Gilford progeria syndrome (HGPS), which is caused by a spontaneous mutation and characterized by premature aging. HGPS phenotypes share certain similarities with several apparently comparable medical conditions, such as aging and atherosclerosis, with the conspicuous absence of neuronal degeneration and cancer rarity during the short lifespan of the patients. Cell lines from HGPS patients are characterized by multiple nuclear defects, which include abnormal morphology, altered histone modification patterns, and increased DNA damage. These cell lines provide insight into the molecular pathways including senescence that require lamins A and B1. Here, we review recent data on HGPS phenotypes through the lens of transcriptional deregulation caused by lack of functional lamin A, progerin accumulation, and lamin B1 silencing.

Key words: aging; Hutchinson–Gilford progeria syndrome; lamin A; lamin B1; *LMNA*; nuclear lamina; progerin; transcription.

Hutchinson–Gilford progeria syndrome

Humans express four major lamin proteins: lamin B1 (LB1), lamin B2 (LB2), and lamins A and C (LA, LC), encoded by *LMNB1*, *LMNB2,* and *LMNA* genes, respectively. Lamins A, LB1, and LB2 are expressed as prelamins that contain a carboxyl-terminal CaaX motif. The cysteine in the CaaX motif undergoes farnesylation, followed by cleavage of the last three amino acids (aaX) and methyl esterification. While LB1 and LB2 remain farnesylated, LA undergoes additional cleavage of the last 15 amino acids and becomes nonfarnesylated.

The most frequent mutation in Hutchinson–Gilford progeria syndrome (HGPS), affecting approximately 90% of patients, is a *de novo* autosomal dominant, single base substitution mutation in *LMNA* (C1824T). This mutation activates a cryptic splice site, which produces a mutant LA protein with an internal deletion of 50 amino acids that disrupts the last cleavage step of prelamin A. This truncated LA, termed progerin, is permanently farnesylated, toxic to cells and displays altered structural and biochemical properties (De Sandre-Giovannoli *et al.*, 2003; Eriksson *et al.*, 2003). While LA is present both at the nuclear periphery and at nuclear interior, progerin localizes predominantly at the nuclear periphery (Capell *et al.*, 2005; Glynn & Glover, 2005). Progerin expression leads to reduction in the protein levels of lamin B1, thus further disrupting the nuclear lamina (Capell *et al.*, 2005; Scaffidi & Misteli, 2005; Taimen *et al.*, 2009; Shimi *et al.*, 2011)

In HGPS patients, progerin affects mostly tissues of mesenchymal origin, including bone, skin, fat, teeth, hair, and blood vessels, while mortality is primarily due to accelerated atherosclerosis. The most prominent HGPS cellular phenotypes are nuclear lobulation, heterochromatin alterations, mitochondrial dysfunction, and chromosomal and telomeres aberrations. Altered nuclear functions include compromised cell-cycle regulation, impaired DNA repair, increased apoptosis and senescence (Merideth *et al.*, 2008; Burtner & Kennedy, 2010; Mehta *et al.*, 2011; Trigueros-Motos *et al.*, 2011; Fig. 1) The molecular mechanisms by which progerin causes tissue-specific effects, and the reasons for the similarities or differences between HGPS phenotypes and those of aging are largely obscure. Noteworthy, the cryptic splice site activated in HGPS to create progerin is also used at low frequency in healthy individuals. Increased progerin levels are found in cells with physiological aging (Scaffidi & Misteli, 2006; McClintock *et al.*, 2007; Olive *et al.*, 2010). Furthermore, reduced lamin B1 protein expression is also observed in human cells undergoing senescence where the loss of LB1 probably leads to activation of either p53 or pRb pathways and has been proposed to serve as a senescence-associated biomarker (Shimi *et al.*, 2011; Freund *et al.*, 2012).

General mechanisms of lamin-regulated gene expression

Gene expression is dependent on chromatin–nuclear lamina interactions, miRNA regulation, sequestration of specific transcription factors (TFs), and interactions with RNA polymerase II (pol II) transcription machinery (Gruenbaum *et al.*, 2003; Malhas & Vaux, 2009; Fiserova & Goldberg, 2010; Mattout *et al.*, 2011; Zwerger *et al.*, 2011; Jung *et al.*, 2012).

Although there are few examples for lamin-dependent gene activation (e.g., NF-κB and TonEBP/NFAT5 transcription pathways), generally lamins and lamin-associated proteins provide a repressive chromatin environment due to specific protein–chromatin interac-

