Aging, metabolism and stem cells: spotlight on muscle stem cells

Laura García-Prat[1,2] and Pura Muñoz-Cánoves[1,2,3]*

[1]Tissue Regeneration Laboratory, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; [2]Cell Biology Group, Department of Experimental and Health Sciences, Pompeu Fabra; University (UPF) y CIBERNED; Barcelona, Spain. [3]ICREA, Barcelona, Spain

* Corresponding author:
Pura Muñoz-Cánoves (pura.munoz@upf.edu)

Abstract

All tissues and organs undergo a progressive regenerative decline as they age. This decline has been mainly attributed to loss of stem cell number and/or function, and both stem cell-intrinsic changes and alterations in local niches and/or systemic environment over time are known to contribute to the stem cell aging phenotype. Advancing in the molecular understanding of the deterioration of stem cell cells with aging is key for targeting the specific causes of tissue regenerative dysfunction at advanced stages of life. Here, we revise exciting recent findings on why stem cells age and the consequences on tissue regeneration, with a special focus on regeneration of skeletal muscle. We also highlight newly identified common molecular pathways affecting diverse types of aging stem cells, such as altered proteostasis, metabolism, or senescence entry, and discuss the questions raised by these findings. Finally, we comment on emerging stem cell rejuvenation strategies, principally emanating from studies on muscle stem cells, which will surely burst tissue regeneration research for future benefit of the increasing human aging population.

Introduction

The incidence of tissue dysfunction and diseases, including cancer, cardiovascular pathologies or diabetes, exponentially increases with age. However, aging is still a largely mysterious process. Why do we age? Which are the molecular mechanisms regulating aging? Is there a limit to how long we can live?

Over the years, many theories have emerged to explain what processes and mechanisms drive aging. In fact, almost every important discovery in molecular or cellular biology has led to a new family of theories of aging. It is widely accepted that the ability of an organism to ensure healthy function during aging depends on mechanisms regulating homeostasis(Goodell and Rando, 2015). In many organs of mature vertebrates, resident stem cells participate in tissue homeostasis and regeneration after injury or disease, with variations in their roles across different tissues and organs(Jones and Rando, 2011,Bell and Van Zant, 2004). Nowadays, we know that the function, and in some cases the number, of adult stem cells declines during the aging process of an organism(Garcia-Prat, Sousa-Victor and Munoz-Canoves, 2013). Their position at the base of cellular lineages makes dysfunction of stem cells potentially more impactful than in other cell types, and their exhaustion is the consequence of integration of multiple types of aging-associated damages(Lopez-Otin, Blasco, Partridge et al., 2013). Hence, some issues still need to be addressed, such as which are the factors that maintain the fitness of stem cell populations over time, what blunts their regenerative potential, and what drives their terminal dysfunction. Investigating on stem cell biology and aging should help clarify these issues and provide the basis of novel strategies to sustain healthy aging(Goodell and Rando, 2015).

In this review, we will discuss stem cell aging as a multifactorial process induced by different alterations in various molecular systems, their exact nature and relative contribution to age-associates dysfunction, while taking into account important tissue-particularities. We will pay special attention to the combined effects of age-dependent

damages such as mutations, DNA damage, epigenetic modifications, senescence arrest and dysregulated metabolism to stem cell aging, focusing particularly on the possible causes that may explain the age-associated dysfunction of stem cells in skeletal muscle.

The aged phenotype in skeletal muscle

Muscle stem cells (also called satellite cells, SCs) are essential for skeletal muscle formation, regeneration and homeostatic turnover caused by daily wear and tear(Yin, Price and Rudnicki, 2013). As their name implies, SCs are located outside the myofiber plasma membrane, in a “satellite” position, surrounded by basal lamina. SCs remain mitotically quiescent throughout life and only activate in response to muscle damage or stress. Upon activation, SCs start to proliferate and their progeny contributes both to the differentiated nuclei within the growing muscle fibers and to replenishment of the SC compartment in a process known as self-renewal(Chang and Rudnicki, 2014). Nonetheless, it has been shown that aged SCs decline in number and functionality(Garcia-Prat et al., 2013,Chakkalakal, Jones, Basson et al., 2012,Conboy, Conboy, Smythe et al., 2003,Roth, Martel, Ivey et al., 2000,Wagers and Conboy, 2005,Shefer, Van de Mark, Richardson et al., 2006,Shefer, Rauner, Yablonka-Reuveni et al., 2010,Zammit, Heslop, Hudon et al., 2002,Day, Shefer, Shearer et al., 2010), which are consequences of a combination of different factors, including mechanistic defects in self-renewing, quiescence and myogenic regenerative capacity, as well as alterations in apoptosis and senescence(Sousa-Victor and Munoz-Canoves, 2016,Blau, Cosgrove and Ho, 2015). Furthermore, it is now widely accepted that aged SCs show a tendency to adopt fibroblastic and adipogenic fates in vitro and in vivo, particularly in diseased aging muscle, which explains the increased levels of higher fat deposition and fibrotic tissue in muscles of aged (and dystrophic) mice(Sousa-Victor, Garcia-Prat, Serrano et al., 2015,Taylor-Jones, McGehee, Rando et al., 2002,Shefer, Wleklinski-Lee and Yablonka-Reuveni, 2004,Brack, Conboy, Roy et al., 2007). Nowadays, the causes of these age-associated changes are under intensive investigation and recent promising studies suggest that stem cell rejuvenation may reverse this aging phenotype at the organismal level.

Blueprints of aging stem cells: focus on muscle stem cells

DNA damage and mutations in old stem cells

Strong evidences indicate that DNA damage contributes to stem cell and tissue aging.
Stem cells, like other cells in the body, are frequently exposed to DNA damaging agents(Friedberg, Aguilera, Gellert et al., 2006). Exogenous sources of DNA damage include ultraviolet (UV) radiation, ionizing radiation (IR) or chemical exposure can damage DNA. Endogenous agents such as reactive oxygen species (ROS) generate DNA damage by oxidative modification of DNA bases or by spontaneous hydrolysis of nucleosides. Both IR and ROS can lead to the formation of DNA double-strand breaks (DSBs). In order to maintain genomic integrity after DSBs, cells activate a highly organized and complex program, called the DNA damage response (DDR)(Nagaria, Robert and Rassool, 2013,Khanna and Jackson, 2001).

It is widely accepted that there is a general age-dependent decline in the efficiency of normal DNA repair mechanisms and an increased accumulation of DNA damage that have important consequences on stem cell functionality(Sperka, Wang and Rudolph, 2012,Rube, Fricke, Widmann et al., 2011). In hematopoietic stem cells (HSCs) and SCs, histone H2AX phosphorylation and comet tails, both of which are indicative of DNA damage, increase with age(Rube et al., 2011,Oh, Lee and Wagers, 2014,Rossi, Bryder, Seita et al., 2007,Sinha, Jang, Oh et al., 2014,Garcia-Prat, Martinez-Vicente, Perdiguero et al., 2016). Furthermore, cycling old HSCs in mice have high levels of replication stress associated with cell cycle defects and chromosome gaps, which are due to decreased expression of mini-chromosome maintenance (MCM) helicase components and altered dynamics of DNA replication forks(Flach, Bakker, Mohrin et al., 2014). Analysis of the functional defects observed in HSCs of mice deficient in DNA repair proteins such as FANCD1(Navarro, Meza, Quintana-Bustamante et al., 2006), MSH2(Reese, Liu and Gerson, 2003) or ERCC1(Prasher, Lalai, Heijmans-Antonissen et al., 2005) has also provided further support for a DNA damage-associated mechanism underlying stem cell aging(Garcia-Prat et al., 2013). Moreover, premature aging syndromes, or progeroid syndromes, are mainly caused by defects in DNA repair genes, strengthening the idea that the aging rate is determined in part by a balance between DNA damage and repair(Oh et al., 2014,Hasty, Campisi, Hoeijmakers et al., 2003).

In old stem cells, however, high levels of damaged DNA may arise from build-up of continuous injuries throughout lifetime, a rise in the injury rate, a decline in the repair rate, or a compound of all (Oh et al., 2014). The levels of ROS also increase during aging in human mesenchymal stem cells and SCs, and the frequency of blood-forming HSCs with low ROS levels declines with age in mice(Garcia-Prat et al., 2016,Stolzing, Jones, McGonagle et al., 2008,Jang and Sharkis, 2007). This excessive cellular ROS concentration leads to aberrant proliferation, malignancy and compromised self-renewal capacity in HSCs and neural stem cells (NSCs), and senescence in SCs(Ito, Hirao, Arai et al., 2004). Mouse lines with ablation of antioxidant genes such as Foxo1, Foxo3a, Foxo4 and Sod2 in HSCs and NSCs results in high levels of ROS and disruption of stem cell quiescence and increase in apoptosis, further demonstrating the correlation between excessive levels of ROS and stem cell dysfunction(Bigarella, Liang and Ghaffari, 2014,Rossi, Jamieson and Weissman, 2008,Paik, Kollipara, Chu et al., 2007,Paik, Ding, Narurkar et al., 2009,Tothova and Gilliland, 2007,Miyamoto, Araki, Naka et al., 2007,Yalcin, Zhang, Luciano et al., 2008,Renault, Rafalski, Morgan et al., 2009,Golden, Hinerfeld and Melov, 2002).
Analysis of DDR pathways in HSCs indicates that, independent of age, quiescent stem cells, such as HSCs and SCs, are restricted to use the error-prone non-homologous end-joining pathway (NHEJ) for repairing DSBs, and this process could introduce mutations and promote genomic instability(Vahidi Ferdousi, Rocheteau, Chayot et al., 2014,Mohrin, Bourke, Alexander et al., 2010). Consistent with this notion, mouse HSCs forced to proliferate show fewer mutational events after exposure to DNA-damaging radiation, suggesting that, in certain instances, a break from quiescence enables the cell to engage the high-fidelity homologous recombination (HR) pathway, which will help to maintain stem cell's genomic integrity(Oh et al., 2014,Beerman, Seita, Inlay et al., 2014). In addition, other studies have demonstrated that in certain instances quiescence can promote accumulation of DNA damage and mutations by allowing the

survival of damaged cells(Sperka et al., 2012). On the other hand, each time that a fast-dividing stem cell replicates its DNA, the likelihood of a mutation increases. For instance, a recent study proposed that the accumulation of mutations through stem cell divisions is a major determinant of lifetime cancer risk(Adams, Jasper and Rudolph, 2015).

