

mice are born with very long telomeres—much longer than human telomeres—mouse telomeres do suffer extensive shortening associated with aging (Flores et al., 2008). In particular, while mouse cells maintain relatively long telomeres during their first year of life, there is a dramatic loss of telomeric sequences at 2 years of age, even in various stem cell populations, and this change is concomitant with the loss of regenerative capacity associated with mouse aging. In addition, telomerase-deficient mice from the first generation (G1Terc^{-/-}) exhibit a significant decrease in median and maximum longevity and a higher incidence of age-related pathologies and stem cell dysfunction compared with wild-type mice (Flores et al., 2005; Garcia-Cao et al., 2006), indicating that, as in humans, telomerase activity is rate limiting for natural mouse longevity and aging. These results suggest that strategies aimed to increase telomerase activity may delay natural mouse aging. Further supporting this notion, it was recently shown that overexpression of TERT in the context of mice engineered to be cancer resistant owe to increase

expression of tumor suppressor genes (Sp53/Sp16/SARF/TgTERT mice) was sufficient to decrease telomere damage with age, delay aging, and increase median longevity by 40% (Tomas-Loba et al., 2008). However, it remains to be seen whether telomerase reactivation late in life would be sufficient to delay natural mouse aging and extend mouse longevity without increasing cancer incidence.

In summary, these proof-of-principle studies using genetically modified mice are likely to encourage the development of targeted therapeutic strategies based on reactivation of telomerase function. Indeed, small molecule telomerase activators have been reported recently and have demonstrated some preliminary health-span beneficial effects in humans (Harley et al., 2010). Identifying drugable targets and candidate activators clearly opens a new window for the treatment of age-associated degenerative diseases.

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HGPS-Derived iPSCs For The Ages

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DOI 10.1016/j.stem.2010.12.014

In this issue of *Cell Stem Cell*, Zhang et al. (2011) generate patient-derived iPSCs for one of the major premature aging diseases, Hutchinson-Gilford Progeria Syndrome (HGPS). These cells are a much-needed new tool to study HGPS, and their use may lead to novel insights into mechanisms of aging.

Some problems in biology are more difficult to study than others. Human aging is certainly one of them. Most conclusions regarding molecular mechanism of human aging rely on mere correlation, and direct experimental testing is generally not feasible. One approach to dissect the molecular basis of human aging is to study naturally occurring premature aging disorders. One of the most dramatic and prominent of such

diseases is Hutchinson-Gilford Progeria Syndrome (HGPS). Zhang et al. (2011) now report the generation of induced pluripotent stem cells (iPSCs) from HGPS cells, providing a powerful new tool to unravel the molecular and physiological mechanisms of premature and normal aging.

HGPS is a truly remarkable disease in many ways. To start with, it affects an unusually wide spectrum of tissues and

leads to the development of highly diverse symptoms ranging from depletion of subcutaneous fat to loss of hair and tendon contractures. The diversity of affected tissues pointed early on to stem cell defects as a likely disease mechanism. Most relevant in patients are vascular defects and recurring strokes, which invariably are fatal in patients in their mid- to late teens (Hennekam, 2006). The disease is exceedingly rare

with only about 200 patients in the world at any time, making access to relevant tissues very difficult. HGPS is also remarkable in how much we know about its molecular and cellular basis. HGPS is caused by a mutation in the *LMNA* gene encoding the intermediate filament proteins lamin A and C, key architectural components of the cell nucleus and both involved in higher-order genome organization (Worman et al., 2010). The disease mutation leads to activation of a cryptic splice site in *LMNA* and the production of a dominant gain-of-function isoform of lamin A, referred to as progerin. This protein is permanently farnesylated at its C terminus and accumulates in the nuclear lamina, where it disrupts normal lamina function.

Progerin is not only relevant to HGPS, but also to normal aging, because the cryptic splice site which creates progerin is also used at low frequency in healthy individuals and progerin can be found in normal tissues (Scaffidi and Misteli, 2006). Further parallels between HGPS and normal aging are suggested, given that several cellular defects such as loss of epigenetic marks and increased DNA damage are observed in both settings. In addition, HGPS patients and normally aged individuals exhibit similar vascular defects. Due to the rarity of the disease and the fragility of the patients it is difficult, however, to obtain relevant biological materials for molecular analysis, and much of what we know about the disease's mechanisms comes from cultured skin cells and animal models. The generation of HGPS-derived iPSCs now reported by Zhang et al. (2011) now provides a much needed source for tissue-specific cell lines with which to probe the effect of progerin on tissue function and differentiation.

