Manifestations and mechanisms of stem cell aging

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Adult stem cells exist in most mammalian organs and tissues and are indispensable for normal tissue homeostasis and repair. In most tissues, there is an age-related decline in stem cell functionality but not a depletion of stem cells. Such functional changes reflect deleterious effects of age on the genome, epigenome, and proteome, some of which arise cell autonomously and others of which are imposed by an age-related change in the local milieu or systemic environment. Notably, some of the changes, particularly epigenomic and proteomic, are potentially reversible, and both environmental and genetic interventions can result in the rejuvenation of aged stem cells. Such findings have profound implications for the stem cell–based therapy of age-related diseases.

Introduction

Stem cells reside in most adult mammalian tissues where they maintain normal tissue homeostasis and participate in tissue repair and regeneration in response to damage (Weissman, 2000; Li and Clevers, 2010). In general, stem cells represent a diminishingly small proportion of the cells within any tissue, rendering them difficult to identify and even more difficult to study. In the past few years, much effort has been focused on identifying molecular markers that would allow the isolation of different types of tissue-specific stem cells (Relaix et al., 2005; Barker et al., 2007; Yan and Owens, 2008). The development of specific methods to isolate functional stem cells is important not only to study the molecular mechanisms that underlie such important stem cell characteristics as multipotentiality and the ability to self-renew but also for the establishment of stem cell–based therapeutics.

The isolation of stem cells away from other local and systemic influences is essential for characterizing and measuring their intrinsic properties and functionality. However, in vivo labeling and tracing of stem cell lineages are equally important and particularly useful in delineating the influences of environmental factors on stem cell function, such as the switch between quiescence and activation or the determination of the fate of differentiating daughter cells. Environmental cues are transmitted to stem cells by their niches, which are composed of the extracellular matrix, cells in direct contact with stem cells, and soluble factors that are secreted or concentrated locally (Schofield, 1978; Voog and Jones, 2010). The niche is profoundly influenced by the systemic milieu and dynamically changing to regulate stem cell function, a feature that is especially relevant with regard to the process of aging (Gopinath and Rando, 2008).

Aging is accompanied by a decline in the homeostatic and regenerative capacity of all tissues and organs (Kirkwood, 2005; Rando, 2006). With age, wound healing is slower in the skin, hair turns gray or is lost, skeletal muscle mass and strength decrease, the ratio of cellular constituents in the blood is skewed, and there is a decline in neurogenesis (Sharpless and DePinho, 2007). As the homeostatic and regenerative activities of these tissues are attributable to the resident stem cells, these age-related changes are reflections of declines in stem cell function (Bell and Van Zant, 2004; Dorshkind et al., 2009; Jones and Rando, 2011). Clearly, in terms of organismal aging, the focus on stem cells is most relevant for those tissues in which normal cellular turnover is very high, such as epithelia of the skin and gut, as opposed to tissues, such as the cerebral cortex and the heart, in which cellular turnover in adults is exceedingly low (Rando, 2006). There is also an increasing interest in the therapeutic potential of stem cells to treat age-related degenerative diseases or conditions, further highlighting the importance of understanding the relationship between stem cell function and the properties of aged tissues. Within this context, it is essential to understand how the local environment influences stem cells, how aging affects stem cell number and function, and the extent to which aspects of stem cell aging may be reversible. This review focuses on manifestations and underlying mechanisms of age-related changes in stem cells and stem cell functionality.

Aging of somatic stem cells

Adult stem cells are exposed to many of the same factors that lead to age-related changes in their replicative or postmitotic progeny, but stem cells must resist those changes as a self-renewing

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Abbreviations used in this paper: HSC, hematopoietic stem cell; NPC, nuclear pore complex; NSC, neural stem cell.

The Rockefeller University Press
J. Cell Biol. Vol. 193 No. 2 257–266
www.jcb.org/cgi/doi/10.1083/jcb.201010131

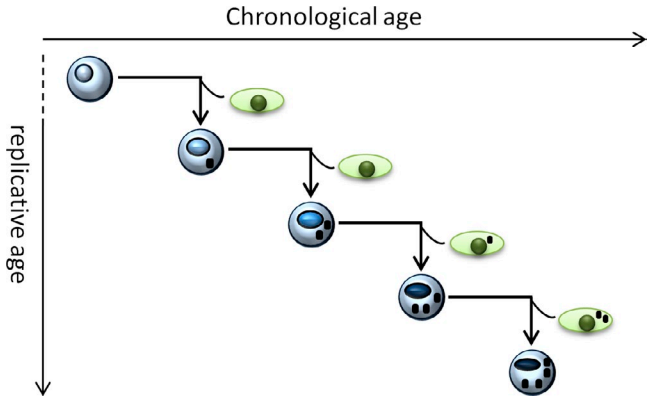

Figure 1. **The chronological and replicative lifespan of stem cells.** During prolonged periods of quiescence and by the process of self-renewal to establish a cellular continuum, stem cells experience chronological aging caused by the accumulation of damaged or aberrant intracellular molecules. During the process of asymmetric cell division and self-renewal, stem cells also experience replicative aging, which is particularly important in tissues with high turnover rates.

population to assure proper function and normal tissue homeostasis across the lifespan (Rando, 2006; Sharpless and DePinho, 2007; Jones and Rando, 2011). As a replicative population that may have prolonged periods of quiescence (Fig. 1), stem cells must possess defense and repair mechanisms that are relevant to both highly proliferative cells and to long-lived postmitotic cells (Rando, 2006).

