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REVIEW

When stem cells grow old: phenotypes and mechanisms of stem cell aging

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ABSTRACT

All multicellular organisms undergo a decline in tissue and organ function as they age. An attractive theory is that a loss in stem cell number and/or activity over time causes this decline. In accordance with this theory, aging phenotypes have been described for stem cells of multiple tissues, including those of the hematopoietic system, intestine, muscle, brain, skin and germline. Here, we discuss recent advances in our understanding of why adult stem cells age and how this aging impacts diseases and lifespan. With this increased understanding, it is feasible to design and test interventions that delay stem cell aging and improve both health and lifespan.

KEY WORDS: Age-related diseases, Hematopoietic stem cells, Multicellular organisms

Introduction

Many tissues have an ability to grow and regenerate during normal physiology or in response to injury – a process made possible by resident stem cells. Adult stem cells are characterized by the ability to self-renew and differentiate into multiple cell types within a tissue. Although they are regarded as immortal, as they are not subject to replicative senescence, it is now appreciated that stem cells are nonetheless susceptible to damage accumulation. Their position at the base of cellular lineages makes their dysfunction potentially more impactful than that of other cell types (see Box 1). In tissues that continually regenerate, understanding stem cell aging is likely to be necessary if we are to understand aging at the organ level. In this Review, we discuss what is known about aging in some major stem cell populations: hematopoietic stem cells (HSCs), intestinal stem cells (ISCs), satellite cells of the skeletal muscle, neural stem cells (NSCs), skin stem cells and germline stem cells (GSCs). We also specifically consider what changes are known to occur in stem cell number and function with age, what aspects of stem cell behavior make them susceptible or resistant to aging, and to what degree a decline in stem cell function contributes to aging (Table 1).

The characteristics and consequences of stem cell aging

Hematopoietic stem cells

The best-characterized adult stem cells are those from the hematopoietic system. HSCs reside in the bone marrow in adult mammals and are responsible for blood formation. In early studies, bone marrow cells from young and old mice were found to display similar reconstitution abilities in transplantation assays (Harrison, 1973; Ogden and Micklem, 1976; Harrison et al., 1978). In fact, serial transplantation studies have shown that normal HSCs can sustain blood formation for multiple lifetimes (Harrison, 1979). Thus, a stark collapse of the hematological system does not occur during normal aging. However, when murine HSCs with long-term repopulating ability were isolated by selecting for c-Kit^{+}, lineage^{-} (multiple markers), Sca-1^{+} cells (KLS) (Ikuta and Weissman, 1992; Spangrude et al., 1988), the number of HSCs was found to steadily increase with age (de Haan et al., 1997; Morrison et al., 1996). Only when function was measured on a per-cell basis were old immunophenotypic HSCs shown to have a greatly reduced ability to engraft and properly differentiate in new hosts (Dykstra et al., 2011; Liang et al., 2005; Morrison et al., 1996). Furthermore, in both mice and humans, the proportion of differentiated blood cells arising from just a few HSC clones increases, suggesting that the number of active, functional HSCs declines with age (Beerman et al., 2010; Genovese et al., 2014; Jaiswal et al., 2014). This discrepancy between immunophenotypically and functionally defined HSCs has been interpreted as a compensatory increase in HSCs in response to declining function and a decline in their ability to differentiate.

Another defining characteristic of HSC aging is a skewed differentiation potential. Aged HSCs are more likely to differentiate towards the myeloid lineage at the expense of the lymphoid lineage (Sudo et al., 2000; Liang et al., 2005; Rossi et al., 2005). This discovery is fully consistent with the observation that the adaptive immune system (mediated in large part by the lymphoid system) declines with age (Linton and Dorshkind, 2004) and with the fact that acute lymphoblastic leukemia, a primarily juvenile disease, declines with age, whereas the incidence of acute myeloid leukemia increases with age (Lichtman and Rowe, 2004). The aging of HSCs might also contribute to moderate anemia observed in the elderly (Guralnik et al., 2004). In heterochronic cell transplantation

Box 1. The evolutionary basis for biological aging

When discussing the etiology of aging, one must be careful not to describe aging as a genetically programmed phenomenon on par with embryonic development or adult homeostasis. Theories in which aging is considered beneficial must invoke group selection and are therefore considered as evolutionarily unstable strategies. Furthermore, predation, starvation, injury and exposure eliminate most wild animals before they reach old age (Kirkwood, 2005). This fact does not preclude the existence of single genes or mechanisms that promote robustness and longevity, but they demand a different evolutionary framework. Two theories predominate. The first is 'antagonistic pleiotropy', the theory that genes that drive aging are selected because they provide an advantage early in life (Williams, 1957). The second is the 'disposable soma' theory, which posits that somatic maintenance is costly and can only be a strategy at the expense of growth and reproduction (Kirkwood, 1977). As such, species with a high degree of predation invest heavily in growth and reproduction at the expense of longevity. Consistent with these theories, many of the mechanisms that drive stem cell aging exist because they ostensibly confer health and survival benefits during development or youth but are deleterious later in life.

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Table 1. Characteristics of stem cell aging vary in cell abundance, behavior and the relative impact of intrinsic and extrinsic mechanisms

Stem cell population	Model	Change in frequency with age	Other aging phenotype	Mechanism
Hematopoietic stem cells (HSCs)	Mouse, human	Increase	Skewed differentiation	Intrinsic
Intestinal stem cells (ISCs)	Fly, mouse	Increase		Both
Satellite cells	Mouse	Decrease	Skewed differentiation	Both
Neuronal stem cells (NSCs)	Mouse, rat	Decrease		Extrinsic
Hair follicle stem cells	Mouse, rat	No change	Lengthened telogen phase	Both
Melanocyte stem cells	Mouse	Decrease		Extrinsic
Germline stem cells (GSCs)	Worm, fly, mouse, rat	Decrease		Extrinsic

experiments, old HSCs transferred to young hosts retain their aged phenotype (Rossi et al., 2005), supporting the notion that HSC aging is largely driven by cell-intrinsic mechanisms.