In contrast, quiescence can also protect stem cells from DNA damage accumulation and functional decline by increased expression of specific stress-protection genes and prevention of cell proliferation and DNA replication(Montarras, L'Honore and Buckingham, 2013,Pallafacchina, Francois, Regnault et al., 2010). Indeed, SCs in quiescence are known to have elevated expression of genes coding for antioxidant enzymes, for solubilization of xenobiotics, for multidrug resistance and for elimination of toxic debris, among others(Montarras et al., 2013). A better understanding of the causes of DNA damage, endogenous sources of ROS and the cellular compartments in which they act is important for clarifying the stem cell regulatory actions and potential therapeutic value of ROS modulating agents.

Epigenetic modifications in old stem cells

Epigenetic regulation is a term used to classify heritable changes of gene expression that are not attributed to changes in DNA sequence and refers mainly to DNA methylation and post-translational histone modifications(Goldberg, Allis and Bernstein, 2007). The epigenetic landscape of stem cells not only regulates the transcriptional program that dictates the function of the stem cells themselves but has also the potential to coordinate cellular differentiation towards distinct effector lineages. Stem cells heritably transmit epigenetic marks to their daughter cells, priming lineage-specific loci for activation or repression in the downstream cell progeny(Beerman and Rossi, 2015). Given that most chromatin changes are reversible, epigenetic alterations are therefore considered good targets for molecular effectors and potential therapies for distinct pathologies. Hence, there has been great interest in understanding the extent to which erosion of these genome-scale regulatory mechanisms lead to dysregulated control of gene expression, and contribute to the decline of stem cell and tissue function with age. Genetic evidence in model organisms supports the notion that aberrant epigenetic regulation affects organismal aging(Goodell and Rando, 2015).
In mammalian cells, DNA methylation is catalyzed by DNA methyltransferases, which coordinate the establishment (DNMT3A and DNMT3B) and maintenance (DNMT1) of methylated nucleotides, and together with regulated DNA demethylation, cell-type- and tissue-specific marks that regulate gene expression are introduced, in order to coordinate cellular (and tissular) functions(Beerman and Rossi, 2015). Recent studies have examined the global DNA methylation profiles of purified stem cells isolated from young and old mice. Interestingly, in old HSCs, altered DNA methylation (either gain or loss) has been associated with genes silenced in the stem cell compartment, which are exclusively transcribed in downstream cellular lineages(Beerman, Bock, Garrison et al., 2013,Sun, Luo, Jeong et al., 2014). Thus, heritable epigenetic marks acquired in the stem cell compartment during aging might alter stem cell differentiation potential by either restricting or allowing access to key lineage-specific genes, and the effects of these altered marks may only be manifested in the transcriptional programs of the differentiated progeny(Beerman et al., 2013). Histone modification is an additional layer

of epigenetic regulation that includes acetylation, methylation, phosphorylation, sumoylation, ubiquitination, and others, that change chromatin structure and accessibility. For instance, acetylation of histone tails alters the charge of the histone, loosening compacted chromatin and allowing a more open and permissive transcriptional state. In a sub-population of HSCs, decreased levels and markedly altered cellular distribution of lysine 16 acetylation on histone 4 (H4K16ac) contrasted with high levels of polarized H4K16ac expression in young HSCs, and was associated with altered HSC function with aging. The altered H4K16ac and function of aged HSCs could be reversed by pharmacological inhibition of Cdc42, which activity increases with age and causes depolarization of planar cell polarity markers in the cytoplasm as well as loss of epigenetic polarity for H4K16ac in the nucleus(Schultz and Sinclair, 2016,Florian, Dorr, Niebel et al., 2012,Geiger, de Haan and Florian, 2013).

Another extensively studied histone mark is methylation of lysine 27 on histone 3 (H3K27), mainly correlated with repression of gene. Interestingly, the overall H3K27me3 levels are unchanged in aged compared to young HSCs, although the coverage and intensity of the H3K27me3 signal in the former cells is higher(Beerman and Rossi, 2015,Sun et al., 2014). Similar findings have been reported in quiescent SCs(Liu, Cheung, Charville et al., 2013). However, how alterations of H3K27me3 influence old stem cell function remains unknown. In addition, some loci contain bivalent domains, which harbor both active H3K4me3 and repressive H3K27me3 marks, and are usually associated to lineage commitment(Beerman and Rossi, 2015). In fact, the bivalent status serves as a priming mechanism that allows stem cells to either activate or repress key lineage-specific genes upon adopting differentiation-related decisions, and not surprisingly, unique bivalent domains have been found in HSCs, hair follicle stem cells, mesenchymal stem cells and SCs(Sun et al., 2014,Liu et al., 2013,Noer, Lindeman and Collas, 2009). During cell differentiation, bivalent domains present in the stem cell usually evolve to H3K4me3- or H3K27me3-only states, thereby restricting lineage commitment(Weishaupt, Sigvardsson and Attema, 2010). Interestingly, gains and losses of bivalent domains were found in aged (compared to young) HSCs and SCs, and this was associated dysregulation of lineage potential in most aging tissues(Beerman and Rossi, 2015,Sun et al., 2014,Liu et al., 2013). Specifically, in SCs, aging-associated gains of novel bivalent domains were attributed to acquision of H3K27me3 repressive marks on loci marked only for activation in young SCs(Liu et al., 2013). Together, these results suggest that DNA methylation and histone modifications play a complex, interactive role in maintaining and safeguarding stem cell functionality during aging.

Environmental and cell-intrinsic alterations of aging stem cells

A stem-cell niche is the extracellular territory of the stem cell that provides the required local microenvironment for stemness maintenance. Stem cells receive influences from other types of cells within the niche, which help to prevent or promote their differentiation(Bentzinger, Wang, Dumont et al., 2013). Thus, aging of the stem cell niche can also critically modify and affect stem cell functionality. During tissue remodeling, changes in the niche structure or in factors derived from systemic or local inflammation can affect stem cells(Brack and Munoz-Canoves, 2015). In resting skeletal muscle, old SCs display an increased propensity to exit the quiescent state and a diminished capacity to self-renew and fully regenerate muscle after injury. These

alterations were attributed to increased niche-derived FGF2 in aged muscle and downregulation of Sprouty1 (a negative regulator of FGF-induced signaling) in quiescent satellite cells. Because Sprouty1 is required for satellite cell self-renewal during regeneration, the dysregulated FGF2/Sprouty1 axis can explain the difficulty of old satellite cells to maintain quiescence(Chakkalakal et al., 2012). Other studies have demonstrated that the levels of TGFβ increase in aged mice, resulting in Smad transcription factor activation, which, by dysregulating the endogenous Notch/Smad3 balance, inhibit SC proliferation and, in consequence, limit the regenerative capacity of aged muscle(Carlson, Hsu and Conboy, 2008). Also, elevated Wnt levels in the circulation of aged individuals promote SC differentiation and reduce the capacity of SCs to self-renew(Brack et al., 2007,Carlson, Conboy, Hsu et al., 2009,Brack, Conboy, Conboy et al., 2008). These pro-aging effects can be reproduced in young SCs by inhibition of Notch signaling, whereas muscle regeneration in aged mice can be partially rescued by delivery of active Notch(Carlson, O'Connor, Hsu et al., 2007,Carlson and Conboy, 2007).

Some of the most compelling evidences for the impact of age-associated changes in the stem cell niche and/or environment have come from elegant experiments that have experimentally restored “youthful” characteristics to the stem cell environment and thus improved regeneration in aged rodents(Sousa-Victor et al., 2015). Heterochronic tissue transplants(Harrison, 1983,Carlson and Faulkner, 1983)[71,72] but, principally, heterochronic parabiosis(Brack and Rando, 2007,Villeda, Luo, Mosher et al., 2011,Conboy, Conboy, Wagers et al., 2005)[73-75]in which young donor components (cells, blood, secreted factors, etc.) are transferred to old hosts, have been shown to rejuvenate old muscle stem cells(Brack et al., 2007,Harrison, 1983,Carlson and Faulkner, 1983,Villeda et al., 2011,Conboy et al., 2005). The heteroparabiosis studies have illuminated a path for discovery of rejuvenating factors for muscle recovery at old age, although some of the identified anti-aging factors remain controversial, such as the growth differentiation factor 11 (GDF-11). One group showed that GDF11 levels decrease during aging, and consistently GDF11 administration improved the function of SCs(Sinha et al., 2014). However, the validity of this work has been questioned by another study, whose authors demonstrated that GDF11 increases with age and it has a negative effect on regeneration of aged muscle(Egerman, Cadena, Gilbert et al., 2015). Other circulating factors that also influence stem cell functions in an age-dependent manner are insulin and IGF-1. Intringuingly, mutation to genes encoding orthologs of insulin/igf1 signaling molecules in model species such as *Caenorhabditis elegans*(Kenyon, Chang, Gensch et al., 1993,Lin, Dorman, Rodan et al., 1997,Ogg, Paradis, Gottlieb et al., 1997) and *Drosophila*(Broughton, Piper, Ikeya et al., 2005,Clancy, Gems, Harshman et al., 2001,Tatar, Kopelman, Epstein et al., 2001) extends life span. In mice, reduced insulin or igf1 signaling, either systematically or in a tissue-specific fashion, slow signs of aging and increases life span(Kenyon, 2010,Berman, Willman, Han et al., 2010). For instance, reduced IGF-1 levels have been associated with improved HSCs self-renewal and better abiltity to generate peripheral blood cells upon transplantation(Cheng, Adams, Perin et al., 2014).