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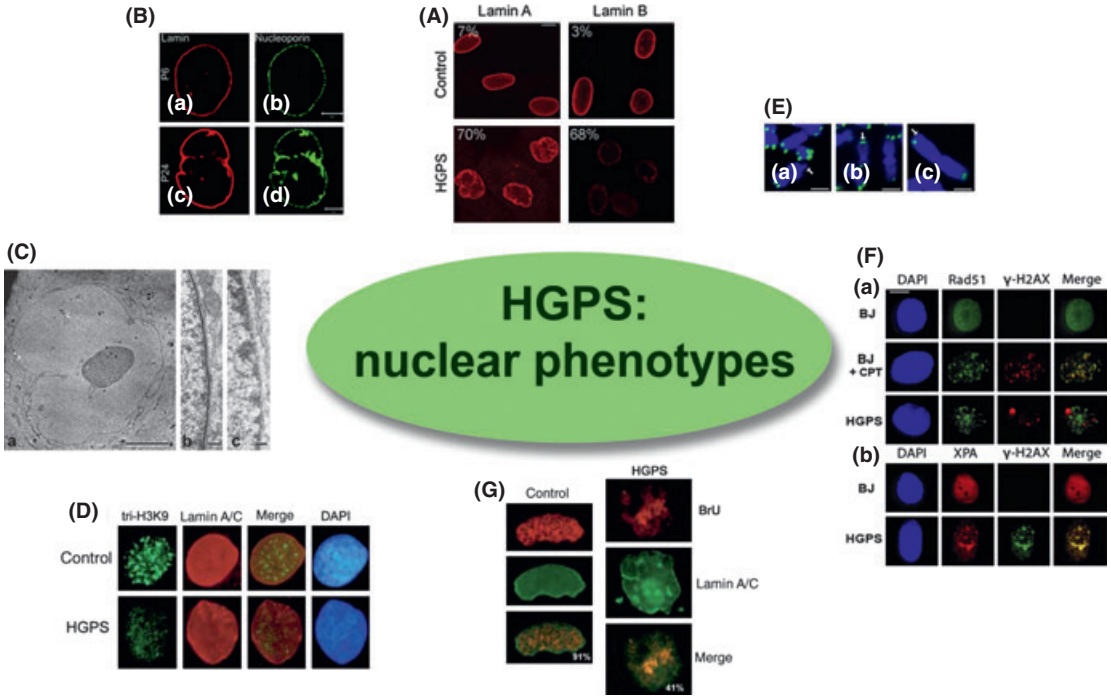

Fig. 1 Nuclear phenotypes in Hutchinson–Gilford progeria syndrome (HGPS) cells. (A) HGPS cells show aberrant nuclei with reduced expression of lamin B. The panel is taken with permission from Scaffidi & Misteli (2005). (B) Changes in nuclear structure and progressive nuclear pore complexes alterations occur at later passage number. (a) and (b) are cells from passage 6, (c) and (d)are cells from passage 24. The panel is taken with permission from Goldman *et al.* (2004). (C) Transmission electron microscope analysis shows nuclear envelope changes in HGPS cells. (a) Nuclear envelope invaginations. (b) Nuclear lamina thickening and peripheral heterochromatin loss. (c) Control nuclei with normal lamina and heterochromatin organization. The panel is taken with permission from Goldman *et al.* (2004). (D) HGPS cells show reduced H3K9me3. The data were taken with permission from Columbaro *et al.* (2005). (E) HGPS cells show chromosomal break (a), sister chromatid fusion (b) and free chromosomal end due to short undetected telomere (c). The panel is taken with permission from Benson *et al.* (2010). (F) HGPS cells have compromised DNA repair. (a) BJ: control cells. BJ + CPT: γ-H2AX colocalization with Rad51 in Camptothecin-treated cells. HGPS: impaired γ-H2AX colocalization with Rad51 in HGPS cells. (b) HGPS: γ-H2AX colocalization with XPA in HGPS cells. The panel was taken with permission from Liu *et al.* (2008). (G) Altered transcript distribution in HGPS cells. Nonuniform distribution of BrU labeling in HGPS cells compared with controls. The data were taken with permission from Columbaro *et al.* (2005).

tions (Lammerding *et al.*, 2004; Favale *et al.*, 2007; Peric-Hupkes & van Steensel, 2010; Puckelwartz *et al.*, 2011). The specific lamin-based complexes control binding to and release of lamin-associated proteins from the nuclear periphery, including emerin, MAN1, LAP2, LBR, SUN-1, and others (Wilson & Foisner, 2010). These lamin-based complexes probably regulate gene silencing and activation of developmentally regulated genes. (Nili *et al.*, 2001; Somech *et al.*, 2005; Holaska & Wilson, 2006; Meister *et al.*, 2010; Bank & Gruenbaum, 2011; Mattout *et al.*, 2011).

The nuclear lamina association with specific gene regulatory sequences requires the transcriptional repressor cKrox in a complex with histone deacetylase 3 and Lap2β, as well as methylation of histone 3 on lysine 9 (H3K9) (Towbin *et al.*, 2012; Zullo *et al.*, 2012). Mutations in lamins perturb lamin–chromatin interactions, spatial arrangement of chromosome territories and intranuclear chromosome folding (Scaffidi & Misteli, 2005; Mewborn *et al.*, 2010; Mattout *et al.*, 2011; Mehta *et al.*, 2011; Puckelwartz *et al.*, 2011; van Steensel, 2011).