Intestinal stem cells

The rapid turnover of the gut epithelium is sustained by ISCs. Most of our knowledge about ISC aging comes from studies on *Drosophila*, where ISCs are easily identified by expression of the transcription factor escargot (Esg) and the Notch ligand delta (Dl). During aging, numbers of immunophenotypic fly ISCs increase several-fold with age, concomitant with a decline in function (Biteau et al., 2008; Choi et al., 2008). This increase in numbers is due to increased ISC proliferation, although it has been argued that the apparent increase in ISCs might also represent an accumulation of functionally differentiated cells that retain stem cell markers (Biteau et al., 2008). An increase in the number of fly ISCs is also induced by treatments that stress the gut, including bacterial infection and paraquat treatment, suggesting that environmental factors contribute to ISC aging (Biteau et al., 2008; Buchon et al., 2009; Choi et al., 2008; Guo et al., 2014; Hochmuth et al., 2011). In support of this hypothesis, ISC aging under normal conditions is associated with the activation of environmental stress response pathways, including the JNK (Biteau et al., 2008), p38-MAPK (Park et al., 2009) and PDGF/VEGF signaling pathways (Choi et al., 2008). Cell-intrinsic causes of ISC aging, such as mitochondrial dysfunction (Rera et al., 2011), could also be at play.

In mammals, two interconvertible populations of ISCs exist: proliferative Lgr5-expressing cells in the base of the crypt, and quiescent label-retaining cells a few positions above the crypt base (Takeda et al., 2011). Before these markers were known, irradiation experiments suggested that, while the intestine becomes more sensitive to damage with age, the total number of clone-forming units (a surrogate for estimating stem cell number) increases (Martin et al., 1998). Some have suggested that the aging of human ISCs contributes to the increase in colorectal cancer incidence with age (Merlos-Suárez et al., 2011; Patel et al., 2009), but this remains speculative because it not yet known how cells expressing mammalian ISC markers change in frequency and function with age.

Satellite cells

The regeneration of skeletal muscle fibers, for example, in response to injury, is driven by a small population of stem cells termed satellite cells (Beauchamp et al., 1999; Mauro, 1961; Sherwood et al., 2004). Unlike HSCs and ISCs, the frequency of satellite cells decreases with age (Brack et al., 2005; Collins et al., 2007; Gibson and Schultz, 1983). The *in vitro* proliferation rate and the *in vivo* engraftment and regeneration potential of satellite cells upon transplantation also decline with age (Bernet et al., 2014; Bortoli et al., 2003; Collins et al., 2007; Cosgrove et al., 2014; Sousa-Victor et al., 2014). Furthermore, like HSCs, aged satellite cells exhibit a skewed differentiation potential, whereby they differentiate towards a fibrogenic lineage rather than a myogenic lineage, largely because of changes in Wnt and TGF-β signaling (Brack et al., 2007; Carlson et al., 2009). It is generally agreed that a loss of satellite cell function contributes to the decrease in recovery from injury observed in the elderly (Cosgrove et al., 2014), but possibly not to sarcopenia, the age-related decrease in the size of muscle fibers (Fry et al., 2015).

There is a large body of data on the molecular mechanisms that underlie satellite cell aging. The heterochronic transplantation of satellite cells from old into young mice indicates that the mechanisms underlying changes in satellite cell regeneration potential are largely cell-extrinsic and include changes in the availability of Wnt, Notch, FGF and TGF-β-superfamily ligands (Brack et al., 2007; Carlson and Faulkner, 1989; Chakkalakal et al., 2012; Conboy et al., 2003, 2005; Sinha et al., 2014), and changes in cytokine signaling through the JAK-STAT pathway (Price et al., 2014). By contrast, the self-renewal defects appear to be cell-intrinsic: an increase in stress-induced p38-MAPK signaling is associated with satellite cell aging (Bernet et al., 2014; Cosgrove et al., 2014), along with an increase in cellular senescence (Cosgrove et al., 2014; Sousa-Victor et al., 2014) – changes that are not reversed after transplantation to a young environment.

Neural stem cells

Although most neurons are post-mitotic, slowly cycling NSCs sustain neurogenesis in specific regions of the mammalian brain during adulthood. Like satellite cells, NSCs decrease in number with age, which, in turn, contributes to decreased neurogenesis (Kuhn et al., 1996; Maslov et al., 2004). Unlike other stem cells, however, the *in vitro* function of aged NSCs on a per-cell basis is not substantially impaired with age (Ahlenius et al., 2009), which implies that cell-extrinsic factors are largely at play. Indeed, heterochronic parabiosis (the joining of the circulatory systems of two animals of different age) and restoring the levels of IGF-1, GH, Wnt3, TGF-β or GDF11 in old mice to those found in young mice improves neurogenesis (Blackmore et al., 2009; Katsimpardi et al., 2014; Lichtenwalner et al., 2001; Okamoto et al., 2011; Pineda et al., 2013; Villeda et al., 2014). An age-dependent change in the senescence of NSCs also contributes to their declining numbers (Molofsky et al., 2006; Nishino et al., 2008) and might underlie learning and memory deficits in the elderly (Zhao et al., 2008a).

Skin stem cells

The skin contains multiple types of stem cells, including hair follicle stem cells (HFSCs) that sustain hair growth and melanocyte stem cells that generate pigment-producing melanocytes. Hair follicles cycle through phases of growth, regression and rest (anagen, catagen and telogen, respectively). The most pronounced change during aging is an increase in the period of rest and, in some cases, a complete loss of hair growth (alopecia) (Keyes et al., 2013). Surprisingly, the frequency of HFSCs does not decline with age (Giangreco et al., 2008; Rittié et al., 2009). Instead, there is a clear loss of function that underlies the lengthening periods of dormancy.