Lately, stem cell transplantation has become the gold standard procedure to study stem cell functions, by challenging the stem cell’s ability to proliferate, differentiate and self-renew(Brack and Munoz-Canoves, 2015). In the muscle field, functional

comparisons of transplanted SCs, isolated from young and aged mice, into pre-injured young muscle, have shown a substantial reduction in the number of aged SCs capable of repopulating the niche and contributing to new muscle fiber formation(Blau et al., 2015). This functional deficit suggests the existence of cell-intrinsic alterations specific of aging SCs. Recent studies provided insights into the mechanisms of SC aging. Studies showed a cell-autonomous increase in the activity of the p38 mitogen-activated protein kinase (MAPK) pathway as a driver of impaired proliferation and self-renewal capacities of aged muscle stem cells, and these deficits could not be rescued by a youthful environment, as demonstrated in transplantation experiments of old SCs into muscles of young hosts(Cosgrove, Gilbert, Porpiglia et al., 2014,Bernet, Doles, Hall et al., 2014). Others attributed the lower regeneration potential to persistent JAK-STAT3 signaling in old SCs, by altering the rate of symmetric- asymmetric divisions(Price, von Maltzahn, Bentzinger et al., 2014,Tierney, Aydogdu, Sala et al., 2014). Notably, transient pharmacologic inhibition or knockdown by siRNAs of p38 MAPK and JAK-STAT3 signaling, was able to restore the regenerative capacity of aged satellite cells after transplantation(Cosgrove et al., 2014,Bernet et al., 2014,Price et al., 2014,Tierney et al., 2014,Almada and Wagers, 2016). Overall these findings demonstrate reversibility of stem cell dysfunction in old animals and add new potential strategies to improve the regenerative capacity of aged tissues through by restoration intrinsic signaling pathways, or mimicking a young extrinsic environment.

Cellular senescence in aging stem cells: opposite roles of senescence throughout lifetime

Senescent cells are dysfunctional cells that have ceased proliferation by permanent withdrawn from the cell cycle(Munoz-Espin and Serrano, 2014). Although classically envisioned as a tumor suppressor mechanism, evidence is emerging of its role in coordinating tissue-remodeling processes(Collado, Blasco and Serrano, 2007). During embryonic development, senescence assists in eliminating unwanted cells, and thereby, contributes to morphogenesis(Munoz-Espin, Canamero, Maraver et al., 2013,Storer, Mas, Robert-Moreno et al., 2013). Senescence also participates in tissue repair after damage. In both situations, senescent cells exist only transiently. However, in aged tissues or in pathological contexts, senescent cells accumulate and this seems to contribute to exacerbate tissue dysfuntion and aging phenotypes. Indeed, senescent markers have been detected in various animal tissues, correlating with chronological aging(Collado et al., 2007,Satyanarayana, Wiemann, Buer et al., 2003,Lechel, Satyanarayana, Ju et al., 2005,Jeyapalan, Ferreira, Sedivy et al., 2007,Jeyapalan and Sedivy, 2008,van Deursen, 2014). Whether and how senescent cells contribute to organismal aging remains still poorly understood. The most striking and compelling prove in this direction came from studies by the van Deursen lab. These authors first demonstrated that removal of senescent cells by genetic manipulation in a progeric mouse model delayed the premature aging phenotype and related diseases(Baker, Wijshake, Tchkonia et al., 2011); more recently, they showed that the clearance of cells expressing $p16^{INK4a}$ (expressed by senescent cells) in wild type mice expands median lifespan, delays tumorigenesis and attenuates age-related deterioration of several organs such as kidney, heart and fat(Baker, Childs, Durik et al., 2016). Consistent with this, another study showed that clearance of senescent cells with a specific inhibitor of the anti-apoptotic proteins BCL-2 and BCL-xL, ABT263, could

rejuvenate old HSCs and muscle stem cells in normally-aged mice(Chang, Wang, Shao et al., 2016).

Senescence has also been found to be a major contributing cause of aging in skeletal muscle stem cells(Sousa-Victor, Gutarra, Garcia-Prat et al., 2014). Indeed, in very old mice (geriatric mice), SCs lose their reversible quiescent state in basal conditions, because of derepression of the gene encoding $p16^{INK4a}$, and adopt a senescent-like state, which impairs the regeneration process after muscle injury, by preventing stem cell activation, proliferation and self-renewal. This upregulation of $p16^{INK4a}$ in geriatric SCs further correlates with reduced levels of phosphorylated retinoblastoma (Rb) protein, and with reduced expression of genes regulated by Rb and the transcription factor E2F(Sousa-Victor et al., 2014,Sousa-Victor, Perdiguero and Munoz-Canoves, 2014). The nature of the initiating triggers of senescence in quiescent muscle stem cells remains largely unknown. A recent study, however, shed light on the upstream events that lead to the senescence phenotype in aged SCs. This study showed that aging SCs accumulated autophagosomes due to impaired autophagy flux for its clearance. This increase in intracellular waste, consisting mainly in damaged proteins and mitochondria, induces high levels of ROS and DNA damage, and provokes the upregulation of $p16^{INK4a}$, leading to muscle stem cell senescence in geriatric mice(Garcia-Prat et al., 2016,Garcia-Prat, Martinez-Vicente and Munoz-Canoves, 2016,Garcia-Prat, Munoz-Canoves and Martinez-Vicente, 2016) (see below). Yet, why proteostasis is perturbed in old SCs remains a mistery. Taken together, cellular senescence appears as a beneficial compensatory response to damage that becomes deleterious and accelerates aging when tissues exhaust their regenerative capacity(Lopez-Otin et al., 2013).

Altered proteostasis, mitochondria function and metabolism in aging stem cells

The quality of the proteome is regulated by a complex network of cellular mechanisms that monitor protein concentrations, folding, cellular localization and interactions, from their synthesis through their degradation. There are evidences that the ability to maintain protein and organelle homeostasis declines during aging. Misfolded, damaged, aggregated or unnecessary proteins are degraded by the proteasome or autophagy systems. When these mechanisms are perturbed, accumulation of damaged proteins and organelles can have deleterious consequences for cells(Vilchez, Simic and Dillin, 2014,Rubinsztein, Marino and Kroemer, 2011). While proteasome activity is poorly characterized in adult stem cells, there is accumulating evidence for the active role of autophagy in the regulation of embryonic stem cells (ESCs), and several adult tissue stem cells, such as HSCs, NSCs and SCs, as well as a number of cancer stem cells (CSCs)(Guan, Simon, Prescott et al., 2013). Autophagy is a process whereby cytoplasmic cell components are degraded by the lysosome(Klionsky, Abdalla, Abeliovich et al., 2012,Cuervo, Bergamini, Brunk et al., 2005). In HSCs, it has been shown that the induction of autophagy upon in vitro stress (by cytokine removal) protects HSCs from apoptosis, and, importantly, that this autophagic activity does not decline in HSCs with aging. Instead, in old HSCs, autophagy is induced in basal conditions compared to young cells, which can potentially be attributable to the untoward effects of the aged bone marrow environment (with growth factor fluctuations and nutrient deprivation)(Warr, Binnewies,

Flach et al., 2013). Thus, aged HSCs rely on ongoing autophagy to maintain their energy levels and survive in cytokine poor conditions.
Autophagy appears to be particularly critical in non-dividing stem cells, which cannot eliminate intracellular toxic debris by mitotic dilution. The first example supporting this notion came from studies in stem cells of skeletal muscle. As discussed above (see senescence section), basal active autophagy was demonstrated in muscle quiescent stem cells, which are G0 arrested. In particular, aged SCs exhibit defective autophagosome clearance, as a consequence of decreased proteolytic activity of lysosomes and/or of impaired ability of lysosomes to fuse with autophagosomes. The accumulation of undigested products (proteins and organelles) inside lysosomes seems to be responsible, at least in part, for reduced autophagosome elimination. This autophagy block results in an accumulation of toxic intracellular waste, mainly composed of altered mitochondria, similar to what it has been described in Atg7-deficient HSC (Garcia-Prat et al., 2016,Garcia-Prat et al., 2016,Garcia-Prat et al., 2016,Mortensen, Soilleux, Djordjevic et al., 2011). Mitochondria are subcellular organelles, which function as power generators within each cell. They represent a central hub within the complex metabolic networks of a cell and many of the mitochondrial metabolism pathways have the potential to generate ROS (see DNA damage and mutations section) and alter the acetyl-CoA, NAD/NADH or AMP/ATP or SAM/SAH ratios, all of which can regulate stem cell self-renewal and homeostasis(Min-Wen, Jun-Hao and Shyh-Chang, 2016,Shyh-Chang, Daley and Cantley, 2013). Indeed, several signaling pathways that ultimately converge on the mitochondria have been implicated in stem cell aging. For example, during the transition from quiescence to proliferation, SCs experience a metabolic switch from mitochondrial fatty acid oxidation to glycolysis. This metabolic switch decreases NAD^+ levels and decreases SIRT1 activity, thus increasing H4K16 acetylation and activation of muscle gene transcription in proliferating myoblast(Ryall, Dell'Orso, Derfoul et al., 2015). In addition, Sirt1 is required for induction autophagy in SCs during the transition from quiescence to activation to provide the nutrients necessary to meet bioenergetic demands(Tang and Rando, 2014). Several studies in HSCs support the relevance of metabolism controlling stem cell fate by perturbing metabolic pathways such as Pten, LKB1, TSC1 or SIRT7, which are required to limit mitochondria biogenesis and OxPhos (by inhibiting NRF1 activity) and maintain a “youthful” state(Yilmaz, Valdez, Theisen et al., 2006,Zhang, Grindley, Yin et al., 2006,Gan and DePinho, 2009,Gan, Hu, Jiang et al., 2010,Mohrin, Shin, Liu et al., 2015,Wrighton, 2015).