Lamins provide a protein scaffold for the organization of TFs. The disruption of the nuclear lamina, especially that of LB1, affects DNA replication and leads to a significant reduction in pol II activity (Moir *et al.*, 2000; Spann *et al.* 2002; Ljungman & Lane 2004; Shumaker *et al.* 2008: Musich & Zou, 2011). Lamins A interacts with several components of pol II transcription machinery, including nuclear actin, nuclear myosin 1, chromatin remodeling complexes, and ribonucleoproteins (Sasseville & Langelier, 1998; Bengtsson & Wilson, 2004; Columbaro *et al.*, 2005; Simon *et al.*, 2010; Euskirchen *et al.*, 2011). The reduction in LB1 levels, the reduction of intracellular TATA-binding protein levels, and the diminished global transcription in progerin-expressing fibroblasts all provide important insights into the role of lamins in transcriptional regulation (Candelario *et al.*, 2011).

Lamin A and actin

Actin polymers and nuclear myosin play a key role in transcription regulation by affecting chromatin remodeling and movement, as well as by forming complexes that facilitate progress of the transcription machinery along the DNA template (Visa & Percipalle, 2010). *In vitro* binding between lamin and actin, and actin polymerization assays mapped two actin-binding sites in the LA tail domain (Simon *et al.*, 2010; Simon & Wilson, 2011). The *in vitro* study showed significant reduction in LA-actin binding and consequently, in LA-induced actin bundling in various diseases resulting

from mutations in the C-tail domain of LA, including the HGPS deletion. Hence, it is possible that lamins, and in particular LA, regulate transcription by directly affecting actin polymerization.

Lamin and progerin interactions with miRNA

miRNAs are small RNA molecules that are involved in post-transcriptional regulation by binding to target mRNAs, usually resulting in translational repression and gene silencing. *LMNA* and *LMNB1* expression is regulated by specific miRNAs. For example, miR-23 down-regulates *LMNB1* (Lin & Fu, 2009) and the brain-specific miR-9 down-regulates LA and progerin, but not lamin C and might thus bear relevance to the absence of primary central nervous system involvement in HGPS (Jung *et al.*, 2012). Lamins can regulate the expression of specific miRNAs. For example, LB1 binds and sequesters Oct1 (Malhas & Vaux, 2009). Insufficient LB1 yields unsequestered active Oct1, which leads to excess production of mir-31. In turn, mir-31 binds and silences p16(Ink4a)/p14(Arf) mRNA which normally inhibit progress through the cell cycle by maintaining the pRb checkpoint (p16/Ink4a) and augmenting p53 levels and function (p14/Arf) (Malhas *et al.*, 2010). LB1 reduction in HGPS cells and over-activation of mir-31 might thus explain, at least in part, the initial phase of hyperproliferation that characterizes HGPF fibroblasts prior to their apoptotic death (Bridger & Kill, 2004).

Lamins, HGPS, and mechanotransduction

Mechanotransduction describes a mechanism by which cells adjust to changes in the mechanical load exerted by their environment in part by activating specific gene expression programs. The mechanotransduction properties of cells require lamins and emerin function and are probably mediated through LINC complexes that link the nuclear lamina with the actin cytoskeleton and the NF-kB transcription pathway (Starr, 2007; Zwerger *et al.*, 2011). Lamin A-deficient cells have decreased nuclear stiffness and increased nuclear fragility (Broers *et al.*, 2004; Lammerding *et al.*, 2004). In response to mechanical stress, these cells display defective mechanotransduction and attenuated NFkB response, with impaired induction of mechano-sensitive and anti-apoptotic genes (Lammerding *et al.*, 2004, 2005; Lammerding & Lee, 2005). Hutchinson-Gilford progeria syndrome fibroblasts, which are characterized by stiffer and less compliant nuclei, are also more sensitive to mechanical strain and react with cell apoptosis/necrosis and impaired strain-induced proliferation response (Lammerding *et al.*, 2004; Dahl *et al.*, 2006; Verstraeten *et al.*, 2008).

In the vascular tree, HGPS cells fail to cope with fluid shear stress and thus contributing to vascular smooth muscle cells (VSMCs) loss and atherosclerosis (Verstraeten *et al.*, 2008; Olive *et al.*, 2010). The increased stiffness of HGPS fibroblasts suggests an increase in mechanical sensitivity and aberrant mechanotransduction of the progerin-expressing cells, including cells of the arterial walls. Notably, evaluation of NFkB in HGPS cells has not been reported yet. Deposition of progerin in the arterial walls might render HGPS cells more sensitive to mechanical strain and contribute to the aberrant mechanotransduction (Olive *et al.*, 2010; Zwerger *et al.*, 2011)

Lamin-regulated transcription mechanisms are altered in HGPS

The normal transcription pattern, particularly of genes encoding TFs, is altered in HGPS cells as indicated by genome-wide expression studies. (Csoka *et al.*, 2004; Scaffidi & Misteli, 2008; Hernandez *et al.*, 2010; Marji *et al.*, 2010; Plasilova *et al.*, 2011). In the next section, we discuss specific mechanisms by which lamins regulate and progerin deregulates gene expression in HGPS cells.