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Consistent with this, aged HFSCs exhibit decreased colony formation ability *in vitro* (Doles et al., 2012; Keyes et al., 2013). The heterochronic transplantation of skin from old to young mice results in decreased telogen length, possibly because of increased levels of the bone morphogenetic protein (BMP) inhibitor follistatin, a factor that promotes entry into anagen (Chen et al., 2014). However, heterochronic parabiosis only modestly restores the colony-forming ability of aged HFSCs, suggesting that cell-intrinsic mechanisms are important. There are several possible mechanisms to explain why HFSC function declines during aging, including increased sensitivity to BMPs (inhibitors of anagen entry) (Keyes et al., 2013), increases in JAK-STAT signaling and a decline in Notch signaling (Doles et al., 2012).

In contrast to the stability of HFSC numbers with aging, the number of melanocyte stem cells in the skin declines dramatically with age. This decline is not due to apoptosis or senescence but rather is due to ectopic differentiation at the expense of self-renewal (Nishimura, 2011). Ionizing radiation has a similar effect on melanocyte stem cell fate decisions, suggesting that genotoxic stress may be the root cause of age-related hair greying (Inomata et al., 2009). These observations indicate that it might be possible to reduce genotoxic stress or suppress the melanocyte differentiation program to retain hair color.

Germline stem cells

The interplay between fertility and longevity underlies much of the evolutionary framework for biogerontology (Kirkwood, 1977). In invertebrates, stem cells sustain fertility throughout much of the lifespan. In *C. elegans*, GSCs are the only stem cell in the animal because the entire soma is post-mitotic, and these cells modestly decline in number with age (Killian and Hubbard, 2005). In *Drosophila*, the numbers of both male and female GSCs also decrease with age (Zhao et al., 2008b). In this context, aging is also associated with an increase in the number of GSCs with misorientated chromosomes, leading to cell cycle arrest (Cheng et al., 2008). This decline in GSC number across species is thought to contribute to decreased fertility with age (Luo and Murphy, 2011).

In mammals, the male germline is maintained by spermatogonial stem cells (SSCs), which similarly decline in number during aging (Paul et al., 2013; Ryu et al., 2006; Zhang et al., 2006). Whether or not females possess germline stem cells is an area of active debate. While males remain fertile throughout life, female oogenesis from oogonia in the developing ovary ceases before birth, although some mammals, including several species of bats, bushbabies and a species of chinchilla, are reported to continue oogenesis postnatally (Antonio-Rubio et al., 2013; Butler and Juma, 1970; Inserra et al., 2013). Some researchers have proposed that these species (and ostensibly all mammals) possess stem cells that can generate oocytes, called oogonial stem cells (OSCs). OSCs have been isolated from mice and rhesus macaques that generate oocytes *in vitro* and, in the case of mice, have been used to make transgenic pups (Hernandez et al., 2015; Zhang et al., 2011). OSCs have also been described in adult human ovaries that can form oocyte-like cells when transplanted into ovarian tissue (White et al., 2012). Other groups, however, have not found evidence for postnatal oogenesis (Lei and Spradling, 2013; Zhang et al., 2014) or have been unsuccessful in their attempts to isolate OCSs (Zhang et al., 2015). Thus, further experiments are required to resolve whether functional OSCs exist in mice and humans and whether their demise contributes to ovarian aging (Dunlop et al., 2013; Garg and Sinclair, 2015).

The causes of GSC aging appear to be largely cell-extrinsic. Mammalian SSCs, for example, can function for much longer than a normal lifetime when transplanted to a young environment (Ryu et al., 2006; Schmidt et al., 2011). Indeed, niche deterioration has received much attention as a mechanism of GSC aging (Boyle et al., 2007; Toledano et al., 2012; Wallenfang et al., 2006; Xie and Spradling, 2000; Zhang et al., 2006; Zhao et al., 2008b). Circulating factors such as insulin also play a role in maintaining GSC number and function (Hsu and Drummond-Barbosa, 2009; LaFever and Drummond-Barbosa, 2005; Mair et al., 2010). Given the fact that GSCs can give rise to an entire new organism, and that the 'aging clock' is reset with each new generation, it is reasonable that GSC decline is largely a result of such external factors.

Causes of stem cell aging

Despite their heterogeneous aging phenotypes, adult stem cells have many characteristics in common. They express telomerase – a requirement for self-renewal; they cycle between phases of quiescence and activation; their chromatin exists in a bivalent state primed for self-renewal or differentiation; they have unique metabolic requirements; they distribute their macromolecules asymmetrically during asymmetric cell divisions; and they reside in niches that regulate their behavior (Cheung and Rando, 2013). All of these traits interact with known mechanisms of aging to manifest the phenotypes described above. Although few, if any, mechanisms can be generalized to all populations of adult stem cells, many commonalities are apparent.

Telomere attrition

Telomere shortening is a hallmark of aging to which even stem cells are not immune. Although stem cells express telomerase, the telomeres of HSCs, NSCs, HFSCs and GSCs do shorten with age (Ferrón et al., 2009; Flores et al., 2008). Overexpression of telomerase reverse transcriptase (TERT), the enzymatic subunit of telomerase, on a cancer-resistant background or late in life in mice increases median lifespan independent of cancer incidence, suggesting that telomere length contributes to late life survival (de Jesus et al., 2012; Tomás-Loba et al., 2008). It is not clear whether this increase in median lifespan is dependent on stem cell activity. In humans, correlational studies support similar conclusions. For example, while telomere length is negatively correlated with age in humans up to 75 years, it is positively correlated with age in the elderly, suggesting that long telomeres contribute to survival in old age (Lapham et al., 2015). Furthermore, telomere length predicted survival in elderly twins, suggesting that telomeres contribute to longevity in humans even when controlling for the influence of genetic background (Bakaysa et al., 2007).

Despite considerable evidence that telomeres play a role in aging, it is not clear how impactful their shortening is for species that begin life with long telomeres and have shorter lifespans, such as mice. For example, laboratory mice lacking telomerase RNA component (TERC) have no abject phenotype for five generations. Only in the sixth generation is stem cell attrition observed, although a skewed HSC lineage potential phenotype can be observed as early as the fourth generation (Ju et al., 2007; Lee et al., 1998).

