Stem Cell Models: A Guide to Understand and Mitigate Aging?

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A ging · Reprogramming · Stem cells · Progeria

Abstract
Aging is studied either on a systemic level using life span and health span of animal models, or on the cellular level using replicative life span of yeast or mammalian cells. While useful in identifying general and conserved pathways of aging, both approaches provide only limited information about cell-type specific causes and mechanisms of aging. Stem cells are the regenerative units of multicellular life, and stem cell aging might be a major cause for organismal aging. Using the examples of hematopoietic stem cell aging and human pluripotent stem cell models, we propose that stem cell models of aging are valuable for studying tissuespecific causes and mechanisms of aging and can provide unique insights into the mammalian aging process that may be inaccessible in simple model organisms.

Aging and death, although despised and feared, have been taken as an inevitable part of life. However, there are forms of life that are potentially immortal. Deep-sea sponges were found to be several thousand years old, as revealed by analysis of their skeletal elements. Based on mortality observed over several years, the fresh-water cnidarian Hydra was calculated to have a life span of more than thousand years, with many scientists considering them immortal. Interestingly, this species can reproduce both asexually and, under stress, sexually. It is generally the former mode of reproduction that is associated with extremely long life span [1]. Therefore, evolutionary selection for fitness is not incompatible with very long life span [2].

Thanks to a short life span and availability of great genetic tools, the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* have been instrumental in understanding the molecular mechanisms underlying the aging process, such as insulin/IGF or mTOR signaling [3]. However, these models have several caveats that are important to consider. Although *C. elegans* and *D. melanogaster* genomes are highly homologous to the human genome (40 and 60%, respectively) and a subset of the aging pathways are known to be conserved in mammals, both species belong to a phylum with a significant divergence from the common ancestor with humans, indicating the existence of genes and pathways critical to human physiology that are not conserved. It is almost certain that there exist pathways that affect human and mammalian aging but are inaccessible using invertebrates. Additionally, worms and flies likely have pathways leading to life span extension that are not conserved in mammals. Despite their great utility in aging research, there is a need for complementary aging mammalian models to complete our understanding of the aging process.

One of the main limitations of *C. elegans* and *D. melanogaster* is that their somatic adult tissues have limited regenerative capabilities associated with a very small or nonexistent stem cell population. Even still, evidence exists that this stem cell population may be important for organismal aging, at least in flies, as interventions that enhance intestinal stem

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cell regeneration are sufficient to extend the life span of the organism [4]. This is likely only the tip of the iceberg, however, and in most tissues these models cannot capture the role of stem cell-mediated tissue regeneration in aging. This may be particularly important when it comes to complex diseases associated with aging, such as cancer or type 2 diabetes [3, 5].

Despite remarkable successes in extending the life span of invertebrate and mammalian models by genetic and pharmacological interventions, and the identification of central longevity pathways, we still do not know which specific molecular process(es) trigger the increase in mortality risk we define as aging, and important questions remain to be answered. Do somatic DNA mutations lead to epigenetic alterations, or is age-related loss of heterochromatin rendering DNA more susceptible for damage [6]? Do systemic changes, such as age-related hormonal or immunologic changes, result in functional decline of parts of the system [7], Or is it cellular aging that drives systemic changes [8] ? Does each tissue age the same way, or are there tissue-specific mechanisms of aging? Most importantly, are there aspects of aging that can be reversed?

The purpose of this short Viewpoint is to illustrate the reasons why stem cell models of aging likely can provide answers to these questions that may be inaccessible using nonvertebrate models. First, stem cells maintain tissue function by replacing damaged cells. Highly regenerative multicellular organisms such as hydra avoid aging with the help of pluripotent stem cells [2]. Most mammalian tissues harbor a pool of stem cells that serve to maintain tissue function and homeostasis throughout life. It is thus conceivable that aging of a given tissue is in part driven by the exhaustion of its stem cell pool, and preventing stem cell aging would keep the tissue young and might result in overall life span extension, as is the case with fly intestinal stem cells [4]. Comparing adult stem cell function from young versus aged organisms provides clues about which specific processes might drive stem cell aging. Second, breakthroughs in reprogramming somatic cells to pluripotency enable us to model aging of various tissues in the dish, within a shorter time than mouse models, with an isogenic background, and in humans.

Hematopoietic Stem Cell Transplantation Assays: Cell-Extrinsic versus Cell-Intrinsic Aging

Most mammalian tissues harbor a small population of stem cells that maintain tissue function throughout life. A decreased regenerative capability is one of the most obvious changes with mammalian aging, suggesting that adult stem cells experience functional decline. It is unclear whether this is due to cell-intrinsic processes, such as accumulating macromolecular damage, or cell-extrinsic changes, such as cross-linking of extracellular matrix molecules, defects of the neighboring niche cells, or changes of systemic hormone and cytokine levels, or a combination of both. Potential consequences are stem cell death or senescence, a failure to differentiate properly in response to external stimuli, and malignant transformation.