The HGPS-derived iPSCs were generated from patient skin fibroblasts using the standard Yamanaka method (Zhang et al., 2011). The derived cells appeared pluripotent since they form teratomas and exhibit gene expression profiles akin to established human embryonic stem cell (hESC) lines. Interestingly, though, the efficiency of iPSC generation from HGPS patient cells was lower than from wild-type control cells. This might be due, as the authors suggest, to early onset of senescence in HGPS cells, but it might also have something to do with

an inhibitory role of progerin on the large-scale chromatin reorganization required during reprogramming. We know that lamins tether chromatin to the periphery and clamp it down into heterochromatin and that progerin solidifies the normally dynamic nuclear lamina (Dahl et al., 2006). ESCs are one of few human cell types that do not express lamins A and C, and at the same time, they lack heterochromatin, possibly as a means to maintain broad genome plasticity. It is conceivable that the presence of progerin in HGPS cells prevents the dynamic reorganization of chromatin required for efficient reprogramming.

The derivation of HGPS-iPSCs is of significant practical importance. The described cells are able to differentiate into five lineages, including vascular smooth muscle cells (VSMCs) and mesenchymal stem cells (MSCs) (Zhang et al., 2011), confirming their multipotency. These cells now offer a useful experimental system to probe the effect of progerin on the differentiation of various cell lineages, something that could not be done before because of the inability to obtain tissue samples from patients. These cells also open the door to performing critical experiments, such as transplantation of HGPS-derived MSCs into the vasculature of animal models to probe the physiological mechanisms that participate in the vascular defects experienced by HGPS patients.

The HGPS-iPSCs, and their derivatives, will also be useful for drug discovery. At present, the only clinical strategy for HGPS is farnesyltransferase inhibitors (FTIs), which prevent the addition of the C-terminal farnesyl group on progerin (Capell and Collins, 2006). While FTIs have been shown to reverse cellular phenotypes and have a positive effect on vasculature and on extension of lifespan in animal models, the nonspecific nature of the drug might become limiting in clinical applications. Lineage-differentiated cell lines derived from HGPS-iPSCs will provide ample and well-controlled biological materials for the search of novel drugs in high-throughput screens.

Although the HGPS-derived iPSCs appear to differentiate normally *in vitro*, they are functionally compromised, providing some insights into disease mechanism (Zhang et al., 2011). HGPS-iPSC-derived cells are hypersensitive to various

forms of stress. Survival of HGPS-iPSC-derived VSMCs was significantly reduced under hypoxic conditions or when subjected to extended electrical stimulation. The latter is potentially relevant to their pathological function because VSMCs undergo extensive mechanical stress *in vivo* due to the pulsing of the vasculature, and the reduced survival and proliferation observed *in vitro* may suggest increased cell death in the vasculature of HGPS patients. HGPS-iPSC-derived MSCs were also functionally compromised *in vivo*. When transplanted into an ischemic hind-limb muscle, they were unable to prevent necrosis, whereas MSCs derived in parallel from control iPSCs did. This failure may be due to the inability of HGPS-derived MSCs to replace vascular cells that are removed due to their normal turnover and/or the poor survival of these cells in the hypoxic environment of the muscle. Although it remains unclear why exactly the HGPS-iPSC-derived MSCs failed to rescue these defects, it is tempting to consider that MSC transplantation may offer a novel therapeutic option for HGPS. An intriguing, albeit distant, goal may be the generation of patient-derived MSCs in which the *LMNA* mutation has been corrected using recombination-based approaches.