In long-lived animals, adult stem cells, particularly those in continuously renewing tissues, undergo many rounds of cell division to maintain normal tissue homeostasis (Fuchs et al., 2001; van der Flier and Clevers, 2009). During each round of DNA replication, processes that underlie replicative aging, including telomere shortening, chromosome rearrangements, and single base mutations (Ben-Porath and Weinberg, 2005), can occur and ultimately lead to cellular senescence (Hayflick, 1965; Campisi and d'Adda di Fagagna, 2007). Experimental manipulations, such as serial transplantation, clearly reveal that adult stem cells have a finite replicative lifespan that can be exhausted (Siminovitch et al., 1964; Waterstrat and Van Zant, 2009). However, as serial transplantation experiments subject stem cells to excessive rounds of cell division, it remains to be determined whether replicative aging alone is sufficient to contribute to the decline of stem cell function in long-lived mammals during normal aging.

Adult stem cells are also susceptible to the kinds of age-related changes, namely chronological aging, that occur in nondividing cells, such as neurons and cardiomyocytes (Busuttil et al., 2007). These changes include the accumulation of damaged macromolecules, such as proteins, lipids, and nucleic acids, some of which may, in fact, aggregate and form stable, long-lived complexes that are toxic to the cell (Rajawat et al., 2009; Koga et al., 2011). Adult stem cells exhibit prolonged periods of quiescence in most mammalian tissues (Li and Clevers, 2010). Damaged macromolecules can accumulate in stem cells during this time, just as in long-lived postmitotic cells. Specific macromolecules or macromolecular aggregates may even be selectively retained in stem cells as they undergo the process of self-renewal by asymmetric cell division (Conboy et al., 2007; Knoblich, 2008). In this sense, the self-renewing progeny represent a kind of cellular continuum and only add to the risk that adult stem cells may suffer from the effects of chronological aging.

Functional manifestations of stem cell aging

Aging in stem cells causes changes in the fate or functionality of stem cell progeny. In some cases, such as neural stem cells (NSCs) and melanocyte stem cells (Maslov et al., 2004; Inomata et al., 2009), these changes may lead to a depletion of the stem cell pool (Fig. 2; Kuhn et al., 1996; Maslov et al., 2004). However, in most stem cell compartments, the number of stem cells does not decline significantly with age (Booth and Potten, 2000; Brack and Rando, 2007; Giangreco et al., 2008); rather, these stem cells experience a change in cell fate with age.

Age-related changes in the fate of stem cell progeny. Adult stem cells may be unipotent, bipotent, or multipotent, giving rise to a restricted diversity of progeny based on the tissue in which they reside (Weissman, 2000; Rando, 2006). The ability of stem cells to produce an appropriate repertoire of tissue-specific progeny is crucial for functional tissue homeostasis and regeneration. The extent to which adult stem cells and their progeny are committed to a particular lineage is determined largely by the epigenome, influencing which genes will be expressed and which will be repressed and ultimately shaping the phenotypic characteristics of the cells (Bernstein et al., 2006; Mikkelsen et al., 2007; Hemberger et al., 2009). The execution of the epigenomic program that influences the fate of stem cell progeny is modulated by environmental factors and mediated by signaling pathways that have important roles in organogenesis during development, including the Wnt, Notch, and Hedgehog pathways (Berger, 2007; Brack et al., 2008; Rittié et al., 2009; van der Flier and Clevers, 2009). With age, untimely activation of these pathways as a result of signals from the "old environment" may lead to aberrant lineage specification of stem cell progeny as has been demonstrated in tissues, such as skeletal muscle, tendon, and the hematopoietic system (Sudo et al., 2000; Taylor-Jones et al., 2002; Brack et al., 2007; Zhou et al., 2010). Accumulation of these abnormal progeny contributes to the gradual deterioration of tissue structure and function associated with aging.

Despite the extremely low turnover rate of cells within the tissue under normal homeostatic conditions, skeletal muscle possesses remarkable regenerative ability upon injury, a process that is mediated by the resident muscle stem cells (satellite cells; Mauro, 1961; Zammit et al., 2006; Le Grand and Rudnicki, 2007). However, satellite cells isolated from aged rodents have a higher propensity to undergo adipogenic or fibrogenic differentiation that may be partially caused by elevated levels of Wnt in the muscle compartment (Taylor-Jones et al., 2002; Brack et al., 2007). As a result, aged animals require a longer time to recover from muscle injury, the regenerated myofibers are smaller in diameter, and there is an increase in tissue fibrosis (Conboy et al., 2005; Brack et al., 2007). Strikingly, this age-related disruption of lineage fidelity can be restored by a youthful systemic milieu generated by parabiotic pairing between old and young mice (heterochronic parabiosis; Conboy et al., 2005;