How to slow or counter stem cell dysfunction with aging? Calorie restriction (CR) appears as the most effective strategy in promoting longevity across the animal kingdom(Barger, Walford and Weindruch, 2003). Yet, the detailed mechanisms underlying the benefits of calorie restriction in specific tissues and stem cells remain unknown(Shyh-Chang et al., 2013). In SCs, CR increases the abundance of SCs in aged muscle and improves their functionality in transplantation experiments, in concert with an increase in mitochondrial abundance and induction of conserved metabolic and longevity regulators(Cerletti, Jang, Finley et al., 2012). CR also improves the function of other types of stem cells including HSCs in mice(Cheng et al., 2014) and GSCs in flies(Mair, McLeod, Wang et al., 2010). In a variety of organisms, the effects of CR appear to be mediated by AMPK signaling to mitochondrial regulators such as SIRT1, PGC1a, FoxO and mTOR during aging(Min-Wen et al., 2016,Shyh-Chang et al., 2013).

The FoxO transcription factors (FoxO1, FoxO3a, FoxO4 and FoxO6) have also classically associated with longevity in most organisms. They can be functionally suppressed by insulin-PI3K-AKT signaling, and activated by mitochondrial ROS to drive the oxidative stress response. Mice lacking FoxO signaling exhibit a pronounce decline in the HSC population due to enhanced ROS levels, which leads to increase cell proliferation and apoptosis(Miyamoto et al., 2007,Tothova, Kollipara, Huntly et al., 2007). This phenotype could be reverse by treatment with antioxidant N-acetyl-L-cysteine (NAC), thus linking nutrient-sensitive signaling and ROS. Moreover, FoxO3a is also important for autophagy induction in HSCs(Warr et al., 2013), and in NSCs, FoxOs cooperate to regulate the quiescent stem cell pool and limit neurogenesis by controlling ROS detoxification, Wnt signaling, cell cycle and differentiation processes(Paik et al., 2009,Renault et al., 2009). In SC, FoxO3a regulates self-renewal after muscle regeneration and prevents precocious differentiation in vitro(Gopinath, Webb, Brunet et al., 2014). However, the roles of FoxOs during stem cell aging remain largely unexplored.

Contrarily to FoxO signaling, the mTOR pathway has been convincingly shown to promote aging in various model organisms, and in addition, its inhibition later in life can significantly extend lifespan and mitigate multiple age-related diseases(Chen, Liu, Liu et al., 2009,Johnson, Rabinovitch and Kaeberlein, 2013). mTOR also plays a major role in governing stem cell fate by increasing mitochondrial activity and repressing autophagy (and mitophagy) via ULK1(Chan, 2009,Cunningham, Rodgers, Arlow et al., 2007). Evidence of the deleterious role of mTOR, particularly in aging, has been obtained from experiments using rapamycin-treated stem cells. mTOR1 inhibition with rapamycin can delay mouse long-term HSC aging by preserving adult long-term HSC self-renewal and hematopoietic capacity, and prevent epidermal stem cell exhaustion(Gan and DePinho, 2009,Li and Bhatia, 2011,Castilho, Squarize, Chodosh et al., 2009,Demidenko, Zubova, Bukreeva et al., 2009). Indeed, rapamycin treatment of aged mice for two weeks is sufficient to restore autophagy flux in quiescent stem cells of skeletal muscle and prevent senescence entry(Garcia-Prat et al., 2016).

Frequently opposed to mTOR signaling, the AMPK pathway, which detects low ATP/AMP levels during CR and Ca2+ signaling during exercise, also regulates the functions of certain stem cell populations. In HSCs, deletion of the AMPK regulator LKB1 leads to HSC exhaustion and defects in hematopoiesis, owing to severe mitochondria dysfunction during aging. AMPK signaling also regulates neural stem cells and their mitochondria during brain development(Gan et al., 2010,Blagih, Krawczyk and Jones, 2012,Gurumurthy, Xie, Alagesan et al., 2010,Nakada, Saunders and Morrison, 2010,Dasgupta and Milbrandt, 2009).

Since all these signaling pathways regulate mitochondria homeostasis as the primary mechanism to modulate cellular and organismal longevity, it would be efficacious to develop technologies to therapeutically target and direct mitochondria repair in stem cells, as unified strategy to combat aging-related degenerative diseases.

Concluding remarks

Because stem cells are among the most long-lived cells, their age-associate decline is a major contributor to organismal aging and associated diseases. Indeed, despite tissue and organ differences, the function of most stem cells declines with age and provokes tissue deterioration and incapacity to repair. A common theme discussed in this review is that both extrinsic and extrinsic factors drive this stem cell age-associated dysfunction. Much progress has been made in recent years on how stem cells of skeletal muscle age, and common hallmarks of aging stem cells have been identified (see Figure 1), with altered metabolism, proteostasis and senescence networks emerging as major drivers of the stem cell blunted regenerative capacity over time, particularly at advanced geriatric age. With a greater understanding of how stem cells regulate their intrinsic homeostasis network will shed light not only on stem cell biology, but also on designing new strategies to combat stem cell decline with aging. Because most stem-cell types rely on similar protective mechanisms throughout life, which become similarly dysfunctional with aging, the rejuvenating strategies recently reported for muscle stem cells, resulting in improved muscle regeneration in old animals, may also apply to stem cells of other critical organs, and therefore have great impact on organism health during the aging process. We anticipate that novel discoveries in basic knowledge on the homeostasis of stem cells, particularly those related to metabolisms and proteostasis maintenance, will improve the treatment of age-associated pathologies. By continuing to shed light on the basic principles by which stem cells age, one day we might envision and put into practice strategies that will even increase human lifespan.

Bibliography

[1] Goodell, M.A. and Rando, T.A., 2015. Stem cells and healthy aging, Science. 350, 1199-204.

[2] Jones, D.L. and Rando, T.A., 2011. Emerging models and paradigms for stem cell ageing, Nat Cell Biol. 13, 506-12.

[3] Bell, D.R. and Van Zant, G., 2004. Stem cells, aging, and cancer: inevitabilities and outcomes, Oncogene. 23, 7290-6.

[4] Garcia-Prat, L., Sousa-Victor, P. and Munoz-Canoves, P., 2013. Functional dysregulation of stem cells during aging: a focus on skeletal muscle stem cells, FEBS J. 280, 4051-62.

[5] Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M. and Kroemer, G., 2013. The hallmarks of aging, Cell. 153, 1194-217.

[6] Yin, H., Price, F. and Rudnicki, M.A., 2013. Satellite cells and the muscle stem cell niche, Physiol Rev. 93, 23-67.

[7] Chang, N.C. and Rudnicki, M.A., 2014. Satellite cells: the architects of skeletal muscle, Curr Top Dev Biol. 107, 161-81.

[8] Chakkalakal, J.V., Jones, K.M., Basson, M.A. and Brack, A.S., 2012. The aged niche disrupts muscle stem cell quiescence, Nature. 490, 355-60.

[9] Conboy, I.M., Conboy, M.J., Smythe, G.M. and Rando, T.A., 2003. Notch-mediated restoration of regenerative potential to aged muscle, Science. 302, 1575-7.

[10] Roth, S.M., Martel, G.F., Ivey, F.M., Lemmer, J.T., Metter, E.J., Hurley, B.F. and Rogers, M.A., 2000. Skeletal muscle satellite cell populations in healthy young and older men and women, Anat Rec. 260, 351-8.