Cellular senescence

A major barrier to oncogenic transformation in stem cells is cellular senescence – a state of irreversible cell cycle arrest induced by short and uncapped telomeres or by stress-induced epigenetic alterations. There is increasing evidence that cellular senescence is a cause of stem cell aging. Given that stem cells are defined by their ability to

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self-renew and differentiate, the induction of senescence in a stem cell would clearly compromise its function. Senescent niche cells might also affect neighboring stem cells by secreting tumor-promoting mitogens and pro-inflammatory cytokines that negatively affect stem cell function through paracrine signaling (Coppé et al., 2008). In HSCs, satellite cells, and NSCs, markers of cell senescence such as $p16^{INK4a}$ accumulate with age, and preventing senescence through various methods of $p16^{INK4a}$ repression improves the function of aged stem cells (Cosgrove et al., 2014; Janzen et al., 2006; Molofsky et al., 2006; Nishino et al., 2008; Signer et al., 2008; Sousa-Victor et al., 2014). However, the frequency of senescent HSCs in aged mice appears to be low, complicating the model (Attema et al., 2009).

DNA damage and mutations

An accumulation in DNA damage and mutations has also been implicated in stem cell aging; the 'mutation accumulation theory' is in fact one of the earliest theories of aging (Medawar, 1952). In HSCs, histone H2AX phosphorylation and comet tails, both of which are measures of DNA damage, increase with age (Beerman et al., 2014; Rübe et al., 2011). H2AX phosphorylation also accumulates with age in satellite cells (Sinha et al., 2014). Moreover, aged HSCs display a history of replication stress and decreased expression of DNA helicases, further sensitizing them to future replication challenges (Flach et al., 2014).

Each time a stem cell replicates its DNA and divides, the likelihood of an oncogenic transformation increases. In fact, the lifetime risk of cancer arising in a tissue correlates with the number of divisions its stem cells have made (Tomasetti and Vogelstein, 2015). Perhaps as a mechanism to protect themselves from acquiring damage, many stem cells, including HSCs and satellite cells, spend large periods of time in a quiescent state. Quiescent cells are protected from replicative damage but are more susceptible to acquiring mutations when DNA damage does occur, largely because double-strand breaks (DSBs) are more likely to be repaired by error-prone non-homologous end joining (NHEJ) rather than by homologous recombination (HR), which provides more accurate repair, during the G0-G1 phase of the cell cycle (Kanaar et al., 2008). Indeed, quiescent HSCs preferentially rely upon NHEJ to repair DSBs, whereas proliferating HSCs rely more heavily upon HR (Beerman et al., 2014; Mohrin et al., 2010). HFSCs also display high rates of NHEJ after irradiation, relative to other skin cells, although this is independent of their position in the cell cycle (Sotiropoulou et al., 2010). Thus, although proliferating stem cells are more likely to encounter DNA damage (Walter et al., 2015), they repair that damage more accurately than do quiescent stem cells.

What are the consequences of DNA damage to stem cells during aging? In the hematopoietic system, mutations that increase proliferation or survival are believed to be selected for with age and, consistent with this, an increase in HSCs with pre-cancerous mutations is observed (Corces-Zimmerman and Hong, 2014; Genovese et al., 2014; Jaiswal et al., 2014; Jan and Snyder, 2012). In some situations, DNA damage may also reduce stem cell numbers by causing them to undergo apoptosis, senescence or differentiation. Evidence for a causal role for DNA damage in the aging process includes the observation that mice with defects in DNA damage repair display some aspects of premature aging (Freitas and de Magalhães, 2011) and enhancing DNA repair through increased expression of SIRT6 increases lifespan (Kanfi et al., 2012), although it is not yet known whether these effects are due to an increase in stem cell longevity.

Epigenetic alterations

The regulation of chromatin state is important for stem cell function. In Waddington's landscape metaphor, stem cells stand at an undifferentiated epigenetic summit above multiple cell fates (Waddington, 1942). Accordingly, loci that regulate cell fate decisions are bivalent, meaning they show both active and repressive chromatin modifications simultaneously (Bernstein et al., 2006). Numerous chromatin and gene expression changes during aging have now been cataloged. In HSCs, DNA methylation, which is a repressive epigenetic mark, decreases with age, although the trend at specific loci varies. This hypomethylation is closely tied to the proliferative history of HSCs, demonstrating why they might normally be programmed to remain quiescent. Interestingly, regions of the genome that have open chromatin in lymphoid cells show increased DNA methylation in aged HSCs, whereas regions of the genome that have open chromatin in myeloid cells show decreased DNA methylation in aged HSCs (Beerman et al., 2013).

H3K4me3, an activating modification, increases with age at loci that regulate HSC self-renewal, potentially underlying the increase in HSC number observed with aging (Sun et al., 2014). Additionally, the levels of H4K16Ac, another activating modification, decrease with age in HSCs; inhibition of CDC42 restores H4K16Ac levels to that of young HSCs and reverses phenotypes of HSC aging in transplantation assays (Florian et al., 2012). In satellite cells, H3K4me3 levels modestly decrease with age, whereas levels of the repressive modification H3K27me3 significantly increase with age, and it has also been shown that the expression levels of histones themselves decrease with age (Liu et al., 2013).

The expression levels of chromatin modifiers, including components of the SWI-SNF and PRC complexes, HDACs including sirtuins, and DNA methyltransferases, also change with age in stem cells (Chambers et al., 2007; Kofman et al., 2013; Rossi et al., 2005). These changes may underpin declining stem cell function. Indeed, the overexpression of EZH2, a component of PRC2, improves long-term repopulating potential in HSCs (Kamminga et al., 2006). Additionally, in aged HSCs, clusters of genes increase in expression levels based on chromosomal location, suggesting that epigenetic dysregulation engenders regional loss of transcriptional silencing (Chambers et al., 2007). Taken together, these findings suggest that changes in epigenetic modifications are a general trait of stem cell aging that impacts function.

