

Regulation of Stem Cell Aging by Metabolism and Epigenetics

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Stem cell aging and exhaustion are considered important drivers of organismal aging. Age-associated declines in stem cell function are characterized by metabolic and epigenetic changes. Understanding the mechanisms underlying these changes will likely reveal novel therapeutic targets for ameliorating age-associated phenotypes and for prolonging human healthspan. Recent studies have shown that metabolism plays an important role in regulating epigenetic modifications and that this regulation dramatically affects the aging process. This review focuses on current knowledge regarding the mechanisms of stem cell aging, and the links between cellular metabolism and epigenetic regulation. In addition, we discuss how these interactions sense and respond to environmental stress in order to maintain stem cell homeostasis, and how environmental stimuli regulate stem cell function. Additionally, we highlight recent advances in the development of therapeutic strategies to rejuvenate dysfunctional aged stem cells.

Introduction

Aging can be defined as a complex, time-dependent process that affects multiple tissues and organs leading to a progressive reduction in physiological integrity and the degeneration of tissue, organ, and organismal function. Understanding the cause of aging has been an area of interest throughout human history. During the past 30 years, aging research has dramatically advanced, beginning with the initial discoveries that certain *Caenorhabditis elegans* mutants live significantly longer than their wild-type counterparts (Klass, 1983). The molecular and cellular mechanisms that extend *C. elegans* lifespan, as well as populations of human centenarians, have been the focus of intensive investigation using interdisciplinary approaches. Several hallmarks of aging (e.g., genomic instability, telomere attrition, epigenetic alteration, cellular senescence, stem cell exhaustion, mitochondrial dysfunction, dysregulated nutrient sensing, loss of proteostasis, and altered intercellular communication) have been described as major contributors to cellular aging. In addition, aging has been identified as the primary risk factor for the development and progression of certain diseases, such as cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases (Lopez-Otin et al., 2013).

Adult stem cells are essential for the maintenance of tissue homeostasis and regeneration. Consequently, the quantitative and qualitative decline in stem cell function during life, known as stem cell exhaustion, has been proposed as one of the drivers of aging (Lopez-Otin et al., 2013). Supporting this notion, age-associated phenotypes can be restored by the induction of stem cell rejuvenation *in vivo* (Rando and Chang, 2012; Lavasani et al., 2012). Therefore, characterizing the mechanisms of stem cell aging will be critical for ultimately understanding the aging process and for developing novel strategies to ameliorate age-

associated phenotypes and treat age-related diseases. Nevertheless, the complexity of stem cell biology (compared with terminally differentiated somatic cells) may complicate our understanding of the molecular mechanisms governing stem cell aging.

The role of metabolism in stem cell aging has recently been the focus of intense research. At this moment, there is increasing evidence that metabolic signal pathways are strongly associated with aging. Evidence supporting this connection suggests that: (1) decreased nutrient signaling can extend lifespan; (2) anabolic signaling accelerates aging; and (3) pharmacological manipulation of metabolic pathways extends organismal lifespan (Fontana et al., 2010; Harrison et al., 2009). In addition, recent evidence suggests that cellular metabolic pathways can alter epigenetic states and that these changes can affect organismal aging and longevity (Tatar and Sedivy, 2016; Berger and Sassone-Corsi, 2016).

Here, we highlight the mechanisms by which metabolic pathways contribute to the regulation of chromatin, and how these specific processes are thought to affect stem cell exhaustion and aging phenotypes. We also discuss fundamental questions regarding stem cell aging that remain to be answered, including what are the genetic or epigenetic drivers of stem cell aging, and whether it might be possible to restore redox homeostasis and nutrient-sensing pathways to reset the epigenetic clock of stem cell aging.

Drivers of Stem Cell Exhaustion during Aging

Adult stem cells play a vital role in maintaining tissue homeostasis through repair and regeneration during life (Goodell and Rando, 2015). Stem cell exhaustion, defined as the decline in stem cell number and function, is observed in virtually all tissues

and organs maintained by adult stem cells, such as the forebrain, bone, and muscle (Conboy and Rando, 2012; Gruber et al., 2006; Molofsky et al., 2006). In addition, age-associated changes in hematopoietic stem cell (HSC) differentiation lead to the production of fewer adaptive immune cells. This potentially leads to anemia and myeloid malignancies in aged organisms (Shaw et al., 2010). Moreover, stem cell exhaustion is frequently observed in human age-related diseases and rare genetic disorders. As an example, a premature depletion of mesenchymal stem cells (MSCs) is observed in patients with Hutchinson-Gilford progeria syndrome (HGPS) (Liu et al., 2011a, 2011b), Werner syndrome (WS) (Zhang et al., 2015), and Fanconi anemia (FA) (Liu et al., 2014); neural stem cells (NSCs) show defects on neuronal differentiation and DNA repair in patients with Parkinson's disease (PD) (Liu et al., 2012c) and xeroderma pigmentosum (XP) (Fu et al., 2016); and HSCs are deregulated in FA (Liu et al., 2014). For these reasons, age-associated stem cell exhaustion is considered a hallmark of aging (Lopez-Otin et al., 2013). The observation that stem cell exhaustion is one of the most significant hallmarks of aging has led to many questions, including: (1) what are the mechanisms underlying stem cell senescence and exhaustion during organismal aging? and (2) is it possible to delay the exhaustion of stem cells or to rejuvenate senescent stem cells to ameliorate age-associated phenotypes? In this regard, many cellular hallmarks of aging act as major drivers of the quantitative and qualitative changes observed in stem cells during aging. These hallmarks, which are in many instances directly related to the role and features of stem cells, include genomic instability, telomere attrition, epigenetic alterations, cellular senescence, mitochondrial dysfunction, loss of proteostasis, and altered intercellular communication. Importantly, a reversal of aging phenotypes can be induced *in vivo* by stem cell rejuvenation, opening the door for potential anti-aging interventions based on approaches aiming at the improvement of stem cell function (Rando and Chang, 2012).

Stem cells are extremely vulnerable and their homeostasis can be challenged by many factors directly leading to decreased proliferation, which is one of the major features of stem cell aging. In aged mice, HSCs exhibit decreased rates of cell division, indicating a general decline in cell-cycle activity (Rossi et al., 2007). Replication stress caused by age-related cell-cycle defects (e.g., DNA damage or chromosome disorganization) can diminish HSC functional activity, leading to decreased blood production and impaired therapeutic potential in transplantation assays (Flach et al., 2014). As part of the underlying mechanisms, increased levels of *p16^{INK4a}* (a cell-cycle regulator that inhibits cell-cycle progression) and the accumulation of DNA damage have been closely associated with declines in stem cell populations during aging (Janzen et al., 2006; Rossi et al., 2007). Consequently, rejuvenation of cell-cycle activity and engraftment capacity are observed in INK4a-deficient aged HSCs (Janzen et al., 2006). In contrast, loss of quiescence and excessive proliferation can also cause premature exhaustion of stem (progenitor) cells due to the accelerated exhaustion of stem cell populations. For example, loss of *p21* leads to the premature exhaustion of HSCs and NSCs in mice (Cheng et al., 2000; Kippin et al., 2005). Moreover, basal levels of autophagy help to maintain mouse satellite cells (muscle stem cells) in a quiescent state, whereas autophagy impairment causes senes-

cence of satellite cells, leading to proteostasis imbalance and mitochondrial dysfunction (Garcia-Prat et al., 2016). Additionally, autophagy has been reported to actively preserve quiescence and stemness of old mouse HSCs by suppressing cellular metabolism (Ho et al., 2017). Conversely, restoration of autophagy in geriatric satellite cells can reverse their senescent phenotype. Moreover, while dietary restriction promotes the proliferation of intestinal stem cells (ISCs) through the cooperation of mammalian target of rapamycin complex 1 (mTORC1) and Sirtuin 1 (SIRT1) (Igarashi and Guarente, 2016), rapamycin (an inhibitor of mTOR) can significantly repress the expansion of ISCs. Telomere attrition represents an additional driver of age-related stem cell exhaustion in multiple tissues (Sharpless and DePinho, 2007; Flores et al., 2005). Lastly, genome instability, as a consequence of multiple types of stress or damage, constitutes another important cause of the dysfunction and decline of adult stem cells during aging. For instance, deficiency of the DNA helicase WRN results in WS, a premature aging syndrome also known as adult progeria, which is characterized by features of physiological aging in young individuals (Kudlow et al., 2007; Lopez-Otin et al., 2013). Deficiency of WRN protein in human MSCs not only triggers activation of the DNA damage response, but also induces the instability of heterochromatin, which has recently been suggested to drive MSC exhaustion and, subsequently, organismal aging (Zhang et al., 2015).

