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Stems Cells and the Pathways to Aging and Cancer

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The aging of tissue-specific stem cell and progenitor cell compartments is believed to be central to the decline of tissue and organ integrity and function in the elderly. Here, we examine evidence linking stem cell dysfunction to the pathophysiological conditions accompanying aging, focusing on the mechanisms underlying stem cell decline and their contribution to disease pathogenesis.

Introduction

Most tissues and organs contain minor populations of primitive stem cells and progenitor cells. These cells are integral first in the developing fetus for the generation of tissues and organs and later in the adult for ongoing tissue maintenance and regeneration after injury. Differences in developmental potential and lineal relationships between stem and progenitor subsets establish a hierarchical structure for primitive stem cell compartments. Stem cells reside at the apex of the hierarchy and give rise to multipotent progenitors, which in turn give rise to progenitors with more restricted lineage potential. Although best exemplified in the hematopoietic system (see the Review by S.H. Orkin and L.I. Zon, page 631 of this issue), the existence of hierarchical relationships between stem and progenitor cells is emerging as a common feature of other tissue-specific stem cell compartments as well (Bryder et al., 2006). All stem cells are capable both of self-renewing to give rise to daughter stem cells and of differentiating into a variety of mature cell types. These are the defining properties of stem cell biology that ensure that tissues can be functionally sustained throughout the lifetime of the organism, while avoiding the onset of hypoplasia and atrophy.

Although certainly one of the most recognizable characteristics of human biology, aging remains one of the least understood. This is largely attributable to the fact that aging is both gradual and inherently complex, with almost all aspects of physiology and phenotype undergoing steady modification with advancing age. The complexity of the aging process does not allow for a single all-encompassing definition, yet decades of study using diverse systems, methodologies, and model organisms have begun to build a consensus regarding the central physiological characteristics of aging. Indeed, such studies have shown that the process of aging is invariably accompanied by a diminished capacity to adequately maintain tissue homeostasis or to repair tissues after injury. When homeostatic control diminishes to the point at which tissue/organ integrity and function are no longer sufficiently maintained, physiologic decline ensues, and aging is manifested. Consistent with this, many of the pathophysiological conditions afflicting the elderly, such as anemia, sarcopenia (loss of muscle mass), and osteoporosis, suggest an imbalance between cell loss and renewal. The fact that homeostatic maintenance and regenerative potential of tissues wane with age has implicated stem cell decline as a central player in the aging process. However, the degree to which aging is attributable to stem cell dysfunction or instead reflects a more systemic degeneration of tissues and organs will likely differ substantially between different tissues and their resident stem cells. Nonetheless, mounting evidence points to stem cells as an important contributing factor to at least some of the pathophysiological attributes of aging in a number of different tissues. It therefore seems likely that a central focus of future research on mammalian aging will involve a careful evaluation of the contribution and mechanisms of stem cell dysfunction on a tissue-by-tissue basis.

That advancing age is accompanied by an increased incidence of cancer is incontrovertible. As cancers arise only after the acquisition of multiple mutagenic events, long-lived cells are the only cells capable of serving as such reservoirs. Stem cells represent ideal cellular targets for the accumulation of precancerous damage as the central properties of stem cells—self-renewal and differentiation—enable mutations acquired in the stem cell compartment to be propagated to both self-renewing progeny and downstream progenitors over the lifetime of the organism. The danger posed by mutagenic accumulation in stem cells is held in check by the activity of tumor suppressor proteins that censor potentially malignant clones by eliciting apoptosis or permanent growth arrest. However, as the homeostatic demands of tissues require ongoing stem and progenitor cell activity, tumor suppressor pathways may inadvertently lead to stem cell attrition and in such a manner contribute to aging.

In this Review we focus on several aspects of stem cell biology, aging, and disease pathogenesis, beginning with an overview of the changing roles that stem cells play during ontogeny. We then discuss studies indicating that stem cell aging contributes to age-dependent physiological decline, with a particular emphasis on mechanisms known to drive cellular aging, and examine how such mechanisms impact stem cell biology. We

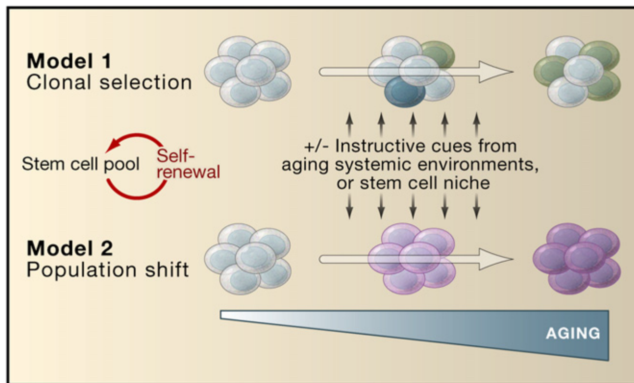

Figure 1. Models of Stem Cell Aging
Stem cell compartments established during fetal development are maintained throughout adult life by self-renewal and not by de novo generation from more primitive progenitors. In one model of stem cell aging (top), functionally distinct clones emerge within the stem cell pool due to differences in the state of their genome and epigenome (such clones may or may not already exist within the fetal stem cell pool). During aging, certain clones expand and come to predominate the pool, whereas other clones fail to thrive. The success or failure of different clones can be related to differences in genetic and epigenetic makeup and clonal fitness to respond and flourish within the aging microenvironment. In a second model (bottom), all stem cells in the stem cell pool are imbued with more or less similar functional capacity, which gradually and coordinately changes over time. Changes in functional potential of stem cells in both models may be cell autonomous or derive from instructive cues from the stem cell niche or aging systemic environment. It should be noted, however, that these models are not mutually exclusive, and both may act in concert or at different stages of ontogeny.

conclude with a discussion of the connection between the increased incidence of cancer with advanced age and how, at least in the hematopoietic system, the clonal progression to cancer operates through the stem cell compartment.

Stem Cell Ontogeny: Age Matters

The question of when physiological aging begins in the context of stem cell biology is germane to this discussion and requires a consideration of stem cell ontogeny. Stem cells are vital to all stages of life, yet the specific roles that stem cells play during different stages of ontogeny change considerably. During early embryogenesis, pluripotent stem cells differentiate to give rise to the three germ layers that establish the body plan (see Review by C.E. Murry and G. Keller, page 661 of this issue). As development proceeds, distinct subsets of stem cells emerge to orchestrate the construction of tissues and organs—processes that are often incomplete at birth and carry over into postnatal life, as is the case with mammalian reproductive tissues. Once tissues are fully established, however, stem cells undergo a fundamental change as their role turns from one of tissue building to one of tissue maintenance, which persists throughout adult life. A fundamental assumption that underlies stem cell models of aging is that at some point during development, all tissue-specific stem cells derive from pre-existing stem cells by self-renewal, and not by de novo generation from more primitive progenitors (Figure 1). In this view, stem cells in both young and old adults alike are ultimately derived only from stem cells of fetal origin through their ability to sustain long-term self-renewal, and thus the "aging" of a stem cell lineage can be thought to commence upon definitive specification of that lineage and subsequent to any additional input from more primitive progenitors.

Coincident with the changing roles that stem cells play during distinct stages of ontogeny, their basic properties change too. This is probably best exemplified in the hematopoietic system where hematopoietic stem cells (HSCs) of fetal origin differ from those of the adult by a number of criteria such as surface phenotype (Morrison et al., 1995; Osawa et al., 1996; Yoder et al., 1997), cell-cycle status (Bowie et al., 2006), and the anatomical location where stem cell activity resides (see Review by S.H. Orkin and L.I. Zon). Different ontological stages are also marked by characteristic changes in HSC developmental potential. For example, fetal murine HSCs have the capacity to give rise to distinct repertoires of T cell (Ikuta et al., 1990) and B cell (Kantor and Herzenberg, 1993) subsets, which is lost in adulthood. More gradual transitions are also at play with fetal HSCs having a greater capacity to give rise to lymphoid lineage cells than stem cells from young adult mice (Morrison et al., 1995), which in turn possess greater lymphoid lineage potential than do HSCs from old mice (Kim et al., 2003; Rossi et al., 2005; Sudo et al., 2000). The timing of these developmental changes suggests the existence of molecular switches that are tightly regulated during ontogeny. Evidence of this is found upon the examination of the global gene expression profiles of stem cells from fetal, young, and old mice, which indicate profound differences in the transcriptome that are reflective of changing developmental potentials at different stages of ontogeny (Ivanova et al., 2002; Phillips et al., 2000; Rossi et al., 2005). Underlying mechanisms likely include the utilization and dependence on different transcriptional networks, which play fundamental roles in distinguishing embryonic, fetal, and adult stem cells. For example, whereas the transcription factor Oct4 is essential for the establishment of embryonic stem cells (Nichols et al., 1998), it is entirely dispensable for self-renewal and maintenance of adult stem cells (Lengner et al., 2007). Similarly, the transcription factor Sox17 is required for the maintenance and function of fetal murine HSCs but not for adult HSCs (Kim et al., 2007). In contrast, other transcription factors such as Gfi-1 (Hock et al., 2004a; Zeng et al., 2004) and Etv6 (Hock et al., 2004b) are more important for self-renewal and maintenance of adult stem cells but are less important during fetal development.