But why does chromatin structure change over time? It is now appreciated that permanent changes to chromatin occur progressively as organisms age in response to cell stress, most notably in response to DNA damage signals (Fig. 1). Of all the different types of DNA damage, the one that has the greatest lasting effect on chromatin is the DSB. From yeast to mammals, DSBs cause a dramatic redistribution of chromatin factors as part of the response to damage that is not fully restored after repair (Chen et al., 2008; Oberdoerffer et al., 2008). Thus, changes in chromatin caused by DNA damage might underlie the skewed lineage phenotypes exhibited by aged stem cells (Beerman et al., 2013). It will be interesting to determine whether long-lived animals and humans are relatively resistant to epigenetic changes induced by DNA damage and the resulting changes in lineage. Conversely, if DSBs are induced in stem cells, will aging be accelerated?

Nutrient sensing and metabolism

The most robust longevity-extending intervention across species is caloric restriction (CR) without malnutrition. Some of the benefits of CR may be exerted through altered stem cell phenotypes. Indeed, CR increases the abundance of satellite cells in muscle (Cerletti et al.,

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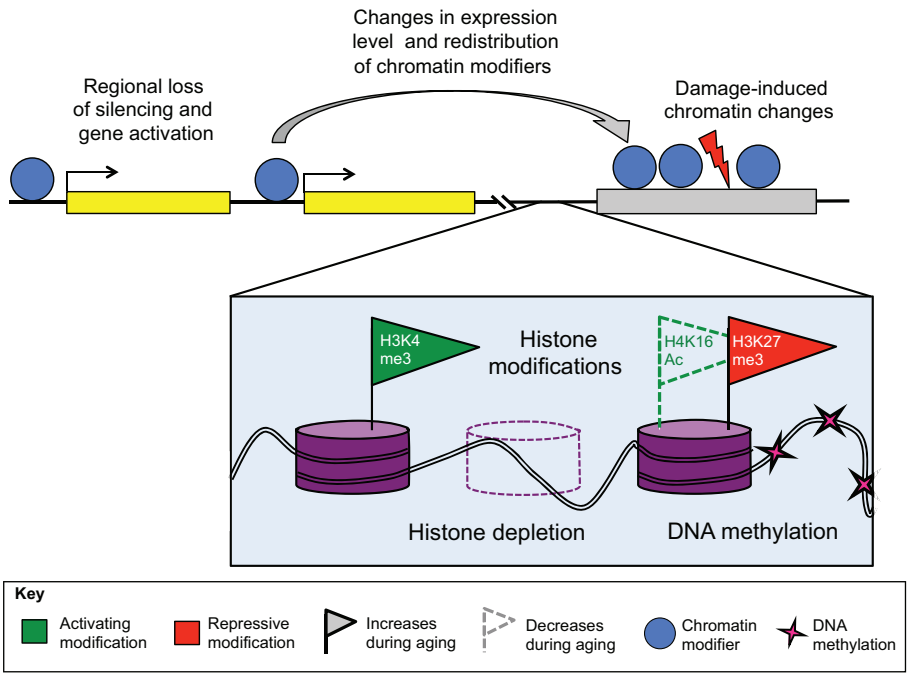

Fig. 1. Chromatin changes associated with stem cell aging. Changes in the structure and makeup of chromatin during aging have been characterized in HSCs and satellite cells. These changes include differences in the levels and distribution of chromatin-modifying enzymes (blue circles), histone modifications (green flag, activating; red flag, repressive; filled flag, increases during aging; empty flag, decreases during aging) and DNA methylation (pink star) patterns. Such changes result in regional loss of transcriptional silencing (yellow), altered cell fate decisions, decreased function and cellular senescence.

2012) and improves the function of many stem cell populations, including HSCs in mice (Chen et al., 2003) and GSCs in flies (Mair et al., 2010). CR also promotes ISC self-renewal in mice by induction of the enzyme BST1 in Paneth cells, which form the niche. BST1 then converts NAD^+ to the paracrine signal cyclic ADP ribose (cADPR), which is sensed by the ISCs (Yilmaz et al., 2012). Pathways and factors thought to mediate lifespan extension by CR (Fig. 2), namely insulin-IGF signaling, TOR signaling, AMPK, sirtuins and FOXO transcription factors have all been implicated in mediating the response of stem cells to CR (Mihaylova et al., 2014).

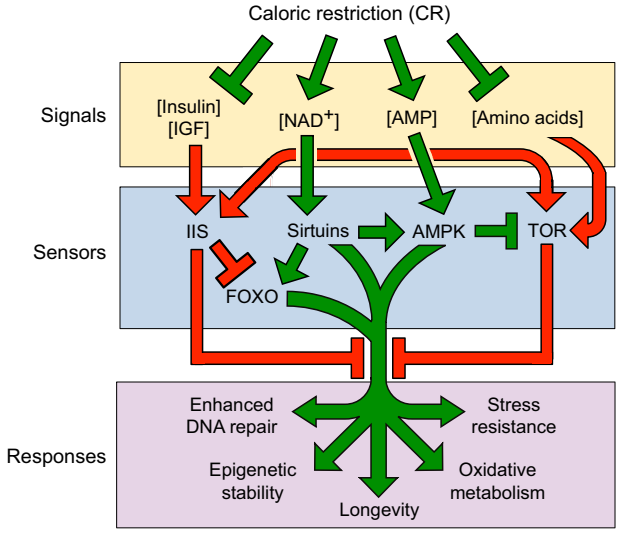

Fig. 2. The molecular effects of caloric restriction. Caloric restriction causes reduced levels of insulin, IGF and amino acids, and increased levels of NAD^+ and AMP. These changes are sensed by the insulin-IGF signaling (IIS) pathway, target of rapamycin (TOR), sirtuins and AMP kinase (AMPK), resulting in enhanced DNA repair, stability of the epigenome, stress resistance, oxidative metabolism and ultimately, longevity. All of these signals, sensors and responses regulate stem cell behavior. Green arrows represent interactions that promote longevity and related phenotypes, and red arrows are interactions that suppress these phenotypes.