Chromatin organization and modifications

Several studies support LA involvement in higher order chromatin organization and anchorage, heterochromatin formation, histone modifications, and transcriptional activity (Dechat *et al.*, 2010; Gonzalez-Suarez & Gonzalo, 2010). The critical role of LA in chromatin organization is evident by the properties of progerin-containing HGPS nuclei that show loss of peripheral heterochromatin and specific changes in chromatin-modifying enzymes such as decreased H3 Lys 27 methyltransferase (EZH2), decrease in the chromatin modifications H3K27m3, H3K9m3, and increased in H4K20m3 (Scaffidi & Misteli, 2006; Shumaker *et al.*, 2006; Shimi *et al.*, 2011). These epigenetic changes affect chromatin organization, transcription, senescence, and DNA repair. Interestingly, the specific profile of HGPS-associated histone modifications oppose those which have been found in several tumors and may thus explain in part the divergent HGPS phenotypes of premature aging and cancer rarity (Dimauro & David, 2009; Chi *et al.*, 2010).

Telomeric heterochromatin

Telomere integrity is essential for maintaining chromosomes integrity and is therefore a key determinant of aging, cancer, and the viability of stem cells (Blasco, 2005). Telomere dysfunction results in genomic instability, activation of DNA damage responses (DDR), mitochondrial dysfunction, and stem cell exhaustion, which have all been implicated in the aging process (Sahin & Depinho, 2010). Interestingly, recent studies suggest possible interconnections between these aging effectors, thus linking DNA damage and metabolic pathways through a proposed 'telomere - p53 – mitochondrial' axis. Dysfunctional telomeres, recognized as DNA damage, activate p53, promote senescence, and compromises mitochondrial function due to PGC1 repression. Mitochondrial dysfunction, in turn, further aggravates functional decline of tissue stem cells and telomere dysfunction, thus setting up a vicious circle. The essential role of short telomeres in premature aging and the suggested telomere–mitochondrion link are demonstrated by Werner syndrome, which encompasses premature aging and a concomitant metabolic disorder of insulin-resistant diabetes (Sahin & Depinho 2012).

Loss of LA expression as well as increased expression of progerin cause gross telomere abnormalities, including telomere attrition and mislocalization. This phenotype is normally observed in cells undergoing senescence including HGPS fibroblasts (Raz *et al.*, 2008; Gonzalez-Suarez *et al.*, 2009; Benson *et al.*, 2010). Intriguingly, a recent report pointed to a synergistic relationship between progerin production and telomere dysfunction during the induction of

cellular senescence in normal human fibroblasts; The progressive telomere damage during cellular senescence plays a causative role in activating progerin production, while expression of progerin causes telomere shortening (Cao *et al.*, 2011). This finding lends insight into the process of normal aging in which progerin levels are increased (Scaffidi & Misteli, 2006; McClintock *et al.*, 2007; Olive *et al.*, 2010). The progerin-induced telomere dysfunction activates DDR and p53-signaling pathways leading to senescence (Benson *et al.*, 2010). In HGPS, the induction of senescence by DDR and p53 might substantially reduce stem cell populations and contribute to premature aging (Gotzmann & Foisner, 2006; Halaschek-Wiener & Brooks-Wilson, 2007). Activation of p53 in HGPS also augments mitochondrial dysfunction and stem cells exhaustion in part by repressing the mitochondrial PGC1 protein (Halaschek-Wiener & Brooks-Wilson, 2007; Sahin & Depinho, 2010; Sahin *et al.*, 2011). Given that telomere attrition and dysfunction and mitochondrial dysfunction are both associated with cardiovascular disease (CVD) and atherosclerosis, they might also be implicated in HGPS-related atherosclerotic CVD (Calado & Young, 2009; Puddu *et al.*, 2009; Viteri *et al.*, 2010). Moreover, in the presence of activated p53, short telomeres suppress tumorigenesis, despite genomic instability, thus potentially contributing to cancer rarity in HGPS (Blasco, 2005).

Chromatin remodeling

Lamins A interacts with the nucleosome remodeling and deacetylation (NuRD) complex through association with RBBP4/7. Site of interaction is localized at the LA region (amino acids 562–664) that overlaps the region deleted in progerin. This interaction is crucial to form and maintain heterochromatin foci, H3K9 methylation and heterochromatin protein 1 gamma (HP1γ) chromatin association (Meshorer & Gruenbaum, 2009; Pegoraro *et al.*, 2009). Indeed, silencing of individual NuRD subunits in normal cells recapitulated aging-associated chromatin defects, including heterochromatin loss. The inability of progerin to bind RBBP4/7 is likely responsible for the reduction in several NuRD proteins and the loss of peripheral heterochromatin in HGPS cells (Meshorer & Gruenbaum, 2009; Pegoraro *et al.*, 2009).

LINC complexes in HGPS

Besides the impaired role of the LINC complexes in mechanotransduction, LA binding to the nuclear membrane SUN1 protein plays a key role in the etiology of HGPS (Crisp *et al.*, 2006; Haque *et al.*, 2006, 2010). Hutchinson-Gilford progeria syndrome cells display increased SUN1 levels at the nuclear envelope as SUN1 preferentially interacts with prelamin A/progerin (Goldman *et al.*, 2004; Haque *et al.*, 2010; Chen *et al.*, 2012). Down-regulation of SUN1 by RNAi in HGPS cells restores nuclear shape, prevents the heterochromatin loss, and inhibits senescence (Chen *et al.*, 2012).

