Trends in Cell Biology

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Review

Quiescence: Good and Bad of Stem Cell Aging

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Stem cells are required for lifelong homeostasis and regeneration of tissues and organs in mammals, but the function of stem cells declines during aging. To preserve stem cells during life, they are kept in a quiescent state with low metabolic and low proliferative activity. However, activation of quiescent stem cells – an essential process for organ homeostasis/regeneration – requires concerted and faithful regulation of multiple molecular circuits controlling biosynthetic processes, repair mechanisms, and metabolic activity. Thus, while protecting stem cell maintenance, quiescence comes at the cost of vulnerability during the process of stem cell activation. Here we discuss molecular and biochemical processes regulating stem cells' maintenance in and exit from quiescence and how age-related failures of these circuits can contribute to organism aging.

Regulation of Quiescence

Quiescence has been identified in various organ systems including muscle stem cells (MuSCs), hematopoietic stem cells (HSCs) [1], hair follicle stem cells (HFSCs) [2], neural stem cells (NSCs) [3], and intestinal stem cells (ISCs) [4–6]. It became apparent over past years that the regulation of quiescence requires a complex interplay of different molecular mechanisms. These include stem cell-intrinsic signaling pathways and epigenetic regulators as well as components of the micromilieu and the blood-circulatory environment [7–9]. Overarching principles of quiescence maintenance have been identified involving the expression of cell cycle inhibitors like p21 and p57 [10–12] as well as tumor suppressor genes like RB and p53 [13–16] in stem cells of different organs/tissues (Figure 1).

Despite residing in quiescence, adult stem cells retain the capacity to rapidly become activated to reenter the cell cycle in response to tissue injury thus act as a cell reservoir guaranteeing tissue homeostasis and regeneration. The activation of quiescent stem cells is a highly complex process involving epigenome modulations and the activation of transcription, RNA processing, protein synthesis, DNA replication, mitochondrial biogenesis, and shifts in metabolic pathways among others (for a review see [17]). The complexity of molecular and biochemical circuits that need to be reactivated during quiescence exit represent a stage of vulnerability, which by itself can contribute to the induction of molecular damage in stem cells. There is evidence that increasing the numbers of activation cycles accelerates DNA damage-driven stem cell aging in progeroid mouse models (see below; [18]). Moreover, aging can lead to disturbances in processes that control the maintenance of stem cell quiescence and the quiescence exit of stem cells in response to injury. Our review focuses on processes that protect stem cells from molecular damage during quiescence, on regulatory mechanisms that control the quiescence exit of activated stem cells. We discuss aging-related processes that undermine quiescence maintenance/exit thus aggravating stem cell and tissue aging. This review mainly focuses on HSCs and MuSCs, simply because these are among the best-studied adult stem cell systems in regard to stem cell quiescence and aging. It is likely the case that some of the general principals of stem cell quiescence and exit also apply to other stem cell systems, but we anticipate that there are organ- and cell type-specific differences that need to be worked out in future research, as, for example, in NSCs [19].

Highlights

The quiescence stage of stem cells has beneficial and adverse effects on stem cell aging.

Stem cell quiescence delays stem cell aging by reducing DNA replication, metabolic activity, gene transcription, and mRNA translation, since all of these activities are accompanied by induction of molecular damage.

Stem cell quiescence comes at the cost of impaired expression of repair factors in quiescence and increased vulnerability in response to stem cell activation requiring the concerted and faithful activation of multiple molecular circuits controlling biosynthetic processes, repair, and metabolic activity.

Aging-associated increases in stem cell-intrinsic accumulation of molecular damage as well as stem cell-extrinsic alterations (e.g., chronic inflammation, niche cell defects) contribute to the deregulation of quiescence maintenance and increasing vulnerability during exit from quiescence.

Epigenetic alterations occur during aging in quiescent and activated stem cells and lead to aberrant expression of developmental genes resulting in alterations of quiescence maintenance, self-renewal, and differentiation.

In conclusion, quiescence protects stem cells against molecular damage but comes at the cost of aging-associated failure in the correct regulation of quiescence maintenance and exit, thus increasing the vulnerability of stem cells during aging.