The regulatory mechanisms underlying the transition of stem cells into old age are less well defined. Nonetheless, important insights have been gained from global gene expression studies of stem cells purified from young and old mice. Such studies have implicated the involvement of higher-order chromatin dynamics and epigenetic regulation in stem cell aging, as suggested by coordinated age regulation of lineage specification genes (Rossi et al., 2005), chromosomal regions (Chambers et al., 2007), and genes involved in chromatin remodeling (Chambers et al., 2007; Rossi et al., 2005, 2007b). Functional evaluation of diverse chromatin regulators supports a role for such factors in stem cell aging. For example, overexpression of the Polycomb group (PcG) protein Ezh2 can extend the replicative capacity of mouse HSCs during serial transplantation (Kamminga et al., 2006). Another PcG protein, Bmi-1, is required for maintaining

self-renewal of adult HSCs (Iwama et al., 2004; Park et al., 2003), neural stem cells (NSCs) (Molofsky et al., 2003), and leukemic stem cells (LSCs) (Lessard and Sauvageau, 2003). Bmi-1 has also been implicated in aging through its capacity to repress the *p16^{Ink4a}-p19^{Arf}* locus (Jacobs et al., 1999), which encodes two tumor suppressors: p16INK4A, an inhibitor of Cdk4/6, and p19/ARF that regulates p53 stability through inactivation of the ubiquitin ligase Mdm2. By repressing the *p16^{Ink4a}-p19^{Arf}* locus, Bmi-1 averts growth arrest or apoptosis of stem cells (Molofsky et al., 2005; Oguro et al., 2006). Intriguingly, Bmi-1-mediated suppression of the *p16^{Ink4a}-p19^{Arf}* locus is dependent upon the presence of Ezh2 (Bracken et al., 2007), suggesting a mechanism through which reduced expression of *Ezh2* in aged HSCs (Rossi et al., 2005) may lead to induction of *p16^{Ink4a}* (Janzen et al., 2006). Together these studies demonstrate that the mechanisms regulating stem cell activity change during ontogeny and suggest that the utilization of different transcriptional networks driven by epigenetic regulation of chromatin accessibility is central to these changes.

Hematopoietic Stem Cell Aging

Advancing age is accompanied by a number of pathophysiological changes in the hematopoietic system, whose etiology suggests loss of homeostatic control and possible stem and progenitor cell involvement. The most clinically significant of these changes are the decreased competence of the adaptive immune system (Linton and Dorshkind, 2004), the increased incidence of myeloid diseases including leukemias (Lichtman and Rowe, 2004), and the onset of anemia in the elderly (Beghe et al., 2004). The ability of human HSCs to give rise to primitive progenitors declines substantially during the ontological transitioning from fetal liver, to cord blood, to adult bone marrow (Lansdorp et al., 1993), suggesting a progressive loss of stem cell activity with age. Consistent with this, a recent meta-analysis conducted using the National Marrow Donor Program (NMDP) registry assessed multiple donor traits (including donor age, cytomegalovirus serologic status, ABO blood group compatibility, race, and sex) on various parameters of bone marrow transplant success in 6978 transplant recipients (Kollman et al., 2001). This study revealed that advanced donor age was significantly associated with overall decreased disease-free survival. These studies suggest that the proliferative and regenerative capacity of human HSCs diminishes with age, and that diminished stem cell activity is largely cell intrinsic. These conclusions have been corroborated and expanded upon in studies of mice.

Models of aging often presuppose that loss of homeostatic control and regenerative potential of tissues is driven by diminution of stem cell reserves, yet extensive evidence indicates that HSC numbers increase substantially with advancing age in common strains of laboratory mice (de Haan et al., 1997; Morrison et al., 1996; Rossi et al., 2005; Sudo et al., 2000). The expansion of the stem cell pool is a cell-autonomous property as HSCs from aged donors exhibit a greater capacity, than young controls, to self-renew to give rise to phenocopies of themselves upon transplantation in young recipients (Pearce et al., 2006; Rossi et al., 2005). Studies have shown that murine HSCs have the ability to reconstitute successive recipients through serial transplantation, indicating that the replicative capacity of HSCs far exceeds that of a normal mouse life span (Harrison, 1979). However, when HSCs from old mice were assayed for bioactivity, numerous functional deficiencies came to light, including altered homing and mobilization properties (Liang et al., 2005; Xing et al., 2006; see Review by D.J. Laird et al., page 612 of this issue), diminished competitive repopulating ability (Kim et al., 2003; Morrison et al., 1996; Rossi et al., 2005; Sudo et al., 2000), and a skewing of lineage potential from lymphopoiesis toward myelopoiesis with age (Kim et al., 2003; Liang et al., 2005; Rossi et al., 2005; Sudo et al., 2000). The latter property correlates with the reduced frequency of lymphoid progenitors in old mice (Miller and Allman, 2003; Rossi et al., 2005), whereas myeloid progenitors are maintained or even expanded in old mice (Rossi et al., 2005). Importantly, the differential capacity of HSCs from old mice to give rise to lymphoid and myeloid progenitors was found to be a transplantable, cell-autonomous property of HSC aging underwritten by the suppression of numerous genes involved in specifying lymphoid fate and the upregulation of genes involved in myeloid specification (Rossi et al., 2005). Cumulatively, these studies suggest that two clinically important pathophysiological features of the aged hematopoietic system, namely, immune system decline and the development of myelogenous disease, may have their origins in age-dependent functional and molecular changes intrinsically arising in stem cells (Figure 2).

A growing body of evidence suggests that the stem cell pool may be comprised of a number of clonal lineages possessing heritable differences in functional capacity (reviewed in Muller-Sieburg and Sieburg, 2006). As aging proceeds, certain clones may out-compete others while still remaining under the homeostatic regulatory mechanisms imposed by the systemic environment or the stem cell niche microenvironment (see Review by S.J. Morrison and A.C. Spradling, page 598 of this issue). For example, the myeloid lineage bias of aged HSCs may result from clonal expansion of stem cell clones with a myeloid bias during aging. The reproducibility of the skewing of lymphoid/myeloid lineage potential in the aged hematopoietic system implies a certain fitness of myeloid-biased clones to thrive under the cues provided by the aging systemic environment or niche microenvironment (Figure 1). In an alternative model, the developmental potential of most or all of the cells in the stem cell population could gradually and coordinately change over time perhaps in a cell-autonomous fashion or in response to instructive cues from the aging environment. In this latter model, stem cell aging may be viewed as an ongoing developmental process with fetal development and old age as the start and end points, respectively (Figure 1). Within this framework it can be argued that changes in lineage potential characteristic of HSC aging may simply reflect the developmental demands of distinct stages of ontogeny. So while the lineage potential of HSCs may be inclined toward the lymphoid system early in life to facilitate the establishment of immunological memory in the adaptive immune system, the myeloid bias of aged stem cells may reflect the need to maintain robust innate immunity to counter the increased risk of infection in the elderly.

