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COMMENTARY

Dedifferentiation rescues senescence of progeria cells but only while pluripotent

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Abstract

Hutchinson-Gilford progeria syndrome (HGPS) is a genetic disease in which children develop pathologies associated with old age. HGPS is caused by a mutation in the *LMNA* gene, resulting in the formation of a dominant negative form of the intermediate filament, nuclear structural protein lamin A, termed progerin. Expression of progerin alters the nuclear architecture and heterochromatin, affecting cell cycle progression and genomic stability. Two groups recently reported the successful generation and characterization of induced pluripotent stem cells (iPSCs) from HGPS fibroblasts. Remarkably, progerin expression and senescence phenotypes are lost in iPSCs but not in differentiated progeny. These new HGPS iPSCs are valuable for characterizing the role of progerin in driving HGPS and aging and for screening therapeutic strategies to prevent or delay cell senescence.

Hutchinson-Gilford progeria syndrome (HGPS) is an autosomal dominant disease characterized by early onset of several pathologies associated with old age, including arteriosclerosis, strokes, loss of subcutaneous fat, and alopecia. However, patients with HGPS do not develop other pathologies associated with aging (such as neurodegeneration), suggesting that the pathophysiology is limited to certain cell lineages, particularly those of mesenchymal origin [1]. The disease is caused by a mutation in the *LMNA* gene encoding the intermediate filament protein lamin A/C, which is critical for nuclear architecture of differentiated cells. The lamins also are important for genome organization, tethering chromatin to the nuclear envelope to help dictate domains of heterochromatin. The HGPS mutation activates a cryptic splice donor site, resulting in synthesis of a dominant negative, incompletely processed form of lamin A, termed progerin [2]. The expression of progerin alters nuclear structure and heterochromatin, affecting cell cycle progression, gene expression, and genomic stability. Progerin is hypothesized to promote sequestration of DNA repair and replication proteins, resulting in a more frequent stalling of replication forks and thereby replication-dependent double-strand breaks [3]. Progerin is farnesylated on its C-terminus, leading to concentration of the truncated protein at the nuclear periphery and leading to rigidity of the normally dynamic nuclear lamina [4], the structure meshwork lining the nuclear membrane. Currently, inhibitors of farnesylation are the only available strategy for treating HGPS but are incompletely effective because they are nonspecific.

Interestingly, the cryptic splice site in *LMNA* is sporadically used in cells from normal individuals, leading to a low-level expression of progerin [5]. Furthermore, fibroblasts from individuals who are more than 80 years old show nuclear abnormalities and changes in heterochromatin markers typical of cells from adolescent patients with HGPS. Thus, progerin may contribute to both accelerated and normal aging. However, establishing a direct causal role for progerin (or anything else) in aging is particularly challenging.

Progerin also leads to activation of NOTCH, a crucial regulator of stem cell differentiation [6]. Thus, constitutive expression of progerin in human mesenchymal stem cells (MSCs) induces aberrant expression of differentiation markers [6]. This supports the notion that HGPS is caused by dysfunction of adult stem cell populations (in particular mesenchymal progenitors), resulting in stem cell exhaustion. But this raises the question of whether patients with HGPS will ever be amenable to autologous stem cell therapy to delay their degenerative symptoms.

The recent development of technology to reprogram somatic differentiated cells into induced pluripotent stem cells (iPSCs) allows the generation of stem cells from virtually anyone, including patients with rare genetic diseases that lead to premature stem cell exhaustion [7]. iPSCs, which proliferate extensively in culture, can be