[11] Wagers, A.J. and Conboy, I.M., 2005. Cellular and molecular signatures of muscle regeneration: current concepts and controversies in adult myogenesis, Cell. 122, 659-67.
[12] Shefer, G., Van de Mark, D.P., Richardson, J.B. and Yablonka-Reuveni, Z., 2006. Satellite-cell pool size does matter: defining the myogenic potency of aging skeletal muscle, Dev Biol. 294, 50-66.
[13] Shefer, G., Rauner, G., Yablonka-Reuveni, Z. and Benayahu, D., 2010. Reduced satellite cell numbers and myogenic capacity in aging can be alleviated by endurance exercise, PLoS One. 5, e13307.
[14] Zammit, P.S., Heslop, L., Hudon, V., Rosenblatt, J.D., Tajbakhsh, S., Buckingham, M.E., Beauchamp, J.R. and Partridge, T.A., 2002. Kinetics of myoblast proliferation show that resident satellite cells are competent to fully regenerate skeletal muscle fibers, Exp Cell Res. 281, 39-49.
[15] Day, K., Shefer, G., Shearer, A. and Yablonka-Reuveni, Z., 2010. The depletion of skeletal muscle satellite cells with age is concomitant with reduced capacity of single progenitors to produce reserve progeny, Dev Biol. 340, 330-43.
[16] Sousa-Victor, P. and Munoz-Canoves, P., 2016. Regenerative decline of stem cells in sarcopenia, Mol Aspects Med.
[17] Blau, H.M., Cosgrove, B.D. and Ho, A.T., 2015. The central role of muscle stem cells in regenerative failure with aging, Nat Med. 21, 854-62.
[18] Sousa-Victor, P., Garcia-Prat, L., Serrano, A.L., Perdiguero, E. and Munoz-Canoves, P., 2015. Muscle stem cell aging: regulation and rejuvenation, Trends Endocrinol Metab. 26, 287-296.
[19] Taylor-Jones, J.M., McGehee, R.E., Rando, T.A., Lecka-Czernik, B., Lipschitz, D.A. and Peterson, C.A., 2002. Activation of an adipogenic program in adult myoblasts with age, Mech Ageing Dev. 123, 649-61.
[20] Shefer, G., Wleklinski-Lee, M. and Yablonka-Reuveni, Z., 2004. Skeletal muscle satellite cells can spontaneously enter an alternative mesenchymal pathway, J Cell Sci. 117, 5393-404.
[21] Brack, A.S., Conboy, M.J., Roy, S., Lee, M., Kuo, C.J., Keller, C. and Rando, T.A., 2007. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis, Science. 317, 807-10.
[22] Friedberg, E.C., Aguilera, A., Gellert, M., Hanawalt, P.C., Hays, J.B., Lehmann, A.R., Lindahl, T., Lowndes, N., Sarasin, A. and Wood, R.D., 2006. DNA repair: from molecular mechanism to human disease, DNA Repair (Amst). 5, 986-96.
[23] Nagaria, P., Robert, C. and Rassool, F.V., 2013. DNA double-strand break response in stem cells: mechanisms to maintain genomic integrity, Biochim Biophys Acta. 1830, 2345-53.
[24] Khanna, K.K. and Jackson, S.P., 2001. DNA double-strand breaks: signaling, repair and the cancer connection, Nat Genet. 27, 247-54.
[25] Sperka, T., Wang, J. and Rudolph, K.L., 2012. DNA damage checkpoints in stem cells, ageing and cancer, Nat Rev Mol Cell Biol. 13, 579-90.
[26] Rube, C.E., Fricke, A., Widmann, T.A., Furst, T., Madry, H., Pfreundschuh, M. and Rube, C., 2011. Accumulation of DNA damage in hematopoietic stem and progenitor cells during human aging, PLoS One. 6, e17487.
[27] Oh, J., Lee, Y.D. and Wagers, A.J., 2014. Stem cell aging: mechanisms, regulators and therapeutic opportunities, Nat Med. 20, 870-80.
[28] Rossi, D.J., Bryder, D., Seita, J., Nussenzweig, A., Hoeijmakers, J. and Weissman, I.L., 2007. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age, Nature. 447, 725-9.
[29] Sinha, M., Jang, Y.C., Oh, J., Khong, D., Wu, E.Y., Manohar, R., Miller, C., Regalado, S.G., Loffredo, F.S., Pancoast, J.R., Hirshman, M.F., Lebowitz, J., Shadrach, J.L., Cerletti, M., Kim, M.J., Serwold, T., Goodyear, L.J., Rosner, B., Lee, R.T. and Wagers, A.J., 2014. Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle, Science. 344, 649-52.

[30] Garcia-Prat, L., Martinez-Vicente, M., Perdiguero, E., Ortet, L., Rodriguez-Ubreva, J., Rebollo, E., Ruiz-Bonilla, V., Gutarra, S., Ballestar, E., Serrano, A.L., Sandri, M. and Munoz-Canoves, P., 2016. Autophagy maintains stemness by preventing senescence, Nature. 529, 37-42.

[31] Flach, J., Bakker, S.T., Mohrin, M., Conroy, P.C., Pietras, E.M., Reynaud, D., Alvarez, S., Diolaiti, M.E., Ugarte, F., Forsberg, E.C., Le Beau, M.M., Stohr, B.A., Mendez, J., Morrison, C.G. and Passegue, E., 2014. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells, Nature.

[32] Navarro, S., Meza, N.W., Quintana-Bustamante, O., Casado, J.A., Jacome, A., McAllister, K., Puerto, S., Surralles, J., Segovia, J.C. and Bueren, J.A., 2006. Hematopoietic dysfunction in a mouse model for Fanconi anemia group D1, Mol Ther. 14, 525-35.

[33] Reese, J.S., Liu, L. and Gerson, S.L., 2003. Repopulating defect of mismatch repair-deficient hematopoietic stem cells, Blood. 102, 1626-33.

[34] Prasher, J.M., Lalai, A.S., Heijmans-Antonissen, C., Ploemacher, R.E., Hoeijmakers, J.H., Touw, I.P. and Niedernhofer, L.J., 2005. Reduced hematopoietic reserves in DNA interstrand crosslink repair-deficient Ercc1-/- mice, EMBO J. 24, 861-71.

[35] Hasty, P., Campisi, J., Hoeijmakers, J., van Steeg, H. and Vijg, J., 2003. Aging and genome maintenance: lessons from the mouse?, Science. 299, 1355-9.

[36] Stolzing, A., Jones, E., McGonagle, D. and Scutt, A., 2008. Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies, Mech Ageing Dev. 129, 163-73.

[37] Jang, Y.Y. and Sharkis, S.J., 2007. A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche, Blood. 110, 3056-63.

[38] Ito, K., Hirao, A., Arai, F., Matsuoka, S., Takubo, K., Hamaguchi, I., Nomiyama, K., Hosokawa, K., Sakurada, K., Nakagata, N., Ikeda, Y., Mak, T.W. and Suda, T., 2004. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells, Nature. 431, 997-1002.

[39] Bigarella, C.L., Liang, R. and Ghaffari, S., 2014. Stem cells and the impact of ROS signaling, Development. 141, 4206-18.

[40] Rossi, D.J., Jamieson, C.H. and Weissman, I.L., 2008. Stems cells and the pathways to aging and cancer, Cell. 132, 681-96.

[41] Paik, J.H., Kollipara, R., Chu, G., Ji, H., Xiao, Y., Ding, Z., Miao, L., Tothova, Z., Horner, J.W., Carrasco, D.R., Jiang, S., Gilliland, D.G., Chin, L., Wong, W.H., Castrillon, D.H. and DePinho, R.A., 2007. FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis, Cell. 128, 309-23.

[42] Paik, J.H., Ding, Z., Narurkar, R., Ramkissoon, S., Muller, F., Kamoun, W.S., Chae, S.S., Zheng, H., Ying, H., Mahoney, J., Hiller, D., Jiang, S., Protopopov, A., Wong, W.H., Chin, L., Ligon, K.L. and DePinho, R.A., 2009. FoxOs cooperatively regulate diverse pathways governing neural stem cell homeostasis, Cell Stem Cell. 5, 540-53.

[43] Tothova, Z. and Gilliland, D.G., 2007. FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system, Cell Stem Cell. 1, 140-52.

[44] Miyamoto, K., Araki, K.Y., Naka, K., Arai, F., Takubo, K., Yamazaki, S., Matsuoka, S., Miyamoto, T., Ito, K., Ohmura, M., Chen, C., Hosokawa, K., Nakauchi, H., Nakayama, K., Nakayama, K.I., Harada, M., Motoyama, N., Suda, T. and Hirao, A., 2007. Foxo3a is essential for maintenance of the hematopoietic stem cell pool, Cell Stem Cell. 1, 101-12.

[45] Yalcin, S., Zhang, X., Luciano, J.P., Mungamuri, S.K., Marinkovic, D., Vercherat, C., Sarkar, A., Grisotto, M., Taneja, R. and Ghaffari, S., 2008. Foxo3

is essential for the regulation of ataxia telangiectasia mutated and oxidative stress-mediated homeostasis of hematopoietic stem cells, J Biol Chem. 283, 25692-705.
[46] Renault, V.M., Rafalski, V.A., Morgan, A.A., Salih, D.A., Brett, J.O., Webb, A.E., Villeda, S.A., Thekkat, P.U., Guillerey, C., Denko, N.C., Palmer, T.D., Butte, A.J. and Brunet, A., 2009. FoxO3 regulates neural stem cell homeostasis, Cell Stem Cell. 5, 527-39.
[47] Golden, T.R., Hinerfeld, D.A. and Melov, S., 2002. Oxidative stress and aging: beyond correlation, Aging Cell. 1, 117-23.
[48] Vahidi Ferdousi, L., Rocheteau, P., Chayot, R., Montagne, B., Chaker, Z., Flamant, P., Tajbakhsh, S. and Ricchetti, M., 2014. More efficient repair of DNA double-strand breaks in skeletal muscle stem cells compared to their committed progeny, Stem Cell Res. 13, 492-507.
[49] Mohrin, M., Bourke, E., Alexander, D., Warr, M.R., Barry-Holson, K., Le Beau, M.M., Morrison, C.G. and Passegue, E., 2010. Hematopoietic stem cell quiescence promotes error-prone DNA repair and mutagenesis, Cell Stem Cell. 7, 174-85.
[50] Beerman, I., Seita, J., Inlay, M.A., Weissman, I.L. and Rossi, D.J., 2014. Quiescent Hematopoietic Stem Cells Accumulate DNA Damage during Aging that Is Repaired upon Entry into Cell Cycle, Cell Stem Cell. 15, 37-50.
[51] Adams, P.D., Jasper, H. and Rudolph, K.L., 2015. Aging-Induced Stem Cell Mutations as Drivers for Disease and Cancer, Cell Stem Cell. 16, 601-12.
[52] Montarras, D., L'Honore, A. and Buckingham, M., 2013. Lying low but ready for action: the quiescent muscle satellite cell, FEBS J. 280, 4036-50.
[53] Pallafacchina, G., Francois, S., Regnault, B., Czarny, B., Dive, V., Cumano, A., Montarras, D. and Buckingham, M., 2010. An adult tissue-specific stem cell in its niche: a gene profiling analysis of in vivo quiescent and activated muscle satellite cells, Stem Cell Res. 4, 77-91.
[54] Goldberg, A.D., Allis, C.D. and Bernstein, E., 2007. Epigenetics: a landscape takes shape, Cell. 128, 635-8.
[55] Beerman, I. and Rossi, D.J., 2015. Epigenetic Control of Stem Cell Potential during Homeostasis, Aging, and Disease, Cell Stem Cell. 16, 613-25.
[56] Beerman, I., Bock, C., Garrison, B.S., Smith, Z.D., Gu, H., Meissner, A. and Rossi, D.J., 2013. Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging, Cell Stem Cell. 12, 413-25.
[57] Sun, D., Luo, M., Jeong, M., Rodriguez, B., Xia, Z., Hannah, R., Wang, H., Le, T., Faull, K.F., Chen, R., Gu, H., Bock, C., Meissner, A., Gottgens, B., Darlington, G.J., Li, W. and Goodell, M.A., 2014. Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal, Cell Stem Cell. 14, 673-88.
[58] Schultz, M.B. and Sinclair, D.A., 2016. When stem cells grow old: phenotypes and mechanisms of stem cell aging, Development. 143, 3-14.
[59] Florian, M.C., Dorr, K., Niebel, A., Daria, D., Schrezenmeier, H., Rojewski, M., Filippi, M.D., Hasenberg, A., Gunzer, M., Scharffetter-Kochanek, K., Zheng, Y. and Geiger, H., 2012. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation, Cell Stem Cell. 10, 520-30.
[60] Geiger, H., de Haan, G. and Florian, M.C., 2013. The ageing haematopoietic stem cell compartment, Nat Rev Immunol. 13, 376-89.
[61] Liu, L., Cheung, T.H., Charville, G.W., Hurgo, B.M., Leavitt, T., Shih, J., Brunet, A. and Rando, T.A., 2013. Chromatin modifications as determinants of muscle stem cell quiescence and chronological aging, Cell Rep. 4, 189-204.
[62] Noer, A., Lindeman, L.C. and Collas, P., 2009. Histone H3 modifications associated with differentiation and long-term culture of mesenchymal adipose stem cells, Stem Cells Dev. 18, 725-36.