It is also worth noting that although pediatric leukemias tend to be lymphoid in origin, the leukemias that manifest in old age often originate in myeloid compartments, suggesting that the malignant capacity of hematopoietic progenitors changes in

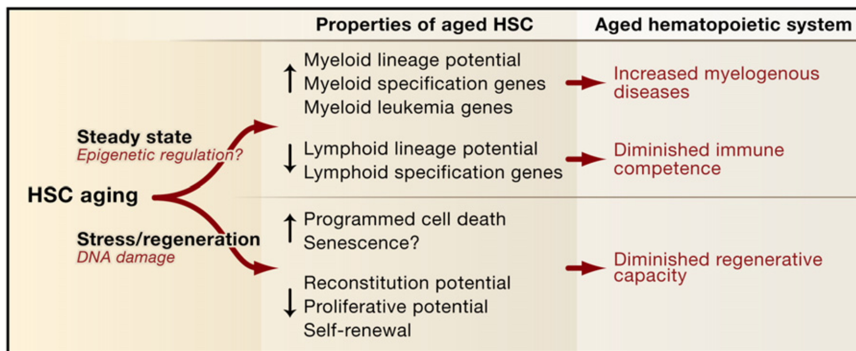

Figure 2. Properties of Aging HSCs
A model of hematopoietic stem cell (HSC) aging under steady-state and stress conditions is presented with the properties of aged stem cells proposed to contribute to age-dependent pathophysiologies. In the steady state, the functional and molecular properties of aged HSCs result in a skewing of lineage potential away from lymphoid toward myeloid fates, which we propose may underlie the immuno-decline and prevalence of myelogenous disease, respectively, in the elderly. Several lines of evidence suggest that these changes may be mediated by epigenetic regulatory mechanisms. Under conditions of stress and regeneration, stem cells exhibit a number of functional deficits that are believed to contribute to the diminished regenerative capacity of the aged hematopoietic system, and which evidence suggests may result from age-dependent accrual of DNA damage.

a manner mirroring the change in lineage potential of HSCs during aging (Kim et al., 2003; Liang et al., 2005; Rossi et al., 2005; Sudo et al., 2000). This raises the possibility that differences in disease spectra arising at different points during ontogeny may be influenced by, or directly result from, changes in the developmental potential of stem cells at a given stage. In support of this, a recent study demonstrated that ectopic expression of the *BCR-ABL* oncogene (which causes chronic myeloid leukemia in humans) in bone marrow cells of young mice led to the concurrent development of a myeloproliferative disease (MPD) and B cell leukemia, whereas expression of the *BCR-ABL* oncogene in marrow cells of old mice gave rise to MPD with little or no lymphoid involvement (Signer et al., 2007). Moreover, the observation that stem cell aging is coupled to elevated expression of numerous genes, such as *Aml1*, *Pml*, and *Eto*, involved in the pathogenesis of myeloid leukemia (Rossi et al., 2005) suggests the possibility that the upregulation of such proto-oncogenes may act synergistically with the myeloid bias of aged stem cells to predispose to myelogenous disease and leukemia (Figure 2). Whether or not the upregulation of such proto-oncogenes in stem cells with age increases their susceptibility to the types of cytogenetic rearrangements and translocations that promote the development of leukemia remains to be determined.

Aging in Other Stem Cell Compartments

Until recently, functional evaluation of stem cells from nonhematopoietic tissues was limited by a paucity of appropriate assays and by the purity of the stem cells in question. Recent technical advances in assay systems, along with the prospective isolation of a wide variety of somatically derived stem cells, are now permitting the types of functional evaluation that have long been afforded the HSC research community. Emerging evidence suggests that diverse tissue-specific stem cell reserves decline with advancing age, and that such a decline bears important pathophysiological consequences for aging of tissues and organs. For example, graying of hair, one of the most recognizable aspects of human aging, appears to result from an age-dependent loss of melanocyte stem cells (MSCs) from the subcutaneous hair follicle bulge region (Nishimura et al., 2005) and may be exacerbated by telomere dysfunction as suggested by the premature graying of telomerase-deficient mice (Rudolph et al., 1999). Epidermal stem cells, on the other hand, which also reside in the bulge region, exhibit reduced mobilization and proliferation upon telomere shortening but do not appear to be diminished (Flores et al., 2005). Conversely, overexpression of the catalytic component of telomerase drives quiescent epidermal stem cells into cycle in a manner that is independent of telomere length (Flores et al., 2005; Sarin et al., 2005). These studies indicate that age-dependent loss of function of two distinct stem cell compartments in the hair follicle may be under the control of both canonical and noncanonical telomerase pathways.

Studies of the murine gastrointestinal tract have shown that cells from old mice at or near the position of the stem cells within the crypts of Lieberkuhn are more susceptible to apoptosis under stress (Martin et al., 1998a) and exhibit reduced regenerative potential despite an age-dependent increase in the number of clonogenic crypt cells (Martin et al., 1998b). Although signaling pathways that control self-renewal, such as the Wnt pathway, are deregulated in the majority of colon cancers, the mechanisms promoting normal crypt stem cell self-renewal have been less well defined. Until recently, there was a relative dearth of information regarding the phenotype and function of normal intestinal stem cells. Recently, small intestine and colon stem cells were identified based on expression of a Wnt target gene, *Lgr5 (leucine-rich-repeat-containing G protein-coupled receptor 5)* (Barker et al., 2007), that was isolated from a panel of Wnt target genes because of its crypt-restricted expression. This discovery provides the impetus for investigating the molecular mechanisms driving normal intestinal stem cell aging and the sequential events required for the development of colon cancer.

In the central nervous system, the generation of new neurons continues throughout life in at least two regions of the brain, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus of the hippocampus (Eriksson et al., 1998; Palmer et al., 1997; see Review by C. Zhao et al., page 645 of this issue). Ongoing neurogenesis is mediated by the activity of NSCs and is believed to be important for sensory functions such as olfaction as well as cognitive functions such as memory and learning. The decline in both sensory and cognitive functions in the elderly thus implicates age-associated

NSC decline as an important contributory factor. In support of this, NSC numbers and proliferative potential are reduced in the SVZ with age (Maslov et al., 2004; Molofsky et al., 2006), correlating with the diminished neurogenesis observed in the olfactory bulb of old mice (Molofsky et al., 2006). Similarly, the diminished proliferative potential of granule cell progenitors in the dentate gyrus of aged rats may contribute to the decline in hippocampal neurogenesis that occurs with aging (Kuhn et al., 1996), which is associated with age-related loss of cognitive ability (Drapeau et al., 2003). The diminishing function of NSCs with age has been linked with increased genomic instability (Bailey et al., 2004) and induction of p16^{Ink4a} expression (Molofsky et al., 2006). As in the hematopoietic system, the spectrum of tumor types in the brain changes with age, with neuroblastomas and medulloblastomas predominating in pediatric cases, and tumors of glial origin tending to predominate in aged individuals. Given that the cellular origin of brain tumors may arise directly from the progeny of CD133^{+} stem cells (Uchida et al., 2000), it will be important to determine if changes in the developmental potential of NSCs during ontogeny underlie the predisposition to the changing spectrum of brain tumors presented during different stages of life (Figure 1).

Extrinsic Regulation of Stem Cell Aging

Stem cells from a variety of tissues reside in close proximity to specialized support cells that extrinsically regulate stem cell self-renewal, differentiation, and, as emerging evidence indicates, aging. For instance, in the fruit fly *Drosophila*, aging of both male and female germline stem cells (GSCs) is regulated, at least in part, by a niche-dependent decline resulting from either diminished signaling via bone morphogenetic proteins (BMPs) in the ovary (Pan et al., 2007) or diminished signaling by a ligand called Unpaired in the testis (Boyle et al., 2007; see Review by S.J. Morrison and A.C. Spradling). In both cases, reduced cadherin-mediated adhesion of GSCs to the niche and weakening of direct contacts between stem cells and niche cells with age correlated with the decline in GSC numbers and function. The systemic milieu has also been shown to regulate stem cell decline during aging as demonstrated by experiments in which exposure of muscle satellite cells of old mice to a young systemic environment through heterochronic parabiosis and the establishment of a surgically conjoined circulatory system was sufficient to restore the regenerative potential of the satellite cells from old mice, in part, through the restoration of Delta-Notch signaling (Conboy et al., 2005). The impaired ability of aged muscle satellite cells to adequately respond to injury has been connected to declining numbers of these cells (Shefer et al., 2006) and the tendency of satellite cells to convert from their normal myogenic fate to a fibrotic fate with age due to elevated Wnt signaling originating from the serum of old mice (Brack et al., 2007). Activated Wnt signaling also appears to suppress stem/progenitor cell numbers in Klotho-deficient mice (Liu et al., 2007), which develop degenerative phenotypes linked to impaired vitamin-D homeostasis. Studies aimed at elucidating the function of Wnt signaling in HSC biology underscore the cell type and context-specific effects of Wnts in development. Differences in model systems have resulted in some studies indicating that Wnt signaling, through the canonical pathway, enhances stem cell self-renewal capacity (Reya et al., 2003; Willert et al., 2003), whereas others suggest alternative effects (Cobas et al., 2004). It has, however, become apparent that too much Wnt signaling through constitutive activation of β-catenin can severely blunt HSC self-renewal, adhesion, and differentiation properties (Kirstetter et al., 2006; Scheller et al., 2006). So although Wnt signaling may be beneficial to stem cells in certain physiological settings, imbalances in Wnt, the activation of noncanonical pathways by Wnt, or ontological differences in the way that Wnt signals are received may suppress stem cell activity in other settings such as aging.

