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Stem cell aging: mechanisms, regulators and therapeutic opportunities

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Abstract

Aging tissues experience a progressive decline in homeostatic and regenerative capacities, which has been attributed to degenerative changes in tissue-specific stem cells, stem cell niches and systemic cues that regulate stem cell activity. Understanding the molecular pathways involved in this age-dependent deterioration of stem cell function will be critical for developing new therapies for diseases of aging that target the specific causes of age-related functional decline. Here we explore key molecular pathways that are commonly perturbed as tissues and stem cells age and degenerate. We further consider experimental evidence both supporting and refuting the notion that modulation of these pathways *per se* can reverse aging phenotypes. Finally, we ask whether stem cell aging establishes an epigenetic 'memory' that is indelibly written or one that can be reset.

Changes in tissue structure are nearly universal in aging animals. Such structural changes are evident at the microscopic and macroscopic levels and are almost invariably accompanied by impairment in normal tissue function and a deficient response to injury. In many tissues, homeostatic tissue maintenance and regenerative responsiveness to injury depend on tissue-specific stem cells—long-lived cells endowed with the capacity to both self-renew and differentiate to produce mature daughters. Stem cells in tissues typically display tissue-specific differentiation patterns, and their ability to balance quiescence with proliferative activity appears to be critical for their survival and maintenance of appropriate physiological and regenerative responses[1]. The life-long persistence of stem cells in the body makes them particularly susceptible to the accumulation of cellular damage, which ultimately can lead to cell death, senescence or loss of regenerative function. Indeed, stem

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cells in many tissues have been found to undergo profound changes with age, exhibiting blunted responsiveness to tissue injury, dysregulation of proliferative activities and declining functional capacities. These changes translate into reduced effectiveness of cell replacement and tissue regeneration in aged organisms.

Understanding the molecular processes controlling stem cell survival, self-renewal, quiescence, proliferative expansion and commitment to specific differentiated cell lineages is crucial to determining the drivers and effectors of age-associated stem cell dysfunction. Furthermore, such knowledge will be essential to inform development of therapeutic interventions that can slow, and perhaps reverse, age-related degenerative changes to enhance repair processes and maintain healthy function in aging tissues.

In this Review, we focus on recent discoveries that highlight the dynamic interplay between cell-intrinsic, environmental and systemic signals that have been reported to drive the loss of stem cell functionality during aging. We further discuss the potential reversibility of these processes as possible therapeutic avenues in age-related disease. Finally, we consider whether aging establishes a genetic or epigenetic ‘memory’ in tissue-specific stem cells or their differentiated daughters, and whether such a memory may be reversible, such that aged stem cells can be reset to a more youthful state. These issues are discussed in the context of conserved cellular processes—accumulation of toxic metabolites, DNA damage, proteostasis, mitochondrial dysfunction, proliferative exhaustion, extracellular signaling and epigenetic remodeling—that clearly affect the activity of both stem cells and non-stem cells with age and may be linked to mechanisms that determine organismal lifespan and healthspan (Fig. 1).

Age-related accumulation of toxic metabolites in stem cells

Reactive oxygen species and stem cell aging

To ensure continued function, tissue-resident stem cells, like many other cell types, must withstand potentially damaging modifications of cellular macromolecules that result from exposure to reactive molecules generated as a byproduct of normal metabolism or from extrinsic paracrine and endocrine mediators. Interestingly, analysis of aged stem cells in diverse tissues points to some common effectors and signaling pathways that contribute to stem cell dysfunction in response to toxic metabolites. Primary among these are pathways induced by reactive oxygen species (ROS), which are produced predominantly as a result of electron ‘leak’ during mitochondrial oxidative phosphorylation and appear to contribute to perturbed stem cell function and fate control in the context of aging[2–5].

The notion that ROS may drive stem cell dysfunction with age draws precedence from the free radical theory of aging, described by Harman in 1972 (ref. 6). This theory proposes that accumulated cellular damage and declining mitochondrial integrity in aged cells leads to elevated ROS production, which in turn drives a vicious cycle that further damages cellular macromolecules and disrupts mitochondrial oxidative phosphorylation, leading to eventual cellular decomposition[6]. Yet the causal role of oxidative damage in the aging process remains controversial, in part because of the absence of a clear correlation between the efficacy of antioxidant defenses and extended cell function or longevity. ROS also have

essential roles in cell signaling and homeostasis[7,8], suggesting a dose-dependent, context-dependent and pleiotropic activity of these reactive mediators that may explain the complex relationship between ROS production, stem cell function and regulation of lifespan and healthspan.

In support of the hypothesis that ROS generation may promote stem cell aging, studies of aged human mesenchymal stem cells have found elevated ROS[9], and the frequency of blood-forming hematopoietic stem cells (HSCs) with low ROS levels declines with age in mice[10]. In addition, in HSCs and neural stem cells (NSCs) of mice, excessive cellular ROS concentrations lead to abnormal proliferation, malignancy and compromised stem cell self-renewal[11–14]. Conditional ablation in the mouse hematopoietic system of the transcription factors FoxO1, FoxO3 and FoxO4, downstream effectors of the insulin and insulin-like growth factor 1 (IGF-1) signaling pathways, induces a marked increase in ROS accumulation in HSCs. ROS induction in these FoxO-deficient mice correlates with disruption of HSC quiescence, increases in HSC apoptosis and defects in hematopoietic repopulating abilities[15]. Similar results have been reported for NSCs in these FoxO-deficient mice[14] and in HSCs and NSCs in mice lacking only FoxO3 (refs. 16–18). A decline in long-term regeneration capacity of HSCs in mice with conditional deletion of phosphatase and tensin homolog (PTEN) and double deficiency of protein kinase B (AKT) 1 and 2 also indicates that signaling through the PTEN–AKT–mammalian target of rapamycin (mTOR) pathway, upstream of FoxO, senses and controls ROS and regulates HSC self-renewal and survival[19–21].

Potential associations of ROS with stem cell dysfunction also have been studied in animal models manipulated to have impaired cellular antioxidant mechanisms. Mice whose hematopoietic system was reconstituted with HSCs lacking the gene encoding superoxide dismutase 2 (*Sod2*), a mitochondrial antioxidant enzyme regulated by FoxO3, showed oxidative stress–mediated hematopoietic abnormalities[22]. A cytoprotective role of SOD2 was also observed in mouse NSCs, and its overexpression increased survival of NSCs both *in vitro* and *in vivo*[23]. Moreover, age-dependent alterations in regulation of SOD2 by FoxO3 and the DNA damage sensing serine/threonine protein kinase ATM (ataxia-telangiectasia mutated) have been shown to contribute to loss of HSC function with aging[11,16, 24]. Studies of $Atm^{-/-}$ mice indicate that HSC self-renewal capacity depends on ATM-mediated inhibition of oxidative stress[25]. Such studies have led some investigators to suggest that ROS accumulation may drive stem cell aging. However, in some situations, ROS can play an adaptive role in stem cell function. For example, in *Drosophila*, hematopoietic stem and progenitor cell differentiation is regulated by physiological ROS signaling[26]. Such observations highlight the need for further studies of the modulators of ROS generation and regulators of ROS activities in young and aged stem cells.