Barrier-to-autointegration factor in HGPS

The distinct effects of LA on peripheral heterochromatin organization are further exemplified by LA interactions with the barrier-to-autointegration factor (BAF). Barrier-to-autointegration factor is involved in tethering heterochromatin to the nuclear envelope via its interactions with DNA, histones, RBBP4, LA, and LEM-domain proteins (Shumaker *et al.*, 2001; Montes de Oca *et al.*, 2009). Notably, the 50-amino acids deletion that characterizes progerin does not interfere with progerin-BAF binding. Binding of BAF to progerin as well as to LA and prelamin A result in loss of BAF cytoplasmic pool, and in its partial dysfunction probably due to loss of BAF interactions with the chromatin-organizing protein RBBP4 (Capanni *et al.*, 2010). Interestingly, a recessive mutation in BAF causes segmental premature-aging syndrome that resembles HGPS but lacks atherosclerosis (Puente *et al.*, 2011).

Altered regulation of specific transcription factors in HGPS

The interactions between lamins and various TFs–regulate tissue-specific transcriptional programs and lead in most cases to transcriptional repression (Mattout-Drubezki & Gruenbaum, 2003). The interactions operate through specific protein–protein interactions, via sequestration of TFs to the nuclear periphery and by affecting nuclear pore complexes (NPCs) positioning and function (Heessen & Fornerod, 2007; Andrés & González, 2009; Marmiroli *et al.*, 2009; Fiserova & Goldberg, 2010). A summary of TFs that are regulated directly or indirectly by lamin A/progerin and their relevance to HGPS pathology is shown in Table 1. PRX1 (Kubben *et al.*, 2010), MEOX/GAX (Csoka *et al.*, 2004), and TWIST2 (Plasilova *et al.*, 2011) appear only in Table 1. Moreover, progerin expression disrupts the Ran gradient and reduces the function of the SUMOylation pathway (Kelley *et al.*, 2011). These effects might contribute to the compromised nucleo-cytoplasmic transport of TFs through the NPC. Furthermore, SUMO pathway disruption might also compromise the regulation of lamin A by SUMO2 (Zhang & Sarge, 2008), SUMO3 (Galisson *et al.*, 2011), and SUMO1 (Simon *et al.*, 2013). Interestingly, Farnesyl transferase inhibitor (FTIs) treatment has been found to prevent progerin effects on the Ran-GTPase system, a fact that might further expand FTIs therapeutic yield (Kelley *et al.*, 2011).

pRb

The retinoblastoma tumor suppressor (pRb) protein regulates numerous TFs and determines cell-fate and differentiation (Burkhart & Sage, 2008). Lamins A and its associated protein LAP2α interact with the pRb, as well as with inhibitor-of-growth-protein1 (ING1). These interactions probably regulate pRb association with HP1, histones, and chromatin remodeling enzymes and contribute to chromatin recruitment to the nuclear periphery (Gonzalo & Blasco, 2005). Genome-wide expression studies identified the LA-pRb-signaling network as a major pathway affected in HGPS (Marji *et al.*, 2010). Indeed, loss of LA activity leads to mislocalization, increased proteasomal degradation and inactivation of pRb (Johnson *et al.*, 2004; Nitta *et al.*, 2007; Andrés & González, 2009; Boban *et al.*, 2010).

TGFβ

Binding of LA and LAP2α to pRb probably modulates TGFβ effects on fibroblasts proliferation. Moreover, LA inhibits TGFβ-induced

Table 1 Possible effects of lamins/progerin-related transcription factors on HGPS phenotypes

TFs	Known status in HGPS	Resultant cellular characteristics	Proposed related clinical characteristics
pRb	Dysfunction	Genomic instability: mitotic defects, chromosomal mis-segregation HC disorganization histone modifications	Premature aging
		Increased apoptosis (via E2F1,E2F3)	Cancer rarity
		Impaired osteogenesis	Bone abnormalities
		Impaired adipogenesis	Lipodystrophy
		Increased fibrosis	Fibrosis: skin, blood vessels
ING1	Aberrant cytoplasmic sequestration Reduced nuclear levels	HC disorganization and loss of peripheral HC Compromised DNA repair	Premature aging
SREBP1	Altered localization Impaired PPARγ signaling	Impaired adipogenesis	Lipodystrophy CVD – PPARγ related
Notch	Altered pathway activation	Stem cell exhaustion	Premature aging
		Mesenchymal stem cells dys: impaired adipogenesis impaired osteogenesis	Lipodystrophy bone abnormalities
		VSMCs pathology	Vasculopathy, CVD atherosclerosis
NFκβ	Altered response to mechanical strain (in LA-deficient cells)	Increased mechanosensitivity Increased apoptosis /necrosis	Premature aging atherosclerosis
		VSMCs pathology	Vasculopathy, CVD, atherosclerosis
Wnt-β-catenin	Down-regulated Altered LEF1 localization & activity	Stem cells exhaustion	Premature aging
		Mesenchymal stem cells dys: impaired adipogenesis impaired osteogenesis	Lipodystrophy bone abnormalities
		Defective ECM	Premature aging Skin abnormalities Abnormal dentition Vasculopathy
		Decreased tumorigenesis	Cancer rarity
Prx1	Preferential progerin binding Down-regulated interaction with Tenascin-C	Defective ECM Altered skeletogenesis	See above: in Wnt Bone abnormalities
		VSMCs pathology	Vasculopathy, CVD, atherosclerosis
MEOX2/GAX	Up-regulated	VSMCs pathology	Vasculopathy, CVD, atherosclerosis
TWIST 2	Down-regulated	Impaired osteogenesis	Bone abnormalities

