

Defining the General Principles of Stem Cell Aging: Lessons from Organismal Models

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Published online: 23 June 2015
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Abstract One of the most obvious characteristics of the aging process is the progressive decline in the regenerative potential of tissues. Adult somatic stem cells are critical for rejuvenating tissues and persist throughout the lifespan of organisms. However, stem cell function declines during the aging process in tissues such as the brain, blood, skin, intestinal epithelium, bone, and skeletal muscle. This demise may contribute to tissue degeneration, organismal aging, and age-related diseases. A series of organismal models have emerged as valuable systems to study stem cell aging in vivo. Here, we review the age-associated changes of stem cells and the different organismal models used to define stem cell aging.

Keywords Aging · *C. elegans* · *D. melanogaster* · Germline stem cells · Organismal models · Somatic stem cells

This article is part of the Topical Collection on *Age-related Stem Cell Modifiers*

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Introduction

Organismal aging is associated to a progressive imbalance in tissue and organ homeostasis and a timely decline in their regeneration capacity, which is accompanied by the loss of physiological integrity that, in turn, results in susceptibility to death. Stem cell exhaustion has been defined as one of the hallmarks of aging and determines the tissue repair ability of organisms [1••]. Stem cells are divided into two groups: embryonic stem cells (ESCs) and adult stem cells. ESCs are pluripotent and are found in early stage of embryos. Since ESCs do not undergo replicative senescence, they are considered to be immortal in culture [2, 3]. Adult organisms have two types of stem cells: (1) adult somatic stem cells with regenerative potential, which are found in several tissues and (2) germline stem cells (GSCs), which can generate gametes for reproduction [4]. GSCs are designed to maintain an unlimited proliferative capacity to fulfill their biological purpose: to be passed from one generation to the next. Adult somatic stem cells are critical for rejuvenating tissues and persist in the adult body of an organism throughout its lifespan. Adult somatic stem cells reside in various adult tissues such as liver, brain, and bone marrow. In contrast to ESCs, they can only differentiate into a limited type of cells. Adult somatic stem cell function decreases with age, and this failure may contribute to age-related diseases [1••, 5, 6]. For instance, neural stem cells (NSCs) from older animals are less able to self-renew and more skewed toward astrocytes [7]. A decline in the proliferation of NSCs and neurogenesis during aging has been correlated with impairment of olfactory discrimination [8]. Notably, postnatally generated neurons are absent in advanced stages of Huntington's disease [9]. Another example is the decline of hematopoietic stem cell (HSCs) function with age. This failure could have a significant impact on organismal aging since decreased hematopoiesis with age results in a

diminished generation of adaptive cells and increased myeloid malignancies and anemia [6]. In this review, we will focus on age-associated changes of somatic and germline stem cells and the emerging models for the study of their aging in vivo.

In Vivo Models for Stem Cell Aging

A series of model organisms have started to reveal the general principles of stem cell aging (Fig. 1). These findings generally come from studies in the budding yeast (*Saccharomyces cerevisiae*), invertebrates (the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*), and rodents (Table 1). *S. cerevisiae* has been used to identify genes that modulate replicative lifespan [10], a process observed in many dividing cells such as adult stem cells. The yeast cell divides asymmetrically by forming a bud which gives rise to the daughter cell. As the cell wall of the daughter cell is formed de novo, the mother cell retains the same wall, evidenced by scars from each budding event on the cell wall surface. Accumulation of budding scars is a marker of replicative senescence. Ultimately, the mother cell is not able to reproduce anymore, and sterility is the final phenotype of yeast aging [10]. In addition, the asymmetric cell divisions invoked by the budding yeast resemble stem cell self-renewal. In yeast asymmetric cell division, the daughter cell inherits a limited amount of damaged macromolecules from the mother cell [11] enabling the generation of a rejuvenated, germ-like, daughter cell lineage. Thus, this phenomenon can be