Regulation of oxidative metabolism has further been linked to stem cell aging through studies of the sirtuin family of NAD-dependent protein deacetylases, which are important regulators of oxidative stress, aging and stem cell function. For example, recent findings suggest a role for SIRT1 in maintaining mesenchymal stem cell growth and differentiation, which have been shown to decline with age[27, 28]. Another member of the sirtuin family, SIRT3, is critical to the maintenance of mitochondria and metabolism and controls ROS

production during HSC aging[29]. In addition, ectopic induction of SIRT3 expression improves the function of aged HSCs by enhancing the antioxidant activity of SOD2 (ref. 30). Together, these studies suggest that aging of stem cells may be reversible by modulation of their metabolic and redox state, which in turn can influence intracellular accumulation of ROS.

Restoring aged stem cell function by targeting toxic metabolites

The antioxidant *N*-acetyl-L-cysteine (NAC), a precursor of glutathione and a direct ROS scavenger, has been used as a therapeutic agent to ameliorate the damaging effects of ROS[31–33]. NAC treatment restores the quiescence and reconstitution capacity of ATM-null HSCs[24], and also improves survival of a distinct population of myogenic stem cells in skeletal muscle, both *in vitro* and *in vivo*[34]. In FoxO-deficient mice, defects in HSC quiescence, survival and repopulating capacity can be reversed by treatment with NAC[15]. As yet, however, it remains unclear whether NAC or other antioxidant treatments have a direct or indirect effect on age-dependent deficits in stem cells or stem cell function, and this remains an area of fertile research.

In aggregate, studies in many tissue systems suggest that aging phenotypes caused by uncontrolled accumulation of ROS can be reversed by reducing ROS levels; however, questions remain regarding the pleiotropic functions of ROS in different cell populations. In addition, as highlighted by a recent study reporting improvement of some aging-related phenotypes by expression of the mitochondria-targeted antioxidant skQ1 (ref. 35), a better understanding of the endogenous sources of ROS and the cellular compartments in which they act is also likely to be important for clarifying the stem cell regulatory actions and potential therapeutic value of ROS modulating agents.

DNA damage in aging stem cells

As discussed above, stem cells persist throughout life in a largely quiescent state. As a result, stem cells in aged tissues experience long-term exposure to genotoxic assaults, from both endogenous and exogenous sources, and an apparent accumulation of DNA damage in aged stem cells has been noted in several studies. For example, aged HSCs and muscle stem cells (also called satellite cells) show an increased number of nuclear foci that stain for the phosphorylated form of the variant histone H2A.X (γH2A.X)[36–38], which serves as a marker of DNA double-strand breaks[39]. Accumulation of DNA damage in these stem cells can also be detected using single-cell gel electrophoresis assays[38,40]. Furthermore, telomeres—specialized chromatin structures that protect chromosome ends to prevent gene erosion and chromosome fusion—are shorter in aged hair follicle stem cells[41]. However, the level of DNA damage in stem cells must be interpreted with caution as regards its role in aging because such damage can be repaired[40] or involved in normal cellular process such as differentiation[42] or asymmetrically passed on to daughter cells[43].

DNA damage and stem cell function

Elevated levels of damaged DNA in aged stem cells could result from an accumulation of damage over time, an increase in the rate of damage, a decrease in the rate of repair in

response to DNA damage, or a combination of these possibilities. Supporting a role for changes in the DNA damage response (DDR), aged human HSC show compromised capacity to repair experimentally introduced DNA damage, such as that produced by ionizing radiation[37]. These observations suggest that DDR pathways, evolved to safeguard genomic integrity and maximize survival of the organism[13,44–46], may show reduced activity in stem cells with age. Indeed, such changes have been observed in other aging cell types[47,48]. Conversely, studies in yeast, flies and mammals have noted an age-associated increase in the activation of transposable elements, contributing to genomic instability[49–51]. Whether these shifts in cellular DNA repair pathways result from age-related dysfunction or represent an age-related strategy to respond to changing cellular physiology remains to be clarified.

The specific relevance for tissue stem cells of age-related alterations in DDR pathways is an area of ongoing interest and investigation. Notably, naturally occurring mutations that disrupt the ability of cells to detect, respond to and repair DNA damage frequently result in progeroid phenotypes, which show some features in common with chronological aging and may include perturbation of stem cell functions in various tissues (see Box 1). Likewise, experimentally induced genetic disruption of DDR pathways in mice also produces cell-autonomous defects in HSC function, as assayed by *in vivo* hematopoietic reconstitution and *in vitro* colony forming assays[11,36,52,53]. Yet recent data suggest that it may be unnecessary to posit a change in DDR mechanisms to explain the accumulation of DNA damage in aged stem cells. Analysis of DDR pathways in HSCs indicates that, independent of age, quiescent HSCs show reduced expression of DNA repair genes and accumulate DNA damage that is repaired only when the cells enter the cell cycle, such as during self-renewal or proliferation of HSC-derived progenitors[40]. Thus, as many HSCs remain quiescent for much of adult life[54,55], the continuing accumulation of damage in these cells may eventually exceed a threshold that impairs stem cell function in aged animals.

Box 1

Progeroid Syndromes

Progeroid syndromes are a group of human hereditary diseases with phenotypes resembling accelerated aging. However, progeroid syndromes are unimodal or segmental, mirroring one or multiple, but never all, of normal aging phenotypes. This distinction has raised some controversy regarding whether knowledge of the molecular bases of progeroid syndromes offers truly useful insights into the process of chronologic aging[185]. Yet studies indicate that, like chronologic aging, most progeroid syndromes are associated with an increased burden of DNA damage that may directly affect the tissue stem cell compartment[186]. The segmental nature of each disease may be partially explained by differences in the specific DNA damage repair strategies used by each tissue and cell type[186]. For example, quiescent HSCs and hair follicle stem cells rely more heavily on non-homologous end joining for repair of double-strand breaks, while actively proliferating intestinal stem cells rely on the homologous recombination pathway[56,187,188]. In Werner syndromes, Bloom syndrome and Rothmund-Thomson syndrome, patients have mutations in the RecQ helicase family of genes, which are

important for the re-initiation of stalled replication forks and suppression of inappropriate homologous recombination. Mesenchymal stem cells, but not neural stem cells, reprogrammed from Werner syndrome iPSCs show premature cellular senescence[189]. In Hutchinson-Gilford progeria syndrome, cells accumulate an abnormally processed nuclear intermediate filament called progerin. Progerin is a product of the lamin A gene, and its accumulation in the nuclear envelope leads to increased DNA damage in reprogrammed mesenchymal stem cells derived from patients with this syndrome[190]. Finally, patients with dyskeratosis congenita have mutations in the *DKC1* gene, an important component of the telomerase complex[191]. Defects in telomere maintainance lead to deprotection of telomeres and, in HSCs, cause failure to sustain bone marrow reconstituting activity[192].