Epigenetic Mechanisms of Stem Cell Aging

There is mounting evidence that epigenetics plays an important role in driving organismal aging (Lopez-Otin et al., 2013; Sen et al., 2016; Benayoun et al., 2015; Booth and Brunet, 2016; Pal and Tyler, 2016). Nucleosomes represent the basic structural units of chromatin and are composed of DNA wrapped around a set of histone proteins. Gene expression is dynamically regulated by the interplay between transcription factors and epigenetic modifiers, which are enzymes capable of directly modifying DNA or the core histone variants, including H2A, H2B, H3, H4, H3.3, macroH2A, and H2A.Z. On the other hand, although heterochromatin domains established during early embryonic development are considered constant throughout an animal's lifespan, aging triggers the loss of constitutive heterochromatin by telomere attrition, transcription changes at boundaries, and disorganization of the nuclear periphery (Figure 1). The disorganization of heterochromatin causes global and local alterations in DNA methylation patterns, which are regularly observed in aging cells as consequences of reduced histone 3 lysine 9 trimethylation (H3K9me3) levels and heterochromatin-associated proteins, such as heterochromatin protein 1 (HP1) (Scaffidi and Misteli, 2005; 2006; Zhang et al., 2015). Furthermore, it has been demonstrated that the aging of human adult stem cells is associated with diverse epigenetic alterations including global loss of H3K9me3, decondensation of centromeric heterochromatin, physical attrition of telomeres, and changes in the nucleolus organizer region related to ribosomal DNA (NOR-rDNA) (Ren et al., 2017; Zhang et al., 2015; Liu et al., 2011a; Kubben et al., 2016; Deng et al., 2015; Ding et al., 2015; Fu et al., 2016; Yang et al., 2017; Wang et al., 2017).

Unlike worms and flies, which are simple models for studying the regulation of aging and longevity at the organismal level, the aging process in mammalian systems is controlled by more

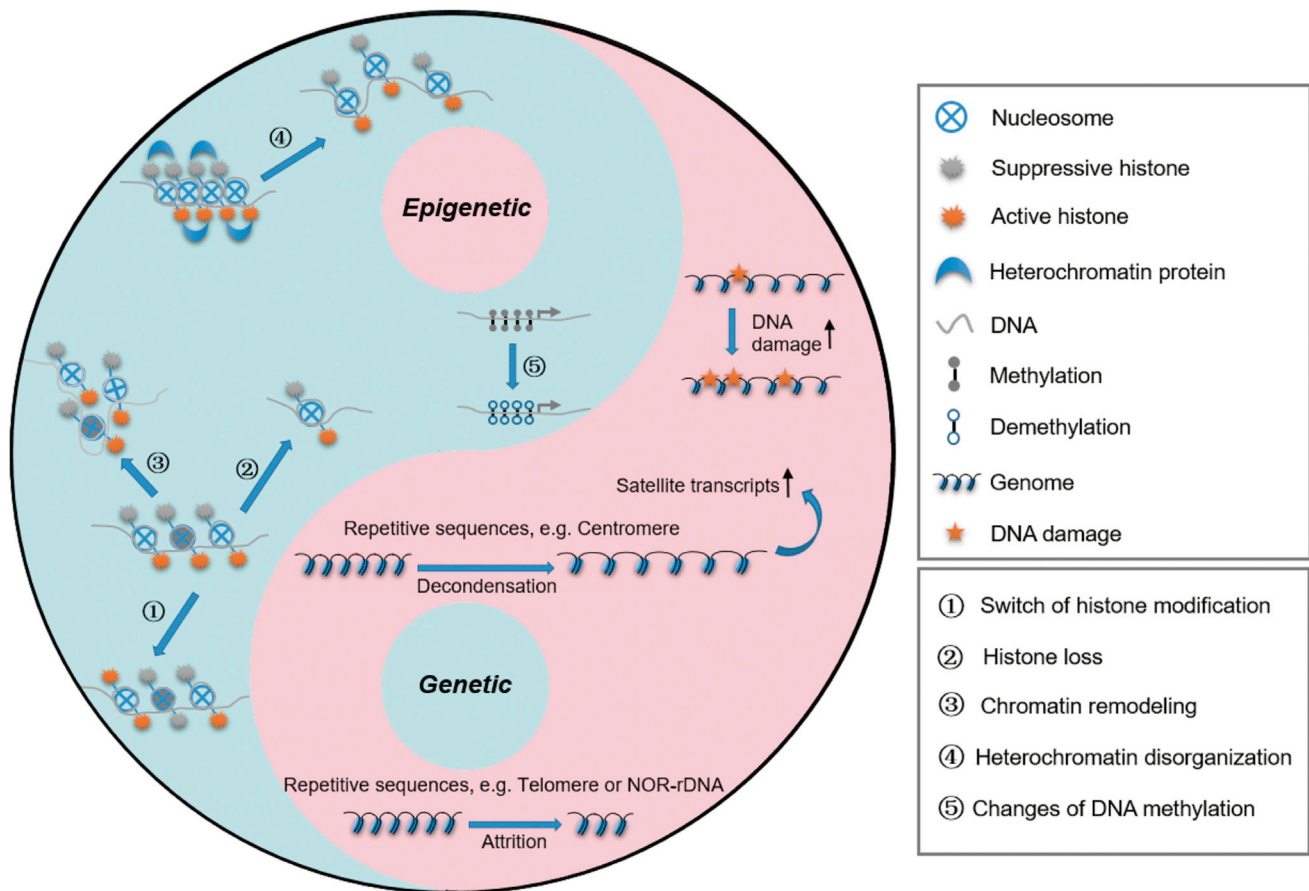


Figure 1. Interplay between Epigenetic and Genetic Instabilities in (Stem) Cell Aging

During aging, major epigenetic changes are observed, including loss of core histones together with changes in histone modifications and chromatin remodeling enzymes. These changes lead to a loss of repressive heterochromatin, chromatin decondensation in centromeres and telomere attrition, and DNA damage.

complex mechanisms in which stem cell aging may play crucial roles. Moreover, multiple lines of evidence suggest that epigenetic changes drive adult stem cell aging in mammals. These include HSCs, muscle stem cells (MuSCs) or satellite cells (Liu et al., 2013), and MSCs (Pan et al., 2016; Zhang et al., 2015; Kubben et al., 2016).