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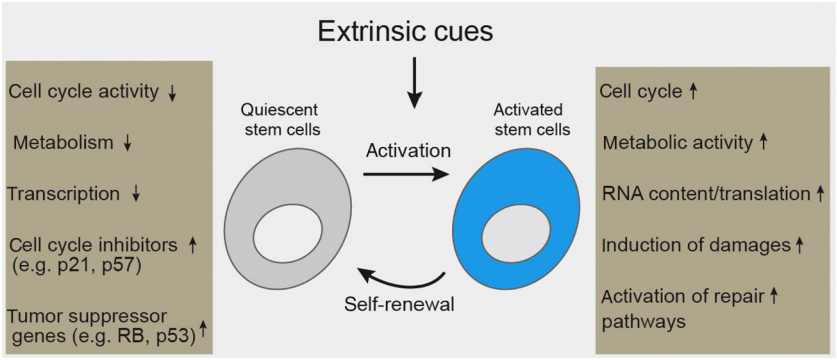

Figure 1. Common Characteristics of Quiescent Stem Cells. Quiescence is not a passive state but rather an actively regulated state. The main function of stem cell dormancy is to reduce the accumulation of molecular damage by lowering metabolic, molecular, and biochemical activities that can lead to the production of toxic metabolites or errors during the synthesis/turnover of (macro)molecules. Quiescent stem cells retain the capacity to reenter metabolic and cell-cycle activity, which is required for organ homeostasis and regeneration, but when these processes are completed stem cells reenter the protective stage of quiescence/dormancy. The concerted regulation of many biochemical and molecular processes is involved in regulating the exit and reentry of stem cells from/into quiescence. Disturbances in the faithfulness of these regulatory circuits contribute to stem cell aging. The figure depicts the main characteristics of quiescent versus activated stem cells.

DNA Damage in Quiescent and Activated Stem Cells

It has been postulated that the cumulative number of cell cycles determines the risk of DNA-replication-driven mutations in tissue stem cells and carcinogenesis in various tissues [20]. Conversely, the nondividing quiescence state of stem cells may reduce the risk of transformation by decreasing the accumulation of DNA replication-induced mutations.

Alterations in the stoichiometry of factors of the DNA replication complex represent a significant source of DNA damage in HSCs of aging mice (Figure 2 and [21]). There is evidence that quiescence itself has negative effects on DNA repair. Quiescent HSCs express low levels of DNA damage repair genes. Accumulating DNA damage is thus repaired more efficiently when quiescent HSCs enter the cell cycle and re-express DNA damage repair genes [22]. In addition, quiescent HSCs exhibit an aging-associated failure to resolve DNA damage signaling due to mislocalization of the PP4c phosphatase, which is required for dephosphorylation of the DNA damage response factor γH2AX [21]. Interestingly, residual γH2AX signaling in aging, quiescent HSCs was located at rRNA-encoding regions (nucleoli), suggesting that it may disturb the proper assembly of ribosomes and protein homeostasis during aging.

Another disadvantage of stem cell quiescence was revealed in studies on skin stem cells and HSCs showing that quiescent stem cells employ nonhomologous end joining (NHEJ) rather than homologous recombination (HR) to repair DNA double-strand brakes [22–24]. HR can be employed only in proliferating cells and thus cannot be used in quiescence. Since NHEJ is more error prone than HR, it is possible that quiescent stem cells have a disadvantage in maintaining genomic integrity.

Overall, stem cell quiescence is protective in preventing DNA replication and thus replication-associated DNA damage. However, stem cell quiescence comes at the cost of DNA repair deficiencies that may increase the risk of acquiring mutations. Moreover, the aging-associated failure to resolve DNA damage signaling [21] may contribute to imbalances of protein expression that increase the vulnerability of aging stem cells during exit from quiescence. Further research should