The mechanisms by which accumulation of DNA damage in aged stem cells may contribute to stem cell dysfunction are still being elucidated. Genotoxic lesions could cause stem cell senescence or apoptosis (Fig. 2) and might directly affect gene regulation, leading to alterations in stem cell self-renewal and differentiation. Some studies suggest that the ensuing loss of homeostasis in aging tissues might further create a microenvironment that favors the selection of stem cells with higher self-renewal but also neoplastic potentials[46].

Interestingly, it has been suggested that the very mechanisms that some stem cells utilize in an attempt to maintain genomic stability could enhance their susceptibility to oncogenic mutations. In particular, because quiescent cells are restricted to using the error-prone non-homologous end joining pathway for repair of double-strand breaks, DNA repair, if it occurs in quiescent HSCs[40], could introduce mutations and promote genomic instability. Consistent with this notion, mouse HSCs that were forced to proliferate showed fewer mutational events after exposure to DNA-damaging radiation, suggesting that in certain instances a break from quiescence that enables engagement of the high-fidelity homologous recombination pathway can help to maintain stem cell genomic integrity[56].

To counter the potential for malignant transformation, an important downstream consequence of activation of the DNA damage checkpoint is induction of cellular senescence—an irreversible cell cycle arrest induced in response to cellular stresses, including telomere shortening[57–59]. An essential effector of senescence, $p16^{INK4a}$, has been suggested to promote aging phenotypes in stem cells, whereas abolishing $p16^{INK4a}$ function can enhance the regenerative potential of stem cells from the brain and bone marrow (see below).

Targeting DNA damage repair pathways to restore stem cell function

Accumulation of DNA damage in stem cells may trigger the production of defective progeny, stem cell senescence or neoplastic transformation[60,61], leading to age-dependent loss of organ function and homeostasis[62] It is therefore reasonable to propose that an increase in the activity of DNA repair pathways might slow or prevent the accumulation of age-related defects in stem cells and thereby promote healthy functioning of aged tissues, particularly if such an intervention can take place before the establishment of DNA damage in the genome. To achieve such a globally enhanced DNA repair system would likely

require the identification of master regulator genes that synthesize inputs from multiple DDR pathways to exert sufficient impact in the correct tissues and at the appropriate time and dosage, in conjunction with additional signaling pathways. This approach has been illustrated in the case of telomerase reactivation. In particular, studies in mice indicate that it is possible to reverse degenerative phenotypes in aged animals rendered genetically deficient in telomerase activity (through inactivation of the telomerase RNA component mTERC) by late life reactivation of mTERC[63]. Analysis of various cell types in these animals showed reduced foci of DNA damage, marked by staining with the DDR protein 53BP1, and decreased apoptosis. In addition, neural stem cells from mTERC-reactivated animals showed enhanced multipotency, suggesting recovered neurogenic function. Further examination of additional DNA damage repair/response pathways under analogous inducible settings will be informative to access the full rejuvenation potential of these pathways.

Yet while upregulation of the telomeric pathway may in theory reverse telomere attrition and rescue the function of aged stem cells and postmitotic somatic cells, this pathway is also used by cancer cells to overcome replicative senescence. Overexpression of the mouse telomerase reverse transcriptase catalytic subunit (mTERTcs) has been shown to induce a variety of malignancies[64–66]. Only when mTERTcs was overexpressed in a tumor-resistant background, produced by increasing gene dosage of p53 and p16/p19, could the balance be tipped in favor of function-promoting effects, resulting in prolonged median lifespan and improved neuromuscular coordination[67]. A recent parabiosis study further demonstrated the potential to correct DNA damage accumulated in aging mouse satellite cells via circulating systemic factors, with associated improvement of stem cell and tissue functions[38]. Whether it will be possible to translate these findings to design effective therapeutic strategies that exploit the beneficial effects of DDR and checkpoint regulation on aging-related phenotypes while mitigating risks of tumorigenesis remains to be seen.

Protein homeostasis and stem cell aging

Just as genome stability is critical for stem cell survival and function, maintenance of the stem cell proteome is equally important for assuring stem cell identity. The cellular processes responsible for the synthesis, folding and turnover of proteins are known collectively as protein homeostasis, or proteostasis, pathways[68–70]. Proteostasis is essential for development and for most cellular functions, including replication of genetic material, catalysis of metabolic reactions, maintenance of cellular architecture, signaling and immune responses[69,70]. Protein quality control is regulated by a complex protein network that monitors the concentration, subcellular location and folding of proteins and includes molecular chaperones and folding enzymes, as well as pathways that drive protein degradation when needed, through the proteasome, lysosome and autophagy pathways[69–71].

Defects in proteostasis commonly lead to aberrant folding, toxic aggregation and accumulation of damaged proteins, which can in turn cause cellular damage and tissue dysfunction[68]. Indeed, age is one of the main risk factors for most diseases associated with protein misfolding, including neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease and amyotrophic lateral sclerosis[72]. This

association suggests that aged cells may be more prone to form and accumulate misfolded protein aggregates[71,73]. Recent studies have reported age-dependent deficits in the activities of both the autophagy-lysosomal and the ubiquitin-proteasome systems, which represent the principal proteolytic systems implicated in protein quality control[74,75]. Yet other studies have challenged the view that proteolytic activity declines with age, showing instead that autophagic potential or proteasome activity remains intact in aged cells[76,77] and attributing age-related disruptions in proteostasis mainly to increasing damage caused by metabolic stress that overwhelms the protective capacity of proteolytic systems[76].

As regards stem cell function, proteostasis has been implicated as an important determinant of stem cell maintenance through studies in HSCs showing that deletion of autophagy-related gene 7 (*Atg7*) increases ROS levels and depletes HSCs[78]. This notion has been further supported by Warr *et al.*, who reported autophagy induction by FoxO3A to be an essential mechanism that protects HSCs from metabolic stress in aging[76]. Also, mTOR, a potent activator of protein translation and inhibitor of autophagy, has been shown to regulate the self-renewal and differentiation of HSCs and intestinal stem cells[79,80].