Table 1 Example of model organisms for stem cell aging

Organism	Examples for in vivo studies
<i>S. cerevisiae</i>	Asymmetric cell division giving rise to rejuvenated daughter cell Model for germline stem cells Resemblance to adult stem cells Study of replicative lifespan Chronological lifespan inducible by dietary restriction Epigenetic changes in aging
<i>C. elegans</i>	Study of germline stem cells
<i>D. melanogaster</i>	Study of germline stem cells, somatic stem cells (mostly intestinal stem cells)
<i>M. musculus</i>	Study of several adult stem cell types (HSCs, NSCs, satellite cells, epidermal stem cells, cardiac stem cells, etc.) Modulation of specific stem cells by either their niche or other tissues in a non-autonomous manner Heterochronic parabiotic experiments to identify the role of the stem cell niches

considered a model for GSCs. One interesting example for successful use of yeast and its extrapolation to stem cell aging is the role of sirtuins, which are histone-deacetylating enzymes involved in gene silencing. The sirtuin family of proteins impinges upon replicative lifespan of yeast [12]. For example, Sir2 was shown to be involved in the stabilization of repetitive DNA in yeast. During aging and DNA damage, Sir2 specifically localizes to the sites of low genomic integrity

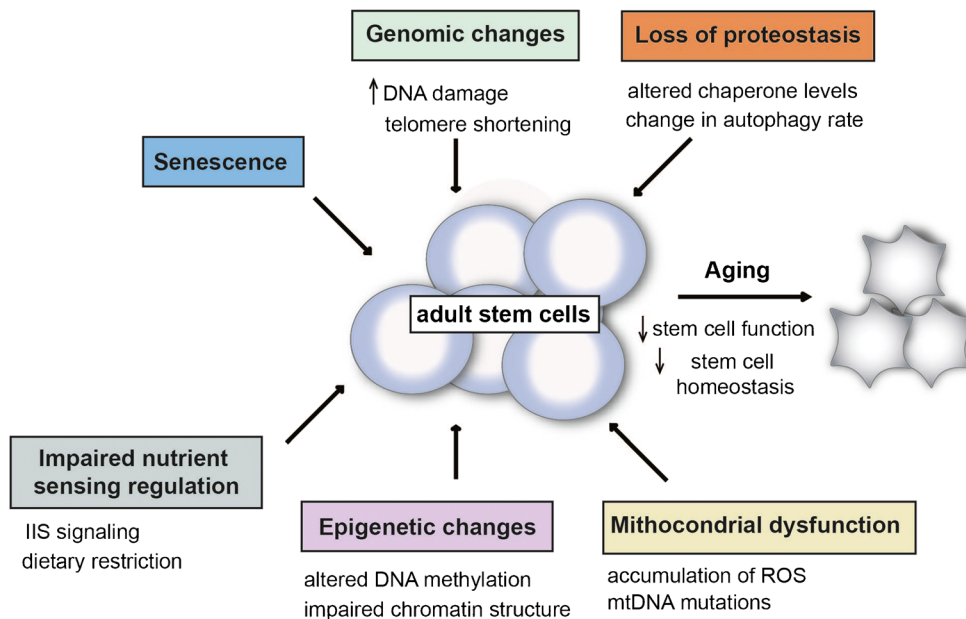


Fig. 1 The impact of aging on adult stem cells. Aging causes a decrease in stem cell functionality that leads to a decline in tissue rejuvenation and repair. Age-associated changes of stem cells include (1) genomic changes, which arise from increased DNA damage as well as a shortening of the telomeres, (2) loss of proteostasis through altered

chaperone levels and deregulation of autophagy rate which might lead to accumulation of damaged proteins, (3) epigenetic changes in methylation and chromatin-remodeling factors, (4) mitochondrial dysfunction as a consequence of ROS accumulation and mtDNA mutations, (5) cellular senescence, and (6) impaired nutrient sensing regulation

(genomic instability), and SIRT1, the mammalian orthologue of Sir2, may play a major role in age-dependent gene expression [13]. Thus, data from yeast have suggested a major role for epigenetic changes in aging. Finally, yeast can also be used as a model for chronological aging [14]. In these studies, yeast cells are induced into a non-dividing state by withdrawal of nutrients.