The scarcity of adult stem cells is a major challenge in identifying the causes for stem cell aging. Nevertheless, studies on adult stem cell aging, especially aging of hematopoietic stem cells (HSC), have provided important information on how the aging The scarcity of adult stem cells is a major challenge in identifying the causes for stem cell aging. Nevertheless, studies on adult stem cell aging, especially aging of hematopoietic stem cells (HSC), have provided important information on how the aging process affects stem cell function, the consequences for the respective tissue and

the organism, and the interventions that might prevent age-related decline (f ig. 1).

Along with intestinal epithelium, blood is one of the most rapidly turned-over tissues. For instance, red blood cells are replaced within 4 months and neutrophils within 1–5 days [9] . Age-related functional decline of hematopoietic cells results in a decreased immune response, anemia and a markedly increased incidence of myeloproliferative disorders [10]. Thanks to bone marrow transplantation (BMT), the first stem cell therapy ever used, the hematopoietic system is among the best-described tissues to date. Virtually all levels and branches of the hematopoietic differentiation tree, with the primitive HSC at its root, can be identified by a distinct set of surface markers [11, 12]. This relatively well-characterized system to study blood regeneration has served as an excellent model for adult stem cell function.

The great advantage of HSC over other adult stem cell models is that stem cell functions can be studied by transplantation assays in vivo [11], making it possible to discern between cell-intrinsic and cell-extrinsic mechanisms of aging. In mice, serial transplantation can be done for at least five times, suggesting that, in a lifetime, HSC function is not limited by replication per se [13] . It should be noted that recent studies have called into question to what extent repopulation of differentiated species in the hematopoietic lineage arise directly from HSC or existing progenitor cells. However, HSC undoubtedly do age: donor age is the most important predictor for therapeutic success of BMT. Higher donor age increases the risk for failed engraftment and graft- versus-host disease, and decreases 5-year survival [14]. Similarly, transplantation of physiologically aged HSC into young mice results in poorer engraftment, early onset of bone marrow failure and myeloid malignancies [15, 16]. While, counterintuitively, HSC numbers increase with aging, aged HSC exhibit poor engraftment and a differentiation defect: lymphoid differentiation decreases in favor of myeloid differentiation (myeloid skewing) [17] . Another feature of the aging HSC population is clonality, whereby a limited number of HSC increasingly dominate the pool and downstream lineage with age [18]. The reasons for this remain unclear, but are likely to provide important insights into the mechanisms driving aging in this context.

Exemplifying the power of the HSC model of aging, several cell-autonomous inducers of HSC aging have been identified, and reversing some of them resulted in phenotypical and functional rejuvenation of HSC. First, aged HSC were observed to suffer increased replication stress caused by decreased expression of MCM DNA helicases; induction of replication stress in young HSC by pharmaceutical means, or by knocking down MCM DNA helicases mimics functional HSC aging [16]. Furthermore, it has been found that a switch from canonical to noncanonical Wnt signaling leads to a loss of cell polarity by activation of the RhoGTPase Cdc42; pharmacological inhibition of Cdc42, or inhibition of upstream noncanonical Wnt signaling, restores polarity and rejuvenates aged HSC [10, 19]. Interestingly, despite telomere attrition that accompanies HSC aging and serial transplantation, telomerase overexpression was not found to prevent HSC aging in serial transplantation assays [20].

While cell-intrinsic mechanisms of HSC aging have been elucidated by transplanting aged HSC into young animals, the HSC microenvironment, or niche, was also found to drive HSC aging. By expressing HSC ligands and secreting cytokines, a complex network of stroma cells, mesenchymal stem cells (MSC), endothelial cells, osteoblasts, immune cells, and sympathetic nerve endings directs HSC quiescence, self-

renewal, differentiation, and mobilization into peripheral blood [21], and its regulatory circuits are far from fully understood. In order to determine the role of an aged environment, young HSC were transplanted into old recipient animals. Interestingly, myeloid skewing, at least in part, is caused by extrinsic factors, i.e. proinflammatory factors and subsequent mTOR activation [15, 22]. mTOR inhibition by systemic rapamycin treatment, which is a robust way to extend life span in mice [23], ameliorates myeloid skewing and increases repopulation efficiency of HSC [24]. Furthermore, loss of regulatory MSC as a consequence of damage to neurons innervating the bone marrow drives and maintains myeloproliferative disorders; restoring regulation circuits by neuroprotective or sympathomimetic drugs blocks proliferation of leukemic stem cells [25, 26].