HSCs

During aging, an increase in DNA damage and a decrease in the DNA repair capacity of HSCs has been shown to result in a progressive loss of HSCs (Nijnik et al., 2007; Rossi et al., 2007). In addition, high levels of replication stress upon re-entry in the cell cycle contribute to the functional decline in HSCs in old organisms (Flach et al., 2014). Despite the clear role of DNA damage and replication stress during HSC aging, epigenetic dysregulation has been shown to be an important contributor to HSC exhaustion. In this regard, epigenetic profiling of young and old HSCs has revealed changes in H3K4me3 levels across self-renewal genes and detected increase in DNA methylation at genes related to differentiation, leading to defects in differentiation during HSC aging (Sun et al., 2014). In addition, the proliferation of mouse HSCs has been shown to be promoted by *Sirtuin 6* (*Sirt6*) deletion, as SIRT6 represses Wnt target genes by interacting with the transcription factor LEF1 and by deacety-

lating H3K56Ac (Wang et al., 2016). Lastly, levels of both H3K27me3 and H3K4me3 increase with age in mouse HSCs (Chambers et al., 2007), and changes in DNA methylation or H3K9 methylation during age induce mouse HSC differentiation (Challen et al., 2014; Mayle et al., 2015; Ugarte et al., 2015).

MSCs

Several studies have highlighted changes in the epigenetic regulation of the genome leading to a loss of self-renewal and osteoblast differentiation during aging in MSCs. As an example, a decreased expression of histone deacetylases (HDACs), together with a downregulation of polycomb group genes and upregulation of *JMJD3* was observed in senescent MSCs (Jung et al., 2010). Similarly, changes in H3 acetylation but not DNA methylation were found to lead to reduced self-renewal and increase osteogenic differentiation accompanied by increased expression of osteogenic genes such as *RUNX2* and *ALP* (Li et al., 2011). On the other hand, inhibition of DNA methyltransferases with 5-azacytidine or small interfering RNA was shown to induce senescence in MSCs by dysregulating not only DNA methylation but also promoting changes in histone marks on promoter regions (So et al., 2011). Importantly, patients with WS, a disease caused by deficiency in WRN protein, who exhibit phenotypes associated with premature cellular aging

(Kudlow et al., 2007), are also characterized by defects in MSCs. MSCs differentiated from WRN-null embryonic stem cells (ESCs) exhibit a global loss of H3K9me3 and heterochromatin disorganization. This is caused by the deconstruction of a heterochromatin-associated complex composed of WRN, SUV39H1, HP1 α , and the nuclear lamina-heterochromatin anchoring protein LAP2. Moreover, decreased levels of WRN and heterochromatin marks are also observed in primary MSCs from older individuals, suggesting that genome instability and heterochromatin disorganization associated with WRN deficiency may be a potential driver of normal MSC aging, as shown in Figure 1 (Zhang et al., 2015). On the other hand, SIRT6 could play dual roles as both an epigenetic modulator and a key regulator of redox homeostasis by coactivating the NRF2 antioxidant pathway in wild-type human MSCs. Supporting this hypothesis, SIRT6 interacts with NRF2 and deacetylates H3K56 at the promoters of NRF2 target genes, leading to the recruitment of the RNA polymerase II (RNAP II) complex and NRF2 transactivation. SIRT6 deficiency in human MSCs increases global levels of H3K56Ac, which impairs the recruitment of the RNAP II complex to promoters of NRF2 target genes, resulting in senescence-associated cellular redox unbalance (Pan et al., 2016; Yang et al., 2017).

MuSCs and Other Stem Cells

Due to the loss of regenerative capacity observed in muscle during aging, muscle stem cell aging has been subjected to intensive study. Interestingly, epigenetic changes during aging have also been described in this adult stem cell compartment. Specifically, an increase in H3K27me3 and a decrease in H3K4me3 are observed in aged MuSCs as they become quiescent (Liu et al., 2013). Similarly to HSCs, increased H3K27me3 levels downregulate genes linked to stem cell function in MuSCs. In the same way, the activity of the H3K27me3 demethylase UTX is required for mouse MuSC-mediated muscle regeneration (Faralli et al., 2016). Nevertheless, there is contradictory evidence that H3K27me3 regulators, such as UTX or JMJD3, can extend or shorten lifespan in model organisms (Labbadia and Morimoto, 2015; Maures et al., 2011; Merkwirth et al., 2016). In addition to H3K27me3 or H3K4me3, there are other chromatin modifications that are altered with age in various adult stem compartments (Benayoun et al., 2015; Booth and Brunet, 2016; Lopez-Otin et al., 2013; Pal and Tyler, 2016; Sen et al., 2016; Avgustinova and Benitah, 2016). Changes in additional histone marks during aging play important roles in the maintenance of stem cell quiescence, self-renewal, and differentiation. As an example, H4K20 methylation maintains MuSC quiescence in mice (Boonsanay et al., 2016). On the other hand, broad H3K4me3 domains promote the self-renewal and differentiation of mouse NSCs (Benayoun et al., 2014). It is also clear that the maintenance of nuclear architecture and chromatin homeostasis is required for proper human stem cell (e.g., NSC) function, and this process is disrupted in age-related diseases such as PD (Liu et al., 2012c). Although epigenetic dysregulation is clearly implicated in these changes observed in stem cell populations during life, the mechanisms by which specific epigenetic marks and their regulators are involved in stem cell aging remain unclear and will need to be the subject of further investigation.

Metabolic Regulation of Aging

The metabolic regulation of aging has recently attracted a lot of attention in the field of aging research. Overall, different lines of evidence suggest that the aging process is controlled by a metabolic clock involving mitochondria and nutrient-sensing pathways (Figure 2) (Lopez-Otin et al., 2013; 2016).

Mitochondria-Based Metabolic Regulation of Aging

Mitochondria are the primary source of cellular energy, and significant damage accumulates in these organelles during genotoxic stress and natural aging. For this reason, mitochondrial metabolism represents a promising target for the amelioration of age-associated phenotypes. Current evidence indicates that mitochondrial DNA (mtDNA) accumulates age-associated somatic mutations as a consequence of (1) the oxidative environment of mitochondria, (2) the absence of protective histones binding to mtDNA, and (3) the lack of efficient repair mechanisms for mtDNA (Linnane et al., 1989). Consequently, mutations and deletions characterize mtDNA from aged organisms and likely contribute to aging (Park and Larsson, 2011). A link between mtDNA damage, aging, and age-related diseases was first suggested when mtDNA mutations were shown to cause human multisystem disorders associated with partial aging phenotypes (Wallace, 2005). Additional evidence derived from the study of mice carrying a mutation in the proofreading exonuclease domain of the mtDNA polymerase γ showed that premature aging and reduced lifespan of these mutant mice is associated with accumulated mtDNA mutations (Kujoth et al., 2005; Trifunovic et al., 2004; Vermulst et al., 2008). Importantly, these mice exhibit impaired mitochondrial function with no significant increase in reactive oxygen species (ROS) levels (Edgar et al., 2009; Hiona et al., 2010). In addition, somatic stem cells (SSCs) in these mice are particularly sensitive to the accumulation of mtDNA mutations (Ahlqvist et al., 2012). Based on these studies, it has been suggested that mtDNA mutations may be tightly associated with cellular senescence and aging at the organism level. On the other hand, further studies will be necessary to determine whether lifespan can be extended by an increase in the repair of mtDNA mutations. In this regard, preservation of mitochondrial function as a consequence of activating the mitochondrial unfolded protein response (UPR^{mt}), a cellular stress response pathway activated as a consequence of the accumulation of misfolded proteins in the mitochondria, by the conserved histone lysine demethylases JMJD-1.2 and JMJD-3.1, promotes longevity in *C. elegans* (Merkwirth et al., 2016). In addition, mitochondrial 1C metabolism-induced redox homeostasis correlates with cell proliferation in mice (Ducker et al., 2016). Furthermore, during natural aging, mitochondrial dysfunction and impairment of muscle fiber integrity are tightly linked with reductions in nicotinamide adenine dinucleotide (NAD⁺) content in mice. Moreover, supplementation of the NAD⁺ precursor nicotinamide riboside can reverse progressive muscle dysfunction (Frederick et al., 2016). Lastly, it has recently been shown that exercise counteracts age-associated secondary damage by protecting against declines in mitochondrial respiration (Cartee et al., 2016). These results suggest that both the maintenance of mitochondrial activity and the rejuvenation of mitochondrial function may represent promising approaches for delaying aging and extending lifespan.