[63] Weishaupt, H., Sigvardsson, M. and Attema, J.L., 2010. Epigenetic chromatin states uniquely define the developmental plasticity of murine hematopoietic stem cells, Blood. 115, 247-56.
[64] Bentzinger, C.F., Wang, Y.X., Dumont, N.A. and Rudnicki, M.A., 2013. Cellular dynamics in the muscle satellite cell niche, EMBO Rep. 14, 1062-72.
[65] Brack, A.S. and Munoz-Canoves, P., 2015. The ins and outs of muscle stem cell aging, Skelet Muscle. 6, 1.
[66] Carlson, M.E., Hsu, M. and Conboy, I.M., 2008. Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells, Nature. 454, 528-32.
[67] Carlson, M.E., Conboy, M.J., Hsu, M., Barchas, L., Jeong, J., Agrawal, A., Mikels, A.J., Agrawal, S., Schaffer, D.V. and Conboy, I.M., 2009. Relative roles of TGF-beta1 and Wnt in the systemic regulation and aging of satellite cell responses, Aging Cell. 8, 676-89.
[68] Brack, A.S., Conboy, I.M., Conboy, M.J., Shen, J. and Rando, T.A., 2008. A temporal switch from notch to Wnt signaling in muscle stem cells is necessary for normal adult myogenesis, Cell Stem Cell. 2, 50-9.
[69] Carlson, M.E., O'Connor, M.S., Hsu, M. and Conboy, I.M., 2007. Notch signaling pathway and tissue engineering, Front Biosci. 12, 5143-56.
[70] Carlson, M.E. and Conboy, I.M., 2007. Regulating the Notch pathway in embryonic, adult and old stem cells, Curr Opin Pharmacol. 7, 303-9.
[71] Harrison, D.E., 1983. Long-term erythropoietic repopulating ability of old, young, and fetal stem cells, J Exp Med. 157, 1496-504.
[72] Carlson, B.M. and Faulkner, J.A., 1983. The regeneration of skeletal muscle fibers following injury: a review., Med.Sci.Sports Exercise. 15, 187.
[73] Brack, A.S. and Rando, T.A., 2007. Intrinsic changes and extrinsic influences of myogenic stem cell function during aging, Stem Cell Rev. 3, 226-37.
[74] Villeda, S.A., Luo, J., Mosher, K.I., Zou, B., Britschgi, M., Bieri, G., Stan, T.M., Fainberg, N., Ding, Z., Eggel, A., Lucin, K.M., Czirr, E., Park, J.S., Couillard-Despres, S., Aigner, L., Li, G., Peskind, E.R., Kaye, J.A., Quinn, J.F., Galasko, D.R., Xie, X.S., Rando, T.A. and Wyss-Coray, T., 2011. The ageing systemic milieu negatively regulates neurogenesis and cognitive function, Nature. 477, 90-4.
[75] Conboy, I.M., Conboy, M.J., Wagers, A.J., Girma, E.R., Weissman, I.L. and Rando, T.A., 2005. Rejuvenation of aged progenitor cells by exposure to a young systemic environment, Nature. 433, 760-4.
[76] Egerman, M.A., Cadena, S.M., Gilbert, J.A., Meyer, A., Nelson, H.N., Swalley, S.E., Mallozzi, C., Jacobi, C., Jennings, L.L., Clay, I., Laurent, G., Ma, S., Brachat, S., Lach-Trifilieff, E., Shavlakadze, T., Trendelenburg, A.U., Brack, A.S. and Glass, D.J., 2015. GDF11 Increases with Age and Inhibits Skeletal Muscle Regeneration, Cell Metab. 22, 164-74.
[77] Kenyon, C., Chang, J., Gensch, E., Rudner, A. and Tabtiang, R., 1993. A C. elegans mutant that lives twice as long as wild type, Nature. 366, 461-4.
[78] Lin, K., Dorman, J.B., Rodan, A. and Kenyon, C., 1997. daf-16: An HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans, Science. 278, 1319-22.
[79] Ogg, S., Paradis, S., Gottlieb, S., Patterson, G.I., Lee, L., Tissenbaum, H.A. and Ruvkun, G., 1997. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans, Nature. 389, 994-9.
[80] Broughton, S.J., Piper, M.D., Ikeya, T., Bass, T.M., Jacobson, J., Driege, Y., Martinez, P., Hafen, E., Withers, D.J., Leevers, S.J. and Partridge, L., 2005. Longer lifespan, altered metabolism, and stress resistance in Drosophila from ablation of cells making insulin-like ligands, Proc Natl Acad Sci U S A. 102, 3105-10.

[81] Clancy, D.J., Gems, D., Harshman, L.G., Oldham, S., Stocker, H., Hafen, E., Leevers, S.J. and Partridge, L., 2001. Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein, Science. 292, 104-6.
[82] Tatar, M., Kopelman, A., Epstein, D., Tu, M.P., Yin, C.M. and Garofalo, R.S., 2001. A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function, Science. 292, 107-10.
[83] Kenyon, C., 2010. A pathway that links reproductive status to lifespan in Caenorhabditis elegans, Ann N Y Acad Sci. 1204, 156-62.
[84] Berman, D.M., Willman, M.A., Han, D., Kleiner, G., Kenyon, N.M., Cabrera, O., Karl, J.A., Wiseman, R.W., O'Connor, D.H., Bartholomew, A.M. and Kenyon, N.S., 2010. Mesenchymal stem cells enhance allogeneic islet engraftment in nonhuman primates, Diabetes. 59, 2558-68.
[85] Cheng, C.W., Adams, G.B., Perin, L., Wei, M., Zhou, X., Lam, B.S., Da Sacco, S., Mirisola, M., Quinn, D.I., Dorff, T.B., Kopchick, J.J. and Longo, V.D., 2014. Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression, Cell Stem Cell. 14, 810-23.
[86] Cosgrove, B.D., Gilbert, P.M., Porpiglia, E., Mourkioti, F., Lee, S.P., Corbel, S.Y., Llewellyn, M.E., Delp, S.L. and Blau, H.M., 2014. Rejuvenation of the muscle stem cell population restores strength to injured aged muscles, Nat Med. 20, 255-64.
[87] Bernet, J.D., Doles, J.D., Hall, J.K., Kelly Tanaka, K., Carter, T.A. and Olwin, B.B., 2014. p38 MAPK signaling underlies a cell-autonomous loss of stem cell self-renewal in skeletal muscle of aged mice, Nat Med. 20, 265-71.
[88] Price, F.D., von Maltzahn, J., Bentzinger, C.F., Dumont, N.A., Yin, H., Chang, N.C., Wilson, D.H., Frenette, J. and Rudnicki, M.A., 2014. Inhibition of JAK-STAT signaling stimulates adult satellite cell function, Nat Med. 20, 1174-81.
[89] Tierney, M.T., Aydogdu, T., Sala, D., Malecova, B., Gatto, S., Puri, P.L., Latella, L. and Sacco, A., 2014. STAT3 signaling controls satellite cell expansion and skeletal muscle repair, Nat Med.
[90] Almada, A.E. and Wagers, A.J., 2016. Molecular circuitry of stem cell fate in skeletal muscle regeneration, ageing and disease, Nat Rev Mol Cell Biol. 17, 267-79.
[91] Munoz-Espin, D. and Serrano, M., 2014. Cellular senescence: from physiology to pathology, Nat Rev Mol Cell Biol. 15, 482-96.
[92] Collado, M., Blasco, M.A. and Serrano, M., 2007. Cellular senescence in cancer and aging, Cell. 130, 223-33.
[93] Munoz-Espin, D., Canamero, M., Maraver, A., Gomez-Lopez, G., Contreras, J., Murillo-Cuesta, S., Rodriguez-Baeza, A., Varela-Nieto, I., Ruberte, J., Collado, M. and Serrano, M., 2013. Programmed cell senescence during mammalian embryonic development, Cell. 155, 1104-18.
[94] Storer, M., Mas, A., Robert-Moreno, A., Pecoraro, M., Ortells, M.C., Di Giacomo, V., Yosef, R., Pilpel, N., Krizhanovsky, V., Sharpe, J. and Keyes, W.M., 2013. Senescence is a developmental mechanism that contributes to embryonic growth and patterning, Cell. 155, 1119-30.
[95] Satyanarayana, A., Wiemann, S.U., Buer, J., Lauber, J., Dittmar, K.E., Wustefeld, T., Blasco, M.A., Manns, M.P. and Rudolph, K.L., 2003. Telomere shortening impairs organ regeneration by inhibiting cell cycle re-entry of a subpopulation of cells, EMBO J. 22, 4003-13.
[96] Lechel, A., Satyanarayana, A., Ju, Z., Plentz, R.R., Schaetzlein, S., Rudolph, C., Wilkens, L., Wiemann, S.U., Saretzki, G., Malek, N.P., Manns, M.P., Buer, J. and Rudolph, K.L., 2005. The cellular level of telomere dysfunction determines induction of senescence or apoptosis in vivo, EMBO Rep. 6, 275-81.