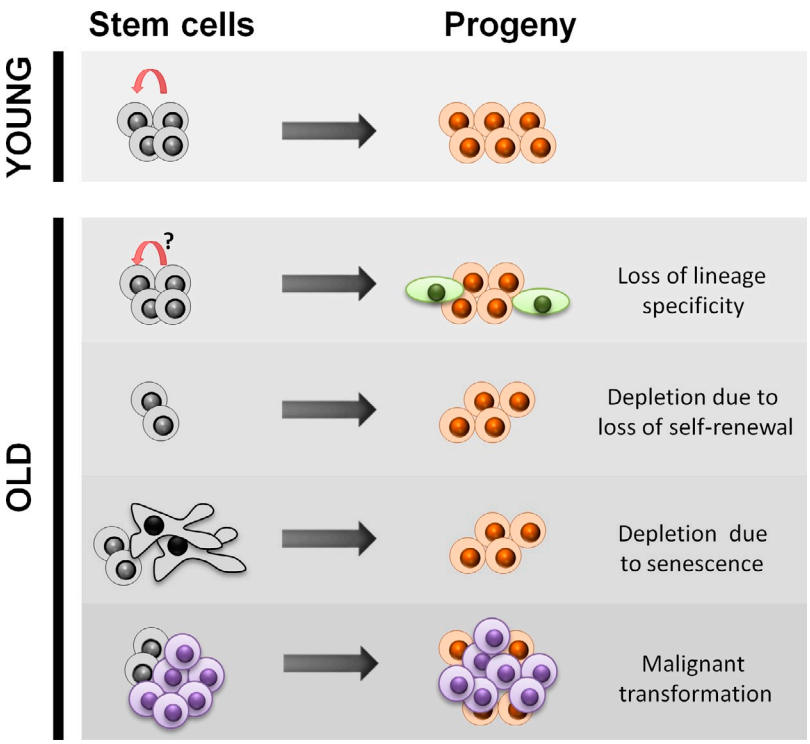

Figure 2. **Decline in stem cell function with age.** In young animals, stem cells divide asymmetrically to self-renew and give rise to lineage-specific differentiated progeny during tissue homeostasis or regeneration. With age, some stem cells lose their lineage specificity and give rise to non-functional progeny, resulting in loss of tissue integrity and decline of physiological function, even though the number of stem cells remains unaffected. Some stem cells lose the capacity for self-renewal, resulting in symmetric cell divisions giving rise to two differentiated daughters and a gradual depletion of the stem cell pool. The senescence of stem cells can also contribute to a loss of functional stem cells. The increase in malignancies with age, particularly in epithelia with high turnover rates, has been proposed to arise from within the stem cell compartment or from early progenitors.

Brack et al., 2007). As such, these age-related changes in satellite cells appear to be driven by an "aged environment" rather than intrinsic changes within the cells. An elevated level of Wnt pathway activators in the circulation may be part of such an aged environment, as inhibition of the Wnt pathway restores the lineage specificity of satellite cells (Brack et al., 2007). Furthermore, the cytokine TGF-β has been found to be expressed at an elevated level in the serum of aged human and mice (Carlson et al., 2009) and may cross talk with the Wnt pathway to influence the satellite cell progeny.

Within the hematopoietic system, the ratios of differentiated progeny change with age. Hematopoietic stem cells (HSCs) from both old humans and old mice show an increased propensity to differentiate along the myeloid rather than the lymphoid lineage (Sudo et al., 2000; Rossi et al., 2005). Such lineage bias is not caused by a change in the differentiation potential of individual HSCs but rather by a preferential selection of distinct subsets of HSCs over time (Cho et al., 2008; Beerman et al., 2010; Challen et al., 2010). The differential responsiveness of these two HSC populations to TGF-β may further enhance the skewed ratio between myeloid versus lymphoid progeny in old individuals (Challen et al., 2010). Although the progeny of the aged HSCs do not include any cells that are not otherwise part of the normal repertoire of cells produced by HSCs, this lineage skewing results in a decreased number of memory B cells and naive T cells (Linton and Dorshkind, 2004; Min et al., 2004) and adversely affects immunological responses (Rink et al., 1998; Grubeck-Loebenstein et al., 2009).