Metazoan models provide the means to study stem cell decline during organismal aging. *C. elegans* and *D. melanogaster* are established models for the study of GSCs. Fly and mouse models are the main organisms used for somatic stem cell aging research. *D. melanogaster* has been mostly used to study the intestinal stem cell (ISCs) population in the midgut, although other somatic stem cell compartments are starting to be identified. Mouse models are suitable for the study of a wide number of stem cell types. These models have helped to establish not only general principles of stem cell aging but also differences between the different somatic stem cells [8, 15]. Although these general age-related changes may induce a decline in stem cell function at some extent, they do not always result in a change in the stem cell number. For instance, the number of NSCs and melanocyte stem cells decreases with age in mammals. However, the number of HSCs increases with age in some mouse strains [16]; whereas, their functionality declines (e.g., HSCs from old mice are skewed to differentiate toward the myeloid lineage [17]). Metazoans also allow the study of the modulation of specific stem cells by either their niche or other tissues in a non-autonomous manner (i.e., through systemic signals in response to environmental and nutrient changes). Of special relevance are heterochronic parabiotic experiments in mice that demonstrated that aging of muscle stem cells (also known as satellite cells) is regulated by systemic factors. In this experimental paradigm, in which aged mice are surgically connected to young mice, muscle stem cells are rejuvenated by the exposure to a youthful environment [18].

Age-Associated Changes of Stem Cells

Somatic stem cell exhaustion is one of the hallmarks of aging [1••]. Besides stem cell exhaustion, López-Otín and colleagues have pointed out other hallmarks of aging (i.e., genomic instability, telomere shortening, epigenetic alterations, loss of proteostasis, mitochondrial dysfunction, cellular senescence, altered intercellular communication, impaired nutrient sensing regulation) [1••]. Somatic stem cells exhibit a series of these age-related changes that could trigger stem cell dysfunction/death and, in turn, a progressive decline in regeneration capacity [15] (Fig. 1). In this section, we discuss age-related cellular changes of somatic stem cells.

Genomic Instability and Telomere Attrition

Similar to other cell types, somatic stem cells also undergo both DNA damage and telomere shortening with age [6]. Markers of DNA damage have been observed in stem cell populations during aging [19, 20]. Increased DNA damage contributes to the decline in the self-renewal ability of both epidermal and melanocyte stem cells during the aging process [21, 22]. In addition to elevated DNA damage, a decrease in DNA damage response (DDR), which is crucial for repair mechanism, has also been reported in stem cells [23, 24]. Increased DNA damage in stem cells may result in their senescence and production of defective daughter cells [25, 26]. It has been shown that deletion of *Atm*, a DNA damage sensor, results in increased ROS levels and loss of quiescence in HSCs. Eventually, this defect causes depletion of the HSC pool [27].

Stem cells may be prone to telomere shortening due to their high self-renewal ability. Both human and mice studies have shown telomere shortening in stem cells during the aging process [28, 29]. The importance of telomere length in stem cell homeostasis has emerged especially in NSCs. In these cells, telomere shortening results in interrupted neuronal differentiation. Moreover, reduction of NSC function has been also observed in telomerase-deficient (*Terc*^{-/-}) mice, a deficiency that results in short telomeres [29].

Epigenetic Alterations

Epigenetic alterations (e.g., histone modifications such as acetylation, ubiquitination, and methylation) contribute to the aging process [1••]. Several studies have suggested an important role of epigenetic alterations in stem cell aging [30, 31]. However, the analysis of epigenomic changes requires a large amount of cells, which is not always feasible for somatic stem cells. Therefore, epigenetic research on stem cells usually depends on measurement of tissue compartments in which these cells are found.