Metabolic status and the formation of damaging reactive oxygen species (ROS) are also thought to play a role in stem cell aging (Fig. 3). Quiescent stem cells generally rely upon glycolysis to generate energy, perhaps because this reduces the abundance of ROS (Renault et al., 2009; Tothova et al., 2007). Many adult stem cells also reside in hypoxic niches, perhaps as part of a further mechanism to limit ROS production (Jang and Sharkis, 2007; Santilli et al., 2010). Proliferating stem cells, however, rely heavily upon oxidative phosphorylation, which predisposes them to oxidative damage and cellular dysfunction. Consistent with this, molecules that scavenge ROS and overexpression of the transcription factor *NRF2*, which regulates the response to oxidative stress, reduce the hyperproliferation phenotype of aged ISCs (Hochmuth et al., 2011; Myant et al., 2013).

Mitochondrial decline in stem and progenitor cells might also underlie age-related changes in stem cell function (Tilly and Sinclair, 2013). In *Drosophila* ISCs, overexpression of the PGC-1 homolog *spargel*, which is a potent regulator of mitochondrial biogenesis, forestalls ISC aging and increases median lifespan (Rera et al., 2011). Recent studies also show that HSCs and satellite cells increase glucose and glutamine metabolism during activation (Oburoglu et al., 2014; Ryall et al., 2015) – an alteration that mimics the Warburg effect of cancer cells. Similarly, in skeletal muscle, aging is associated with pseudohypoxia and Warburg-like metabolism, which compromise cellular function (Gomes et al., 2013) and promote oncogenic transformation (Wu et al., 2014).

Cell polarity and proteostasis

Stem cells utilize diverse mechanisms to prevent the accumulation of damaged components such as the asymmetric segregation of damaged proteins and enhanced proteostasis. Stem cells can divide symmetrically, producing two daughter cells with the same fate, or asymmetrically, producing a daughter stem cell and a differentiating cell. During asymmetric divisions, components of the cell are distributed unevenly. Given that the daughter stem cell is likely to persist much longer than the differentiating cell, mechanisms have evolved to enrich the daughter stem cell with undamaged components. These mechanisms are similar to those observed in

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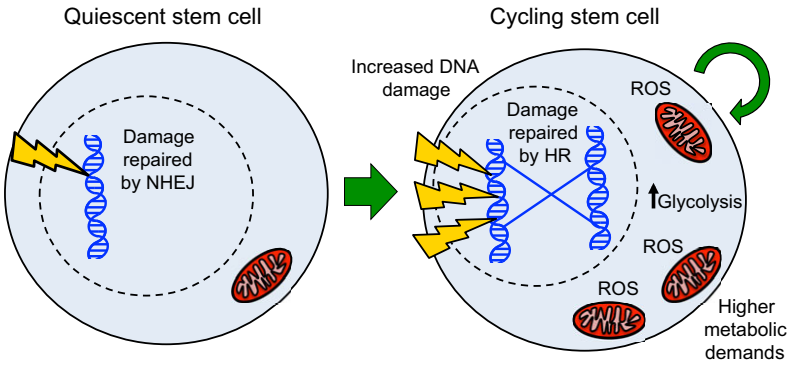

Fig. 3. Differences between quiescent and cycling stem cells in DNA repair and metabolism. The proliferation rate of a stem cell affects its exposure and responses to mechanisms of aging. Although quiescent stem cells are less susceptible to DNA damage, when it does occur they are more likely to utilize error-prone non-homologous end-joining (NHEJ) as a repair mechanism, whereas cycling stem cells are more likely to use homologous recombination (HR). Cycling stem cells also have higher metabolic demands than quiescent stem cells, including both higher rates of glycolysis and increased oxidative metabolism.

budding yeast, wherein damaged DNA, replicating circular DNA, carbonylated proteins and damaged organelles are sequestered, in this case in the mother cell, so that the daughter cell remains youthful (Aguilaniu et al., 2003; Higuchi et al., 2013; Lindner et al., 2008; Sinclair and Guarente, 1997).

In animals, the most well-known example of such asymmetric distribution is the 'immortal strand hypothesis', where the parental strand of DNA is sequestered in the daughter stem cell, whereas the strand synthesized during S phase, which might contain errors from replication, is directed to the differentiating daughter cell (Cairns, 1975). According to this hypothesis, the same DNA strands present at birth in stem cells are kept over the course of a lifetime. Such non-random strand segregation could be a means to avoid mutations or it could serve as a mechanism to control the inheritance of epigenetic state (Lansdorp, 2007). This process appears to occur in satellite cells (Conboy et al., 2007; Rocheteau et al., 2012; Shinin et al., 2006), whereas it does not appear to occur in HSCs (Kiel et al., 2007; Wilson et al., 2008). There is disagreement as to whether it occurs in other stem cell populations such as ISCs (Escobar et al., 2011; Falconer et al., 2010; Potten et al., 2002; Schepers et al., 2011), HFSCs (Huh et al., 2011, 2013; Waghmare and Tumbar, 2013), NSCs (Karpowicz et al., 2005) or GSCs (Karpowicz et al., 2009; Yadlapalli and Yamashita, 2013; Yadlapalli et al., 2011) (reviewed in Yennek and Tajbakhsh, 2013).

Stem cells have also been shown to asymmetrically segregate damaged proteins and mitochondria (Bufalino et al., 2013; Katajisto et al., 2015; Rujano et al., 2006). Any such asymmetric division requires that a cell be polarized (Fig. 4), and several studies demonstrate that aged GSCs and HSCs are less able to perform such polarized divisions, suggesting that loss of polarity contributes to stem cell aging (Cheng et al., 2008; Köhler et al., 2009). Data from HSCs suggest that changes in age-related Wnt signaling are a cause of this loss of polarity (Florian et al., 2013).

Stem cells might also maintain high levels of autophagy and proteasome activity to clear damaged proteins. For example, autophagy is greater in HSCs and skin stem cells than in surrounding differentiated cells (Salemi et al., 2012). Although proteasome activity has yet to be characterized in adult stem cells, it has been shown that human embryonic stem cells exhibit high proteasome activity (Vilchez et al., 2014) and that fly oocytes, which require similar long-term proteome-protection mechanisms to stem cells, maintain 26S proteasome activity with age despite declining activity in the soma (Fredriksson et al., 2012).