[97] Jeyapalan, J.C., Ferreira, M., Sedivy, J.M. and Herbig, U., 2007. Accumulation of senescent cells in mitotic tissue of aging primates, Mech Ageing Dev. 128, 36-44.
[98] Jeyapalan, J.C. and Sedivy, J.M., 2008. Cellular senescence and organismal aging, Mech Ageing Dev. 129, 467-74.
[99] van Deursen, J.M., 2014. The role of senescent cells in ageing, Nature. 509, 439-46.
[100] Baker, D.J., Wijshake, T., Tchkonia, T., LeBrasseur, N.K., Childs, B.G., van de Sluis, B., Kirkland, J.L. and van Deursen, J.M., 2011. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders, Nature. 479, 232-6.
[101] Baker, D.J., Childs, B.G., Durik, M., Wijers, M.E., Sieben, C.J., Zhong, J., Saltness, R.A., Jeganathan, K.B., Verzosa, G.C., Pezeshki, A., Khazaie, K., Miller, J.D. and van Deursen, J.M., 2016. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan, Nature. 530, 184-9.
[102] Chang, J., Wang, Y., Shao, L., Laberge, R.M., Demaria, M., Campisi, J., Janakiraman, K., Sharpless, N.E., Ding, S., Feng, W., Luo, Y., Wang, X., Aykin-Burns, N., Krager, K., Ponnappan, U., Hauer-Jensen, M., Meng, A. and Zhou, D., 2016. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice, Nat Med. 22, 78-83.
[103] Sousa-Victor, P., Gutarra, S., Garcia-Prat, L., Rodriguez-Ubreva, J., Ortet, L., Ruiz-Bonilla, V., Jardi, M., Ballestar, E., Gonzalez, S., Serrano, A.L., Perdiguero, E. and Munoz-Canoves, P., 2014. Geriatric muscle stem cells switch reversible quiescence into senescence, Nature. 506, 316-21.
[104] Sousa-Victor, P., Perdiguero, E. and Munoz-Canoves, P., 2014. Geroconversion of aged muscle stem cells under regenerative pressure, Cell Cycle. 13, 3183-90.
[105] Garcia-Prat, L., Martinez-Vicente, M. and Munoz-Canoves, P., 2016. Autophagy: a decisive process for stemness, Oncotarget. 7, 12286-8.
[106] Garcia-Prat, L., Munoz-Canoves, P. and Martinez-Vicente, M., 2016. Dysfunctional autophagy is a driver of muscle stem cell functional decline with aging, Autophagy. 12, 612-3.
[107] Vilchez, D., Simic, M.S. and Dillin, A., 2014. Proteostasis and aging of stem cells, Trends Cell Biol. 24, 161-70.
[108] Rubinsztein, D.C., Marino, G. and Kroemer, G., 2011. Autophagy and aging, Cell. 146, 682-95.
[109] Guan, J.L., Simon, A.K., Prescott, M., Menendez, J.A., Liu, F., Wang, F., Wang, C., Wolvetang, E., Vazquez-Martin, A. and Zhang, J., 2013. Autophagy in stem cells, Autophagy. 9, 830-49.
[110] Klionsky, D.J., Abdalla, F.C., Abeliovich, H., Abraham, R.T., Acevedo-Arozena, A., Adeli, K., Agholme, L., Agnello, M., Agostinis, P., Aguirre-Ghiso, J.A., Ahn, H.J., Ait-Mohamed, O., Ait-Si-Ali, S., Akematsu, T., Akira, S., Al-Younes, H.M., Al-Zeer, M.A., Albert, M.L., Albin, R.L., Alegre-Abarrategui, J., Aleo, M.F., Alirezaei, M., Almasan, A., Almonte-Becerril, M., Amano, A., Amaravadi, R., Amarnath, S., Amer, A.O., Andrieu-Abadie, N., Anantharam, V., Ann, D.K., Anoopkumar-Dukie, S., Aoki, H., Apostolova, N., Arancia, G., Aris, J.P., Asanuma, K., Asare, N.Y., Ashida, H., Askanas, V., Askew, D.S., Auberger, P., Baba, M., Backues, S.K., Baehrecke, E.H., Bahr, B.A., Bai, X.Y., Bailly, Y., Baiocchi, R., Baldini, G., Balduini, W., Ballabio, A., Bamber, B.A., Bampton, E.T., Banhegyi, G., Bartholomew, C.R., Bassham, D.C., Bast, R.C., Jr., Batoko, H., Bay, B.H., Beau, I., Bechet, D.M., Begley, T.J., Behl, C., Behrends, C., Bekri, S., Bellaire, B., Bendall, L.J., Benetti, L., Berliocchi, L., Bernardi, H., Bernassola, F., Besteiro, S., Bhatia-Kissova, I., Bi, X., Biard-Piechaczyk, M., Blum, J.S., Boise, L.H., Bonaldo, P., Boone, D.L., Bornhauser, B.C., Bortoluci, K.R., Bossis, I., Bost, F., Bourquin, J.P., Boya, P., Boyer-Guittaut, M., Bozhkov,

P.V., Brady, N.R., Brancolini, C., Brech, A., Brenman, J.E., Brennand, A., Bresnick, E.H., Brest, P., Bridges, D., Bristol, M.L., Brookes, P.S., Brown, E.J., Brumell, J.H. et al., 2012. Guidelines for the use and interpretation of assays for monitoring autophagy, Autophagy. 8, 445-544.

[111] Cuervo, A.M., Bergamini, E., Brunk, U.T., Droge, W., Ffrench, M. and Terman, A., 2005. Autophagy and aging: the importance of maintaining "clean" cells, Autophagy. 1, 131-40.

[112] Warr, M.R., Binnewies, M., Flach, J., Reynaud, D., Garg, T., Malhotra, R., Debnath, J. and Passegue, E., 2013. FOXO3A directs a protective autophagy program in haematopoietic stem cells, Nature. 494, 323-7.

[113] Mortensen, M., Soilleux, E.J., Djordjevic, G., Tripp, R., Lutteropp, M., Sadighi-Akha, E., Stranks, A.J., Glanville, J., Knight, S., Jacobsen, S.E., Kranc, K.R. and Simon, A.K., 2011. The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance, J Exp Med. 208, 455-67.

[114] Min-Wen, J.C., Jun-Hao, E.T. and Shyh-Chang, N., 2016. Stem cell mitochondria during aging, Semin Cell Dev Biol. 52, 110-8.

[115] Shyh-Chang, N., Daley, G.Q. and Cantley, L.C., 2013. Stem cell metabolism in tissue development and aging, Development. 140, 2535-47.

[116] Ryall, J.G., Dell'Orso, S., Derfoul, A., Juan, A., Zare, H., Feng, X., Clermont, D., Koulnis, M., Gutierrez-Cruz, G., Fulco, M. and Sartorelli, V., 2015. The NAD(+)-dependent SIRT1 deacetylase translates a metabolic switch into regulatory epigenetics in skeletal muscle stem cells, Cell Stem Cell. 16, 171-83.

[117] Tang, A.H. and Rando, T.A., 2014. Induction of autophagy supports the bioenergetic demands of quiescent muscle stem cell activation, EMBO J.

[118] Yilmaz, O.H., Valdez, R., Theisen, B.K., Guo, W., Ferguson, D.O., Wu, H. and Morrison, S.J., 2006. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells, Nature. 441, 475-82.

[119] Zhang, J., Grindley, J.C., Yin, T., Jayasinghe, S., He, X.C., Ross, J.T., Haug, J.S., Rupp, D., Porter-Westpfahl, K.S., Wiedemann, L.M., Wu, H. and Li, L., 2006. PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention, Nature. 441, 518-22.

[120] Gan, B. and DePinho, R.A., 2009. mTORC1 signaling governs hematopoietic stem cell quiescence, Cell Cycle. 8, 1003-6.

[121] Gan, B., Hu, J., Jiang, S., Liu, Y., Sahin, E., Zhuang, L., Fletcher-Sananikone, E., Colla, S., Wang, Y.A., Chin, L. and Depinho, R.A., 2010. Lkb1 regulates quiescence and metabolic homeostasis of haematopoietic stem cells, Nature. 468, 701-4.

[122] Mohrin, M., Shin, J., Liu, Y., Brown, K., Luo, H., Xi, Y., Haynes, C.M. and Chen, D., 2015. Stem cell aging. A mitochondrial UPR-mediated metabolic checkpoint regulates hematopoietic stem cell aging, Science. 347, 1374-7.

[123] Wrighton, K.H., 2015. Stem cells: SIRT7, the UPR and HSC ageing, Nat Rev Mol Cell Biol. 16, 266-7.

[124] Barger, J.L., Walford, R.L. and Weindruch, R., 2003. The retardation of aging by caloric restriction: its significance in the transgenic era, Exp Gerontol. 38, 1343-51.

[125] Cerletti, M., Jang, Y.C., Finley, L.W., Haigis, M.C. and Wagers, A.J., 2012. Short-term calorie restriction enhances skeletal muscle stem cell function, Cell Stem Cell. 10, 515-9.