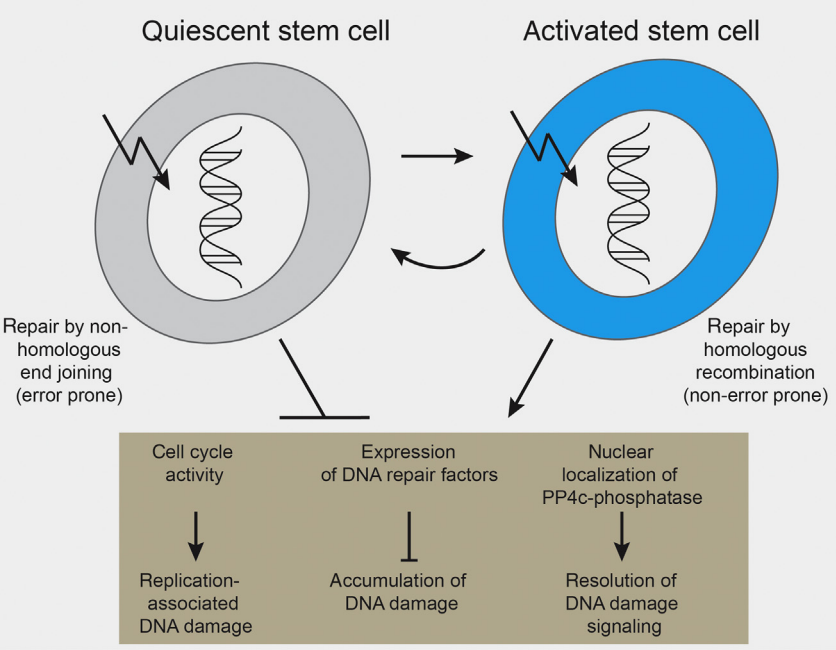

Figure 2. Quiescent and Activated Stem Cells Employ Different DNA Repair Pathways. Whereas quiescent stem cells use nonhomologous end joining (NHEJ), damaged DNA in activated stem cells is repaired by homologous recombination. Quiescence represents a protective state by reducing replication-associated DNA damage; however, it may also increase molecular damages by employing the error-prone NHEJ repair mechanism, by reducing the expression of DNA repair factors, and by aging-associated impairments in resoultion of DNA damage signaling.

clarify the positive and negative effects of quiescence on protecting stem cells from the accumulation of DNA damage and mutations and how this balance of positive versus negative effects of stem cell quiescence may tip during aging.

Inflammatory Signaling in Aged Stem Cells

The accumulation of DNA damage in tissues can also have effects on the micromilieu in stem cell niches or on the systemic circulation of factors that influence stem cell and organism aging. DNA damage leads to the induction of cellular senescence, an irreversible cell cycle-arrest state, which blocks the proliferation of damaged or dysfunctional cells (Figure 3). It has been shown that senescent cells show a strong increase in the secretion of inflammatory cytokines, a phenotype referred to as the 'senescence-associated secretory phenotype' (SASP) [25]. Accumulation of senescent cells in aging tissues appears to be an important source of chronic increases in inflammatory signals during aging. Continuous elevation of inflammation has been associated with tissue dysfunction and the development of disease in aging humans (e.g., see [26,27]), suggesting that inflammation is an important factor in human aging. Animal experiments proved that the accumulation of senescent cells contributes to tissue aging [28] and to the development of aging-associated diseases including cancer (for a review see [29]).

Moreover, it has been shown that geriatric MuSCs fail to maintain the reversible quiescent state and convert to a p16INK4a-dependent pre-senescent state [30]. This cellular shift from quiescence to senescence impairs the regenerative capacity of geriatric MuSCs. The dysregulation of the senescence pathway appears to be an intrinsic defect in quiescent MuSCs from aging