CVD, cardiovascular disease; ECM, extra cellular matrix; HC, heterochromatin; HGPS, Hutchinson–Gilford progeria syndrome; ING1, inhibitor-of-growth-protein1; VSMCs, vascular smooth muscle cells.

fibroblast proliferation by its effects on PP2A, which promotes pRb and Smads 2/3 dephosphorylation (Van Berlo *et al.*, 2005). Lamins A also associates with MAN1 (Mansharamani & Wilson, 2005), which also regulates Smads phosphorylation and sequestration (Lin *et al.*, 2005; Pan *et al.*, 2005; Van Berlo *et al.*, 2005; Ishimura *et al.*, 2006; Cohen *et al.*, 2007). Disruption of these interactions might lead to uncontrolled fibroblasts proliferation and fibrosis, thus contributing to HGPS-related sclerotic skin abnormalities and atherosclerotic CVD (Blobe *et al.*, 2000; Merideth *et al.*, 2008). Notably, the prominent adventitial fibrosis is a major characteristic of the 'atypical' HGPS atherosclerotic CVD along with primary loss of medial VSMCs, deposition of progerin and changes in the composition of the extracellular matrix (Merideth *et al.*, 2008; Olive *et al.*, 2010).

Runx2

pRb also affects osteogenesis and bone development by binding and potentiating Runx2, which is involved in the development and maintenance of bone and cartilage (Thomas *et al.*, 2001). Lamins A silencing impairs osteoblastogenesis indicating that it is critical for bone development (Akter *et al.*, 2009; Rauner *et al.*, 2009). Nevertheless, direct LA–Runx2 interaction has not been documented

SREBP

Aberrant interaction of progerin with TFs can also account for impaired adipogenesis normally controlled by the SREBP1- and PPARγ transcriptional pathways (Capanni *et al.*, 2005; Siersbaek *et al.*, 2010; Duband-Goulet *et al.*, 2011). Nevertheless, SREBP1 binds with high affinity to prelamin A, as well as to progerin resulting in SREBP1 sequestration at the nuclear envelope with concomitant reduction in its intranuclear availability and thus in reduced transcriptional activity of its target genes. In addition, PPARγ is down-regulated and adipocyte differentiation is inhibited in cultured pre-adipocytes (Capanni *et al.*, 2005; Maraldi *et al.*, 2008). As PPARγ also has anti-inflammatory and anti-atherosclerotic activities (Takano & Komuro, 2009), its suppression might enhance CVD in HGPS patients.

Inhibitor-of-growth-protein1

The inhibitor-of-growth-protein1 (ING1) is a member of the ING family of proteins. The ING proteins play a key role in cell-cycle progression, apoptosis, cell aging, and DDR. Inhibitor-of-growth-protein1 functions in the nucleus as an epigenetic regulator by binding to epigenetic determinants mostly on histone H3 and to either histone acetylase or deacetylase complexes (Soliman & Riabowol, 2007). Inhibitor-of-growth-protein1 binds to the N-terminal region of LA rod domain (amino acids 1–406) (Han *et al.*, 2008). The inability of progerin to bind ING1 at physiological levels suggests that the tail region of lamins profoundly affects the ability of the rod domain to interact with ING1. Lamins A association stabilizes ING1 and targets it to the nucleus (Han *et al.*, 2008). In cells, where ING1-LA association is abrogated, ING1 protein levels are markedly reduced, and it is aberrantly sequestered in the cytoplasm. Re-expression of LA in LA-null cells restored ING1 expression and nuclear localization (Han *et al.*, 2008). A model has been proposed according to which tethering by LA has a dual function: it stabilizes ING1 as well as spatially regulates its epigenetic activity (Soliman & Riabowol, 2007; Han *et al.*, 2008). The reduced ING1-LA association in HGPS cells can help to explain the loss of peripheral chromatin in these cells. Nevertheless, the precise role of LA-ING1 association in cellular senescence, heterochromatin formation, and its linkage to different HGPS phenotypes remains largely unknown.

Altered regulation of adult stem cells in HGPS

Recent studies suggest that aberrant regulation of adult stem cell likely affects premature aging in HGPS. Lamins A associates with the Notch co-activator SKIP, regulates SKIP availability and Notch transcriptional activity (Scaffidi & Misteli, 2008). Progerin has a reduced affinity to SKIP leading to an increase in its intranuclear availability and activation of downstream effectors of Notch, which in turn alter the differentiation pathways of mesenchymal stem cells. Indeed, Notch pathway components are markedly overexpressed in several mesenchymal cell line isolated from HGPS patients, while progerin interference with the Notch signaling pathway inhibits mesenchymal stem cell differentiation (Scaffidi & Misteli, 2008). The aberrant regulation of the Notch signaling pathway could account for the phenotypes of mesoderm-derived adipose, bone, and vascular smooth muscle tissues in HGPS (Rosen & MacDougald, 2006; Gridley, 2007; Canalis, 2008; Kurpinski *et al.*, 2010; Andersson *et al.*, 2011).