One of the examples of epigenetic changes in stem cells indicates that DNA methylation levels increase in a site-specific manner in HSCs with age [30]. Chambers and colleagues [31] showed that epigenomic changes in aged stem cells are related with alterations in chromatin-modifying enzymes and their cofactors. Microarray data from HSCs from young and old mice have revealed a decrease in the expression levels of switch/sucrose nonfermentable (SWI/SNF) chromatin-remodeling complex subunits, specific histone deacetylases (HDAC1, 5, and 6), and a DNA methyltransferase (DNMT3b) [31]. In addition, age-dependent changes in histone modification could switch expression of self-renewal genes in HSCs [32•].

Loss of Proteostasis

The integrity of the proteome (proteostasis) declines in a progressive manner with age [1••]. During the aging process, cells accumulate damaged and misfolded proteins. These damaged proteins impair cell functionality and tissue homeostasis and, therefore, must be scavenged. The accumulation of damaged proteins with age is associated to a decline in the intracellular ability of maintaining proteostasis. Several nodes of the proteostasis network such as autophagy-lysosomal potential, proteasomal activity, and chaperone function decline during organismal aging [33–37]. This demise in the proteostasis network could also determine stem cell exhaustion (for extensive review, see [8]).

A series of cellular responses such as the heat shock (HSR) or the endoplasmic reticulum (UPR^{ER}) and mitochondrial (UPR^{mt}) unfolded protein responses are activated to maintain the quality of the proteome when aberrant proteins accumulate. These responses are essential mechanisms to ensure proper cytosolic protein folding and reduce proteotoxic stress. Activation of these mechanisms and increased levels of chaperones are associated with enhanced protection against proteotoxic stress [38]. However, their activity also decreases during the aging process [39, 40]. Supporting evidence suggests that chaperones such as heat shock proteins (HSPs) may play a role in stemness and differentiation. HSPA8 (a non-inducible HSP) negatively influences the stability of proapoptotic Bim mRNA, increasing HSCs survival and preventing their differentiation [41]. Bim is required for apoptosis during hematopoiesis. HSP70 indirectly triggers erythropoiesis by preventing caspase-3-mediated cleavage of GATA-1 [42], an essential transcriptional factor for differentiation and maturation within the erythroid lineage. Several components of the UPR^{ER} have a key role during differentiation. For instance, XBP1 induces osteogenic and plasma differentiation [43], IRE1 increases lymphopoiesis of B cells [44], and CHOP promotes differentiation of B cells, erythrocytes, osteocytes, and chondrocytes [45–48]. Moreover, the UPR^{ER}, as a stress-coordinated pathway, has an important role in the modulation of differentiation of mouse intestinal epithelial stem cells [49•]. The transition from stem cell to transit-amplifying cells of the intestine is accompanied by induced activity of the UPR^{ER}. Perk-eIF2 α induces endoplasmic reticulum stress that, in turn, promotes loss of stemness. In organoid cultures of primary intestinal epithelium, inhibition of Perk-eIF2 α leads to accumulation of stem cells [49•]. Taken together, these observations make it difficult to correlate high levels of HSPs or proteotoxicity protection with somatic stem cell differentiation, and further insights into the impact of these pathways on stem cell identity are necessary. In addition, it will be fascinating to define whether the levels of chaperones and/or the ability to activate proteotoxic cellular stress responses decrease in somatic stem cells with age. Notably, a

recent study has shown that a decline in the UPR^{mt} contributes to HSC aging [50]. Inactivation of SIR7, a regulator of the UPR^{mt}, reduces quiescence, increases mitochondrial protein folding stress, and compromises the regenerative potential of HSCs. Moreover, SIR7 expression is decreased in HSCs, whereas SIRT7 up-regulation enhances the regenerative potential of aged HSCs [50].