Depletion of stem cell pools. Certain types of age-related dysfunction of stem cells, such as loss of the ability to self-renew and the activation of senescence pathways, can lead to the depletion of the stem cell pool. Within specific stem cell compartments, such as melanocyte stem cells in the skin and NSCs in the brain, an age-related decline in stem cell number appears to be responsible for specific aging phenotypes in those tissues (Kuhn et al., 1996; Maslov et al., 2004; Nishimura et al., 2005; Inomata et al., 2009; Renault et al., 2009). The mammalian skin contains at least three types of stem cells at the bulge area in hair follicles that orchestrate skin homeostasis: epidermal stem cells that give rise to keratinocytes, hair follicle stem cells that are responsible for hair growth, and melanocyte stem cells that pigment new hair (Blanpain and Fuchs, 2009). Depletion of melanocyte stem cells in the hair follicles and appearance of mature pigmented melanocytes in the stem cell niche have been reported in both aged mice and humans (Nishimura et al., 2005; Inomata et al., 2009), leading to one of the most visible phenotypic changes during aging, hair graying. Aging or genotoxic stress induces the accumulation of DNA damage in melanocyte stem cells that results in the loss of stem cell self-renewal (Inomata et al., 2009). Both daughter cells of a melanocyte stem cell with high levels of DNA damage tend to differentiate into pigment-producing melanocytes, resulting in the gradual depletion of the stem cell pool. Although the loss of melanocyte stem cell self-renewal occurs independently of checkpoint proteins p53, $p16^{INK4a}$, and $p19^{ARF}$, defective DNA repair sensitizes melanocyte stem cells to premature differentiation under a much lower level of genotoxic damage (Inomata et al., 2009), highlighting the importance of genome maintenance for stem cell longevity.

Depletion of NSCs, possibly also related to a specific loss of capacity for self-renewal, appears to be responsible for declining neurogenesis with age (Molofsky et al., 2006; Nishino et al., 2008; Renault et al., 2009). In rodents, the number of NSCs significantly decreases with age in both the subventricular zone of the lateral ventricle and the subgranular zone of the dentate gyrus in the hippocampus, correlating with a gradual

loss of olfactory function and cognitive function, respectively (Kuhn et al., 1996; Bondolfi et al., 2004; Maslov et al., 2004). Compared with NSCs from young rodents, NSCs from aged rodents exhibit a marked decline in their self-renewal activity as demonstrated in both in vitro neurosphere assays and in vivo tracing studies (Renault et al., 2009; Lugert et al., 2010). Mechanisms for the age-related loss of self-renewal of NSCs have been investigated in mouse models that lack the expression of important regulators of NSC homeostasis, including the Polycomb ring finger protein Bmi-1, the chromatin-associating protein HMGA2, and the longevity-associated transcription factor FoxO3 (Molofsky et al., 2003; Nishino et al., 2008; Renault et al., 2009). As revealed by these studies, the Bmi-1 and HMGA2 proteins regulate NSC self-renewal by suppressing the expression of cell cycle inhibitors $p16^{INK4a}$ and $p19^{ARF}$ (Molofsky et al., 2005; Nishino et al., 2008), and the FoxO proteins affect NSC homeostasis by regulating the expression of cell cycle regulatory proteins, such as cyclin D1, inhibitor of DNA binding 1, and polo-like kinase 2 (Paik et al., 2009). In addition, the FoxO proteins regulate the expression of several soluble Wnt antagonists and fine tune the activity of the Wnt pathway (Paik et al., 2009; Renault et al., 2009), thereby potentially maintaining NSC homeostasis during aging by attenuating Wnt signaling that is likely to be elevated in the aging environment (Brack et al., 2007; Liu et al., 2007). Although these mouse models have proven to be valuable in delineating pathways that are involved in NSC homeostasis and the precocious NSC depletion in these germline knockout mice recapitulates aspects of NSC aging, it remains to be determined whether these proteins play causal roles in NSC depletion during normal aging.

Senescence of stem cells. One potential fate of stem cells and their progeny that has a profound negative impact on tissue homeostasis and regeneration is the state of senescence. The expression of senescence markers, such as senescence-associated β-galactosidase, HP-1 (heterochromatin protein-1) foci, and $p16^{INK4a}$ markedly increases with age in many tissues in several mammalian species (Sharpless and DePinho, 2007). Senescent epidermal stem cells, HSCs, NSCs, and pancreatic β cells have been observed in mouse models for segmental progeroid syndromes or those in which critical genes for stem cell homeostasis are deleted (Krishnamurthy et al., 2004; Liu et al., 2007; Burtner and Kennedy, 2010). Considered a biomarker of aging, $p16^{INK4a}$ appears to contribute to this replicative failure of stem cells (Krishnamurthy et al., 2006; Molofsky et al., 2006; Melzer et al., 2007). Overexpression of $p16^{INK4a}$ suppresses HSC function and pancreatic β cell proliferation in young animals, resembling the functional decline observed in aging (Janzen et al., 2006; Krishnamurthy et al., 2006). Likewise, genetic deletion of $p16^{INK4a}$ attenuates the age-related decline in proliferation and function of NSCs, HSCs, and pancreatic β cells (Janzen et al., 2006; Molofsky et al., 2006). Therefore, it has been proposed that specific populations of stem cells may exhibit an age-related decline in number as a result of stem cell senescence (Sahin and Depinho, 2010), although there are few examples in which stem cell senescence has been directly demonstrated during normal aging. Even in the epidermis, where senescent keratinocytes accumulate with age, direct evaluation of the stem cell compartment has not revealed senescent epidermal stem cells even though their transiently amplifying progeny may undergo senescence (Liang et al., 2004; Stern and Bickenbach, 2007; Giangreco et al., 2008; Charruyer et al., 2009).