Proteostasis was also shown to be fundamental to maintenance of identity in human embryonic stem cells (hESCs). FOXO4 promotes proteasome activity in hESCs by regulating PSDM11 expression, thereby encouraging proteostasis and maintenance of pluripotency markers, such as *POU5F1*, *OCT4*, *NANOG*, *SOX2*, *UTF1*, *DPPA4*, *DPPA2*, *ZFP42* and *TERT*[81]. In addition to its known roles in promoting longevity and stem cell function in tissues throughout the body, FoxO transcriptionally activates several protein-folding chaperone genes[82–84] some of which may regulate aging[84–87]. However, a direct link between chaperone function and stem cell aging has not yet been demonstrated. It will be important to characterize the mechanisms that regulate proteostasis in stem cells to determine if they differ from those in other cells and to assess how they may influence changes in stem cell function during aging.

Reversing stem cell aging by improving protein homeostasis

Although no studies yet have directly addressed whether stimulation of autophagy or proteasome activity has particular beneficial effects for aged stem cells, pharmaceutical inhibition of mTOR by rapamycin treatment has been reported to restore the self-renewal and hematopoietic potential of aged HSCs[88]. Likewise, data from mice genetically manipulated to preserve chaperone-mediated autophagy (CMA), a form of autophagy involving chaperone-dependent selection of substrates, show improved cellular homeostasis, enhanced resistance to stressors and preservation of organ functions with age[89]. Overexpression of heat shock protein 70 (HSP70), a chaperone protein that recognizes damaged proteins in CMA, also protects against age- and disease-related tissue degeneration in brain, heart and skeletal muscle[90–92]. These studies provide intriguing insights into potential therapeutic interventions for age-related dysfunction, although the specific role, if any, of CMA and chaperone proteins in stem cell aging is a subject for future research.

Mitochondrial dysfunction and stem cell aging

A direct relationship between mitochondrial dysfunction and aging has been suggested by many studies (for review, see ref. 93), but dissecting the role of mitochondria in stem cell aging has been challenging, in part because the rarity of tissue stem cells renders traditional biochemical and bioenergetics analyses generally inapplicable in these cells. Mitochondria are often referred to as the cell's powerhouse and contain their own small genome, which encodes key proteins required for their primary function of generating ATP by oxidative phosphorylation. Age-dependent reductions in mitochondrial function leading to respiratory chain dysfunction have been observed in several cell systems and have been thought to result largely from accumulation of mutations in mitochondrial DNA (mtDNA)[94]. The free radical theory of aging points to elevated ROS as a principal cause of mtDNA mutation, as mitochondria represent the primary sources of ROS in the cell[5].

An alternative view on mtDNA mutagenesis suggests that replication errors mediated by mitochondrial DNA polymerase γ may instead drive mtDNA mutation[95]. Supporting this view, a genetically modified mtDNA mutator mouse, harboring a proofreading-defective mtDNA polymerase γ, displays severe respiratory chain deficiency and premature aging phenotypes[96,97]. Furthermore, hematopoietic progenitor cells of the mtDNA mutator mice are affected during fetal development, and their neural stem cells exhibit declining quiescence and decreased self-renewal capacity in response to mtDNA mutation accumulation[98]. Another study reported decreased proliferation and increased apoptosis of epithelial crypt cells in mtDNA mutator mice and impaired *in vitro* development of stem cell–derived organoids[99]. The abnormal phenotypes observed in somatic stem cells from mtDNA mutator mice can be rescued by supplementation with NAC, implying that mtDNA mutations alter the oxidative state of stem cells and that this impairs their functional capacity[98]. In addition to these animal studies, several studies have related mtDNA defects to human stem cell aging[100–102]. However, in adult mouse HSCs, rapidly accumulating mtDNA mutations induced by the presence of proofreading-defective mtDNA polymerase have little functional effect on the HSC pool and instead cause differentiation blocks and/or disappearance of downstream progenitors[103]. This discrepancy between HSC phenotypes observed in these animals and those observed in chronologically aged animals suggests that mtDNA mutations may not be a primary driver of tissue stem cell aging[103]. Still, the mechanisms by which mtDNA mutations do affect stem cell function and their role in stem cell aging in other tissues requires further investigation.

Aside from primary mitochondrial lesions such as mtDNA mutation, secondary alterations in mitochondrial function driven by age-related cellular and metabolic changes may also contribute to the aging process[93]. Nutrient sensing and energy homeostasis have been implicated as connecting mitochondrial function and longevity[104,105]. In HSCs, an age-dependent decrease in nutrient uptake capacity has been observed with age, implying that the nutrient sensing pathway is involved in stem cell aging[76]. In fact, several signaling pathways discussed above that have been reported to regulate stem cell activity during aging function in nutrient sensing as well. For instance, the liver kinase B1 (LKB1)-adenine monophosphate-activated protein kinase (AMPK) pathway senses cellular energy levels and activates glucose uptake when necessary by inhibiting mTOR in HSCs[106]. In addition,

activation of the Phosphoinositide 3-kinase (PI3K)–AKT pathway by growth factors leads to inhibition of FoxO transcription factors[107], which cross talk with AMPK to control the balance between oxidative phosphorylation and glycolysis in response to intracellular and environmental cues[108]. Finally, Peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1) a key regulator of mitochondrial energy metabolism, increases mitochondrial biogenesis and delays the age-dependent decline in intestinal stem cells and gut function in *Drosophila*[109], although its role in mammalian stem cells has yet to be tested.

Improving mitochondrial function in aged stem cells

Enhanced mitochondrial function has been associated with improved stem cell function and tissue regeneration in mammals. Short-term calorie restriction (CR) increases the frequency and function of skeletal muscle stem cells in both young and old mice, potentially by increasing mitochondrial content and promoting oxidative metabolism[110]. CR has been widely employed as a dietary intervention to prevent or reduce the severity of aging phenotypes, and long-term CR can delay the onset of age-related pathologies and extend lifespan in many species, from yeast to primates[111]. CR can also enhance or maintain stem cell function during aging by increasing the expression of brain-derived neurotrophic factor in the brain and reducing the mTOR complex 1 (mTORC1) pathway in intestine[79,112]. Signaling pathways related to nutrient sensing and uptake, such as SIRT1, AMPK and FoxO, may represent molecular effectors of the benefits of CR on stem cell activity[113,114], although a complete understanding of how CR affects stem cell function must await further studies. CR effects are not likely to be limited to enhanced mitochondrial function, however, as CR also modulates mitochondrial hormesis, autophagy induction, production and scavenging of ROS, and DNA damage[115–117]. However, resveratrol, a naturally occurring small molecule that induces metabolic benefits resembling those of CR, does increase mitochondrial biogenesis, in part through activation of SIRT1 and PGC-1α[116].