Niche deterioration and circulating factors

Stem cells require support cells that constitute the niche to maintain proper function. Thus, aging of the stem cell environment can also critically modulate stem cell function. Consistent with this, studies utilizing heterochronic transplantation and parabiosis experiments show that aging in satellite cells (Brack et al., 2007; Carlson and Faulkner, 1989; Chakkalakal et al., 2012; Conboy et al., 2005; Sinha et al., 2014), NSCs (Katsimpardi et al., 2014; Villeda et al., 2011, 2014), and GSCs (Ryu et al., 2006) is largely driven by cell extrinsic mechanisms. In the case of fly GSCs, the cells forming the niche themselves decline in abundance with age, possibly because of decreased self-renewal (Pan et al., 2007; Toledano et al., 2012; Wallenfang et al., 2006), as do factors regulating the adhesion of GSCs to their niche (Boyle et al., 2007; Pan et al., 2007). Aged niche cells can also fail to send proper signals to stem cells, namely through morphogen and growth factor signaling, thereby affecting cell fate decisions. For example, increased Fgf2 in the aged satellite cell niche of mouse muscle impairs self-renewal (Chakkalakal et al., 2012). Markers of inflammation – a hallmark of aging – also increase in the aging niche, for example in HFSCs, and impair stem cell function (Doles et al., 2012).

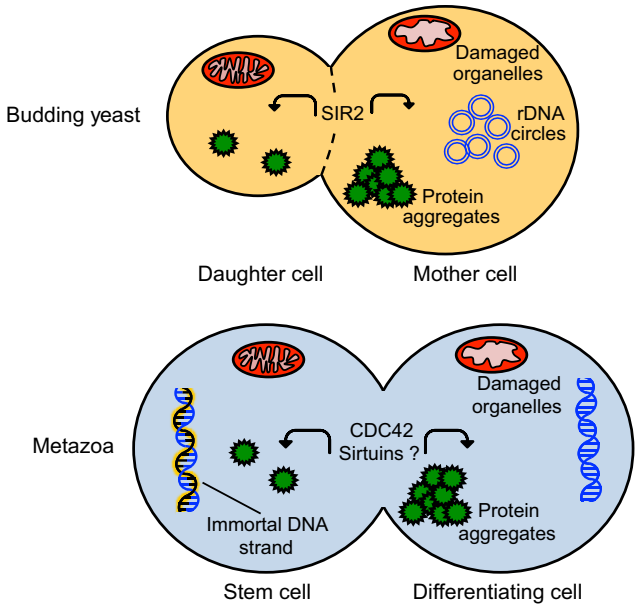

Fig. 4. Asymmetric cell division as a mechanism for removing cellular damage. In budding yeast, extrachromosomal DNA, carbonylated proteins and damaged organelles are sequestered in the mother cell, allowing the daughter cell to inherit more 'youthful' components in a process requiring the SIR2 deacetylase (a homolog of the mammalian sirtuins). During divisions of mammalian stem cells, similar processes maintain a 'youthful' state, in this case in the stem cell. Such polarized divisions, which require the activity of CDC42, become less frequent with increasing age.

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The other major cell-extrinsic influences on stem cell aging are changes in the concentrations of circulating factors. Many of these factors have been identified by characterizing the rejuvenating effects of serum or blood from young or calorically restricted animals on aged cells. Among such factors are insulin and IGF-1. Reduced signaling from these molecules is believed to mediate much of the longevity-extending effect of CR in mice. A main line of evidence comes from growth hormone receptor knockout mice. These mice have low levels of circulating IGF-1 and receive no additional longevity benefit from multiple types of CR (Arum et al., 2009; Bonkowski et al., 2006, 2009). In support of a beneficial role for reduced levels of insulin and IGF-1, dampening of insulin-IGF signaling specifically in fly ISCs improves gut homeostasis and extends lifespan, whereas reduced IGF-1 levels are associated with improved HSC self-renewal (Biteau et al., 2010; Cheng et al., 2014). These results in mice and flies are seemingly in opposition to the fact that insulin, IGF-1 and growth hormone increase the number of NSCs and GSCs in aged animals (Blackmore et al., 2009; Hsu and Drummond-Barbosa, 2009; Lichtenwalner et al., 2001). One explanation is that the increase in NSCs and GSCs might come at the expense of long-term self-renewal or impact homeostatic processes that are not yet known.

A number of other circulating factors that modulate stem cell function during aging have also been identified. For example, TGF-β, the levels of which increase during aging in mouse and human sera, impairs the function of satellite cells and NSCs (Carlson et al., 2009; Pineda et al., 2013). By contrast, growth differentiation factor 11 (GDF11) has been suggested to improve the function of satellite cells and NSCs, and its levels appear to decrease during aging (Katsimpardi et al., 2014; Loffredo et al., 2013; Sinha et al., 2014). The validity of the effects of GDF11 on satellite cells, however, has been questioned by other studies, although it is worth noting that the dose of GDF11 and the skeletal muscle injury models used in the various studies differed (Egerman et al., 2015). Whether GDF11 actually declines with age has also been questioned, based in part on the argument that GDF11 detection methods cross-react with myostatin (Egerman et al., 2015), although a recent study using additional methods and controls also reports that GDF11 declines with age in mice (Poggioli et al., 2015).

Cell survival, selection and fitness

One of the key unsolved mysteries in the aging field is how individual stem cells are able to function over the lifetime of an organism. A leading hypothesis is that they utilize mechanisms to prevent or reverse damage or epigenetic changes, thus acting as reservoirs of youth. As described above, stem cells use numerous mechanisms to prevent damage accumulation and epigenetic changes, including maintenance of long telomeres, enhanced proteostasis and avoidance of ROS production (Flores et al., 2008; Jang and Sharkis, 2007; Salemi et al., 2012). They also express increased levels of ABC transporters, which protect them against toxic substances (Kim et al., 2002).