Whether other types of stem cells in the skin undergo senescence with age has also been investigated (Liu et al., 2007). Normal cycling of hair follicle stem cells is regulated by local induction of Wnt proteins in the niche, and Wnt activity diminishes as the cells return to quiescence (Fuchs et al., 2001; Andl et al., 2002). This temporal regulation of the Wnt pathway appears to be critical in the homeostatic maintenance of hair follicle stem cells. Constitutive activation of the Wnt pathway in transgenic mouse models causes persistent proliferation of hair follicle stem cells followed by detectable signs of premature senescence and disappearance of stem cells that correlate with precocious hair loss (Liu et al., 2007; Castilho et al., 2009). In spite of the lack of direct demonstration, it is possible that hair follicle stem cells may likewise undergo senescence during normal aging, which, as noted in this paragraph, may be associated with increases in Wnt activity (Brack et al., 2007; Liu et al., 2007).

Malignant transformation of stem cells. Another fate that has been proposed for tissue-specific stem cells, which would not only affect the functionality of the population but could dramatically impact organismal longevity, is malignant transformation. Whether cancers arise from adult stem cell compartments is a great challenge to demonstrate experimentally. This question has particular relevance in the context of stem cell aging, as age is the number one risk factor for cancer (Danaei et al., 2010). At the molecular level, cancer develops from a few cells harboring genomic mutations that enable their escape from the stringent cell cycle control (Hahn and Weinberg, 2002). Whether the cellular precursors for cancer are bona fide tissue-specific stem cells that have acquired such mutations remains to be shown (Gupta et al., 2009).

One indirect line of evidence in support of the hypothesis that stem cells themselves may undergo malignant transformation is that, in many cancers, the molecular pathways that sustain tumor growth are the same as those that support tissue-specific stem cells. For instance, the proliferative capacity of human acute myeloid leukemia stem cells requires Bmi-1, the Polycomb group gene that also regulates the self-renewal of normal HSCs (Bonnet and Dick, 1997; Lessard and Sauvageau, 2003). MicroRNA-200c suppresses the expression of Bmi-1 and inhibits the self-renewal of both normal mammary stem cells and breast cancer cells (Shimono et al., 2009). A Wnt pathway regulator, PLAGL-2 (pleomorphic adenoma gene-like 2), regulates the self-renewal of NSCs by suppressing their differentiation and also promotes the undifferentiated state of glioblastoma cells (Liu et al., 2010b; Zheng et al., 2010). Enforced expression of the nuclear receptor Tailless antagonizes the age-dependent NSC depletion in mice and results in increased incidents of glioma formation in aged mice (Liu et al., 2010b). Although these findings are consistent with the idea that normal stem cells can give rise to certain types of malignant tumors whose incidence increases with age, conclusive experimental evidence for such conversion requires lineage-tracing studies to label stem cells and monitor their activity during aging.

Intrinsic changes in aging stem cells

Among the cell-intrinsic changes that may mediate age-related changes in stem cell function are alterations at the level at the genome, the epigenome, and the proteome, each of which is considered in the following sections.

Genomic changes. With age, somatic cells in many, if not all, tissues accumulate measurable genomic lesions, including single- and double-strand DNA breaks, chromosomal translocations, telomere shortening, and single base mutations (Akbari and Krokan, 2008; Wang et al., 2009). DNA repair systems have evolved to maintain genomic integrity, and it has been proposed that the intrinsic DNA repair activity and fidelity in different species may influence the rate of aging (Hart and Setlow, 1974). Mutations in proteins involved in DNA repair, such as the WRN (Werner Syndrome ATP-Dependent) helicase and the ATM (Ataxia Telangiectasia Mutated) kinase, have been associated with segmental progeroid syndromes in humans and mice that have features of accelerated aging in multiple tissues and organs (Savitsky et al., 1995; Gray et al., 1997; Kudlow et al., 2007), providing evidence for the crucial role of DNA repair machinery for normal tissue homeostasis.

Because of their replicative nature, various types of stem cells have been evaluated for telomere shortening. In the skin, shorter telomeres in hair follicle stem cells from old mice parallel a decline in their clonogenic potential (Flores et al., 2008). Additionally, telomere shortening has been observed in stem cells in the small intestine, cornea, testis, and brain in 2-yr-old compared with 2-mo-old mice (Flores et al., 2008). Interestingly, telomeres in old stem cells are still longer than those in the other somatic cells in these tissues (Flores et al., 2008; Wang et al., 2009), suggesting that stem cells divide at a much slower rate than their proliferative progeny or that they have evolved mechanisms to protect against telomere shortening.