A recent study reported that reduced levels of NAD^+ contribute to the mitochondrial decay associated with skeletal muscle aging and that SIRT1 modulates this process[118]. The imbalance between nuclearly and mitochondrially encoded respiratory chain subunits caused by declining nuclear NAD^+ was shown to disrupt oxidative phosphorylation in aged mice, whereas raising nuclear NAD^+ levels restored mitochondrial function in old mice. These data indicate that age-related mitochondrial dysfunction is reversible and contributes to dysfunction in aged tissues. On the basis of this result, one may extrapolate that ectopic expression of the single-subunit yeast alternative NADH dehydrogenase in *Drosophila* intestinal stem cells delays the onset of intestinal aging and extends lifespan by regulating the NAD^+/NADH ratio in aged cells[119]. It will be interesting to determine whether the roles of NAD^+ and NADH dehydrogenase in age-dependent mitochondrial dysfunction are conserved in stem cells of different tissues and different species and whether similar strategies to manipulate the NAD^+/NADH ratio in humans could have therapeutic benefit.

Stem cell depletion and senescence in aged tissues

Progressive loss of stem cell functionality caused by the age-related cellular permutations reviewed above typically leads to depletion of the functional stem cell pool in aged animals. Age-dependent reduction in stem cell number or perturbed cell-cycle activity has been reported in skeletal muscle stem cells, neural stem cells and germline stem cells[110, 120–122]. Depletion of the stem cell pool with age may occur because these cells lose self-renewal activity and terminally differentiate, thereby exiting the stem cell pool, or because they undergo apoptosis or senescence induced by exposure to cellular stress, although it is not exactly clear what mechanisms inform the choice between apoptosis and senescence. Regulation of cell number and cycling activity is different for aging HSCs, which actually show increases in cell number, possibly due to a reduced capacity for asymmetric division[123], with no difference in cycling activity[124]. Yet aged animals still experience a functional depletion of the HSC pool, as aged HSCs show reduced hematopoietic reconstituting activity and skewed differentiation potential such that they overproduce myeloid lineage cells and underproduce lymphoid lineage cells.

Unlike apoptotic cells, senescent cells remain alive, despite “an essentially irreversible arrest of cell division”[125]. Generally, senescent cells upregulate cell cycle inhibitors like p53/p21 and $p16^{INK4a}$ (also called Cdkn2a)[126]. Furthermore, senescent cells secrete bioactive mediators, including degradative enzymes, inflammatory cytokines and growth factors, which may further drive stem cell dysfunction with aging[127]. One study reported that stem cells from geriatric skeletal muscle lose the capacity to enter reversible quiescence and become “pre-senescent” as a result of dysregulation of $p16^{INK4a}$ (ref. 120). Silencing $p16^{INK4a}$ in geriatric satellite cells restores their quiescence and regenerative potential, suggesting that this pre-senescent state is potentially escapable[120]. Old $p16^{INK4a-/-}$ HSCs also exhibit increased cell cycle activity and enhanced engraftment[128], although these enhancements come with a price, as $p16^{INK4a-/-}$ animals show an increased incidence of malignancy. Whether the impact of $p16^{INK4a}$ deficiency reflects a direct role in HSCs themselves remains somewhat controversial, however, as some groups have failed to detect $p16^{INK4a}$ expression in HSCs freshly isolated from young or aged mice[129]. Given these data, it will be interesting to determine whether $p16^{INK4a}$ expression may be upregulated *in vivo* or *in vitro* in response to HSC activation.

Stem cell exhaustion may also be driven by an imbalance of stem cell quiescence and proliferation. Maintaining this balance is crucial to sustain the stem cell pool through multiple rounds of tissue regeneration, as demonstrated by studies in the *Drosophila* intestine, and in HSCs and neural stem cells of p21-null mice[109,130,131]. Excessive proliferation caused by loss of cell cycle regulation results in accelerated exhaustion of these stem cell pools. In a similar vein, increased fibroblast growth factor 2 (FGF2) signaling in the aged muscle stem cell niche also drives loss of quiescence in satellite cells, leading to impaired muscle regenerative capacity[132]. Changes in intracellular levels of ROS may also determine the balance between quiescence and proliferation of HSCs, and perhaps other adult stem cells[133]. Low levels of ROS are required for quiescence and stem cell maintenance, whereas ROS induction leads to proliferation and differentiation programs[26]. When combined with the prevailing notion that ROS increases with aging, this result

provides a possible explanation for how stem cells lose quiescence and are depleted in aged tissues.

The balance between quiescence and proliferation in aged stem cells might also be disrupted by an increased demand for replacement of mature cells in aged tissues, as suggested by observations in disease models such as Duchenne muscular dystrophy (DMD). Repetitive damage, caused by continual degeneration of structurally unsound DMD fibers, elicits constant demand for regeneration, diminishing the regenerative capacity of muscle stem cells as a consequence of replicative aging[134]. By analogy, repeated demands on stem cells to generate replacement cells over an entire lifetime may cause an accumulation of cell-autonomous defects, including telomere erosion, mutations and epigenetic dysregulation, and eventual replicative exhaustion.

Replenishing the stem cell pool in aged tissues

Rescuing stem cells from senescence or activating them from a quiescent state could potentially enhance regenerative potential in aged tissues; however, such strategies must maintain the balance of stem cell self-renewal and differentiation. Stem cell transplantation also has been actively explored as a method for the replenishment of regenerative cells in the context of degenerative diseases such as bone marrow failure, muscular dystrophy, diabetes and neurological diseases[135–138].

The therapeutic paradigm of stem cell transplantation is to infuse stem cells from an unaffected donor or gene-corrected autologous cells into a patient. For example, treatment of severe combined immunodeficiency, an X-linked immune cell disorder caused by mutations in the gene encoding the common γ-chain cytokine receptor subunit, via gene correction would seek to repair the gene encoding the common γ-chain in the patient's own cells and then transfer these back to the patient to reconstitute a 'corrected' hematopoietic system. Currently, HSC transplantation is the only stem cell–based therapy widely accepted and used clinically, and it provides an effective cure for hematologic diseases such as leukemia and lymphoma[135]. Studies in mice suggest that stem cell transplantation may someday be possible for other tissues as well. Several groups have reported successful replenishment of the stem cell pool by satellite cell transplantation into dystrophic or irradiated/injured muscles in mice[120,139,140], although the rarity of satellite cells in muscle tissue has been a challenge for translating muscle stem cell transplantation to clinical practice[141] Importantly, given the many cell non-autonomous signals that contribute to stem cell aging (see below), it is unlikely that simple replacement of aged stem cells with younger ones will be sufficient therapeutically. Instead, strategies that attempt to restore the function of residual stem cells or that combine such strategies with stem cell transplant should be considered. For example, recent studies of aged muscle stem cells have shown that inhibition of the p38 mitogen-activated protein kinase signaling pathway in endogenous muscle stem cells can restore muscle regenerative potential in aged mice[142] and can be combined with *ex vivo* culture on soft hydrogels to enhance the transplantation efficacy of these cells[140].