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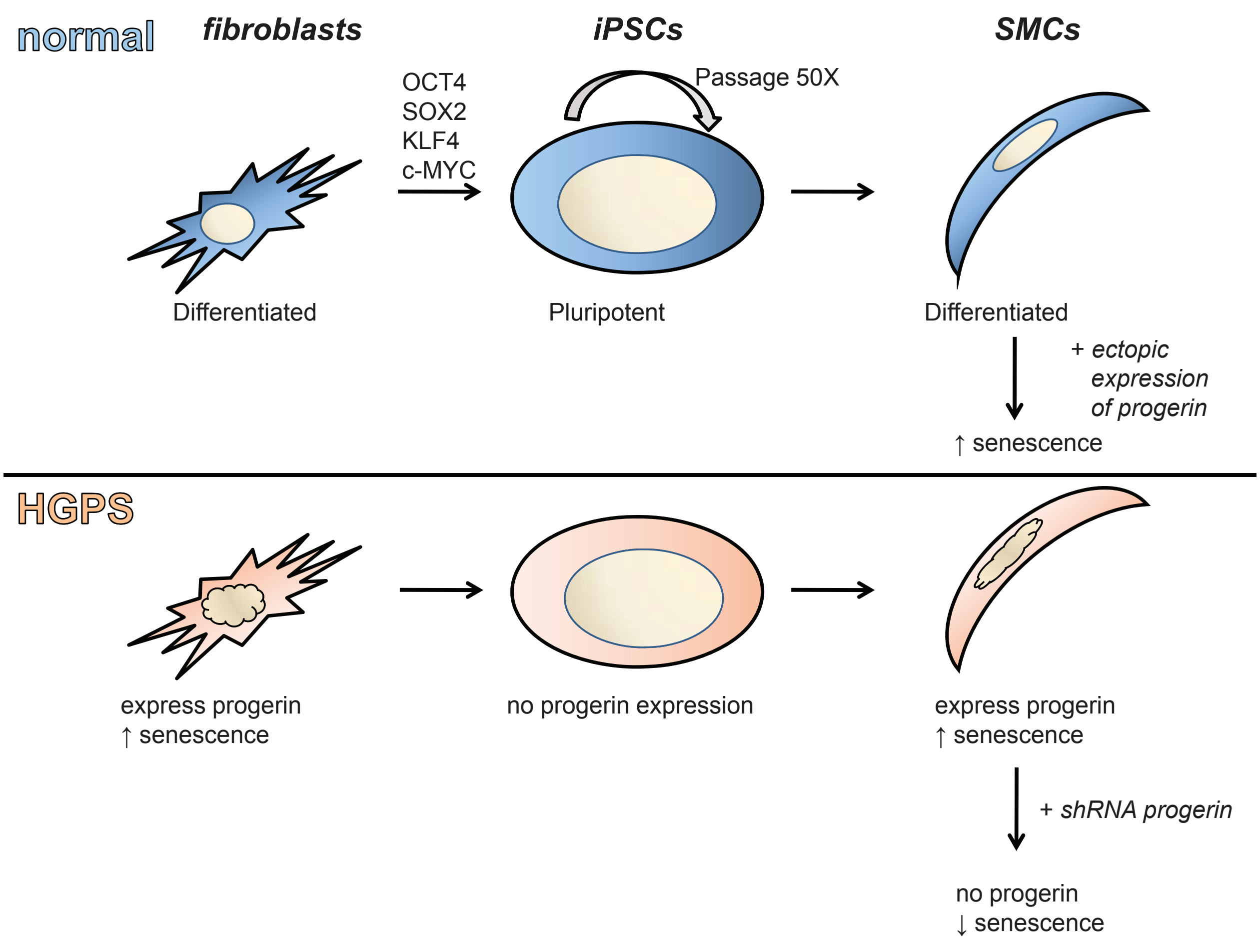

Figure 1. Dedifferentiation of fibroblasts into pluripotent stem cells rescues senescence phenotypes of Hutchinson-Gilford progeria syndrome (HGPS) human cells. Normal (in blue) human fibroblasts can be dedifferentiated into induced pluripotent stem cells (iPSCs) by transduction with retroviruses encoding the transcription factors OCT4, SOX2, and c-MYC plus the transcriptional regulator KLF4. iPSCs can be expanded while maintaining their pluripotent state (passage 50×) or can be induced to differentiate into five different lineages, including smooth muscle cells (SMCs). Fibroblasts from HGPS patients (in orange) express increased levels of progerin and have numerous senescence phenotypes (decreased proliferation, abnormal nuclear morphology, and altered expression of heterochromatin markers) in comparison with normal fibroblasts. Conversion of these cells to iPSCs attenuates expression of progerin and senescence phenotypes so that HGPS iPSCs can be serially passaged and remain pluripotent. However, when induced to differentiate into SMCs, these iPSCs re-express progerin and undergo premature senescence. If progerin expression is knocked down in the HGPS iPSC-derived SMCs, senescence is again attenuated. Conversely, ectopic expression of progerin in SMCs derived from normal iPSCs induces senescence. This establishes that progerin drives senescence of differentiated mesenchymal cells. shRNA, short hairpin RNA.

used to examine the pathogenesis of disease by differentiating cells into specific lineages. For example, motor neurons differentiated from iPSCs produced from patients with spinal muscular atrophy are smaller, have impaired pre-synaptic maturation, and degenerate more rapidly than motor neurons produced from normal iPSCs [8]. Disease-specific iPSCs are ideal for screening drugs that improve production of fully functional differentiated cells. In addition, the iPSCs can be forcibly differentiated into specific types of progenitor cells, such as MSCs, of therapeutic value.