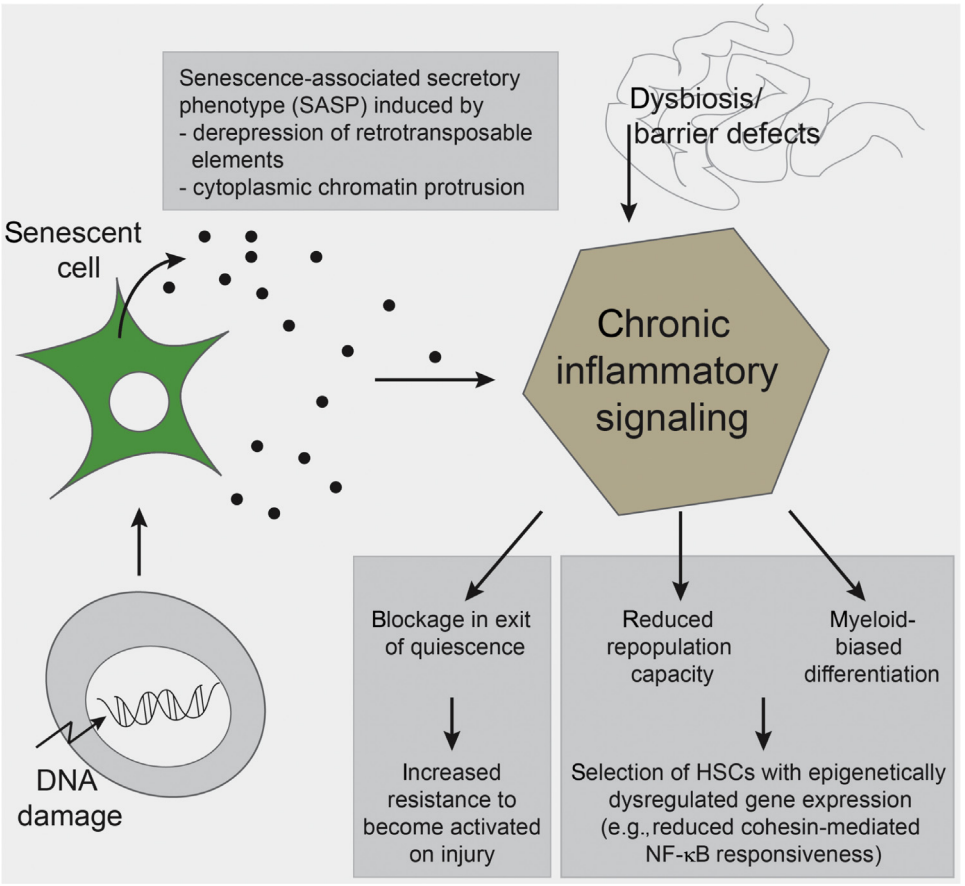

Figure 3. Chronic Inflammation Impairs Stem Cell Function During Aging. DNA damage (e.g., short telomeres) triggers senescence. Senescent cells in turn secrete elevated levels of inflammatory factors leading to chronic activation of inflammatory signaling, which compromises the function of adult stem cells. The mobilization of retro-transposable elements and protrusions of chromatin into the cytoplasm aggravate the senescence-associated secretory phenotype (SASP). In addition, dysbiosis and barrier defects contribute to aging-associated increases in inflammation.

mice. It has been shown that increasing levels of ROS due to defects in autophagy can lead to depression of the p16INK4a locus in old MuSCs [31]. The inactivation of p16INK4a in geriatric MuSCs restores the reversibility of the quiescent state [30] and p16INK4a deficiency has been shown to improve the repopulation capacity of HSCs by increasing the resistance of HSCs to undergo apoptosis in response to activation [32].

Given the important role of stem cells in tissue aging, it will be of special interest for future studies to delineate the influence of senescence and elevated inflammation on the functional decline of stem cells. It has been shown that the stem cell-intrinsic induction of senescence checkpoints contributes to tissue atrophy by impairing the maintenance and functionality of stem cells; for example, in the hematopoietic system and in the intestinal epithelium in mouse models of telomere shortening-induced aging [33]. Moreover, senescent cells in the stem cell niche were shown to produce SASP factors that impair the functionality of HSCs in aging mice and the removal of senescent cells improved HSC function, and thus health and lifespan in aged mice [34].

Additional factors that increase inflammatory signaling appear to contribute to organism aging. Recently, it has been demonstrated that derepression of retrotransposable elements triggers an interferon response in aging cells and tissues [35]. In addition, cytoplasmic chromatin protrusions were shown to activate the SASP by induction of cGAS/STING signaling [101]. Furthermore, studies on the aging of *Drosophila melanogaster* revealed evidence that an aging-associated dysbiosis in commensal gut bacteria leads to inflammation-mediated impairments in stem cell differentiation in the intestinal epithelium, resulting in barrier defects and systemic inflammation that limits the lifespan of flies [36]. There is emerging evidence that age-associated changes in the gut microbiome also contribute to increases in morbidity and mortality in vertebrate aging [37]. Of note, barrier dysfunction of the intestinal epithelium and the blood–brain barrier occur in aging mice, and are observed in humans, which may contribute to the development of aging-associated pathologies [38].