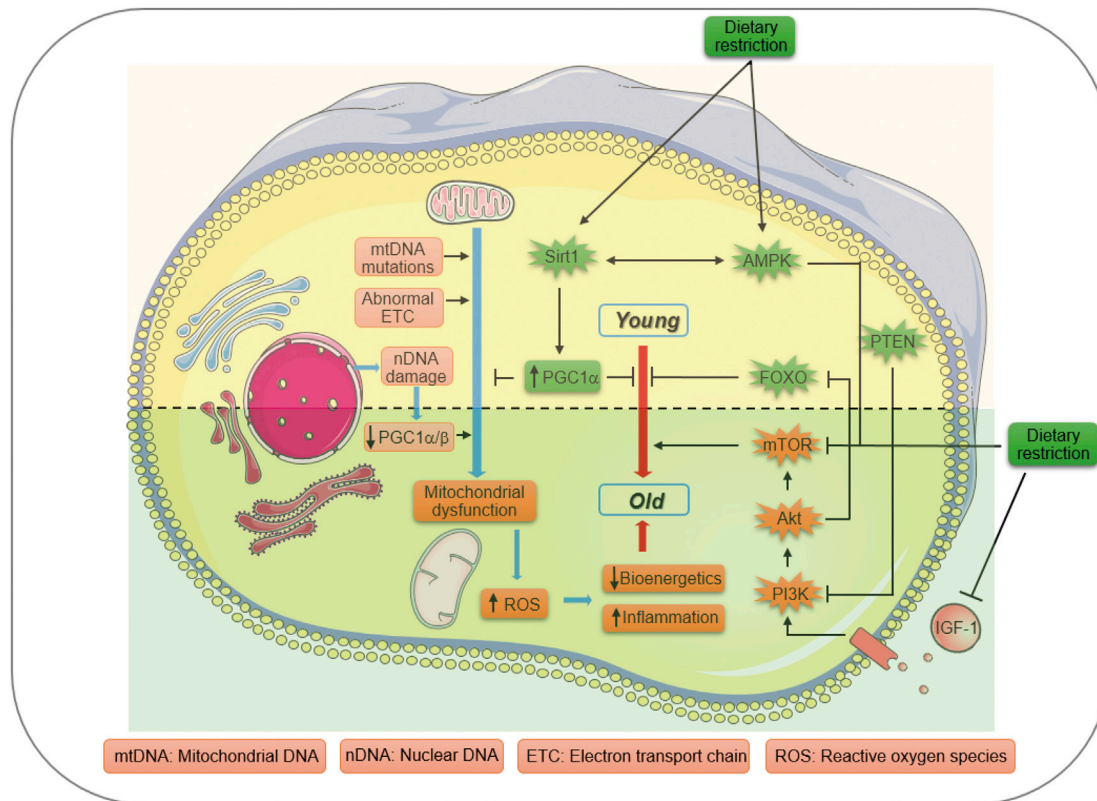


Figure 2. Metabolic Mechanisms Underlying (Stem) Cell Aging

Major metabolic pathways play a key role on cellular aging. On one hand, the mitochondrial respiratory chain is the major source of reactive oxygen species (ROS) that cause oxidative damage to the nuclear and mitochondrial DNA, resulting in further mitochondrial dysfunction and loss of cellular homeostasis. On the other hand, major nutrient sensor pathways including IGF-1 and mTOR promote growth and decrease stress resistance. Caloric restriction improves age-associated phenotypes and leads to lifespan extension by major remodeling of metabolic pathways, including upregulation of mitochondrial function through PGC1 α and downregulation of IGF-1 and mTOR.

Nutrient-Sensing Pathways Affect the Metabolic Regulation of Aging

Anterior pituitary-produced growth hormone (GH) and its secondary mediator, insulin-like growth factor 1 (IGF-1), are important components of the somatotrophic axis in mammals. Since the same intracellular signaling pathway is affected by IGF-1 and insulin, IGF-1 and insulin signaling are together named the “insulin and IGF-1 signaling” (IIS) pathway. The IIS pathway is a highly conserved aging-controlling signaling pathway (Barzilai et al., 2012; Fontana et al., 2010; Kenyon, 2010). Current evidence indicates that the function of the components of the IIS pathway (GH, IGF-1, and insulin) and their relevant downstream intracellular effectors (AKT, mTOR, and FOXO) are tightly associated with longevity in both animal models and humans, suggesting that trophic and bioenergetic pathways play key roles in regulating aging and longevity (Barzilai et al., 2012; Fontana et al., 2010; Kenyon, 2010).

Lifespan is consistently extended in worms, flies, and mice by manipulation of the IIS signaling pathway. In addition, the IIS pathway plays an important role in the extension of lifespan in worms and flies by dietary restriction (Fontana et al., 2010). On the other hand, an additional study showed that the levels of GH and IGF-1, key components of the IIS pathway, decrease during physiological and premature aging (Schumacher et al.,

2008). By contrast, it has been reported that increasing levels of the tumor suppressor PTEN (phosphatase and tensin homolog) drive general reductions in the signaling intensity associated with the IIS pathway. Ultimately, this increased intensity leads to an improvement in mitochondrial oxidative metabolism and an increase in energy expenditure (Ortega-Molina et al., 2012; Garcia-Cao et al., 2012). Together with data from different mouse models exhibiting reductions in IIS activity (e.g., Pten-overexpressing mice and hypomorphic phosphatidylinositol 3-kinase [PI3K] mice), it has been shown that the extension of mouse lifespan is accompanied with decreased levels of IIS activity (Ortega-Molina et al., 2012; Foukas et al., 2013). Interestingly, PTEN deficiency in human NSCs increases activity of the PI3K-AKT signaling pathway and subsequently promotes glycolysis, whereas human MSCs with PTEN depletion present accelerated cellular senescence. This suggests that PTEN inactivation and subsequent activation of the PI3K-AKT pathway may play different roles in regulating the aging process of different human adult stem cells (Duan et al., 2015). Therefore, decreased IIS activity represents a common feature of mice with both physiological and accelerated aging. In contrast, constitutive reduction of IIS activity promotes lifespan extension by decreasing cellular growth rates, metabolic levels, and levels of cellular damage (Lopez-Otin et al., 2013).

In addition to the IIS pathway, which is involved in glucose sensing, three other nutrient-sensing systems have been linked to aging: mTOR, AMP-activated protein kinase (AMPK), and sirtuins. In general, these pathways sense high amino acid concentrations and low-energy states by detecting high levels of AMP or NAD⁺ (Houtkooper et al., 2010). mTORC1 and mTORC2 are two mTOR multiprotein complexes that regulate many aspects of anabolic metabolism (Laplane and Sabatini, 2012). In yeast, worms, and flies, lifespan is extended following the genetic downregulation of mTORC1 activity. mTOR inhibition can also phenocopy dietary restriction (Johnson et al., 2013). The lifespan of mice can also be increased by inactivation of only mTORC1 (Lamming et al., 2012). Moreover, S6K1-deficient mice are also long lived (Selman et al., 2009). In contrast, aging of mouse hypothalamic neurons leads to an increase in mTOR activity, which subsequently results in age-related obesity (Yang et al., 2012). Together with data involving the IIS pathway, these results indicated that IIS and mTORC1 pathway-induced trophic and anabolic activities are major accelerators of the aging process.