When misfolded proteins cannot be “rescued” by chaperones and stress responses, they are degraded through the proteasome and/or autophagy. Increased levels of proteasome components result in enhanced replicative lifespan and resistance to proteotoxic stress in yeast [51]. Although the impact of increased proteasome activity as a determinant of ESC function has been shown, whether somatic stem cells also have an enhanced proteasome activity remains to be elucidated. However, maintenance of proteasomal activity in somatic stem cells may critically impact organismal aging. On the other hand, more insights into the impact of autophagy in somatic stem cells have been shown. In vitro studies with somatic stem cells such as human mesenchymal stem cells (hMSCs) [52], HSCs, dermal stem cells (DSCs), and epidermal stem cells [53] suggest that autophagy activity is increased in these cells compared to their differentiated counterparts. In fetal and postnatal mouse HSCs, a deficiency in essential autophagy genes such as FIP200 or Atg7 increases ROS levels and deregulates proliferation, suggesting that autophagy is required for stemness in fetal and postnatal mouse HSCs [54–57]. Moreover, deletion of ATG7 triggers the depletion of HSC pools [56]. FoxO3, a forkhead transcription factor linked to both longevity and stem cell homeostasis [58], maintains the expression of pro-autophagy genes in adult mouse HSCs to allow a quick autophagic response upon stress [59••]. Notably, old mouse HSCs have higher basal levels of autophagy activity, a characteristic required for their cloning efficiency, and are able to induce autophagy like young HSCs. However, autophagy activity in young HSCs is not required for their cloning efficiency [59••]. This observation is controversial as previous data showed the opposite effect [54–57]. The difference might be that one study [59••] used a drug to block autophagy in normally developed adult mouse HSCs, whereas the latter study used a genetic model to block autophagy that causes severe defects early in life leading to death, thus looking at fetal/early stages. The higher levels of autophagy activity in old adult mouse HSCs were due to an attenuated nutrient (2-NBD glucose) uptake [59••]. That old adult mouse HSCs maintain an autophagy potential similar to young mouse HSCs and exhibit higher levels of autophagy for their survival confronts the traditional view where compromised autophagy is established as a determinant of aging [34].

In NSCs or cardiac stem cells (CSCs), autophagy activity increases upon their differentiation [60–62]. This enhanced autophagy might be due to a specific increase in the

requirements of their differentiated counterparts, such as neurons, to recycle their cellular components. During the initial period of neuronal differentiation (E15.5 mouse embryos), the expression of the autophagy genes *Atg7*, *Becn1*, *Ambra1*, and *LC3* are increased *in vivo* in the mouse embryonic olfactory bulb (OB) [60]. Blocking autophagy either genetically or chemically impairs NSCs and CSCs differentiation [60–62]. Inhibition of autophagy by wortmannin or 3-MA decreases neurogenesis of OB-derived stem cells [60]. In addition, *Ambra1* loss-of-function mice show decreased neural markers in the E13.5 OB [60]. However, *FIP200*, an essential component of autophagy, is required for NSC proliferation [63]. *Ex vivo* treatment of E8.5 mouse embryos with the autophagy activator rapamycin increases the expression of cardiomyocyte markers in the second heart field [61, 62]. Taken together, these observations suggest a higher degree of protection, at least, in somatic stem cells to cytotoxic stresses. Consistent with this idea, impairment of autophagy in epidermal stem cells, DSCs, and HSCs leads to increased susceptibility to cytotoxic stress such as etoposide, doxorubicin, or UV [53].

Accumulation of ROS and Mitochondrial Dysfunction

Several studies indicate that increased accumulation of ROS determines stem cell dysfunction and fate decision during the aging process [64–66]. In mice, the amount of HSCs with low ROS levels decrease in an age-dependent manner [67]. Furthermore, increased ROS levels result in disruption of proliferation and self-renewal of HSCs [68]. The treatment with *N*-acetyl-L-cysteine (NAC), an oxidative stress inhibitor, rescues the defects in HSC and NSC function in a progeroid mice model [69, 70].