With the HSC environment being pivotal in regulating HSC function, it comes as no surprise that, so far, no significant life span extension has been achieved by transplanting young HSC [27]. Interestingly, restoring bone health by transplanting young MSC increased mouse life span significantly [28]. It remains to be determined if restoration of a functional HSC niche and, consequently, improved HSC function play into this life span-extending effect.

In conclusion, HSC transplantation assays elucidated mechanisms of adult stem cell aging and provided ways to rejuvenate blood by reversing HSC aging. Importantly, research needs to be directed at understanding aging in other adult stem cell populations to a depth similar to that known for HSCs. A more dramatic way to rejuvenate not only HSC but also other somatic cells may be reprogramming.

Almost 10 years ago, using transient overexpression of only four transcription factors, the Yamanaka lab succeeded in reprogramming mouse and human fibroblasts to embryonic-like, or induced pluripotent, stem cells (iPSC) [29] . By vast remodeling of the epigenetic landscape, reprogrammed somatic cells reactivate telomerase, remodel mitochondria, switch from oxidative phosphorylation to glycolysis, and restore transcription profiles so that they are almost indistinguishable from embryonic stem cells [30] .

Since this seminal discovery, somatic cells from a variety of tissues and species have been reprogrammed to iPSC. Interestingly, the more differentiated the donor cell is, the harder it is to reprogram [31]. This suggests that, in order to obtain reprogrammed, pluripotent stem cells, most of the epigenetic memory needs to be erased, and that somatic stem cells, which are easier to reprogram, are indeed younger on the developmental axis [32].

Thinking about the relevance of reprogramming for aging, a major question is whether aged cells are more difficult to convert to the pluripotent state. Aging is accompanied by global DNA hypomethylation, hypermethylation of CpG islands and a loss of heterochromatin. In *Saccharomyces cerevisiae*, *C. elegans* and *Drosophila*, restoration of heterochromatin extends life span [33–35]. iPSC seem to ‘remember’ their tissue of origin by DNA methylation [36]; therefore, do they also remember their age? Due to the low reprogramming efficiency and high variability among iPSC clones generated from the same donor cell, results obtained so far are inconclusive. In addition, a low reprogramming efficiency may obfuscate the effects of aging as there is always the possibility of small set of nonaged cells

simulate aging of any tissue of interest by introducing factors that are hypothesized to cause aging, and comparing the outcome with what we see in physiological aging (fig. 2). Thus far, mainly mutations known to induce diseases of accelerated aging have been utilized to model physiological aging.

To give an example, Parkinson's disease (PD) was modeled by ectopic progerin expression in patient iPSCderived dopaminergic neurons. Progerin is a truncated form of the nuclear structure protein lamin A. It is expressed in physiologically aged tissues, and induces premature aging-like phenotypes in Hutchinson-Gilford progeria syndrome (HGPS). HGPS patients display features of aging, such as osteolysis, lipodystrophy, alopecia and severe arteriosclerosis, starting at the age of 2 or 3 years, and typically succumb to cardiovascular disease in their second decade. By overexpressing progerin, dopaminergic neurons generated from familial PD patient-iPSC expressed neuromelanin, a factor exclusively expressed in old neurons, and mimicked the cellular disease phenotype such as dendrite degeneration and Lewy-body inclusions. Without progerin expression, despite the presence of the PD-inducing mutation, dopaminergic neurons did not show a comparable PD phenotype [39] . While in HGPS, progerin levels are very low in neurons and the nervous system is not affected, in this study, ectopic progerin expression serves to mimic the aging process and provides researchers with a cellular model for drug screening. The extent to which this 'in vitro aging with progerin approach is more broadly applicable remains to be determined.

Furthermore, iPSC generated from HGPS fibroblasts may help to study tissue-specific effects of (accelerated) aging. HGPS mainly affects mesodermal tissues, such as adipose tissue, bone, and vasculature, either for extrinsic reasons (mesodermal tissues are

within an aged population that give rise to the isolated iPS cells.

A number of studies observed decreased A number of studies observed decreased old donors; once generated, some iPSC lines were functionally unaffected, while others displayed signatures of aging [37]. One recent study reprogrammed young and aged mouse HSC and did not observe any functional differences in hematopoiesis in mice generated from old or young cells. This suggests that, at least for HSCs, genetic, i.e. irreversible effects of aging, play a minor role, and aging can be reversed [37, 38]. In order to give a decisive answer if aging can be reprogrammed, improvements in reprogramming efficiency and reproducibility are needed.

iPSC as a Stem Cell Model for Aging

The fact that somatic cells can be converted to a pluripotent state suggests that, merely by rebuilding the epigenetic landscape, any cell can go back in time. Moreover, iPSC technology opens new avenues to study diseases, and aging itself: with human cells, in a tissue-specific manner, and with an isogenic background. Currently,
a skin biopsy or a blood sample is sufficient to generate a potentially indefinite supply of functional, differentiated cells of any kind, from one donor.