Reprograming of somatic cells into a desired type of stem cell could provide an alternative source of cells for transplant. In addition to the advantage of being highly amenable to genetic correction[143,144], this strategy has the advantage of generating potentially unlimited

numbers of immune-compatible cells for transplant. Induced expression of *PAX7* and exposure to defined chemical cocktails[145–147] in human pluripotent stem cells has been shown to generate engraftable muscle progenitor cells that replenish the satellite cell compartment and sustain formation of dystrophin-positive myofibers in dystrophin-deficient mice[145,147]. Similarly, transplantation of neural stem or progenitor cells derived from human induced pluripotent stem cells (iPSCs) in a mouse of model of spinal cord injury was reported to stimulate continual motor function recovery[148,149]. However, there remain important obstacles that must be overcome to realize the full potential of stem cell–based therapy for these and other disorders, including the generation of sufficient numbers of cells for transplantation, the promotion of stem cell survival and engraftment in host tissues, and potential immunological barriers to long-term engraftment of allogeneic or even gene-corrected autologous cells after transplantation[141].

Extracellular signals and stem cell aging

The stem cell niche

Stem cells reside in specialized microenvironments, called niches, that promote their maintenance and regulate their functions[150–152]. Aging of niche cells and age-dependent alterations in the acellular components of stem cell niches can cause maladaptive changes in stem cell function. In *Drosophila*, the number of cap cells and hub cells, which serve as support cells for germline stem cells (GSCs) in the testes and ovaries, respectively, decreases with age. This loss of the stem cell niche impairs bone morphogenetic protein (BMP) signaling from the niche that is necessary for GSC maintenance[122,153]. Reduced levels of E-cadherin and Unpaired also contribute to aging of GSC niche by weakening the association between GSCs and cap or hub cells; overexpression of these signaling molecules rescues the age-dependent decline in GSC frequency[122,153]. Similarly, expression of glial cell–derived neurotrophic factor, which is required for normal regulation of spermatogonial stem cell self-renewal and differentiation, is markedly reduced with age in Sertoli cells, the nurse cells of the testes[154]. Moreover, consecutive transplantation of spermatogonial stem cells to the testes of young male mice was sufficient to maintain stem cell function for extended periods of time, underscoring a major role of aging of the niche in GSC functional decline and suggesting the potential for reversing aging phenotypes by targeting the niche[155].

The significance of niche cell support has been documented for other tissue stem cells as well. Paneth cells, found in close association with intestinal stem cells in humans and mice, are an important source of niche factors, including epidermal growth factor, Wnt3A and Notch ligand[79,156], although the effect of aging on their activity is yet to be elucidated. In skeletal muscle, a complex network of interactions between satellite cells and their niche is emerging. For example, immune cells serve as critical regulators of satellite cell function during muscle regeneration, orchestrating inflammatory responses and ensuring appropriate proliferation of myogenic cells to prevent their premature differentiation[157]. Likewise, fibro/adipogenic progenitors (FAPs), stromal constituents of skeletal muscle, facilitate muscle regeneration and serve as a principal source of soluble extracellular matrix regulators[158,159]. The presence of FAPs in the muscle is essential for proper muscle repair after injury[158], and

this regulatory relationship is likely to involve direct communication between FAPs and satellite cells, as co-culturing FAPs with satellite cells enhances the rate of differentiation of primary myogenic progenitors[159]. Muscle fibers also serve as an important source of niche signals to regulate satellite cell function with age. Aged muscle fibers fail to adequately induce the Notch ligand delta, and they overproduce fibroblast growth factor 2 (ref. 132,160). Both of these alterations contribute to acquired loss of satellite cell responsiveness in older animals.

Systemic factors

In addition to locally produced signals, aging also causes changes in circulating factors that can profoundly impact tissue stem cells. In old mice, elevated transforming growth factor-β (TGF-β) that accumulates in aged muscle impedes regeneration and satellite cell proliferation[161]. Chronic elevation of inflammatory mediators in aged tissue also may contribute to age-related dysfunction[162]. Activation of the NF-κB pathway is a known transcriptional signature that mediates chronic inflammation in aged skin, skeletal muscle, bone and nervous system[163], although the direct effects of NF-κB activation on stem function in these tissues is still under investigation. Accumulation of senescent cells in aging tissues can be another driver of chronic inflammation, as these cells secrete inflammatory factors, growth regulators, proteases and other signaling molecules[127], affecting neighboring cells in the local environment and promoting senescence and inflammation.

Targeting extracellular signals to reverse stem cell aging

Strong evidence that aging of stem cells involves dominant signals from the local and systemic environment comes from studies in mice in which surgical and genetic systems have been used to modulate the aged tissue and systemic milieu. In particular, exposure of old skeletal muscle to a youthful systemic environment through heterochronic parabiosis, which creates a conjoined circulatory system between young and old animals, can promote efficient satellite cell activation in old muscle[164]. This surgical intervention also enhances rates of neurogenesis, remyelination and other measures of neural function in the old parabiont, suggesting that changes in stem cell function in the aging central nervous system are also affected by blood-borne factors[165–167]. Intriguingly, at least part of the ‘rejuvenating’ effect of a young systemic environment appears related to the higher levels of circulating growth differentiation factor 11 (GDF11) and oxytocin present in young blood as compared to aged[38,166,168,169]. Treatment of aged mice with either recombinant GDF11 or oxytocin reverses dysfunction of aged satellite cells and restores robust regenerative function in aged mice[38,169]. GDF11 supplementation in old mice further reverses age-related hypertrophy in cardiac muscle[168], enhances neural stem cell and neuronal function[166] and improves physical activity[38]. These studies raise the possibility that manipulation of blood-borne factors could provide an attractive strategy for the treatment of age-related muscle disease.