Another well-characterized hallmark of aging is mitochondrial dysfunction [1••]. This demise can be caused by mitochondrial DNA mutation or changes in mitochondrial function such as alterations in nutrient sensing and energy metabolism [71]. mtDNA mutagenesis in mice affects HSCs during the fetal development stage. mtDNA mutations also affect the number of quiescent NSCs *in vivo* and their self-renewal ability *in vitro* [69]. Furthermore, HSCs exhibit a decrease in their capacity of nutrient uptake in an age-dependent manner [59••].

Senescence

Cellular senescence is defined as irreversible cell cycle arrest. Indirect evidence indicates that signaling pathways related with cellular senescence increase with age in somatic stem cells [15]. However, senescence of these cells has not been clearly shown. Notably, a mutant mouse strain deficient in *Klotho*, a protein involved in mineral homeostasis, shows senescence markers in stem cells of the gut and the skin [72]. These cells show foci of γ -H2AX staining, a hallmark of

senescence induced by DNA damage. Similarly, aged HSCs and muscle stem cells have increased levels of γ -H2AX foci [19, 20]. However, DNA damage could also lead to apoptosis. Therefore, it cannot be ruled out that these cells are undergoing programmed cell death.

Impaired Nutrient Sensing Regulation

The use of invertebrate models for aging research led to the discovery of several longevity pathways [37]. Among these signaling networks, we find evolutionary conserved pathways that have emerged as regulators of somatic stem cell function [8]. One of these pathways is the reduced insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS). IGF-1 is the secondary mediator of the somatotrophic axis in mammals, which is regulated by the growth hormone. The intracellular signaling pathway of IGF-1 is the same as the one triggered by insulin. The major downstream effector of IIS is the FOXO family of transcription factors. Notably, FOXOs regulate the functionality of somatic stem cells such as HSCs and NSCs. A combined deficiency of *FoxO1/FoxO3/FoxO4* depletes the HSC and NSC pools in mice [73, 74]. Deficiency of *FoxOs* increases ROS levels in both NSCs and HSCs [73, 74], which may increase protein misfolding. *FoxOs* protect from oxidative stress, promoting the expression of antioxidant enzymes [75]. *FoxO3* is essential for regulating this process in mice, since *FoxO3* deficiency alone increases ROS levels and depletes the pool of HSCs and NSCs [76, 77]. Notably, the loss of *FoxO3* causes increased neurogenesis during development followed by NSC depletion in adulthood of mice [76]. Interestingly, *FoxO3*-bound genes thoroughly overlap with those bound by the proneuronal bHLH transcription factor *ASCL/MASH1* in cultured neural progenitor cells [78]. *FoxO3* represses the expression of specific *ASCL1* neurogenic targets restraining neurogenesis. Therefore, *FoxO3* may contribute to maintain the homeostasis of the NSC pool by negatively regulating neurogenesis. In flies, IIS is also required for ISC proliferation and may regulate division of these cells during aging and tissue repair [79, 80]. Insulin-like peptides (dILP)-expressing cells in the brain modulate ISC proliferation and age-associated dysplasia [80]. Furthermore, dFOXO modulates the aging of muscle in flies [81, 82]. Circular muscle releases factors that induce ISC proliferation and, therefore, is considered a component of the ISC niche [83]. Thus, IIS may also modulate ISC function through their niche.