In contrast, the AMPK and sirtuin nutrient-sensing systems mediate nutrient scarcity and catabolism. Consequently, upregulation of these pathways induces healthy aging. AMPK activity efficiently downregulates mTORC1 (Akers et al., 2012), and the activation of AMPK by the drug metformin promotes lifespan extension in both worms and mice (Anisimov et al., 2011; Mair et al., 2011; Onken and Driscoll, 2010). Simultaneously, PPAR γ coactivator 1 α (PGC-1 α), which participates in mitochondrial biogenesis, can be activated by either the SIRT1 (Rodgers et al., 2005) or TORC (transducer of regulated CREB binding protein)-CREB (cAMP response element-binding protein) pathway (Wu et al., 2006), and the activation of PGC-1 α subsequently results in the enhancement of antioxidant defenses and the improvement of fatty acid oxidation (Fernandez-Marcos and Auwerx, 2011). Lastly, SIRT1 and AMPK can activate each other as part of a positive feedback loop that integrates two low-energy sensing systems into a unified response (Price et al., 2012).

All of these results suggest that the dysregulation of nutrient sensing is one of the major hallmarks of aging (Lopez-Otin et al., 2013), and that rejuvenating nutrient-sensing pathways may be a promising avenue to delay aging and promote healthy longevity. Caloric restriction has the capacity to regulate both mitochondrial metabolism and nutrient-sensing pathways. Interestingly, caloric restriction has been reported to increase lifespan and/or healthspan in many eukaryote species, including nonhuman primates (Colman et al., 2009; Fontana et al., 2010; Mattison et al., 2012). In addition, recent studies have shown that the lifespan extension by dietary restriction in *C. elegans* modulates both pre-mRNA splicing homeostasis and the TORC1 pathway (Heintz et al., 2016), whereas in mice caloric restriction increases lifespan by maintaining mitochondria energy metabolism and proteostasis (Mitchell et al., 2016), and antagonizes accelerated aging and genomic stress in DNA-repair-deficient mice (Vermeij et al., 2016). It is clear that cellular metabolism is deeply involved in the control of longevity, but the mechanisms underlying the regulation of aging by both mitochondria and nutrient-sensing pathways remain unknown.

The Link between Metabolic and Epigenetic Pathways

A prominent discussion has emerged in recent years concerning how metabolism regulates cellular processes through epigenetic modulation. In worms, mitochondrial stress can be experimentally induced either by disrupting the electron transport chain (ETC) (Dillin et al., 2002; Shao et al., 2016) or by expressing a polyglutamine tract-containing protein in neurons alone (Brignull et al., 2006). Following the induction of mitochondrial stress during development, adults express a number of protective genes (e.g., mitochondrial chaperones and quality control proteases), and the mitochondrial unfolded protein response (UPR^{mt}) is subsequently activated in response to mitochondrial stress by (1) the transcription factors ATFS-1 and DVE-1, (2) the cofactor UBL-5, and (3) the quality control protease CLPP-1. By inducing mitochondrial stress during larval development, adult lifespan is increased by nearly 2-fold (Dillin et al., 2002). In fact, although mitochondria clearly participate in both the aging process and the functional regulation of human stem cells (Xu et al., 2013), how mitochondria affect aging, especially stem cell aging, is still far from settled. Recently, it has been suggested that mitochondrial stress-mediated regulation of lifespan is associated with epigenetic events that regulate the function of the genome, leading to an extension in lifespan (Merkwirth et al., 2016; Tian et al., 2016; Han et al., 2017; Lin and Wang, 2017). On the other hand, recent reports have found that UPR^{mt} is not sufficient for lifespan extension in *C. elegans*, and moreover could be implicated in the maintenance and propagation of mutant mitochondrial genomes (Lin et al., 2016; Matilainen et al., 2017). Lastly, the mammalian transcription factor ATF5 has been reported to activate UPR^{mt} and subsequently promote organelle recovery during mitochondrial stress in both worms and mammals (Fiorese et al., 2016).

Mechanistically, the experimental knockdown of cytochrome C1 (*cyc-1*), *jmjd-1.2*, and *jmjd-3.1* (conserved histone lysine demethylases required to activate the UPR^{mt}) revealed that these factors are involved in regulating the extension of lifespan (Merkwirth et al., 2016). *Jmjd-1.2* and *jmjd-3.1* act upstream of the UPR^{mt} and play an important role in both the initial establishment and propagation of the UPR^{mt} from developmental stages to adulthood. Consequently, this longevity response can be abrogated by dysfunction of either *jmjd-1.2* or *jmjd-3.1* only. Similarly, *Phf8* and *Jmjd3* (mouse homologs of *jmjd-1.2* and *jmjd-3.1*) can regulate the expression of UPR^{mt}-related genes (Merkwirth et al., 2016). In addition, the regulation of UPR^{mt} requires another important protein, *Lin-65*, which is a synMuvB protein that interacts with chromatin modifiers (Tian et al., 2016). In response to ETC stress (induced by expression of a polyQ containing protein in neurons of *C. elegans*) (Brignull et al., 2006), the accumulation of *Lin-65* in the nucleus of intestinal cells subsequently induces chromatin remodeling that drives UPR^{mt} activation, thereby promoting stress-induced longevity. The mitochondrial stress-induced signaling retrogradely elicits nuclear responses, resulting in imbalanced responses on the mitochondrial side to induce UPR^{mt} (Houtkooper et al., 2013). With the specific induction of “stress” during the L3-to-L4 transition, which is the rapid proliferation phase of mitochondria within the germline compartment, UPR^{mt} is triggered by mitochondria-induced epigenetic changes in peripheral tissues of adult worms and the lifespan is also extended.

Cells sense and respond to environmental challenges via specific epigenetic modulations that involve signaling pathway components, including enzymes that modify histones and DNA. These enzymes modify histones or DNA in the presence of different cofactors, including intracellular phosphate, acetyl, and methyl groups. For these reasons, whereas there is an unclear link between cellular metabolism and epigenetic regulation, multiple epigenetic responses to environmental alterations have been revealed by a number of remarkable studies. Among these studies, the function of the fat mass and obesity-associated (*FTO*) gene represents a close link between metabolism and epigenetics. The *FTO* gene encodes an N⁶-methyladenosine (m⁶A) demethylase (Gerken et al., 2007). A SNP within *FTO* affects its response to food intake and subsequently causes obesity. Mechanistically, *FTO* proteins carrying this SNP interact with the homeobox gene, *IRX3*, to aggravate the process of obesity. This indicates that a single gene that causes metabolic dysfunction can induce an epigenetic alteration to directly influence another gene with a similar function (Smemo et al., 2014). In fact, pathways that regulate chromatin always utilize enzymes that function to control either nucleosome positioning or composition, or to modulate DNA and histones with small chemical units. To modify DNA or histones these enzymes use cofactors, which include cellular metabolites such as acetyl-coenzyme A (acetyl-CoA) for acetyltransferases (Ariyannur et al., 2010; Takahashi et al., 2006; Wellen et al., 2009), S-adenosyl methionine (SAM) for methyltransferases (Dolinoy et al., 2007; Kera et al., 2013), ATP as a donor of acetyl, methyl, or phospho groups for kinases (Becker and Workman, 2013), and NAD⁺ for deacetylases (Finkel et al., 2009; Gibson and Kraus, 2012; Schreiber et al., 2006), as well as flavin adenine dinucleotide (FAD) or α -ketoglutarate for demethylases (Hou and Yu, 2010; Metzger et al., 2010; Shi and Tsukada, 2013). Additionally, mitochondrial metabolism controls key regulators of epigenetic enzymes involved in histone acetylation and histone and DNA methylation through the generation of metabolites in the tricarboxylic acid (TCA) cycle including citrate, succinate, fumarate, and 2-hydroxyglutarate (2HG) (Anso et al., 2017; Martinez-Reyes et al., 2016; Matilainen et al., 2017). Along the same lines, the levels of acetyl-CoA act as metabolic regulators and induce autophagy as well as lysosomal biogenesis through induction of histone acetylation and upregulation of gene expression (Li et al., 2017; Marino et al., 2014; Mews et al., 2017). This regulation has been shown to play an important role on the establishment of hippocampal memory (Yang et al., 2017). Similarly, ATP is also used by chromatin remodeling complexes to move or restructure nucleosomes (Becker and Workman, 2013). Moreover, certain enzymes including sirtuins or ADP-ribose polymerases (PARPs) that regulate epigenetic states can directly respond to changes in cellular metabolism.