Pluripotent stem cells have already been used to model genetic diseases such as amyotrophic lateral sclerosis and more complex diseases like autism-related disorders in order to identify molecular disease mechanisms and screen for drugs. Since, developmentally speaking, iPSC and iPSC-derived differentiated cells are in a fetal stage, as revealed by transcriptional profiling, studying late-onset diseases or even aging using iPSC models challenge us to simulate the aging process [30]. At the same time, and in analogy to what has been done with HSC, it raises the possibility to

more exposed to mechanical stress), or for tissue-specific, cell-intrinsic reasons (MSC, the progenitors for mesodermal tissues, express the highest levels of progerin). iPSC derived from HGPS fibroblasts were found to be functionally indistinguishable from their control counterparts. This is consistent with the observation that embryonic stem cells do not express lamin A. Upon differentiation, progerin was expressed in a tissue-specific manner. Consistent with mesodermal tissue being predominantly affected in HGPS, MSC and vascular smooth muscle cells (VSMC) expressed the highest progerin levels, followed by fibroblasts, endothelial cells, and neurons. High progerin levels correlated with a loss of MSC regenerative capability, and VSMC mortality under mechanical stress [40, 41] . While studies using cell culture models and mouse models of HGPS pointed in this direction, these results are intriguing evidence for a cell-autonomous mechanism of (premature) aging, and in addition, provide a model to study the molecular mechanism of how progerin affects cellular functions in different tissues.

One premature aging disease linked to multiple potential aging pathways, including dysfunctional DNA repair, epigenetic changes and telomere dynamics, is Werner syndrome (WS), or adult progeria. The disease is caused by a loss-of-function mutation of the DNA helicase WRN. Symptoms include bilateral cataracts, lipodystrophy, osteoporosis and arteriosclerosis in early adulthood. In contrast to HGPS, there is a high rate of cancer, diabetes mellitus, and autoimmunity and brain atrophy have been observed. Causes of death are predominantly cardiovascular disease, malignancy, and bacterial infection. Interestingly, WRN expression has been found to decrease with physiological aging. Similar to HGPS, mainly mesodermal tissues are affected. To study the effects of WS in a tissue-specific way, Belmonte and coworkers [42] introduced the WS mutation into normal hESC before differentiating them into different cell types. As for HGPS-iPSC, WS-hESC are functionally normal. However, MSC differentiated from WS-hESC undergo premature senescence, increased DNA damage response, telomere attrition and loss of heterochromatin. Using this model, WRN protein was found to be crucial in stabilizing heterochromatin, and restoring heterochromatin partly restored the function of WS MSC. These results suggest that, both in WS and in normal cells from mesodermal tissues, loss of heterochromatin might have a major role in promoting aging.

Conclusions

We have described examples of how studies of adult and pluripotent stem cells are being used to model and study the mammalian aging process. While this remains a relatively nascent field, with the exception of HSC studies that are more mature due to the long history of human BMT, it has already demonstrated great promise.

While a full exploration would be beyond the scope of this text, data from the last decade have suggested that lessons in HSC are at least partly applicable to other stem cell populations. For instance, adult muscle stem cells (or satellite cells) age in a manner that is both cell in- and extrinsic, as do neuronal stem cells [43]. Importantly, the identification of common niche aging factors may be vital in the development of intervention strategies, as serum factors promoting aging in multiple tissues may be modifiable.

Certainly, the relative lack of adult stem cells in nonvertebrate model organisms is a limitation, and new short-lived vertebrate models could provide valuable insights, combining the ability to interrogate adult

stem cell function and organismal aging in the same context. The African Killifish, for example, offers great promise in this context, with a very rapid life span. Further development of this model organism is of high importance for the field of aging.

Given the dramatic rise of the 'over 65' population globally and the accompanying epidemic of associated chronic diseases, understanding the human aging process at the mechanistic level and developing interventions to slow aging and extend health span may be the most important medical challenge of this century. Studying functional changes in adult stem cell populations with aging and utilizing stem cell reprogramming to model aging in vitro are vital approaches to be further explored as a means to address these critical questions. Of course, stem cells have their limitations, and ultimately the path to a holistic understanding of again will involve integration of hypotheses from a wide range of animal models and direct studies in humans to the extent that is possible.

Disclosure Statement

The authors declare no conflict of interest.

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