Although much of the functional decline that accompanies HSC aging is cell intrinsic, the influence of the stem cell niche on HSC biology is not in question, and thus it would be surprising if HSC aging were not extrinsically modulated to some degree. Along these lines it is noteworthy that caloric restriction (CR), the only intervention known to extend longevity across species, significantly improved HSC function in aged mice, even after transplantation into non-CR recipients (Chen et al., 2003). CR has also been reported to suppress myeloid leukemic development in an irradiation-induced mouse model of leukemia, coincident with a decline in the numbers of primitive hematopoietic stem/progenitor cells, the presumed targets of disease development in this model (Yoshida et al., 2006). These provocative findings suggest that stem cell numbers, aging, and diseases of stem cell origin may be modulated by pathways believed to be involved in mediating the antiaging effects of CR across species, such as the insulin-like growth factor (Igf1) pathway (Kenyon, 2005) or the Sir2 family of deacetylases (Chen and Guarente, 2007).

DNA Damage and Its Contribution to Stem Cell Aging

One mechanism believed to be central to the aging of cells and tissues is cumulative damage to cellular macromolecules such as proteins, lipids, RNA, and DNA. Of these, none has been more intimately linked to aging than DNA damage (reviewed in Lombard et al., 2005). This is likely due to the fact that in contrast to other cellular polymers such as proteins and RNA, DNA is neither appreciably turned over nor recycled. Furthermore, the sheer magnitude of genomic insult that cells must endure is believed to be staggering, with some estimates suggesting that every cell in the human body incurs upwards of several thousand DNA lesions daily due to spontaneous depurination and hydrolysis alone (Lindahl, 1993). At the cellular level, genomic damage is imparted by a variety of sources including reactive oxygen species (ROS), the natural by-products of oxidative metabolism, which are considered a major source of damage contributing to aging. It is clear, however, that in addition to ROS, a much broader range of extrinsic and intrinsic sources, such as UV irradiation, alkylating agents, telomere attrition, and DNA replication errors, can also infringe upon genomic integrity. In order to curtail the biological impact of such damage, cells have evolved a number of pathways that recognize, respond to, and repair different types of lesions (reviewed in Hoeijmakers, 2001). Due to the imperfect nature of these repair systems, however, a certain degree of DNA damage evades repair and accumulates with age (Hamilton et al., 2001), although the rates at which damage accrues in different cell types appear to be tissue specific (Dolle

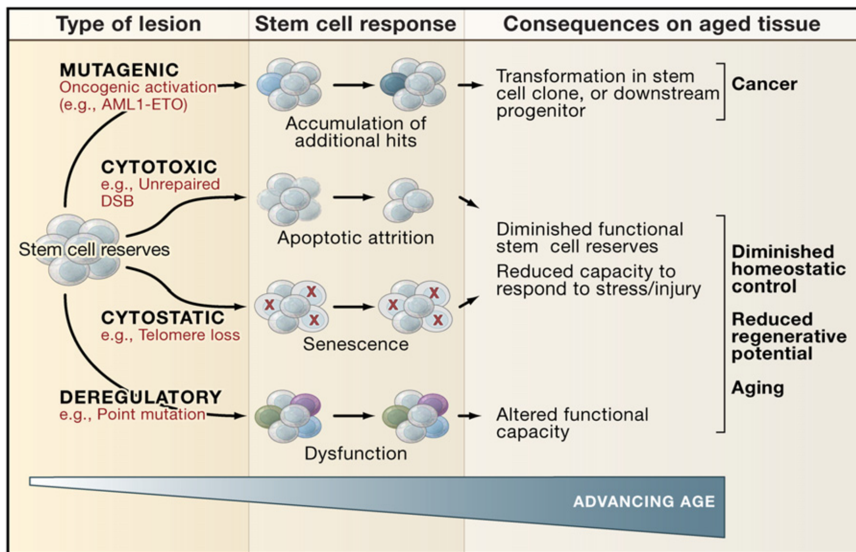

Figure 3. DNA Damage in the Stem Cell Compartment
Heritable DNA damage accrued in stem cells elicits different responses depending upon the nature and extent of the lesions. Examples of each type of lesion are shown, although the cellular response and outcomes are not mutually exclusive. Mutagenic lesions in stem cells can lead to transformation of stem cells or their progeny upon acquisition of a full repertoire of oncogenic lesions. Cytostatic and cytotoxic lesions lead to stem cell senescence or apoptosis, respectively, which over time could lead to diminution of the stem cell pool. Other types of damage can lead to deregulation of the mechanisms governing stem cell biology and to dysfunction. Cumulatively, cytostatic, cytotoxic, and deregulatory lesions can lead to diminished homeostatic control and reduced regenerative potential, the physiological hallmarks of aging.

et al., 1997). Compelling genetic evidence that genomic damage contributes to the pathophysiology of aging has emerged from the study of human segmental progeria syndromes (often referred to as premature aging disorders) such as Werner syndrome, Cockayne syndrome, Trichothiodystrophy, Bloom syndrome, and Ataxia telangiectasia. These diseases have all been found to have deficits in pathways that mediate DNA-damage responses or DNA-damage repair (reviewed in Martin, 2005). This concept has been strengthened in studies of mouse strains harboring DNA-damage repair, response, and maintenance deficiencies, which exhibit a spectrum of degenerative phenotypes reminiscent of ''accelerated'' aging (Lombard et al., 2005). It should be noted, however, that there is no clear consensus as to whether human progeria syndromes, or the murine strains that model them, accurately reflect aging at an accelerated pace or simply exhibit pathologies that share certain phenotypes characteristic of aged individuals.

Stem cells are subject to a similar array of insults as somatic cells and are therefore presumed to accrue damage with age. Unlike somatic cells, however, lesions arising in the stem cell compartment—except those resulting in growth arrest, or cell death—can be propagated both to daughter stem cells and to downstream lineages through the processes of self-renewal and differentiation. In such a manner, the impact of damage accrued in individual stem cells can be potentiated with possible ramifications for all levels of the developmental hierarchy. Although certain types of genomic lesions can modulate cellular behavior directly, the consequence of DNA damage on the physiology of tissues is largely believed to be attributable to the cellular response. Depending upon the nature and extent of the damage, cytostatic, cytotoxic, or mutagenic lesions arising in stem cells have the potential to drive cells to senescence, apoptosis, or transformation, respectively (Figure 3). If diminution of the functional stem cell reserves surpasses levels of self-renewal and mature cell production, then diminished homeostatic control and reduced regenerative potential—the physiological hallmarks of aging—would be predicted to ensue. Alternatively, if unrepaired or imprecisely repaired DNA damage proves sufficiently mutagenic, transformation of stem cells or their progeny could occur. From the point of view of clonal selection, stem cells persisting with unrepaired or mutagenic lesions can act as substrates for additional hits, which in turn could propel the clonal selection process even further if lesions imparting a growth or survival advantage are acquired. However, as in other cell types, potent tumor suppressor pathways are active in stem cells to ensure that cells harboring potentially dangerous lesions are removed from the stem cell pool. In the context of stem cells and progenitor cells that must proliferate throughout life, the action of tumor suppressor pathways also provides an avenue through which stem cell activity may be prematurely truncated during aging (Figure 4).