On the other hand, abnormal metabolism can drive cellular senescence by direct regulation of histone and DNA modifications (Finkel et al., 2009; Gibson and Kraus, 2012; Schreiber et al., 2006). As an example, differentiation of MSCs from induced pluripotent stem cells (iPSCs) derived from patients with HGPS recapitulates premature aging phenotypes characterized by morphological, genomic, and epigenetic changes (Schreiber and Kennedy, 2013; Kudlow et al., 2007; Burtner and Kennedy, 2010; Kubben et al., 2016). These genetic and

epigenetic instabilities were linked to dysregulated redox metabolism in a recent study showing that increased chronic oxidative stress due to progerin-mediated repression of the NRF2 antioxidant transcriptional pathway drives human MSCs to senescence (Kubben et al., 2016). In summary, a variety of metabolites have been functionally linked to enzymatic activities involved in different levels of epigenetic modifications, such as chromatin remodeling and DNA methylation (Katada et al., 2012; Lu and Thompson, 2012). Furthermore, the “niches” of chromatin-associated metabolites have been used to explain locus-specific epigenetic modifications (Katada et al., 2012), which are tightly associated with the aging process, particularly in stem cells.

Metabolite-Epigenetic Mechanisms Underlying Stem Cell Aging

While different epigenetic states are regulated at multiple levels, how cell function and homeostasis are controlled by metabolic and epigenetic mechanisms in a number of cell types, particularly stem cells, remains unknown. It is noteworthy that cofactors of epigenetic enzymes that create different forms of chromatin modifications (e.g., DNA methylation/demethylation, histone acetylation/deacetylation, and histone methylation/demethylation) are almost all metabolites that are derived from cellular metabolism (Berger and Sassone-Corsi, 2016). This direct connection suggests that changes in metabolism in response to environmental stimuli could globally affect the epigenome of stem cells to affect stem cell function, or disrupt homeostasis and induce stem cell senescence (Figure 3). Firstly, it is well known that lifespan and healthspan are regulated in many cases both by enzymes that directly use metabolites as substrates, such as with sirtuins, or by crucial modulators of cellular metabolism, including components of the mTOR and insulin-FOXO pathways (Chandel et al., 2016). Importantly, several studies have recently shown that these pathways affect, at least partially, stem cell homeostasis with age via chromatin/transcription modulation. Sirtuins are directly regulated by cellular metabolites and function as important modulators in the maintenance of somatic stem cell homeostasis. In mouse NSCs, *Sirt1* inactivation causes abnormal expression of metabolism-associated genes, especially genes involved in amino acid metabolism, resulting in the abnormal expansion of oligodendrocyte progenitors (Rafalski et al., 2013). In MuSCs, SIRT1 senses the changes of cellular energetic state via NAD⁺ and epigenetically regulates stem cell fate (Ryall et al., 2015). In human MSCs, *SIRT6* deficiency impairs transcription of NRF2 target genes by upregulating H3K56 acetylation and thereby triggering disrupted redox homeostasis and senescence of stem cells (Pan et al., 2016). Furthermore, the NRF2-HO-1 antioxidant pathway is a vital metabolic system for modulating redox homeostasis during the aging process. Kubben et al. (2016) also showed that increased chronic oxidative stress caused by abnormal nuclear lamina-mediated mislocalization of NRF2 and its impaired transcriptional activity contributes to the accelerated aging of HGPS MSCs. These cells suffer from the detrimental effects of progerin (Pacheco et al., 2014; Rosengardten et al., 2011; Scaffidi and Misteli, 2008) and exhibit decreased capacity to survive under oxidative stress (Liu et al., 2011a, 2012a; Zhang et al., 2011). Accordingly, NRF2-activating compounds, including oltipraz,

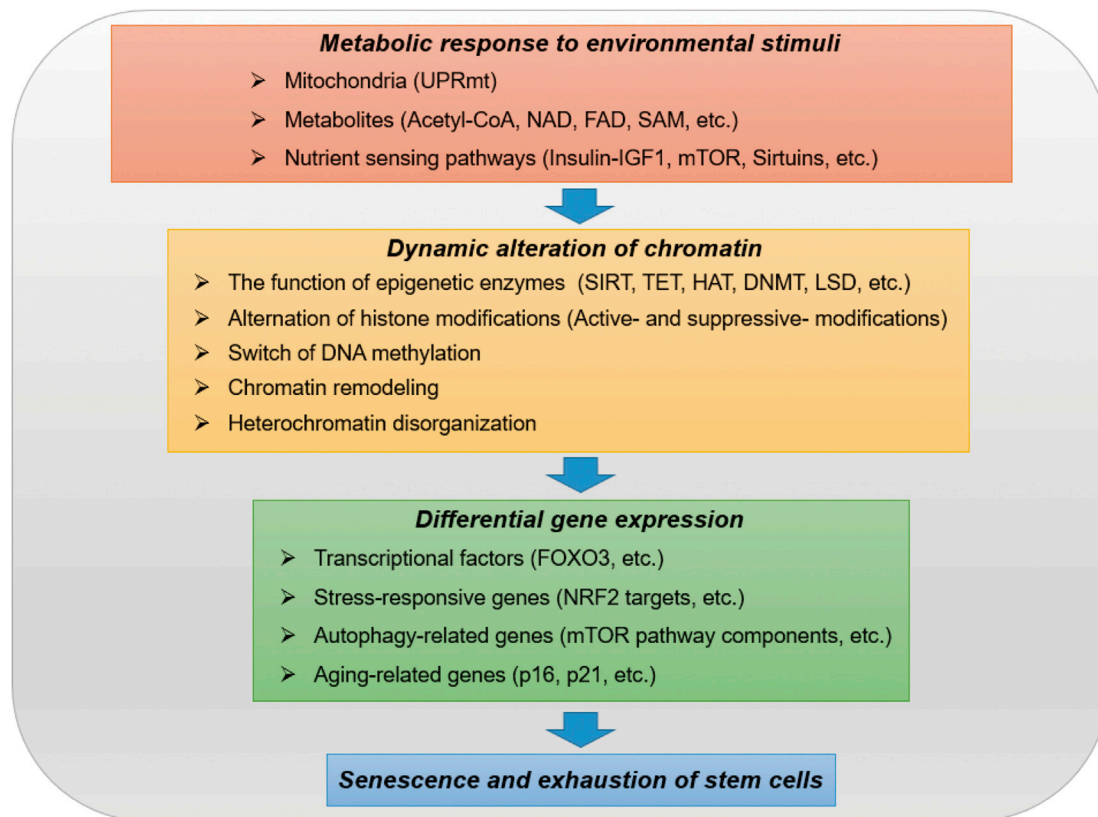


Figure 3. Putative Metabolic-Epigenetic Cascades Underlying (Stem) Cell Aging

Metabolic dysregulation during aging leads to changes in mitochondrial function, metabolites and nutrient-sensing pathways that directly affect epigenetic regulation of gene expression through changes in histone and DNA modification, chromatin remodeling, and loss of heterochromatin. These epigenetic changes may lead to transcriptional changes in old (stem) cells potentially resulting in senescence and stem cell exhaustion. These mechanisms offer a direct link between metabolic and epigenetic dysregulation as major drivers of stem cell aging.