Another hypothesis is that, rather than each cell being kept pristine, some stem cells decline with age and the healthiest stem cells are selected for. Indeed, rather than declining evenly, a subset of satellite cells is thought to account for most muscle regeneration in old animals (Collins et al., 2007). Moreover, the skewed differentiation potential observed in aged HSCs is most likely a result of the increased survival of individual clones with more myeloid potential, rather than an across-the-board shift in pre-determined fate decisions (Cho et al., 2008; Beerman et al., 2010). It has also been suggested that stem cell selection during aging is an active process. In flies, cells display their relative fitness with various isoforms of the membrane protein Flower; cells exhibiting lower fitness than their neighbors are culled, and enhancing this process extends maximal lifespan (Merino et al., 2015). Whether similar systems regulate tissue homeostasis and longevity in mammals remains to be explored.

The impact of stem cell aging on longevity

It is generally accepted that stem cell exhaustion contributes to the decline in health during aging, but to what degree do stem cells contribute to determining lifespan? The strongest evidence for stem cells as regulators of longevity comes from GSCs in *C. elegans*. Laser ablation or genetic manipulation to eliminate GSCs almost doubles worm lifespan (Arantes-Oliveira et al., 2002; Hsin and Kenyon, 1999). This phenomenon requires that the gonad is intact to induce changes in gut cells and neurons involving DAF-16/FOXO, the nuclear hormone receptor DAF-12, DAF-18/PTEN and the transcription factor HSF1, among other factors, and metabolic changes in fat (Berman and Kenyon, 2006; Hansen et al., 2005; Hsin and Kenyon, 1999; Lin et al., 2001; Wang et al., 2008). This suggests that the loss of GSCs induces secretion of hormones that shift the soma towards maintenance, an idea consistent with the 'disposable soma' theory (see Box 1) of aging (Kirkwood, 2005). There is some evidence that GSC ablation can extend lifespan in flies and even mammals, suggesting that such a phenomenon might be conserved (Kenyon, 2010).

Further evidence for stem cells as regulators of longevity comes from studies of ISCs in flies. Overexpression of a PGC-1α homolog or a heat-shock response transcription factor and moderate repression of insulin-IGF or JNK signaling in ISCs is sufficient to extend median lifespan (Biteau et al., 2010; Rera et al., 2011). This increase in lifespan is thought to occur directly through improved gut homeostasis, rather than through indirect signals, as is the case with worm GSCs. Such a model of improved stem cell function that leads to improved tissue function is what one would predict if stem cell aging is a crucial driver of tissue and organ aging.

Rejuvenation through reprogramming

One remarkable aspect of aging is that it is not cumulative over generations: each newborn animal begins life at both a chronological and biological age of zero. Although longevity can be an inherited and even an imprinted phenotype (Greer et al., 2011), age is reset with each new generation. So what unique aspect about the germline allows age to be reset between generations?

There is an increasing body of data suggesting that epigenetic changes drive the aging process and that age is reset with each subsequent generation via extensive epigenetic changes during germline specification and early development. For example, in the context of DNA methylation, primordial germ cells 'wipe-clean' their genome of methylation marks (with the notable exception of imprinted loci) early in development; this global hypomethylation is retained in oocytes and is applied to the paternal genome post-fertilization, with methylation levels at their lowest in the developing embryo pre-gastrulation (Smith et al., 2012). In the same vein, DNA methylation changes correlate with age in somatic tissues. Indeed, the DNA methylation status of only a handful of loci in any of multiple tissues including blood is sufficient to accurately infer age, and is a better predictor of late-life mortality than any of a number of risk factors (Hannum et al., 2013; Horvath, 2013; Marioni et al., 2015).

Thus, one might predict that passing a somatic cell through a pluripotent state would reset age and phenotypes thereof, and this appears to be the case. For example reprogramming aged HSCs to a

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pluripotent state and then differentiating them back to HSCs results in functional rejuvenation (Wahlestedt et al., 2013). Similarly, generating induced pluripotent stem cells (iPSCs) from aged human fibroblasts, and then differentiating them to NSCs generates NSCs with a young phenotype, whereas the direct lineage conversion of aged fibroblasts to NSCs retains the aged phenotype (Mertens et al., 2015). Of course, such reprogramming factors are hardly a fountain of youth because transitioning one's own cells through a pluripotent state is a recipe for cancer, not rejuvenation. Ideally, any intervention that forestalls or reverses aging would change phenotypes thereof without altering cell identity.

Future directions

So how then might stem cells be rejuvenated to forestall or reverse aging? Recent studies showing the rejuvenating effects of GDF11 on cardiac muscle, satellite cells and NSCs are one promising area of ongoing research, although this is not without controversy (Egerman et al., 2015; Katsimpardi et al., 2014; Loffredo et al., 2013; Poggioli et al., 2015; Sinha et al., 2014). Another potential road to aging reversal is through manipulation of longevity pathways such as mTOR inhibition by rapamycin and sirtuin activation by STACs (Fig. 2). Indeed, rapamycin analogs were recently shown to boost the immune system of elderly people (Mannick et al., 2014) and raising NAD^+ levels was shown to rejuvenate skeletal and cardiac muscle (Cantó et al., 2012; Gomes et al., 2013; North et al., 2014). The *ex vivo* rejuvenation of stem cells followed by re-transplantation might be another fruitful avenue, as was recently demonstrated in several studies with satellite cells (Bernet et al., 2014; Cosgrove et al., 2014; Sousa-Victor et al., 2014). The possibility of deleting senescent or otherwise dysfunctional stem cells also warrants further investigation (Baker et al., 2011).

As stem cells are among the longest-living cells within an organism, stem cell aging is highly relevant as a driver of organismal aging, health and longevity. Although phenotypes and mechanisms vary widely, all stem cell populations, it appears, decline in function with age. With a greater understanding of stem cell aging and its reversal, it may one day be possible to rejuvenate tissues mid-life and in the elderly, thereby increasing human fertility, healthspan and even lifespan.

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Competing interests

D.A.S. is a consultant to and inventor on patents licensed to Ovascience.

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