The importance of intact telomeres in stem cells has been demonstrated in both telomerase knockout mice and mice that carry short telomeres in the presence of telomerase (Lee et al., 1998; Flores et al., 2005; Hao et al., 2005). In the late generation of mice that lack the gene encoding the essential RNA component (Terc) of the telomerase holoenzyme, stem cells in many tissues are depleted or functionally compromised (Lee et al., 1998). Epidermal stem cells in the hair follicles in these mice appear defective in proliferation and migration (Rudolph et al., 1999; Flores et al., 2005). Loss of renewal and differentiation of NSCs and increased apoptosis in the intestinal stem cell compartment are also apparent in these mice (Rudolph et al., 1999; Ferrón et al., 2004). Interestingly, although the enzymatic activity of telomerase is required to maintain telomere integrity, the protein component (Tert) of the telomerase holoenzyme may be able to regulate stem cell function independently of telomere maintenance. Expression of an enzymatically inactive Tert in $Terc^{-/-}$ mice is sufficient to activate hair follicle stem cells and stimulate hair growth (Sarin et al., 2005). Although the studies involving mice with genetically manipulated short telomeres provide strong evidence for the essential role of telomerase in maintaining stem cell function and tissue homeostasis, it remains to be determined whether telomere shortening, with or without a change in telomerase activity, contributes to stem cell dysfunction during normal aging.

Accumulation of DNA mutations may also be a common feature of stem cell aging. With age, elevated levels of DNA damage have been reported in epidermal stem cells and HSCs (Rossi et al., 2007; Sotiropoulou et al., 2010). DNA repair processes are important for maintaining stem cell homeostasis during aging, as demonstrated in several genetically modified mouse models that are defective for DNA repair (Ito et al., 2004; Ben-Porath and Weinberg, 2005; Nijnik et al., 2007; Rossi et al., 2007; Ruzankina et al., 2007). Stem cells from these mice exhibit a diminished capacity for self-renewal and proliferation with age, resulting in increased apoptosis or senescence in the stem cell compartment and depletion of functional stem cells (Ruzankina et al., 2007). On the other hand, quiescent stem cells appear to preferentially use nonhomologous end joining to repair DNA breaks (Mohrin et al., 2010), which is an error-prone mechanism as its action does not rely on a DNA template (Weinstock et al., 2006). The chromosomal deletions and insertions that result from misrepaired double-strand DNA breaks can lead to increased genomic instability and aberrant activation or inactivation of functional genes (Akbari and Krokan, 2008). As such, DNA repair processes may paradoxically lead to an increase in the accumulation of genomic mutations in quiescent stem cells with age. In addition, DNA repair causes recruitment of certain chromatin-remodeling enzymes to foci of damage and, thus, results in redistribution of these enzymes across the genome (Oberdoerffer et al., 2008; Conde et al., 2009). Therefore, the impact of genomic changes can be amplified by the subsequent changes in the epigenome.

The immortal strand hypothesis was proposed as a mechanism for stem cells to minimize the accumulation of replication-induced mutations (Cairns, 1975). According to this hypothesis, concurrent with the asymmetrical division of a stem cell, segregation of sister chromatids occurs nonrandomly. For each pair, the chromatid with the older ("immortal") template strand is selectively inherited by the self-renewing daughter stem cell, whereas the chromatid with the newer template strand is inherited by the differentiating daughter cell. Therefore, the original genetic information that arose with the formation of a stem cell pool will be selectively retained and preserved in the stem cells, minimizing the accumulation of replication-induced mutations in the stem cell population (Rando, 2006). Such asymmetrical segregation of sister chromatids according to template strand age has been observed in certain stem cell populations, including muscle satellite cells and NSCs (Karpowicz et al., 2005; Shinin et al., 2006; Conboy et al., 2007; Ferron et al., 2010). The mechanisms that regulate nonrandom DNA segregation, the specific physiological conditions in which it occurs, and the effect it has on cell fate decisions remain to be determined (Falconer et al., 2010; Charville and Rando, 2011).

Epigenomic changes. Unlike acquired DNA mutations, epigenomic changes, including DNA methylation and post-translational modifications of histones, are dynamically maintained by a balance among chromatin-remodeling complexes and are, thus, reversible (Goldberg et al., 2007). Given the influence of cell extrinsic factors on the epigenome and the reversibility of chromatin modifications, epigenomic changes may underlie the stochastic aspects of aging (Herndon et al., 2002;

Fraga et al., 2005; Kirkwood, 2005) and certain environmental influences that delay or even apparently reverse aging, such as the lifespan-extending effect of dietary restriction and the rejuvenation of aged stem cells by exposure to a young environment (Conboy et al., 2005; Dorshkind et al., 2009; Fontana et al., 2010). In yeast, lifespan extension by dietary restriction appears to require Sir2 (Lin et al., 2000), a histone deacetylase that has been shown to extend the lifespan in several model organisms (Longo and Kennedy, 2006). In *Caenorhabditis elegans*, members of the H3K4 methyltransferase complex affect lifespan in a germline-dependent manner (Greer et al., 2010). Although such data suggest that aging can be modulated at the epigenetic level, it should be noted that some epigenomic modifications are secondary to DNA damage itself. For example, phosphorylation of H2AX is induced by double-strand DNA breaks (Rogakou et al., 1998). There is also evidence that levels of acetylation of H3K56 (histone 3 lysine 56) and methylation of H3K79 (histone 3 lysine 79) increase after DNA damage (Conde et al., 2009; Tjeertes et al., 2009). Thus, the presence of particular histone marks can be indicative of permanent genomic changes.