can rescue the accelerated attrition of HGPS iPSC-derived MSCs (Kubben et al., 2016). Additionally, SIRT7 plays an important role in modulating the UPR^{mt} in response to mitochondrial stresses in HSCs, and *SIRT7* expression is downregulated with age. It has been reported that the ability of SIRT7 to maintain HSC homeostasis partially depends on its role as a repressor of NRF1 genomic targets (Mohrin et al., 2015). Interestingly, SIRT1 also regulates autophagy, a key process for MuSC activation from the quiescent state (Tang and Rando, 2014). Conversely, the transcription factor FOXO3 plays an important role in maintaining the quiescence state of NSCs and MuSCs (Gopinath et al., 2014; Paik et al., 2009; Renault et al., 2009; Webb et al., 2013). Also, FOXO3 helps HSCs antagonize complete starvation caused by the rapid induction of cellular autophagy (Warr et al., 2013), by regulating genes involved in autophagy (Webb et al., 2016). As a key nutrient-sensing pathway, mTOR signaling has been reported to influence lifespan and suppress autophagy (Kennedy and Lamming, 2016). When cells are in a nutrient-rich environment, the function of mTOR pathway is required for inducing quiescent MuSCs and HSCs into a stable state for more rapid activation (Rodgers et al., 2014). Garcia-Prat et al. (2016) showed that dysregulated autophagy triggers mitochondrial dysfunction and that the resulting oxidative stress causes young satellite cells to enter into senescence. Conclu-

sively, autophagy involving both catabolic and anabolic processes may play an important role in regulating the process of stem cell aging by linking metabolism to epigenetic changes.

Metabolites can also serve as cofactors to regulate stem cell aging. For example, NAD⁺, a cofactor of epigenetic enzymes, such as sirtuin deacetylases (Verdin, 2015), becomes limiting during the aging process, subsequently changing global levels of histone acetylation (Imai and Guarente, 2014). Recently, it has been shown that decreased levels of cellular NAD⁺ can elevate global levels of H4K16 acetylation and further induce a myogenic program during the process of MuSC activation (Ryall et al., 2015). These results suggest that a metabolic switch from fatty acid oxidation to glycolysis occurs in the transition from quiescence to proliferation of skeletal MuSCs, in accordance with reductions in intracellular NAD⁺ and SIRT1 activity. As another example, α -ketoglutarate functions as a cofactor for both TET enzymes (implicated in DNA demethylation) and Jumonji family proteins (histone demethylases), potentially regulating various chromatin events in different types of stem cells. In particular, phosphoserine aminotransferase 1 (*Psat1*) knock-down induces changes in α -ketoglutarate levels that affect ESC differentiation (Hwang et al., 2016). Likewise, SAM links H3K4 methylation to one-carbon metabolism as a cofactor for histone methyltransferases (Mentch et al., 2015) and threonine

metabolism (Shyh-Chang et al., 2013). More importantly, the altered one-carbon metabolism induces changes in SAM levels, which are tightly linked to age-related alteration of histone methylation. Nevertheless, we do not know the extent to which SAM levels vary with age in different cell types, including stem cells. In conclusion, different metabolites can alter or maintain certain epigenetic states, thereby driving long-lasting changes in gene expression and modulating stem cell states. Moreover, future investigations of metabolic and epigenetic mechanisms underlying stem cell aging will increase our understanding of aging and potentially open the door toward developing potential strategies for treating age-related diseases or extending lifespan.

Potential Strategies Based on the Metabolic-Epigenetic Axis for Slowing Stem Cell Aging

Many attempts have been made over the past decades to conquer organismal aging. For example, connecting the circulatory systems of an old and young mouse, a procedure called heterochronic parabiosis, has the capacity to revert aging phenotypes of stem cells in old mice (Conboy et al., 2005; Villeda et al., 2014). Age-related disorders in both the prematurely aging mouse model and natural aging mice can be delayed by the inducible clearance of p16^{INK4a}-positive senescent cells (Baker et al., 2011, 2016; Ogrodnik et al., 2017; Baar et al., 2017; Jeon et al., 2017; Chang et al., 2016). In addition, dysregulation of metabolic systems, including nutrient-sensing pathways, is thought to cause the senescence and exhaustion of stem cells in many investigated organisms. For example, decreased serum levels of IGF-1 may cause an organism to maintain stem cell quiescence during the aging process, similar to the effect of p16^{INK4a} induction. In contrast, the maintenance of nutrient metabolism plays key roles in directly regulating the proliferation of adult stem cells and potentially antagonizing stem cell aging. Cheng and coworkers reported that the protection, self-renewal, and regeneration of HSCs can be regulated by fasting-induced reductions in signaling levels of the IGF1-PKA pathway (Cheng et al., 2014; Longo and Panda, 2016). Also, Igarashi and colleagues showed that calorie restriction reshapes nutrient sensing in ISCs by inducing mTORC1 and SIRT1, and subsequently promotes the proliferation of ISCs. Interestingly, dietary restriction has been used to rejuvenate the activity of intestinal and MuSCs (Cerletti et al., 2012; Yilmaz et al., 2012). Along these lines, transplanting muscle-derived stem cells from young mice into progeroid mice extends lifespan and rescues degenerative phenotypes (Lavasanli et al., 2012). In this regard, it may be possible that secreted factors from healthy young cells provide therapeutic benefit and help rejuvenate aged organisms by induction of metabolic changes or epigenetic remodeling of old cells. It has been clearly shown that systemic factors from young mice can be used to rescue the dysfunction of neural and muscle stem cell in old mice (Conboy et al., 2005; Villeda et al., 2011).

Since the processes of both stem cell aging and organismal aging seem to be regulated by metabolic and epigenetic mechanisms, it may be possible to use various components of this regulatory axis to slow stem cell aging and finally delay organismal aging (Figure 4). Several recent studies also provide possibilities to conquer either senescent phenotypes of adult stem cells or recover organismal aging by metabolites and their asso-