To study the biology of HGPS, two groups [9,10] recently generated iPSCs from fibroblasts of patients with HGPS by transduction with retroviral vectors encoding OCT4, SOX2, KLF4, and c-MYC. HGPS fibroblasts express progerin and have altered nuclear morphology, reduced proliferation, and loss of heterochromatin markers, relative to their normal counterparts. Interestingly, iPSCs could be generated from early-passage, but not late-passage, HGPS fibroblasts [9] and were generated with less efficiency from HGPS fibroblasts compared with normal fibroblasts [10], suggesting that progerin-positive fibroblasts from normal, aged individuals may pose a challenge to dedifferentiation. The HGPS iPSCs lost expression of progerin and had morphology, prolifera-tion, and heterochromatic markers similar to those of normal iPSCs (Figure 1). Thus, the defects associated with HGPS are lost in pluripotent iPSCs.

However, upon differentiation of the HGPS iPSCs toward embryoid bodies, progerin was again upregulated. Differentiation of the HGPS iPSCs toward smooth muscle cells (SMCs) resulted in altered nuclear morphology, loss of certain heterochromatic markers, and premature senescence. Knockdown of progerin in HGPS iPSCs by using short hairpin RNA (shRNA) attenuated replicative senescence. In contrast, expression of progerin in primary human SMCs induced nuclear abnormalities while attenuating proliferation. These data strongly support the conclusion that progerin is directly responsible for the cellular senescence phenotypes associated with HGPS. Furthermore, this demonstrates the utility of iPSCs for discriminating between factors that drive a specific phenotype (in this case, cell senescence and accelerated aging) as compared with being a passive biomarker or consequence of change.

Zhang and colleagues [10] examined the differentiation capacity of HGPS iPSCs in detail, confirming the multipotency of these cells. MSCs and vascular smooth muscle cells (VSMCs) derived from HGPS iPSCs are sensitive to stress such as substratum deprivation, serum starvation, electrical stimulation, and hypoxia. Moreover, in contrast to normal MSCs, MSCs derived from the HGPS iPSCs are unable to repair ischemic muscle damage caused by ligation of the femoral artery. Interestingly, neural-derived progenitor cells derived from HGPS iPSCs expressed a lower level of progerin in comparison with VSMCs and MSCs. This is consistent with the lack of neurodegeneration in patients with HGPS and the fact that most symptoms in HGPS are related to defects in tissues of mesenchymal origin.

These bodies of work illustrate the utility of iPSCs for identifying mechanisms of pathology caused by inherited mutations because of the potentially reversible expression of mutant protein in differentiated and dedifferentiated cells. In this case, the authors demonstrate that the mutant form of lamin A, progerin, drives senescence of differentiated, but not pluripotent, cells in HGPS. In addition, HGPS iPSCs offer a unique reagent for characterization of the effects of progerin on cellular differentiation, nuclear morphology, epigenetic regulation of gene expression, genomic instability, stem cell function, and cellular senescence. This is applicable not only to patients with HGPS but also to normal, older individuals.

Indeed, the HGPS iPSCs have already been used to provide insight into differences in expression of progerin between cell lineages and response of cells to stress. The HGPS iPSCs can also be used to identify therapies that affect the expression, splicing, farnesylation, and function of progerin or to correct nuclear lamina fluidity by screening for drugs that correct differentiation defects. Correction of the HGPS mutation by homologous recombination or knockdown of the dominant progerin by using shRNA could give rise to progenitor cell populations able to treat some of the pathologies associated with HGPS. Importantly, given the possible role of progerin in natural aging, the development of strategies to reduce the level or activity of progerin could be applied to treating degeneration associated with aging in the general population.

Abbreviations
HGPS, Hutchinson-Gilford progeria syndrome; iPSC, induced pluripotent stem cell; MSC, mesenchymal stem cell; shRNA, short hairpin RNA; SMC, smooth muscle cell; VSMC, vascular smooth muscle cell.

Competing interests
JCG is a stockholder of Diamyd Medical AB, a public pharmaceutical company in Stockholm, Sweden. The other authors declare that they have no competing interests.

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