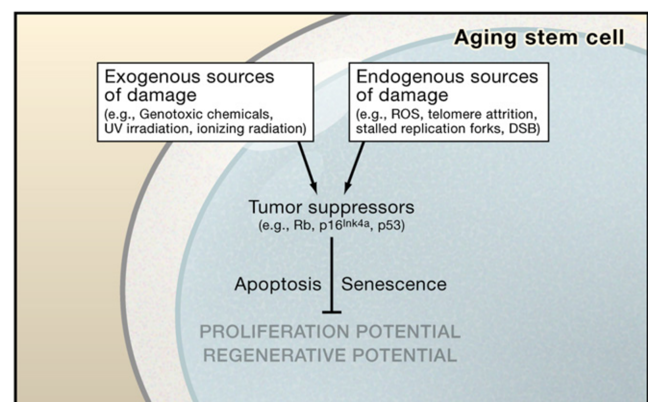

Figure 4. Stem Cell Aging and Tumor Suppressor Pathways
Cellular and genomic damage resulting from endogenous and exogenous sources activate tumor suppressor pathways including those mediated by the tumor suppressor proteins p53 and Rb to ensure that potentially dangerous lesions do not lead to malignancy. As aging advances and damage accrues, the activity of such tumor suppressors increases and in so doing has the potential to negatively modulate stem cell function through the induction of apoptosis or senescence.

Evidence that DNA damage contributes significantly to stem cell decline has principally been generated in the context of the hematopoietic system using mice bearing mutations in mediators of diverse DNA-repair pathways. For example, reduced repopulating activities have been reported in bone marrow transplantation experiments using mice with mutations in *FancD1/Brca2* (Navarro et al., 2006), *Msh2* (Reese et al., 2003), and *Lig4* (Nijnik et al., 2007). That DNA damage impacts stem cell function during aging was demonstrated in a series of experiments evaluating HSC reserves and functional capacity in young and old mice deficient in several different DNA-repair pathways (Rossi et al., 2007a, 2007c). Surprisingly, whereas HSC reserves were preserved during aging in these strains, stem cell function was severely attenuated when quiescent stem cells were forced into the cell cycle under conditions of stress or regeneration. The observation that stem cells from older mutant mice performed poorly compared to those from young mutant mice suggested that DNA damage may be accumulating in quiescent stem cells during aging and contributing to the diminished ability of old stem cells to regenerate tissues after injury (Figure 2). Consistent with this, purified quiescent HSCs from old wild-type mice—known to be deficient in regenerative capacity—were found to accrue considerable DNA damage with age as shown by the accumulation of γH2AX foci, a marker of DNA damage (Rossi et al., 2007a). These findings help to explain why mice bearing mutations in diverse DNA-repair/genomic maintenance pathways exhibit relatively normal hematopoiesis under steady-state conditions yet readily reveal stem cell functional impairment upon challenge (Carreau et al., 1999; Haneline et al., 1999; Navarro et al., 2006; Reese et al., 2003; Rossi et al., 2007a; Samper et al., 2002). It is unlikely that DNA damage underlies all aspects of stem cell aging, however, as the myeloid/lymphoid lineage-skewing characteristic of advanced aging in repair-competent HSCs (Kim et al., 2003; Liang et al., 2005; Rossi et al., 2005; Sudo et al., 2000) was not recapitulated during aging of DNA-repair-deficient mouse strains in which stem cell lineage potential has been evaluated (Rossi et al., 2007a) (Figure 2).

Although it seems clear that DNA damage contributes to the diminished capacity of stem cells to adequately respond to stress, the contribution of damage in stem cells to the general steady-state decline of aged tissues and organisms is less clear. Stochastic destabilization of transcription resulting from global DNA damage has been implicated in the age-dependent decline of certain postmitotic somatic cells (Bahar et al., 2006), yet this mechanism does not appear to be active in other cell types including stem cells (Warren et al., 2007). An alternative possibility is suggested by a recent study in which the DNA-damage response protein ATR (ataxia telangiectasia and Rad3 related) was conditionally ablated in adult mice to generate tissues that were mosaic for ATR deficiency (Ruzankina et al., 2007). These mice undergo rapid massive attrition in multiple tissues with high cell turnover yet were rescued by the emergence of stem cells and progenitor cells that had evaded ATR ablation resulting in restoration of tissue homeostasis to near normal levels. Within a few months, however, the mice developed several age-related phenotypes concomitant with homeostatic imbalances and also showed reduced stem/progenitor cell numbers in certain compartments including hair follicular bulge stem cells and bone marrow LSK (lineage$^-$Sca1$^+$ckit$^+$) cells (a population containing hematopoietic stem and progenitor cells). These results suggest that excessive replicative stress of ATR-competent stem/progenitor cells in response to a massive homeostatic catastrophe may lead to their premature exhaustion. On the other hand, the fact that HSCs can be serially transplanted through multiple successive recipients and still retain functional capacity even after prodigious activity does not support the idea that such a mechanism could exhaust stem cell functional reserves in animals over the course of one lifetime (Harrison, 1979). Moreover, given that HSC reserves do not diminish but rather quite substantially increase with advanced age in common laboratory strains of mice (Morrison et al., 1996; Rossi et al., 2005; Sudo et al., 2000), it seems likely that the loss of LSK cells in aged ATR mosaic mice may be a result of lasting consequences of ATR deficiency in niche cells (Ruzankina et al., 2007). Consistent with this, telomerase deficiency has been shown to lead to a lasting decline in the ability of the bone marrow microenvironment to support HSC engraftment and normal lineage potential (Ju et al., 2007). These studies highlight the importance of extrinsic signaling pathways and the aging of the environment for stem cell aging.

Stem Cell Aging and Reactive Oxygen Species

Prolonged exposure to ROS has long been postulated to contribute to aging. Indeed, this is the essential axiom of the oxidative stress or free radical hypothesis of aging posited by Harman in 1956 (Harman, 1956). Some lines of evidence support Harman's hypothesis, including studies demonstrating that oxidative lesions accumulate with age and the finding that increased longevity associated with caloric restriction is associated with reduced oxidative damage (Sohal and Weindruch, 1996). Furthermore, genetic manipulation of pathways that control cellular responses to oxidative stress have been shown to extend longevity in flies (Orr and Sohal, 1994) and in rodents (Holzenberger et al., 2003; Migliaccio et al., 1999). The argument behind this postulate has typically been framed in the context of damage accumulation in the mitochondrial genome and progressive mitochondrial dysfunction and is supported by genetic studies using mice bearing a proofreading-deficient mitochondrial DNA (mtDNA) polymerase, which exhibit a spectrum of degenerative phenotypes reminiscent of aging (Kujoth et al., 2005; Trifunovic et al., 2004). Recent studies have, however, cast doubt on this paradigm with the suggestion that point mutation accrual in mtDNA may not determine the rate of aging in wild-type mice (Vermulst et al., 2007). Moreover, a recent meta-analysis of data from 68 randomized trials showed no evidence that antioxidant dietary supplements had any beneficial effects on mortality but rather demonstrated, quite startlingly, that several common antioxidant dietary supplements actually increased the risk of death (Bjelakovic et al., 2007). Many lines of evidence have nonetheless pointed to myriad deleterious consequences of ROS in cells such as limiting the replicative capacity of certain cell types and the induction of permanent growth arrest. Importantly, a number of recent studies have provided clear evidence of a role for ROS in the preservation of stem cell function. In an elegant series of studies, the DNA-damage response protein ATM (ataxia telangiectasia-mutated) was shown to be vital for

regulating ROS levels and their effects on stem cells. HSCs from ATM-deficient mice had elevated intracellular levels of ROS with a concomitant decrease in functional capacity that could be restored upon treatment with the antioxidant N-acetyl-L-cysteine (NAC) (Ito et al., 2004). Loss of HSC activity in ATM-deficient mice in response to ROS is dependent upon activation of p38 MAP kinase (Ito et al., 2006) and induction of *p16^Ink4a^* (Ito et al., 2004). In another study, HSCs from mice lacking Foxo transcription factors (*Foxo1/Foxo3/Foxo4* triple mutants) showed enhanced short-term yet diminished long-term repopulation activity associated with increased cycling, apoptosis, and reduced stem and progenitor cell reserves (Tothova et al., 2007). Strikingly, Foxo-deficient HSCs showed elevated intracellular ROS levels that could be reduced upon treatment with the antioxidant NAC, which additionally restored HSC pool size, cell-cycle profile, and apoptosis to normal levels. In a related study, Foxo3a deficiency alone was sufficient to diminish HSC function, elevate intracellular ROS, disrupt stem cell quiescence, and decrease the size of the HSC compartment during aging (Miyamoto et al., 2007). Cumulatively, these studies identify intracellular management of ROS levels—by ATM, p38 MAP kinase, and $p16^{Ink4a}$ as well as by Foxo transcription factors—as an important mechanism contributing to the preservation of HSC reserves and function during aging.