ciated epigenetic regulators. As an example of rejuvenation of stem cell aging by direct regulation of epigenetics and gene expression, resveratrol is reported to rescue declines in adult stem cells in prematurely aging mice and significantly extend mouse lifespan by increasing the interaction between SIRT1 and Lamin A (Liu et al., 2012a). Oltipraz, a Food and Drug Administration-approved NRF2 activator, rescues the accelerated exhaustion of HGPS iPSC-derived MSCs by reactivating the expression of NRF2 target genes (Kubben et al., 2016). In line with this, gene editing at a single nucleotide within NRF2 gene renders genetically enhanced stem cells with increased self-renewal activity, delayed cellular senescence, better regenerative ability *in vivo*, and resistance to neoplastic transformation, thereby allowing for the generation of superior and safer human stem cells for cell replacement therapies (Yang et al., 2017). In another case, pharmacological interventions that regulate cellular metabolic levels have also been used in an attempt to restore the amount and function of stem cells in aged animals. In particular, the mTOR inhibitor rapamycin has been used to induce the functional recovery of oocytes and various stem cells and to extend mouse lifespan (Yilmaz et al., 2012; Castilho et al., 2009; Chen et al., 2009; Dou et al., 2017), which was considered to be induced by some forms of epigenetic reprogramming (Bitto et al., 2016). This hypothesis has been supported by the finding that rapamycin may prevent several histone marks, such as H3K27me3, H3R2me2, H3K79me3, and H4K20me2, from dramatically decreasing with age in mouse brain tissues (Gong et al., 2015). Additionally, NAD⁺ rescues the induction of senescence in MuSCs, at least in part, by activating sirtuins, and the NAD⁺ precursor nicotinamide riboside can delay aging in adult stem cells and extend mouse lifespan (Zhang et al., 2016). More interestingly, vitamin C (VC) may play dual roles as both a redox regulator and an epigenetic modulator, rescuing the attrition of WS MSCs (Li et al., 2016). On one hand, VC treatment can effectively reduce ROS levels in WS MSCs; notably, increased ROS levels have recently been proposed as a driver of heterochromatin disorganization (Frost et al., 2014). On the other hand, VC can promote the proliferation of WS MSCs, likely by regulating epigenetic reprogramming (Young et al., 2015). In addition, since activation of AMPK by the drug metformin extends lifespan in both worms and mice (Anisimov et al., 2011; Mair et al., 2011; Onken and Driscoll, 2010), it will be interesting to explore the effects of metformin, currently being tested as the first geroprotective drug in humans, on stem cells and their epigenome during aging (Yang et al., 2017). Moreover, elimination of senescent cells, which accumulate in old organisms secreting inflammatory cytokines, growth factors, and proteases, by genetic or pharmacological approaches (senolytics), has been shown to improve age-associated phenotypes and rejuvenate HSCs, highlighting its potential as a novel route for anti-aging interventions (Baker et al., 2011, 2016; Chang et al., 2016; Baar et al., 2017; Jeon et al., 2017; Ogrodnik et al., 2017). Lastly, we have recently reported that partial reprogramming of the epigenome *in vivo* by short-term cyclic expression of Yamanaka factors (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*) can improve cellular and physiological phenotypes of prematurely aging mice, including the loss of adult stem cells, leading to an extension of lifespan (Ocampo et al., 2016a, 2016b). Mechanistically, due to the metabolic and epigenetic remodeling observed during cellular

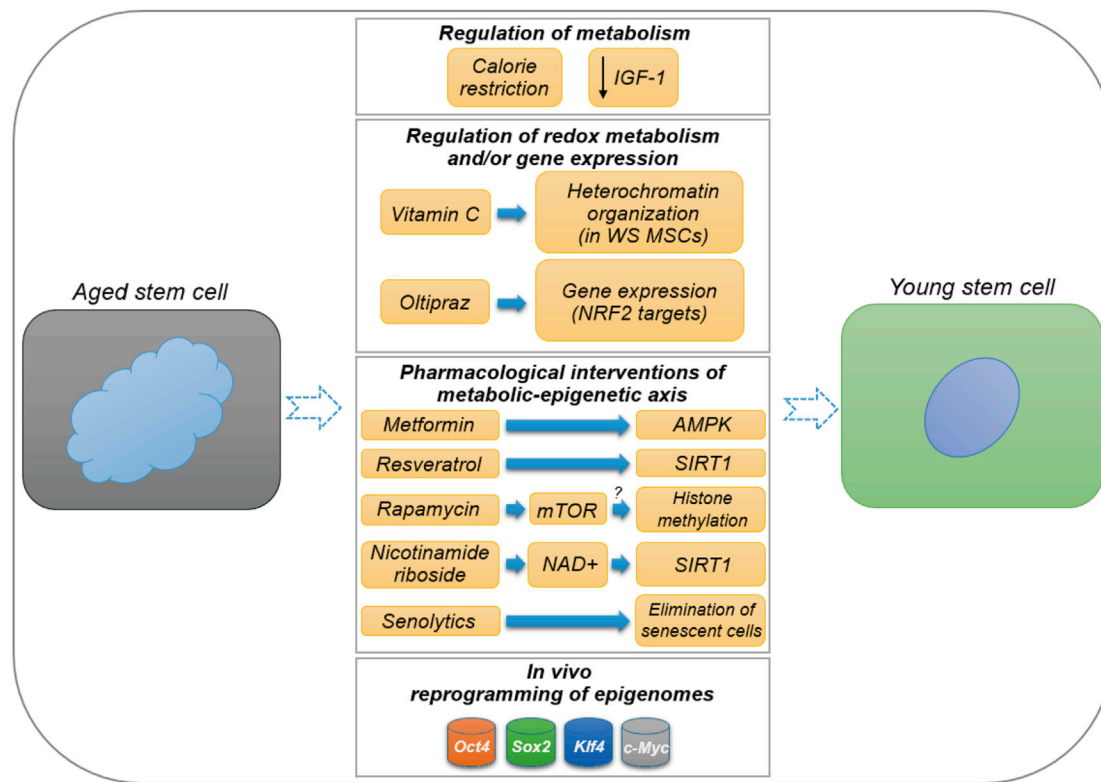


Figure 4. Potential Strategies for the Amelioration of Stem Cell Aging

Genetic, environmental, and pharmacological interventions aiming at the modulation of metabolic pathways or restoration of epigenomes to a younger state may allow for the rejuvenation of aged stem cells to a younger state.

reprogramming, it is possible that partial reprogramming induces the rejuvenation of stem cell populations and restores cellular metabolism and epigenetic regulation in multiple tissues. These observations, together with an epigenetic rejuvenation of HGPS patient fibroblasts by reprogramming toward pluripotency (Christen et al., 2010; Tiscornia and Izpisua Belmonte, 2010; Liu et al., 2011b; Liu et al., 2011a, 2012b; Zhang et al., 2013; Liu et al., 2012b; Li et al., 2012; Yang et al., 2014), suggest that it may be possible to regulate lifespan by directly manipulating the stem cell epigenome using metabolites or small molecules.

In the future, uncovering the metabolic and epigenetic mechanisms by which various metabolites, precursors, and their derivatives regulate stem cell aging may lead to interesting strategies for conquering organismal aging.

Conclusion and Future Outlook

While exciting progress has been made in regulating the aging process of adult stem cells by manipulating the crosstalk between metabolic processes and epigenetic modification, it remains unclear how the metabolic-epigenetic regulatory axis dynamically changes with age. In addition, how this axis is affected by environmental stimuli and how it affects cellular function of different types of adult stem cells is not clear. Due to the fact that several physiological processes, including circadian rhythms, exercise, and sleep, also play critical roles in regulating stem cell homeostasis, how the metabolic-epigenetic regulatory axis controls these processes must also be addressed to

comprehensively understand the global regulation of stem cell aging. Moreover, to slowdown aging and treat age-related diseases, novel technologies and strategies must be used to uncover deeper mechanisms of stem cell aging. These strategies may include: (1) studying the regulatory mechanisms of stem cell aging by knocking out or inducing the expression of adult stem cell-specific metabolic or epigenetic factors; (2) understanding the crosstalk between metabolic and epigenetic mechanisms of human aging by combining human stem cell technologies with gene-editing tools; (3) further exploring the metabolic and epigenetic mechanisms that regulate adult stem cell aging by combining multilayer-omic analyses; (4) rescuing organismal aging by increasing the quantity or activity of adult stem cells *in vivo*; and (5) extending the lifespan and healthspan by clearing tissue-specific senescent stem (or progenitor) cells. Collectively, understanding the mechanisms by which metabolic and epigenetic factors affect aging will be critical in developing novel strategies for maintaining youthful cellular function of adult stem cells, conquering age-related phenotypes in old tissues that are induced by the dysfunction of aged stem cells, and ameliorating organismal age-associated phenotypes.

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