In addition to systemic factors, recent work suggests that targeting senescent cells and their products in aging tissues could also help restore stem cell function. Using an inducible genetic model for senescent cell ablation, one study demonstrated that life-long clearance of senescent cells from progeroid mouse tissues, via ablation of $p16^{Ink4a}$-expressing cells,

delays the onset of pathologies in multiple aging tissues, including the fat, muscle and eye[170]. Clearance of senescent cells only late in life did not improve age-related pathologies but did attenuate their progression[170]. These results have driven several groups to search for pharmacological strategies that could similarly induce the removal of senescent cells from aged tissues. As the benefits of senescent cell removal are apparent in multiple tissues, these so-called ‘senolytics’, if identified, could prove useful in a number of settings of age-associated pathology, including sarcopenia and metabolic disease.

Epigenetic memory of aging in stem cells

Many studies point to epigenetic regulation as important in determining stem cell function and further indicate that alterations in the epigenome that occur with aging can impinge on cellular processes in aged organisms and their stem cells. The acetylation and methylation status of DNA changes with age, while deletion or overexpression of enzymes responsible for these modifications can alter organismal longevity[171]. For example, the ASH-2 trithorax complex mediates trimethylation of histone H3 on Lys4 (H3K4me3), and its deletion increases *Caenorhabditis elegans* lifespan, consistent with excessive H3K4me3 being associated with an aging epigenome[172,173]. Interestingly, a ‘longevity memory’ of ASH-2 mutant parents is observed in homozygous wild-type offspring for up four generations[174]. This intriguing observation demonstrates that the aging epigenome functions like a heritable trait, but also can be modified. Pharmacologic treatment that alters histone modification such as acetylation status also shows an effect on longevity in aging and progeroid mouse models, suggesting that potential future therapeutic interventions targeting epigenetic regulators may be feasible[175,176]. Recent studies also have linked epigenetic variations with aging in stem cells. For example, aging HSCs show site-specific increases in DNA methylation that are particularly targeted to regions of the genome important for lineage-specific gene expression[177], and perturbations of histone modifications (for example, H3K4me3) that may alter the expression of HSC self-renewal genes[178].

Resetting epigenetic memory in aged stem cells

The development of adult frogs from the transfer of tadpole intestinal somatic nuclei into oocytes showed that cellular memory of somatic cells can be reversed and even erased[179]. Evidence suggests that a similar approach might be applied to reset the molecular memory of aging cells. Somatic cell nuclear transfer of senescent fibroblasts from aged bovine donors results in cloned calves with fibroblasts that have restored population doubling and increased telomere maintenance compared to age-matched control animals[180]. It has been further proposed that reprogramming aged somatic cells into iPSCs or iPSC-like cells followed by redifferentiation to the desired somatic cell types, may help reset the memory of aged somatic cells[181,182]. This possibility has been supported in part by studies in which aged hematopoietic progenitors were reprogrammed to produce iPSCs. These iPSCs were then used to create new HSCs in a chimeric embryo system and yielded complete rejuvenation of hematopoietic activity[183]. Thus, age-related epigenetic changes offer a model wherein ‘aging memory’ could potentially be altered or erased to restore stem cell function; however, it remains unclear whether more clinically viable rejuvenation strategies, such as modulating circulating factors or deleting senescent cells, which do not involve

reprogramming to a fully pluripotent state, actually fully reset the aged epigenome. It may be that stem cells exposed to such restorative interventions adopt some functional features of younger cells but still retain an aging memory. Further analysis of shifts in epigenetic signatures of stem cells exposed to such interventions will help to answer this important question.

Conclusion

Stem cell aging is affected by many different cell-intrinsic and cell-extrinsic pathways, which often show cross-talk in the determination of stem cell function. This interdependence makes it difficult to place particular weight on any one pathway with respect to aging mechanisms; however, certain signals do appear more broadly involved than others, at least given available data, and this could imply that they are more important regulators of aging stem cells. For example, the mTOR pathway has been shown to be a major regulator of both ROS levels and autophagy in HSCs[20,78,80,] (Fig. 3). Likewise, mitochondrial function, largely regulated by sirtuins, not only affects stem cell functions directly but also causes secondary effects on ROS and nutrient-sensing activities, which further modulate stem cell phenotypes[29,30,108,113,184].

Stem cell–autonomous phenotypes, such as DNA and protein damage caused by accumulated toxic metabolites, as well as non-autonomous stresses resulting from extracellular signals, contribute to a decline in stem cell function and depletion of the stem cell pool. However, in some cases at least, these aging phenotypes can be reversed to restore the regenerative function of stem cells. Such restorative interventions hold promise for the treatment of many diseases, including sarcopenia, heart failure and neurodegeneration, although whether these strategies truly restore stem cell function to a youthful state or instead induce a state of ‘pseudo-youth’ in which the reprogrammed cells retain an epigenetic memory of their true age remains an open and important question. Nonetheless, observations of the reversibility of stem cell aging have generated excitement about the development of ‘rejuvenating’ interventions that could extend the healthy years of life. Interventions that hold particular promise include those that target aging mechanisms that commonly affect tissues impaired by age-related diseases and dysfunction. For example, based on studies genetically targeting senescent cells for removal in progeroid mice[170], the development of senolytics, compounds that induce (by direct targeting or mobilization of the immune system) the removal of tissue-ensconced senescent cells holds promise for improving stem cell and organ function across a range of tissue systems. Likewise, the demonstration that common blood-borne signals can influence stem cell behavior and aging phenotypes in the muscle, brain and heart, coupled with the accessibility of these circulating mediators for intervention, should encourage further preclinical and translational studies modulating the systemic microenvironment to enhance maintenance and repair in aged tissues. Finally, the availability of nutritional and pharmacological agents that can target derangements in metabolism of aged cells to improve stem cell function and regenerative potential provides another near-term opportunity for new therapeutic strategies. Ultimately, strategies to combat diseases of aging will likely achieve greater success by focusing on high-level integrators of tissue physiology that can evoke remodeling of aging tissue architecture at the cellular and molecular level.

Improving the healthspan of elders takes on increasing urgency as human lifespans continue to increase, and even small gains in healthspan could dramatically lessen the impact of an aging population on the health care system and economy. Although clearly there is much still to be discovered about stem cell function, aging and the pathways to therapy, what we know so far clearly encourages further studies. By continuing to clarify the fundamental mechanisms by which stem cells age, ongoing research will develop interventions that someday may change the way we age.