[126] Mair, W., McLeod, C.J., Wang, L. and Jones, D.L., 2010. Dietary restriction enhances germline stem cell maintenance, Aging Cell. 9, 916-8.

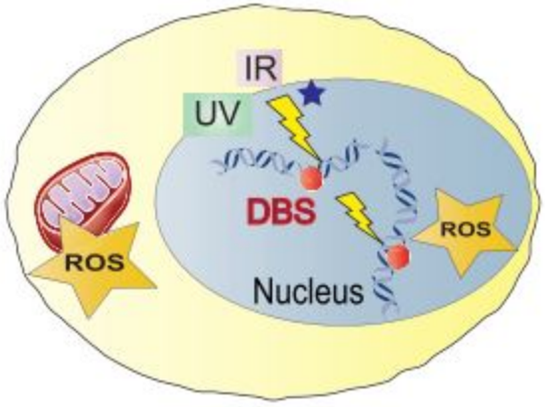
[127] Tothova, Z., Kollipara, R., Huntly, B.J., Lee, B.H., Castrillon, D.H., Cullen, D.E., McDowell, E.P., Lazo-Kallanian, S., Williams, I.R., Sears, C., Armstrong, S.A., Passegue, E., DePinho, R.A. and Gilliland, D.G., 2007. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress, Cell. 128, 325-39.

[128] Gopinath, S.D., Webb, A.E., Brunet, A. and Rando, T.A., 2014. FOXO3 promotes quiescence in adult muscle stem cells during the process of self-renewal, Stem Cell Reports. 2, 414-26.
[129] Chen, C., Liu, Y., Liu, Y. and Zheng, P., 2009. mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells, Sci Signal. 2, ra75.
[130] Johnson, S.C., Rabinovitch, P.S. and Kaeberlein, M., 2013. mTOR is a key modulator of ageing and age-related disease, Nature. 493, 338-45.
[131] Chan, E.Y., 2009. mTORC1 phosphorylates the ULK1-mAtg13-FIP200 autophagy regulatory complex, Sci Signal. 2, pe51.
[132] Cunningham, J.T., Rodgers, J.T., Arlow, D.H., Vazquez, F., Mootha, V.K. and Puigserver, P., 2007. mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex, Nature. 450, 736-40.
[133] Li, L. and Bhatia, R., 2011. Stem cell quiescence, Clin Cancer Res. 17, 4936-41.
[134] Castilho, R.M., Squarize, C.H., Chodosh, L.A., Williams, B.O. and Gutkind, J.S., 2009. mTOR mediates Wnt-induced epidermal stem cell exhaustion and aging, Cell Stem Cell. 5, 279-89.
[135] Demidenko, Z.N., Zubova, S.G., Bukreeva, E.I., Pospelov, V.A., Pospelova, T.V. and Blagosklonny, M.V., 2009. Rapamycin decelerates cellular senescence, Cell Cycle. 8, 1888-95.
[136] Blagih, J., Krawczyk, C.M. and Jones, R.G., 2012. LKB1 and AMPK: central regulators of lymphocyte metabolism and function, Immunol Rev. 249, 59-71.
[137] Gurumurthy, S., Xie, S.Z., Alagesan, B., Kim, J., Yusuf, R.Z., Saez, B., Tzatsos, A., Ozsolak, F., Milos, P., Ferrari, F., Park, P.J., Shirihai, O.S., Scadden, D.T. and Bardeesy, N., 2010. The Lkb1 metabolic sensor maintains haematopoietic stem cell survival, Nature. 468, 659-63.
[138] Nakada, D., Saunders, T.L. and Morrison, S.J., 2010. Lkb1 regulates cell cycle and energy metabolism in haematopoietic stem cells, Nature. 468, 653-8.
[139] Dasgupta, B. and Milbrandt, J., 2009. AMP-activated protein kinase phosphorylates retinoblastoma protein to control mammalian brain development, Dev Cell. 16, 256-70.

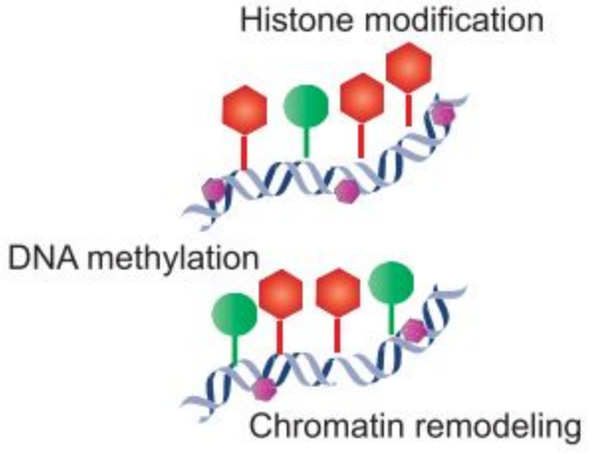
Acknowledgements

Work in the authors' laboratories was supported by SAF2015-67369-R (MINECO, Spain), Convenio UPF-CNIC, AFM, E-Rare/Eranet, MDA, Fundació Marató-TV3, DPP-NL.

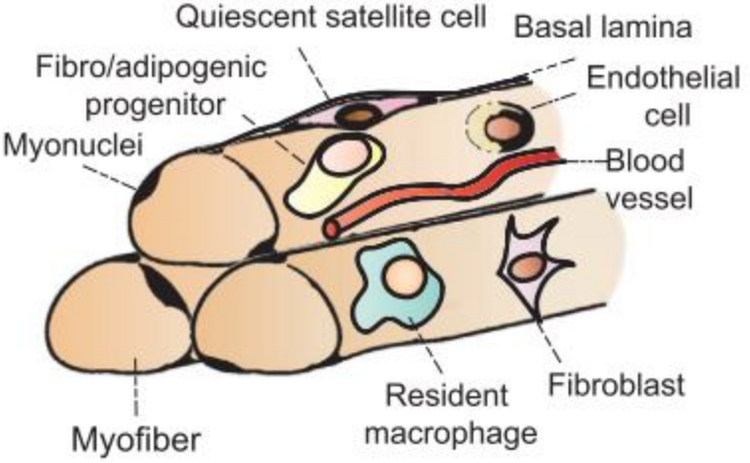
Blueprints of aging stem cells



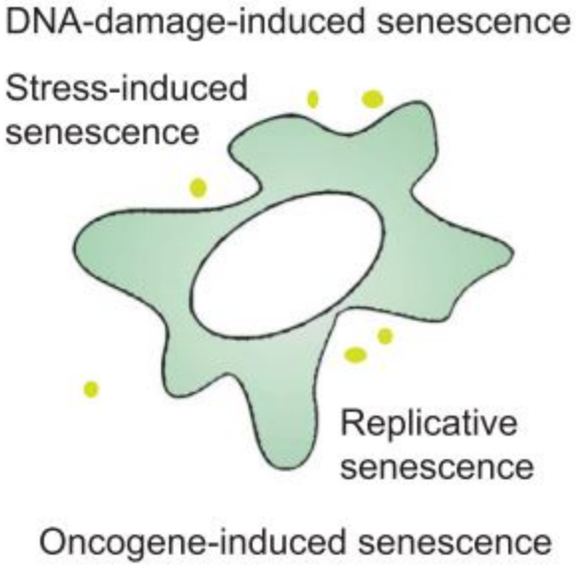
DNA damage and mutations



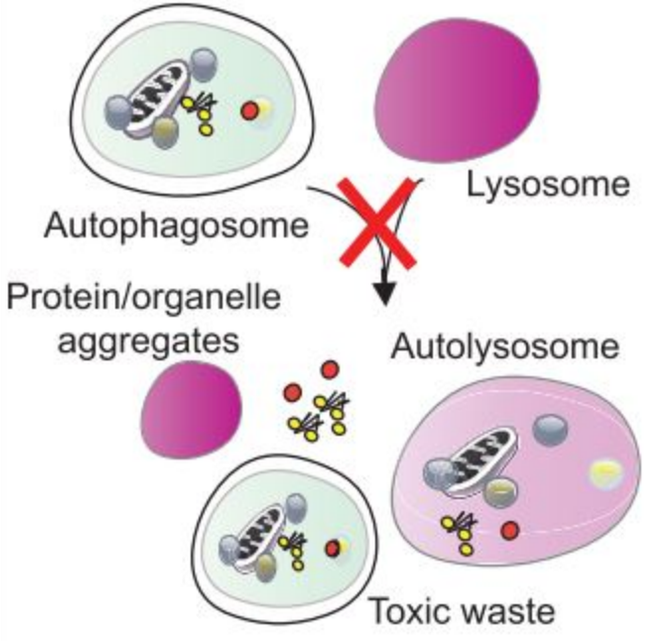
Epigenetic alterations



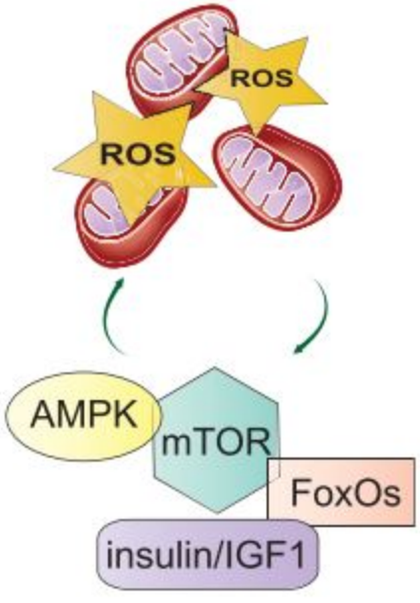
Stem cell niche



Cellular senescence



Loss of proteostasis



Mitochondrial dysfunction and altered metabolism

Fig. 1. Blueprints common to most stem cells that contribute to their numerical and functional decline with aging.