Dietary restriction (DR), defined as reduced food-intake without malnutrition, also extends lifespan in multiple species [36, 84]. The target of rapamycin (mTOR) is required for mediating the effects of DR on longevity. mTOR associates with other proteins to form two different complexes: mTORC1 and mTORC2. mTORC1 activity is inhibited by DR resulting in longevity [85]. Recent findings indicate a role of DR on stem cell proliferation. DR increases functionality of

mouse ISCs via a non-cell-autonomous mechanism acting through adjacent Paneth cells of the ISC niche. DR down-regulates mTORC1 activity in Paneth cells, but not in ISCs, eventually creating an environment where ISC function is enhanced [86]. Decreased activity of mTORC1 in Paneth cells up-regulates the levels of bone stromal antigen 1 (Bst-1), a protein that promotes cell proliferation in bone marrow. Regulation of Bst-1 levels by mTORC1 is required for the enhanced ISC function upon DR. Similarly, the regenerative potential of muscle stem (satellite) cells increases upon DR in both young and old mice [87]. The number of satellite cells per fibers is enhanced after DR treatment during 3 months. DR also increases neurogenesis [88], but the effects are not compatible for in vivo application since a high intake deprivation is necessary. Taken together, these data could help understand the pro-longevity effects of DR suggesting an impact on stem cell function.

Germline Stem Cells

Both *C. elegans* and *D. melanogaster* are well-established models for the study of GSCs. In the female germline stem cell niche of flies, the primary niche-associated factor decapentaplegic (DPP) promotes GSC self-renewal. Interestingly, GSCs themselves regulate the restriction of DPP function through activation of the epidermal growth factor receptor (EGFR) mitogen-activated protein kinase (MAPK) in the nearby somatic cells [89]. In aged flies, dILPs regulate the homeostasis and proliferation of GSCs through autonomous and non-autonomous mechanisms in both females and males [90–92]. Furthermore, IIS declines in the ovary from aged females [90]. Thus, age-associated changes in the levels of local or circulating dILPs may contribute to the decline in the number and functionality of GSCs. In addition, DR also enhances GSC maintenance [93].

Notably, signals from the GSCs regulate organismal lifespan in both worms and flies [94]. Genetic ablation of the germline, but not the entire gonad, leads to lifespan extension in worms [95]. This phenotype may be caused by increased proteome stability within the post-mitotic soma [96, 97]. Inhibiting germline proliferation induces an increase in the somatic levels of the proteasome subunit RPN-6 that, in turn, promotes proteasome activity and extends lifespan [96, 97]. Increased levels of RPN-6 are modulated by DAF-16, the worm FOXO orthologue [98, 99]. Extended lifespan induced by the lack of germline could be explained by the disposable soma theory of aging in reverse. Hypothetically, the need for repairing and preventing damage to the germline dominates resource allocation strategies, while the somatic tissues age and deteriorate [100]. Abrogation of the germline would stimulate a re-allocation of metabolic resources to the somatic tissues.

Conclusions

In this review, we have discussed the different general principles that might define adult stem cell aging. Besides the insights gained into the aging of these cells, the molecular mechanisms underlying stem cell exhaustion are still unknown. It will be fascinating to continue exploring the interconnectedness between stem cell exhaustion and the other candidate hallmarks of aging. Although cumulative evidence suggests that delaying age-associated changes in stem cells could have beneficial effects in the health and lifespan of an organism, more research in this field is needed to facilitate the development of specific adult stem-cell-based therapies. However, considering the links between enhanced stem cell proliferation and malignant cells, some detrimental effects of this approach should not be discarded. Additionally, it is important to remark the contribution of the decline of post-mitotic and progenitor cells to age-associated diseases and loss of tissue homeostasis. Thus, it is important to understand the interplay between stem, progenitor, and post-mitotic cells to develop efficient healthspan-promoting therapies.

Acknowledgments This work was supported by the Deutsche Forschungsgemeinschaft (DFG) (DFG Research Grant VI742/1-1), the University of Cologne Advanced Postdoc Grant, the Cologne Graduate School of Ageing Research, the Alexander von Humboldt Foundation, and the European Commission (FP7-PEOPLE-2013-CIG).

Compliance with Ethics Guidelines

Conflict of Interest The authors declare that they have no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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