The Wnt/β-catenin pathway regulates mesenchymal stem cell proliferation and differentiation with specific roles in skeleton and cartilage development (Day *et al.*, 2005; Chen *et al.*, 2008). In a mouse progeria model, the extracellular matrix (ECM) of adult mice is defective and does not support fibroblasts cell proliferation. The resulting cell senescence is due to the reduced nuclear localization and transcriptional activity of the Wnt-signaling target Lef1, a phenomenon that is also observed in fibroblasts derived from HGPS patients (Hernandez *et al.*, 2010). Microarray studies on HGPS fibroblasts and mesenchymal stem cells confirm profound misregulation of ECM genes expression (Csoka *et al.*, 2004; Scaffidi & Misteli, 2008; Zhang *et al.* 2011). The gene expression alterations in HGPS fibroblasts suggest excess of ECM deposition, resulting from increased expression of ECM components and decreased expression of ECM-remodeling enzymes (Csoka *et al.*, 2004). These findings support the hypothesis that defective ECM triggers the skeletal, dental, skin, and vascular abnormalities in HGPS (Csoka *et al.*, 2004; Hernandez *et al.*, 2010). Importantly, since inappropriate activation of the Wnt pathway has been related to cancer (Klaus & Birchmeier, 2008), its down-regulation in HGPS might contribute to cancer rarity despite accelerated aging.

Compromised DNA damage repair in HGPS cells

Hutchinson-Gilford progeria syndrome cells accumulate DNA double-strand breaks (DSBs) and show a compromised DDR attributed mainly to progerin expression, lack of functional LA, and ROS accumulation (Musich & Zou, 2011; Richards *et al.*, 2011). The LA roles in chromatin organization and its interactions with histone H2A, emerin, ING1, and PCNA as described previously help explain its involvement in the DDR (Mattout-Drubezki & Gruenbaum, 2003; Shumaker *et al.*, 2006; Han *et al.*, 2008).

It has been shown that in LA-null cells, the loss of LA induces transcriptional up-regulation of the cysteine protease Cathepsin L (CTSL). This observation suggests a direct or indirect repression of CTSL by LA (Gonzalez-Suarez *et al.*, 2011). In addition, up-regulation of CTSL was also reported in mouse model of progeria (Varela *et al.*, 2008). By mediating transcriptional regulation of the CTSL, LA directly regulates the levels of major nonhomologous end joining and homologous recombination repair proteins such as 53BP1 and the p130/E2F4 repressor complexes, which in turn, regulate the transcription of the DNA repair proteins RAD51 and BRCA1 (Gonzalez-Suarez *et al.*, 2011; Redwood *et al.*, 2011). Notably, LA also stabilizes 53BP1 by preventing its proteolysis (Gonzalez-Suarez *et al.*, 2009). Indeed, cell lines that express progerin or lack LA activity display impaired DDR pathways (Liu *et al.*, 2005; Manju *et al.*, 2006; Gonzalez-Suarez *et al.*, 2011; Redwood *et al.*, 2011). Progerin further enhances DSBs accumulation and impaired DDR by its aberrant interaction with PCNA and lack of interaction with ING1, which leads to peripheral sequestration of several replication and DNA repair factors and stalled replication forks that collapse into DSBs (Musich & Zou, 2009, 2011). In addition, the progerin-mediated loss of various NuRD subunits further compromises the DDR by perturbing NuRD roles in recruitment of DNA repair proteins, and in promoting transcriptional repression, which facilitates the repair process (Lai & Wade, 2011). In conclusion, progerin-mediated DNA damage accumulation and compromised DDR bear direct relevance to HGPS-related premature aging and deregulated transcription. Additionally, as DDR goes awry, the resultant persistent damage signaling activates p53, thus promoting senescence and tumor suppression (Collado *et al.*, 2007).

Cancer in HGPS

Hutchinson–Gilford progeria syndrome is characterized by cancer rarity (Hennekam, 2006; Burtner & Kennedy, 2010). It is possible