Recent technological advances, particularly in ultra high-throughput sequencing, have allowed detailed studies of epigenomic changes across the genome (Bernstein et al., 2006; Hemberger et al., 2009). Changes in both DNA methylation and histone modifications have been investigated as epigenomic markers of aging (Sedivy et al., 2008; Calvanese et al., 2009). Age-associated global changes in DNA methylation are found in human, rat, and mouse tissues, including liver, spleen, lung, brain, fat, and different sections of the gastrointestinal tract (Christensen et al., 2009; Maegawa et al., 2010; Thompson et al., 2010). Despite the global decrease found in various cell types during aging, hypermethylation has also been found to occur in specific genes whose promoters are rich in CpG islands (Zhang et al., 2002; Maegawa et al., 2010). In the colon, age-related increases in the methylation of CpG islands have been linked to the silencing of tumor suppressor genes and neoplasia (Jones and Baylin, 2007). Recently, dysregulation of acetylation of H4K12 (histone 4 lysine 12) has been found to be associated with cognitive decline (Peleg et al., 2010). Although these studies have identified detectable epigenomic changes in mammalian cells with age, no direct evidence indicates whether they may play a causal role in cellular aging of whether they are merely consequences of the aging process.

Direct measurement of epigenomic changes in stem cells with age is challenging, as most current assays that detect epigenetic modifications require a large number of cells. The link between aging and epigenomic changes has been investigated in some tissue compartments in which stem cells reside. Therefore, such measurements may reflect changes in the stem cells themselves but may also reflect changes in cells within the stem cell niche. Increased DNA methylation was reported in both intestinal and colon crypts in the gut epithelium from old humans and mice (Yatabe et al., 2001; Maegawa et al., 2010). As noted previously, a significant portion of this hypermethylation occurs at CpG islands in the promoter regions of genes (Yatabe et al., 2001). Interestingly, quantitative simulation of the experimental DNA methylation data revealed that age-related DNA methylation appeared to increase more rapidly in the colon than the small intestine, correlating with the faster cell division rate in the former (Kim et al., 2005). This observation suggests that DNA methylation, as one important determinant of the epigenome, may be an indicator of the replicative age of cells.

The age-related epigenomic changes in stem cells can be a result of altered expression of chromatin-modifying enzymes or their cofactors (Chambers et al., 2007). Microarray analysis of HSCs from young and old mice has revealed an age-dependent reduction in the expression of subunits in the SWI/SNF (switch/sucrose nonfermentable) chromatin-remodeling complex, histone deacetylases HDAC1, 5, and 6, and a DNA methyltransferase, DNMT3b (Chambers et al., 2007). Increased genomic instability and inappropriate transcription have been historically associated with aging (Maslov and Vijg, 2009). Several genes clustered on chromosomal regions coordinately change their expression with age, suggesting an overall loss of transcriptional regulation at these regions (Chambers et al., 2007). The age-related change in the expression of chromatin-remodeling proteins, in combination with loss of nucleosome occupancy and redistribution of histone-modifying enzymes on the chromatin caused by DNA damage in old cells (Oberdoerffer et al., 2008; Dang et al., 2009; Feser et al., 2010), can collectively lead to alterations in chromatin configuration that affect the accessibility of large chromosomal regions and, thus, introduce transcriptional noise (Busuttil et al., 2007; Feser et al., 2010). It will be interesting to determine whether and to what degree the rejuvenation of old stem cells, as by exposure to a young environment (Conboy et al., 2005), is mediated by restoring the balance among different chromatin-remodeling complexes.

Proteomic changes. Maintenance of the intracellular proteome requires timely removal of improperly folded or damaged proteins that can otherwise impede normal cellular function (Koga et al., 2011). Autophagosomes, chaperones, lysosomes, and the ubiquitin–proteasome system are all important cellular processes and machineries that maintain protein homeostasis (Rajawat et al., 2009; Koga et al., 2011). Together, they sense and remove misfolded or aberrant proteins in cells and ensure a functional proteome. With age, the protein homeostatic machinery becomes less efficient and less effective (Rodriguez et al., 2010; Koga et al., 2011), and these functional declines would only accentuate the negative effect of proteomic changes during aging.

Age-related increases in the levels of damaged proteins have been well documented in long-lived postmitotic cells, such as neurons, cardiomyocytes, and skeletal myofibers, and in some cases, these damaged proteins form aggregates or inclusion bodies that can cause proteotoxicity to cells (Rodriguez et al., 2010). It has been postulated that proliferative cells are less prone than are postmitotic cells to impairment caused by the accumulation of aberrant proteins and metabolic wastes, as their accumulation is predicted to be diluted by cell division and the synthesis of new cellular constituents during mitosis (Kirkwood, 2005). One example is the age-related deterioration of nuclear pore complexes (NPCs) that results in an increase in nuclear permeability and the leakage of cytoplasmic proteins into the nucleus in neurons (D'Angelo et al., 2009). This NPC deterioration is caused by oxidation of the scaffold nucleoporin complex