Telomere Attrition

The maintenance of telomeres (specialized nucleoprotein structures at the ends of chromosomes) by the enzyme telomerase represents a specialized form of genomic maintenance and is of well-documented importance for cancer. Most human cancers possess the ability to maintain telomere length indefinitely either by overexpressing telomerase or by utilizing an alternative telomere lengthening pathway (ALT) that relies upon exchange of telomeric repeats (Muntoni and Reddel, 2005). In normal untransformed cells, when telomere attrition reaches a critical point, canonical DNA-damage response pathways mediated by the tumor suppressor proteins p53 or $p16^{Ink4a}$ are activated leading to the elimination or permanent growth arrest of these cells. Telomere dysfunction therefore represents a potent tumor suppressor mechanism meant to limit the proliferative capacity of malignant cells and to prevent uncontrolled growth. However, activation of tumor suppressor pathways may be a potential avenue by which stem cell activity is prematurely blunted with age (Figure 4). This may be particularly relevant to stem cell compartments in tissues, such as the blood, gut, and skin, with high turnover. Perhaps as a mechanism to counter this, telomerase activity is primarily restricted to primitive stem and progenitor cell compartments (Harrington, 2004). Nonetheless, as telomere shortening still takes place in human HSCs during aging (Vaziri et al., 1994) and during serial transplantation of murine HSCs (Allsopp et al., 2003a, 2003b), telomerase expression in stem cells may slow down telomere shortening but not stop it altogether. Importantly, expression of the catalytic component of telomerase was able to prevent telomere shortening in HSCs during serial transplantation but was insufficient to extend their serial transplant capacity, suggesting that telomere-length-independent mechanisms may ultimately limit the replicative capacity of murine HSCs (Allsopp et al., 2003b). Indeed, direct experimental evidence demonstrating that telomere shortening attenuates stem cell function or contributes to organismal aging in mice or humans possessing functional telomerase activity is limited.

There is evidence, however, that in the absence of functional telomerase, telomere attrition both diminishes stem cell and progenitor cell activity and reduces life span. For example, the clinical presentation of pancytopenia and bone marrow failure in patients suffering from aplastic anemia or dyskeratosis congenita with mutations in the catalytic component (Yamaguchi et al., 2005) or the RNA component (Fogarty et al., 2003; Vulliamy et al., 2002) of telomerase suggests clear hematopoietic stem and progenitor cell involvement. Mice deficient in telomerase activity have a reduced life span upon telomere shortening and develop progressive pathologies in proliferative tissues such as the gut, blood, and skin that are underwritten by diminished homeostatic maintenance capability and reduced regenerative potential (Blasco et al., 1997; Herrera et al., 1999; Lee et al., 1998; Rudolph et al., 1999). When directly assayed, stem and progenitor cell compartments were particularly affected in these mice with HSCs exhibiting reduced replicative capacity in serial transplantation assays (Allsopp et al., 2003a) and diminished repopulating ability (Rossi et al., 2007a; Samper et al., 2002). The proliferative potential of neural stem and progenitor cells in late generation telomerase-deficient mice was also diminished both in vitro (Ferron et al., 2004; Wong et al., 2003) and in vivo (Ferron et al., 2004). The impact of telomere attrition in several stem cell compartments appears dependent on the Cdk inhibitor p21, whose absence partially rescues stem/progenitor cell activity and at the same time extends the longevity of telomerase-deficient mice without promoting cancer (Choudhury et al., 2007). Similarly, ablation of the mismatch repair gene *Pms2* extends life span and abrogates the degenerative phenotypes of telomerase-deficient mice in part by attenuating p21 induction (Siegl-Cachedenier et al., 2007). Interestingly, loss of either p21 or Pms2 appears to rescue proliferation defects but not apoptosis in cells from telomerase-deficient mice, suggesting that these molecules may be common to a pathway that signals cell-cycle arrest in response to dysfunctional telomeres. In contrast, loss of p53 acts positively upon the germ cell compartment in young telomerase-deficient mice yet exacerbates tumorigenesis later in life, which is likely due to the fact that p53 deficiency rescues both the proliferative defect and apoptosis associated with telomere dysfunction (Chin et al., 1999). A different outcome is observed in mice lacking the DNA-damage repair protein ATM on a telomerase-deficient background. In these animals, telomere dysfunction is exacerbated within several stem and progenitor cell compartments and longevity is compromised, yet there is diminished formation of T cell lymphomas normally associated with ATM deficiency (Wong et al., 2003). These studies highlight the complex interplay between signals emanating from dysfunctional telomeres and tumor suppressor pathways that regulate stem cell biology, aging, and cancer.

Stem Cell Aging and Tumor Suppressor Pathways

Somatic cells respond to potentially deleterious lesions generated by exogenous and endogenous sources by activation of the retinoblastoma (Rb) and p53 tumor suppressor pathways, which act to permanently arrest or kill damaged cells. The role

that inactivation of these pathways plays in the development of cancer is unequivocal, yet it is becoming increasingly evident that activation of these pathways may also contribute significantly to aging (Figure 4). Elevated expression of the Rb effector *p16^{Ink4a}* has been demonstrated in numerous tissues with age (Krishnamurthy et al., 2004; Zindy et al., 1997) and has emerged, along with senescence-associated β-galactosidase activity (SA-β-gal) (Dimri et al., 1995), as one of the principal biomarkers of aging. Several studies have demonstrated not only that *p16^{Ink4a}* increases in several different stem cell compartments with age but also that this induction has functional consequences. For example, NSCs in the SVZ of the mammalian brain diminish in number and function with age (Maslov et al., 2004; Molofsky et al., 2006), concomitant with increasing *p16^{Ink4a}* expression (Molofsky et al., 2006). Importantly, these phenotypes were partially mitigated in p16^{Ink4a}-deficient mice, with a corresponding improvement in neurogenesis in the olfactory bulb of old mice (Molofsky et al., 2006). In a related study, *p16^{Ink4a}* expression was elevated in HSCs from old mice, whereas age-associated repopulating deficits and serial-transplant capacity were improved in aged p16^{Ink4a}-deficient mice (Janzen et al., 2006). Taken together these studies establish a causal role for p16^{Ink4a} in the reduced functional capacity of aged HSCs and NSCs. It is important to note that neither of these studies has established that *p16^{INK4a}* induction leads to cellular senescence in either of these stem cell compartments. In fact, evidence from clonal culture experiments indicates that HSCs from aged mice have a comparable capacity to give rise to progeny as HSCs from young mice (Morrison et al., 1996; Sudo et al., 2000). Moreover, loss of *Bmi-1* and derepression of the *p16^{Ink4a}-p19^{Arf}* locus are not sufficient to impart a senescent phenotype to stem cells, as demonstrated by experiments in which clonally cultured HSCs that lack *Bmi-1* were just as capable of entering the cell cycle and giving rise to daughter cells as wild-type control cells (Iwama et al., 2004). These studies suggest that cellular senescence and permanent growth arrest may not be a significant physiological outcome of normal HSC aging. Consistent with this, p16^{Ink4a} deficiency in the hematopoietic system appears to allay stress-associated apoptosis rather than cellular senescence (Janzen et al., 2006). Thus the question of whether or not stem cell aging is accompanied by increased cellular senescence remains an important unresolved issue.