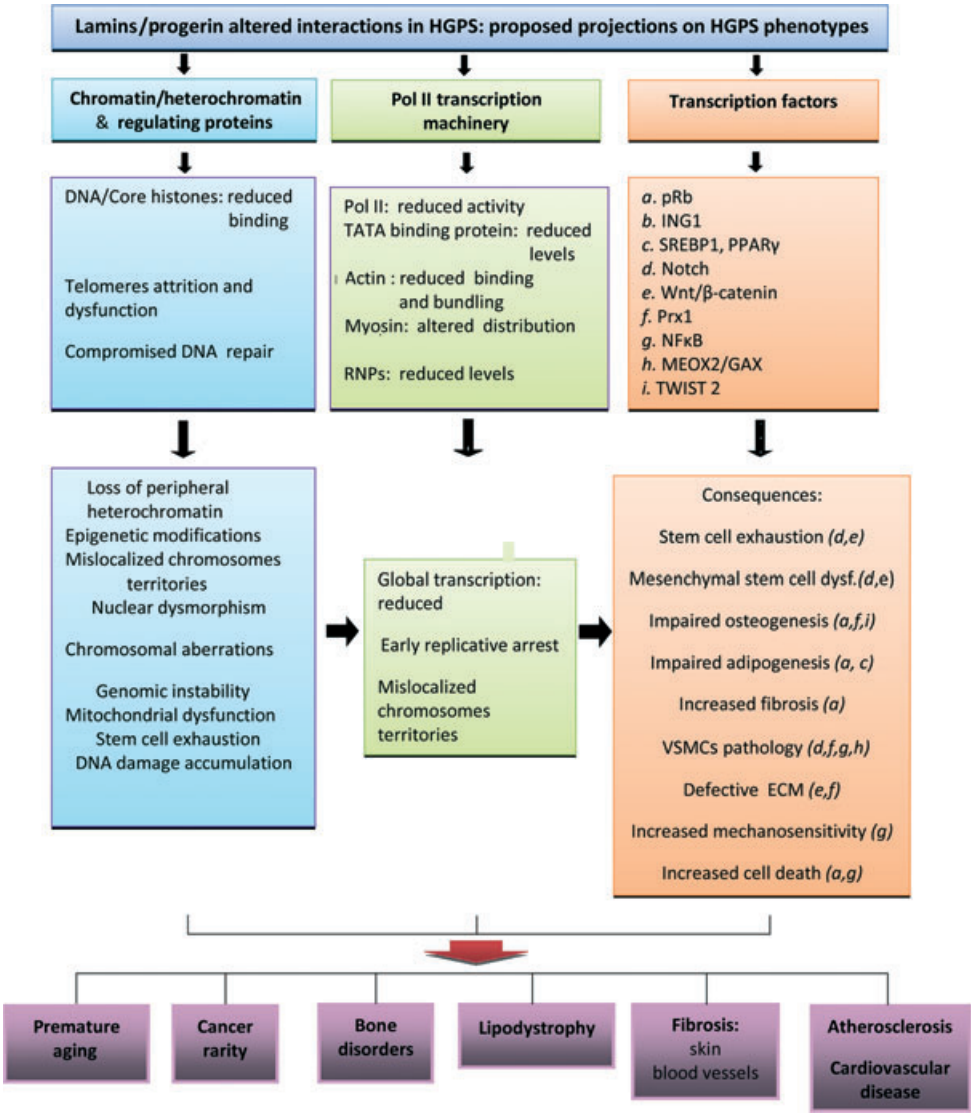

Fig. 2 A scheme depicting the altered interactions and the related cellular effects of lamins and progerin in HGPS cells, and the projection on major HGPS phenotypes. VSMCs, vascular smooth muscle cells; ECM, extracellular matrix**;** actin: nuclear actin; myosin: nuclear myosin; RNPs, ribonucleoproteins. The letters in parenthesis in the box titled 'consequences' refer to the lettered transcription factors in the box above it, whose dysfunctions may contribute to the cellular effects.

that cancer predisposition is masked by the short lifespan of the patients. Nevertheless, a unique constellation of several factors might explain the divergent HGPS phenotypes of premature aging and cancer rarity.

The potential pro-cancerous effects of DNA damage accumulation and compromised DNA repair might be counteracted by the HGPS-associated p53 activation, short telomeres, and the specific profile of histone modifications (Ljungman & Lane 2004; Campisi & d'Adda di Fagagna, 2007). Interestingly, the two cases of cancer reported thus far in HGPS patients refer to osteosarcoma (King *et al.*, 1978; Shalev *et al.*, 2007), which is known, in its genetic as well as its sporadic occurrence, to be related mainly to p53 or pRb dysfunction (Lerner & Antman, 2012).

Concluding remarks

The data reviewed hereby support the concept that the lamins-associated transcriptional regulation in HGPS cells is impaired and can explain major HGPS phenotypes (Fig. 2). Additionally, it may explain the absence of primary neuronal degeneration, the rarity of cancer, and the various differences from atherosclerotic CVD. The proposed concept integrates suitably with current molecular aging theories (Blagosklonny *et al.*, 2010) and indicates a pivotal role for p53 in HGPS as it promotes senescence/aging, suppresses tumorigenesis and activates autophagy through its negative regulation of the mTOR pathway.

Transcriptional deregulation in HGPS appears to be closely related to lack of normal functional LA, LB1 silencing, and disruption of lamins network integrity. It seems to play a major role in HGPS pathogenesis, adding thus to progerin insults. Moreover, in addition to current therapies, aimed to eliminate progerin and inhibit its farnesylation, there might be a place for therapeutic interventions addressing specific alterations in TFs-controlled pathways and their downstream effects. Such potential therapeutic agents include rapamycin (inhibits mTORC1 pathway), tyrosine-kinase inhibitors (block fibrosis via TGFβ signaling), statins (activate PPARγ), and ROS

scavengers (decrease levels of unrepaired DSBs which interfere with transcription).

The famous saying 'inside every old person is a young person wondering what happened' is tragically true in HGPS in view of the speedy progression of devastating aging-phenotypes and fatal outcome. Identifying NL-associated transcriptional deregulation as a possible molecular mechanism underlying HGPS phenotypes might help understand some of the disease's mysteries, and offer an intriguing concept for future research.

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