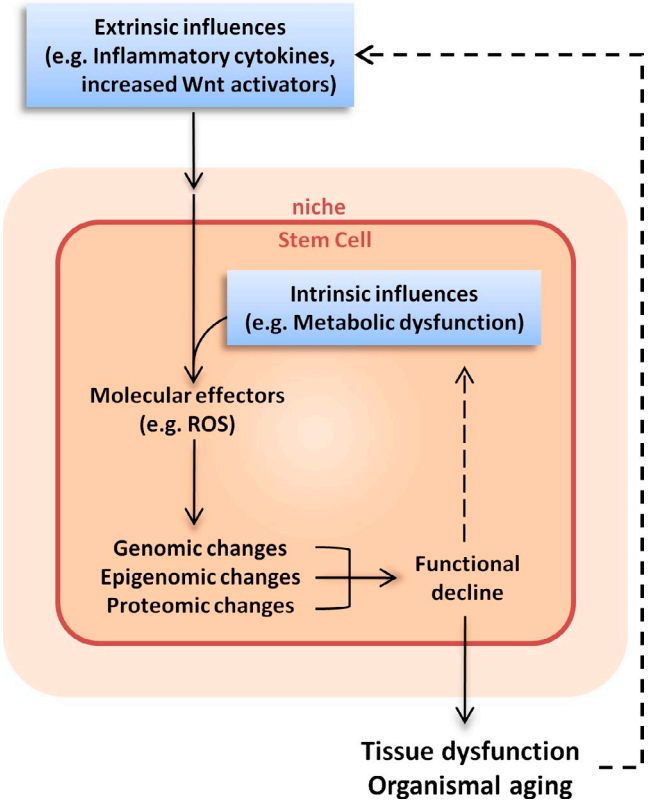

Figure 3. **Extrinsic and intrinsic influences on stem cell aging.** Age-related changes in the systemic milieu, for example, an increased level of inflammatory cytokines or Wnt pathway activators in the circulation, can lead to changes of signaling cascades and molecular changes within cells. For stem cells, the effects of extrinsic influences may also be transmitted indirectly via detrimental changes in the niche. Meanwhile, intrinsic changes, such as changes in mitochondrial activity or metabolic rate, can also occur during cellular aging. The extrinsic and intrinsic influences converge at intracellular molecular effectors, such as reactive oxygen species (ROS), which can cause either reversible changes (such as protein oxidation) or irreversible changes (such as DNA mutations) to macromolecules in the cell. The combinatorial effects of genomic, epigenomic, and proteomic changes lead to a decline in cellular function, which in turn contributes to tissue dysfunction and organismal aging. Because dysfunctional stem cells give rise to abnormal differentiated cells in the tissue, stem cell aging exacerbates the extrinsic influences of aging, thereby also contributing to the aging process of the tissue and organism.

Nup107–160, which is not replaced in postmitotic cells because of its inactive transcription. However, in proliferating cells, the active expression of Nup107–160 allows replacement of this damaged nucleoporin during cell division when the NPCs reassemble (D'Angelo et al., 2009).

Stem cells with low turnover rates, such as satellite cells in skeletal muscle (Morgan and Partridge, 2003), are likely to be more susceptible to proteotoxicity than stem cells with a higher turnover rate. During replicative aging of yeast, damaged protein aggregates are selectively retained in mother cells during budding to ensure the youthfulness of daughter cells (Erjavec et al., 2008; Kaeberlein, 2010). The polarizome, which is composed of the formin Bni1p and myosin motor protein Myo2pa, is a key component of the cellular machinery that ensures asymmetric segregation of protein aggregates during cytokinesis associated with yeast budding (Liu et al., 2010a). Adult mammalian stem cells may use similar mechanisms to protect themselves from the accumulation of damaged protein aggregates, perhaps selectively segregating damaged proteins to their differentiating rather than their self-renewing progeny.

Conclusion

Although stem cells exhibit age-related changes at the genomic, epigenomic, and proteomic levels, the functional consequences of these changes remains to be determined. In contrast, age-related changes in the local or systemic environment have been directly implicated in the decline in stem cell function with age. Ultimately, understanding the age-related changes in stem cell functionality will require a distinction among intrinsic irreversible changes (such as genomic mutations), intrinsic reversible changes (such as epigenomic alterations), and extrinsic influences from the local and systemic environment (Fig. 3). These distinctions are further complicated by the fact that stem cells, in turn, influence the niche in which they reside. All of these issues are brought to bear in the consideration of stem cell therapeutics for diseases and disorders of aging. Whether focusing on the potential of endogenous stem cells or of exogenous transplanted stem cells as therapeutic vehicles, the complex interactions between the cells and their environment are likely to be critical determinants of the success of such therapeutic approaches. Understanding the mechanisms by which stem cell functionality declines with age will be essential to enhance tissue repair in the elderly and to solve the challenges of stem cell therapeutics for age-related diseases.

T.A. Rando is funded by the National Institutes of Health (grants R37 AG23806 and R01 AR056849 and a National Institutes of Health Director's Pioneer Award), the Glenn Foundation for Medical Research, the Department of Veterans Affairs (Merit Review), and the American Federation for Aging Research (Breakthroughs in Gerontology Award).

Submitted: 26 October 2010
Accepted: 28 March 2011

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