The question of how p53 impacts aging and, in particular, stem cell aging is more complex. Loss of p53 predisposes to a spectrum of neoplasms (Donehower et al., 1992), whereas mice overexpressing a short isoform (Maier et al., 2004) or truncated activated form of p53 (Tyner et al., 2002) exhibit suppression of tumorigenesis yet develop early degenerative phenotypes reminiscent of aging. Conversely, mice with increased but normally regulated expression of p53 and its positive regulator Arf are resistant to tumorigenesis, live longer, and have reduced levels of age-associated damage to proteins, lipids, and DNA (Matheu et al., 2007). Mice with increased p53 activity resulting from a hypomorphic allele of *Mdm2* are also tumor resistant yet age normally (Mendrysa et al., 2006). Thus, modulation of p53 activity can have either proaging or antiaging effects depending on context. In accordance with these results, several studies have indicated that modulation of p53 expression in stem cells may also differentially impinge upon stem cell behavior and aging. For example, in *p53*-deficient mice, there were increased numbers of HSCs and they performed better in competitive transplantation assays and were more capable of giving rise to phenocopies of themselves in primary transplant recipients than controls, indicating an elevated self-renewal capacity (TeKippe et al., 2003). In contrast, stem and progenitor cells in mice expressing a truncated p53 protein with elevated activity showed reduced proliferative and repopulating capacity compared to wild-type controls, whereas the same cells from *p53$^{+/-}$* mice exhibited increased activity (Dumble et al., 2007). Although apoptosis of stem cells and progenitor cells was not addressed in either of these studies, p53-mediated apoptosis is critically involved in the physiological regulation of HSC population size and function. For example, mice carrying a mutant form of the Rad50 DNA-repair protein experience precipitous bone marrow failure due to hypermorphic signaling through an ATM-Chk2-p53-dependent apoptotic pathway (Bender et al., 2002; Morales et al., 2005). Conversely, enforced expression of the antiapoptotic protein BCL-2 in the hematopoietic compartment of mice expands HSC numbers and improves repopulating potential (Domen et al., 2000), in a manner similar to that seen with p53 deficiency (TeKippe et al., 2003). Cumulatively, these studies illustrate the tenuous balance between tumor suppression and stem cell aging (Figure 4) yet at the same time also provide hope that aging may be delayed without a concomitant increase in cancer incidence by the careful manipulation of such pathways in stem cells.

Cytoprotection and the Paradox of Remaining Quiescent

The vast majority of somatic cells comprising tissues and organs are terminally differentiated and postmitotic, yet stem cells must retain mitotic potential throughout the lifetime of an animal in order to be able to respond to homeostatic demands. In accordance with the biological importance of such a duty, stem cells from a diverse array of tissues seem to have evolved a number of mechanisms aimed at maintaining genomic integrity beyond that of somatic cells. For instance, stem cells from a variety of tissues express high levels of ABC transporter proteins that are known to have physiological roles in cytoprotection through their capacity to actively pump genotoxic and xenobiotic compounds out of the cell (Zhou et al., 2001). Another cytoprotective property that has been reported for certain tissue-specific stem cells is their ability to differentially segregate newly synthesized chromosomes to more differentiated progeny at mitosis while retaining the original and possibly error-free chromosome within the stem cell (reviewed in Lansdorp, 2007). This mechanism does not, however, appear to be universal to all stem cell compartments (Kiel et al., 2007), and indeed the notion that asymmetric strand segregation is used by stem cells to limit exposure to replication errors has recently been challenged (Lansdorp, 2007). Nonetheless, the fact that many tissue-specific stem cells primarily reside in the quiescent (G_0) phase of the cell cycle throughout their adult lifetime achieves the same end by limiting passage through the cell cycle and the possibility of attendant DNA replication errors. Moreover, quiescent stem cells are also relatively metabolically inactive with concomitantly low generation of endogenous free radicals and ROS (Tothova et al., 2007).

The benefits afforded by residing in a quiescent state, however, appear to come at a cost for stem cells. HSCs from old mice accumulate considerably more damage-induced γH2AX foci in their genomes than more actively proliferating progenitors (Rossi et al., 2007a). DNA damage accrued in proliferating progenitor cells is either repaired or the cells are eliminated by apoptosis as they pass through the cell cycle, whereas quiescent stem cells appear to only undergo apoptosis when they are forced into the cell cycle. These results suggest that quiescence itself might be a critical factor involved in facilitating the accrual of DNA damage in stem cells (Rossi et al., 2007c). This may result from the fact that the cellular machinery responsible for eliciting DNA-damage responses for repair, growth arrest, or apoptosis is so intimately linked to cell-cycle progression and cell-cycle-dependent checkpoint control mechanisms (reviewed in Zhou and Elledge, 2000). Consistent with this, cultured cells driven to quiescence by serum starvation must re-enter the cell cycle in order to repair premutagenic DNA adducts (Bielas and Heddle, 2000) because global genomic DNA repair was suppressed under experimentally induced quiescence (Bielas and Heddle, 2004). Whether or not the repair properties of serum-starved cells in culture will extend to the physiology of stem cells is clearly an important and unresolved question. Nonetheless, the finding that stem cells specifically accumulate DNA damage with age suggests a mechanism whereby stem cells themselves may serve as a reservoir for the acquisition of mutations required for disease pathogenesis or oncogenic transformation (Rossi et al., 2007a).

At the Interface of Cancer, Aging, and Stem Cell Regulation

Cancer is the leading cause of death for individuals younger than 85 (http://seer.cancer.gov/). However, mortality rates have decreased by less than 1.5% per year despite the introduction of a plethora of new anticancer therapies over the last decade. Because a multiplicity of molecular events that correspond with stem cell aging also occur in tumors in the elderly, research to elucidate molecular aberrations in stem and progenitor cells during aging may provide insights into cancer formation and point the way toward new therapeutic targets. Many cancers including colon, breast, brain, head and neck, pancreatic, and hematopoietic malignancies contain minor populations of tumor-initiating cells or cancer stem cells (CSCs) (reviewed in Clarke and Fuller, 2006). CSCs share many of the functional properties of normal stem cells including the potential for unlimited self-renewal. In a manner similar to normal stem cells, CSCs have the potential to give rise to most or all of the cell types within the tumor and are ultimately responsible for ongoing tumor maintenance. Moreover, CSCs, like normal stem cells, are believed to express high levels of many ABC/MDR transporter proteins that mediate drug efflux, a property that may enable CSCs to evade the effects of chemotherapeutic drug regimens, ultimately contributing to relapse. In fact, the leading cause of death in cancer patients continues to be the acquired or intrinsic resistance of tumor cells to therapy. The extent to which CSCs are derived directly from transformed stem cells or are the result of transformative events that impart stem cell-like properties onto more committed progenitors is only just beginning to be elucidated and is likely to vary among different cancers. However, significant deregulation of the mechanisms governing self-renewal, survival, and cell-fate decisions is implied as these are normally tightly regulated processes.

Aberrant Self-Renewal: The Emergence of Leukemic Stem Cells

One of the most instructive diseases for understanding the correlation between stem cell aging and the acquisition of the complement of mutations required for malignant transformation is chronic myeloid leukemia (CML), which is generally a disease of the elderly. CML was the first malignancy to be associated with a characteristic chromosomal abnormality and its constitutively active tyrosine kinase fusion product, BCR-ABL. CML was also the first cancer shown to be derived from a stem cell and to be treated therapeutically with a drug rationally designed to inhibit the BCR-ABL oncoprotein (reviewed in Wong and Witte, 2004). The molecular mechanisms driving progression of human CML from its chronic phase through myeloid blast crisis have been extensively investigated, but only recently has the role of stem cell and progenitor cell biology been evaluated (Jamieson et al., 2004). In chronic phase CML, quantitative PCR analysis has shown that the *BCR-ABL* fusion transcript was present at the highest levels in phenotypic HSCs, whereas blast crisis CML was marked by amplified *BCR-ABL* expression in more committed granulocyte-macrophage progenitors (GMPs) (Jamieson et al., 2004). After the transition to blast crisis, GMPs were found to have gained the capacity to self-renew and to transfer blast crisis leukemia to immunodeficient mice, in part, as a consequence of self-renewal driven by aberrant Wnt/β-catenin signaling (Jamieson et al., 2004). In mouse models, expression of BCR-ABL was not sufficient to confer the properties of LSCs on committed progenitors, whereas ectopic expression of the MOZ-TIF2 oncogene that induces self-renewal was sufficient (Huntly et al., 2004). In another mouse model, ectopic expression of the *MLL-ENL* oncogene in HSCs, common myeloid progenitors (CMPs), or GMPs led to immortalization of the GMPs (Cozzio et al., 2003). Interestingly, expression profiling of LSCs generated through transformation of committed progenitors by ectopic expression of the *MLL-AF9* oncogene revealed that the expression signature of the parental progenitors was more or less retained, whereas the self-renewing leukemic clones had acquired a gene expression signature more characteristic of stem cells (Krivtsov et al., 2006). These results suggest that LSCs can emerge from committed hematopoietic progenitor cells without widespread reprogramming of gene expression, through the activation of only a critical subset of self-renewal genes. Moreover, these studies reveal that the potential of different oncogenic products to impart self-renewal capacity on leukemic clones is developmental stage specific.