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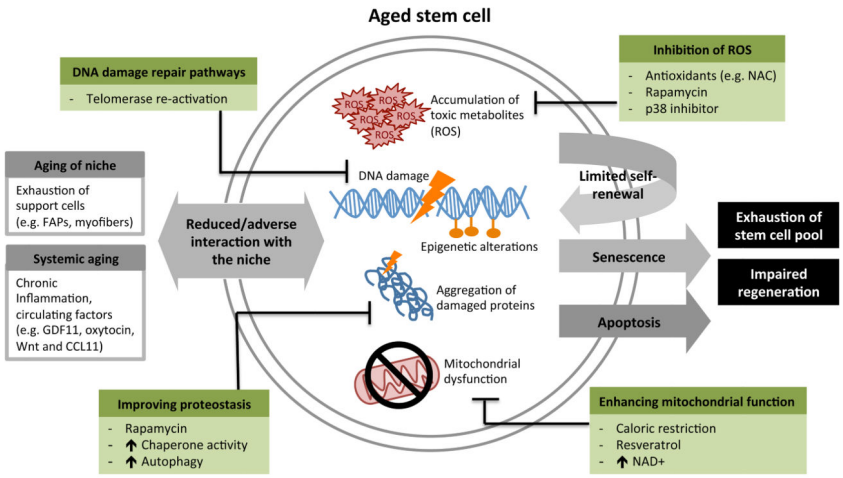

Figure 1.

Common pathways contributing to stem cell loss and dysfunction in the aging process. Common aging phenotypes within the stem cell are shown in orange, in the niche in pink, and the strategies by which to target and hopefully reverse these mechanisms in blue.

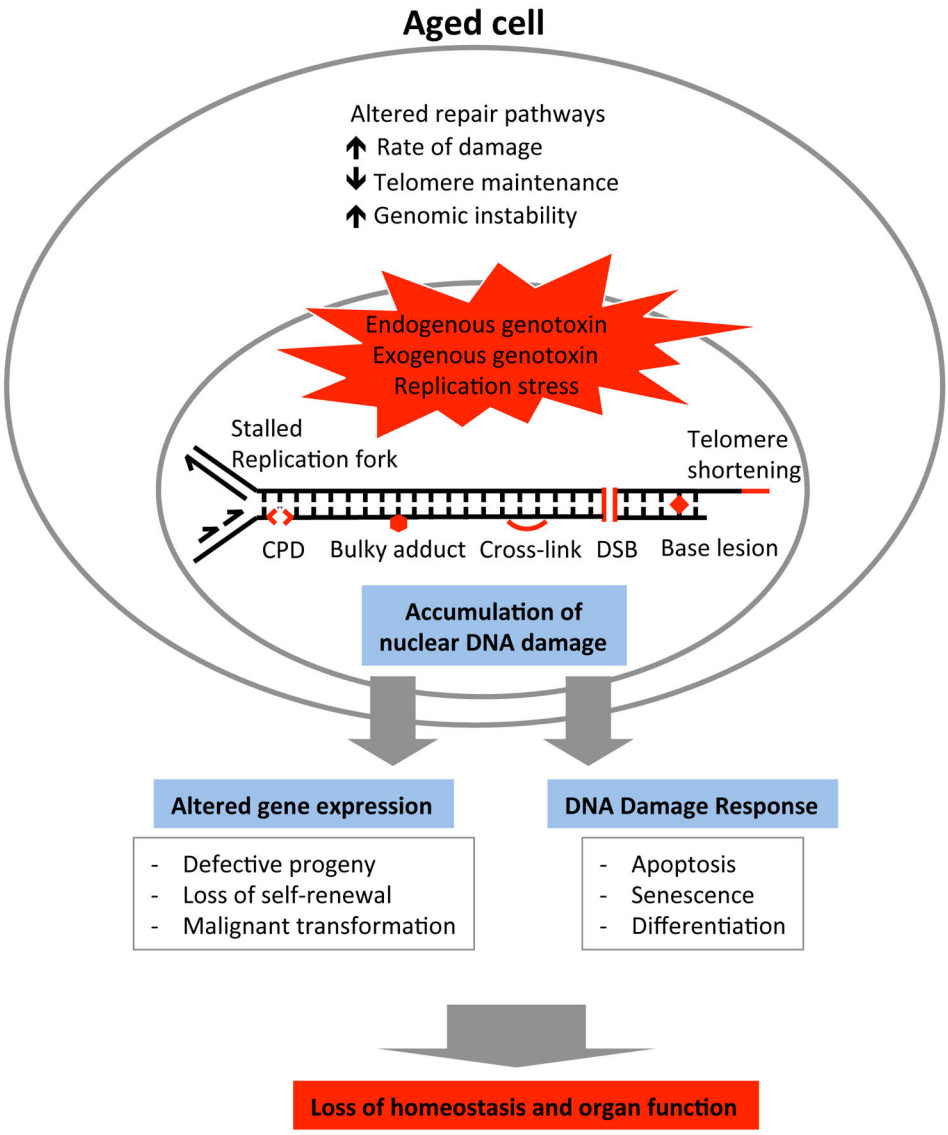

Figure 2.

Types of DNA damage in the aging genome that may affect stem cell function. Products of and effectors of DNA damage known to increase and accumulate during aging are shown, along with their effects on stem cell aging. CPD, cyclobutane pyrimidine dimer; DSB, double-strand break.

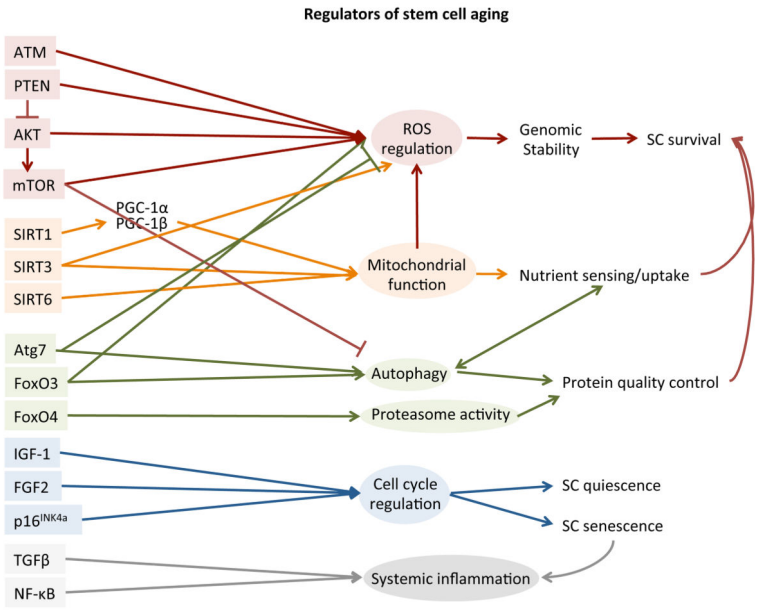

Figure 3.

Signaling pathways involved in aging of stem cells. Major signaling pathways related to aging of stem cells are listed in groups according to stem cell functions that they regulate.