These observations on muscle regeneration are also directly relevant to our thinking about normal aging. Loss of regeneration capacity has become a prevailing, albeit quite obvious, model for aging (Sharpless and DePinho, 2007). If tissue cells, and particularly stem cells, which are lost from a tissue due to normal turnover, are not replaced efficiently, tissues will, of course, deteriorate. It appears that in the case of HGPS, and likely in normal aging, tissue stem cells become increasingly unable to keep up with regeneration of lost tissue cells. This pattern may arise for several reasons. Tissue stem cell numbers may be reduced due to increased apoptosis, in the case of HGPS possibly due to their inability to cope with stress, for example, under hypoxic conditions in tissues. In addition, tissue stem cells might fail to self-renew, or they may produce fewer and functionally impaired offspring. The HGPS-derived iPSCs should be useful in further resolving the relevance of these various pathways to organismal aging.

HGPS is an extraordinary disease, and the generation of patient-derived iPSCs is a significant milestone. This step continues the remarkable progress made in the last few years. After discovery of the disease-causing gene in 2003, it only took four years to initiate several clinical trials. Much has been learnt along the way about the biology of HGPS and its relevance to normal aging. The generation of iPSCs from HGPS patients now heralds another wave of rapid progress with

implications for HGPS disease mechanisms, for aging in general, and potentially as a tool to develop novel strategies to combat vascular disease.

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A Roundabout Way to the Niche

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DOI 10.1016/j.stem.2010.12.011

A new player in hematopoietic stem cell (HSC)-niche interactions is introduced in this issue of *Cell Stem Cell*. Smith-Berdan et al. (2010) demonstrate that Robo4 is involved in HSC engraftment and mobilization and does so in cooperation with Cxcr4 to guide stem cells to and secure them in the niche.

Bone marrow (BM) transplantation has been used for treatment of hematopoietic disorders for some fifty years and represents a paradigm for all future stem cell therapies. A number of cytokines, especially granulocyte colony-stimulating factor (G-CSF), are known to mobilize hematopoietic stem and progenitor cells (HSPCs) from their BM niches into the peripheral blood (PB) (Papayannopoulou and Scadden, 2008). Indeed, mobilization is the preferred method for obtaining transplantable HSC. Despite the number of currently available HSPC mobilizing agents, a significant number of donors mobilize poorly. Therefore, identifying novel and more efficient mobilization approaches is of paramount clinical importance.

Understanding the molecular framework of how the niche regulates retention and release of stem cells provides the ground on which to base alternative mobilization strategies. The basic processes of transplantation are homing to, engraftment in, and retention of HSCs in the niche. Mobilization may thus be under-

stood as the process of breaking the bonds of stem cell retention in the BM niche or enhancement of the existing means that allow HSCs to enter the PB. The cellular milieu and molecular mechanisms that mediate these processes are starting to be revealed but, at best, remain poorly understood (Garrett and Emerson, 2009). The Cxcr4/Cxcl12 axis has been identified as critically important in homing, engraftment, and retention in the BM (Lapidot et al., 2005). Previous work has shown that the Cxcr4 antagonist AMD3100 can mobilize both mouse and human HSPCs and has found use clinically as an adjunct therapy for poor G-CSF mobilizers (Broxmeyer et al., 2005). In this issue of *Cell Stem Cell*, Smith-Berdan et al. show that Roundabout 4 (Robo4), a neuronal guidance molecule, regulates engraftment and mobilization and, in cooperation with Cxcr4, localizes HSCs to the niche.

Previous profiling studies by the senior author had revealed that Robo4 was expressed at high levels in long-term HSCs (Forsberg et al., 2005). In the present

work, the authors show that Robo4 becomes downregulated upon differentiation, consistent with the observations of Shibata et al., who also demonstrated that repopulating cells segregated to the Robo4⁺ fraction of HSPCs (Shibata et al., 2009). Notably, Smith-Berdan et al. also found that Robo4 expression was dramatically downregulated in mobilized HSCs. To determine a functional role for Robo4 in HSCs, the authors investigated Robo4 knockout mice. Robo4^{-/-} mice appear normal but have defects in vascular integrity and angiogenesis (Jones et al., 2008). An analysis of the stem cell compartments revealed that Robo4^{-/-} mice had a specific decrease of HSCs in the BM with a reciprocal increase in PB, suggesting poor BM retention. Upon transplantation, Robo4^{-/-} HSCs engrafted poorly, but those that did engraft contributed to a normal spectrum of blood cell lineages. In addition, the ability of Robo4^{-/-} HSC to make spleen colonies was normal, suggesting that the engraftment defect was likely because of a specific impairment of