Although the mutational events that drive leukemogenesis often act in a cell-autonomous fashion, recent studies have revealed an equally important contribution to myeloproliferative disorder and leukemia pathogenesis through dysregulation of the microenvironment. This was shown by experiments in which wild-type bone marrow cells developed into myeloproliferative disease upon transplantation into environments deficient in either the retinoic acid receptor gamma (RARγ) (Walkley et al., 2007a) or retinoblastoma protein (Walkley et al., 2007b). In

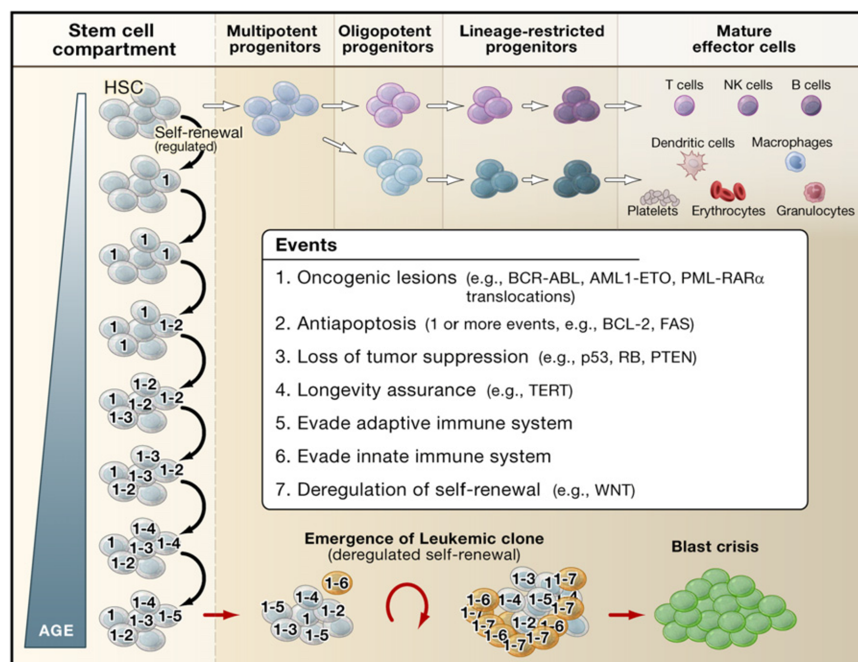

Figure 5. Model of Leukemic Progression
In this model, HSCs serve as the reservoir for the accumulation of the genetic and epigenetic events that eventually lead to blast crisis and leukemia. Stem cell self-renewal and differentiation enable heritable mutations acquired in the stem cell compartment to be propagated to both self-renewing progeny and downstream progenitors over the lifetime of the organism. Stem cells with heritable lesions act as substrates for additional hits, which in turn can promote selection of preleukemic clones if lesions imparting a growth or survival advantage are acquired. In this model, seven events are listed as the full complement of events required for the progression of preleukemic clones to frank leukemia, but the actual number of events may vary depending upon the type of cancer and the nature of the lesions involved as it is possible that multiple oncogenic properties could be conferred upon a preleukemic clone through the acquisition of a single hit. Although the mutagenic events accrue in stem cells, the eventual emergence of leukemic clones occurs at the stage of progenitor cells downstream of HSCs that acquire the capacity for unlimited self-renewal. In chronic myeloid leukemia (CML), this can be the granulocyte/macrophage progenitor, whereas in acute myeloid leukemia, the leukemic clone can emerge at the level of a multipotent progenitor or at a stage further downstream depending upon the nature of the oncogenic lesions involved.

both cases, loss of trabecular bone in the RARγ or retinoblastoma-deficient settings suggests that aberrant extrinsic signals emanating from the stem cell niche may be important in setting the stage for disease pathogenesis.

The cancer stem cell model of tumorigenesis predicts that in order to be clinically effective in the long-term, cancer therapies must target the small subset of CSCs that are responsible for maintaining and spreading the tumor. The fact that many pathways critical to tumorigenesis are so intimately linked to normal stem cell self-renewal processes has raised the possibility that cancer therapies targeting such pathways might also inadvertently ablate normal stem cells. One such self-renewal pathway, mediated by the tumor suppressor protein Pten, is differentially used by both normal and LSCs (Yilmaz et al., 2006; Zhang et al., 2006). This mechanistic distinction allowed for targeted therapy of the Pten pathway—through suppression of mTOR by rapamycin—leading to the depletion of LSCs without damage to the normal stem cell pool (Yilmaz et al., 2006).

Cumulatively, these studies underscore the importance of the cell-intrinsic and -extrinsic mechanisms that straddle the interface of normal and leukemic stem cell regulation. Elucidating these mechanisms will inform diagnostic and prognostic strategies as well as the development of new therapies.

Aberrant Fate Determination and Disease

Aberrant cell-fate decisions are a well-documented feature of aging and are also a hallmark of cancer. Developmental stage-specific differentiation abnormalities in cancer have been most highly studied in leukemias with balanced translocations that result in inappropriate expression of transcription factors that regulate critical cell-fate decisions. In one-third of cases of B-progenitor acute lymphoblastic leukemia (ALL), PAX5, an essential B-lineage commitment transcription factor, is inactivated. An important initiating event in B-progenitor ALL is the expression of the *TEL-AML1* oncogene that acts by repressing transcription, suggesting that deregulated transcription factor expression and altered cell-fate decisions play a critical role in the pathogenesis of lymphoid malignancy (reviewed in O'Neil and Look, 2007). Similar abnormalities in cell-fate decisions are important drivers of myeloid malignancies in the elderly. Indeed, a single nucleotide polymorphism (SNP) within a highly conserved distal enhancer greatly reduces expression of the PU.1 transcription factor within myeloid progenitors in complex karyotype acute myeloid leukemia (AML) by blocking binding of the chromatin-remodeling transcriptional regulator SATB1 (Steidl et al., 2007).

Further evidence that aberrant cell-fate decisions in primitive progenitors contribute to cancer pathogenesis comes from an analysis of AML patients harboring t8;21 translocations generating the *AML-1-ETO* oncogene, the expression of which leads to decreased expression of the critical granulocytic differentiation factor, CEBP/α (Pabst et al., 2001). Although the *AML-1-ETO* oncogene is expressed in HSCs (Miyamoto et al., 2000), the cells capable of transferring leukemia to immunocompromised mice are enriched within a $CD34^{+}38^{-}$/lo subset of blood and bone marrow cells (Bonnet and Dick, 1997), which represent a primitive multipotent hematopoietic progenitor cell subpopulation downstream of HSCs (Majeti et al., 2007). This suggests that although HSCs harbor the primary lesion, additional oncogenic events are required to drive transformation into AML. Consistent with this, ectopic expression of the AML-1-ETO fusion protein in mice was not sufficient to induce leukemogenesis unless combined with direct

mutagenesis using the mutagen ENU (Yuan et al., 2001). Together with the finding that HSCs can accumulate DNA damage during aging (Rossi et al., 2007a), these results suggest a model in which the primary oncogenic lesion generating the AML-1-ETO fusion product occurs in HSCs, which then serve as substrates for the acquisition of additional hits eventually leading to the transformation of downstream progenitors (Figure 5).

Together, these studies provide evidence that leukemogenesis is a multistage process originating in stem cells, with upwards of seven independent genetic and epigenetic events required for transition to blast crisis or acute leukemia. In this model, patients with disease would also be expected to have preleukemic clones containing n = 1, n = 2, and n = 3 events in addition to leukemic clones possessing the full complement of oncogenic events (Figure 5). Analysis of LSCs from such patients at the genomic, gene expression, epigenetic, and proteomic levels should reveal the full life history of these leukemic clones and shed light on how to combat their formation and progression.

Epilogue

The number of elderly adults is at a historical high, and concomitant increases in the prevalence of age-related degenerative and malignant conditions will place a heavy burden on future health care resources. The need to develop therapeutic strategies to treat pathophysiological conditions in the elderly is therefore medically, socially, and economically crucial. Characterization of normal stem cell aging is the critical first step toward achieving these goals as such research should be able to identify the mechanisms underlying stem cell functional decline and inform strategies for intervention. Of particular importance is the development of targeted therapies that will obviate the high mortality rates associated with cancer, the most frequently fatal disease of aging.

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