Endotoxin exposure and NF-κB induction – an important signaling axis in response to bacterial infection – have been reported to affect the function of HSCs in a way that mimics HSC aging, with a reduction in repopulation capacity and a shift towards myeloid differentiation [39,40]. Interestingly, HSCs from old mice display elevated expression of inflammatory markers and increased NF-κB signaling activity [41,42]. It remains to be analyzed whether intestinal barrier defects and/or microbiota dysbiosis contribute to this phenotype of HSC aging.

Together, various mechanisms contribute to increases in chronic inflammatory signaling in aging. Signaling pathways and consequences of aging-associated increases in inflammation on stem cell aging remain to be delineated, especially how this may involve changes in quiescence maintenance/exit. Interestingly, dysregulated inflammatory signaling has been shown to lock old NSCs in a quiescent state [19]. The inflammatory signals are released from the niche (along with Wnt signaling molecules, as discussed below) and keep NSCs in a quiescence state in the old brain. Once the NSCs exit quiescence, they show a very high degree of functional and molecular similarity independent of the age of the mice. Thus, despite being locked into quiescence and blocked from activation, old NSCs appear to function normally if they accomplish reentry into an activation cycle [19]. These data suggest that inflammation may promote the switch of stem cell quiescence into stem cell senescence, which has been described in MuSCs of aging mice [30]. In the aging hematopoietic system, several lines of evidence support the concept that increases in inflammatory signaling drive myeloid-skewed differentiation and suppression of lymphopoiesis. Inflammation-induced lineage skewing has also been described for NSCs, leading to blockage of neurogenesis and induction of the generation of oligodendrocytes as well as astrocytes [43–45].

Telomerase-knockout mice show premature aging and a shortened lifespan due to telomere dysfunction-induced impairments in stem cell function and organ homeostasis [33,46,47]. Interestingly, telomere dysfunction induces systemic increases in inflammation [48] that resemble the secretion of inflammatory cytokines by senescent cells in culture (see above; [25]). Systemic increases in inflammation signaling in aging telomere-dysfunctional mice lead to myeloid-biased differentiation of HSCs, which was rescued by transplantation of HSCs into wild-type mice [48]. Vice versa, the balance in differentiation capacity and the engraftment of wild-type HSCs were strongly impaired on transplantation into telomere-dysfunctional mice [48]. Also, in wild-type mice and in aging humans, inflammation increases during aging, and it was shown that the transplantation of HSCs from old mice into young mice reverts aging-associated myeloid-skewed differentiation [49,50]. It will be of interest for future studies to delineate the influence of cell-extrinsic alterations of the aging organism on stem cell function and how these processes are reverted by re-exposure of aged stem cells to a young environment. Most likely these reversible aging phenotypes of tissue-resident stem cells are to a large extent induced by epigenetic modifications that appear to be reversible by exposure to a young environment.

It is also possible that aging-associated alterations in the stem cell environment select for mutant clones or drifted populations of stem cells that have a growth advantage in the aged environment, which is suboptimal for the function and growth of normal, young stem cells. Along these lines, there is evidence that chronic inflammation selects for subpopulations of stem cells with an altered chromatin structure resulting in inflammation resistance coupled with changes in gene transcription and differentiation defects. Specifically, studies on aging mice revealed evidence that the cohesin complex mediates NF-κB signaling in HSCs in response to inflammation [41]. Cohesin-dependent NF-κB signaling results in stem cell differentiation. Accordingly, increases in inflammation or aging-induced NF-κB signaling were found to promote the selection of subpopulations of HSCs with reduced cohesin expression, thus being resistant to inflammation-induced loss of differentiation and self-renewal [41]. Since cohesin expression is reduced in myeloid-biased HSCs, this selection appears to contribute to the myeloid skewing of hematopoiesis during aging, which is associated with the induction of myeloid differentiation programs in inflammation-exposed HSCs [51]. Together these findings indicate that HSC-extrinsic increases in inflammation that occur outside the HSC (extrinsic: in the niche or in the blood circulation) can promote the age-dependent selection of HSC subpopulations with altered differentiation.

Altered Metabolic Control in Aged Stem Cells

Mitochondrial Metabolism

Stem cell quiescence is associated with low metabolic activity and low rates of oxygen consumption [52–54]. However, there are differences in how this stage of low metabolic activity is maintained in different stem cell compartments. Quiescent HSCs primarily use glycolysis for ATP production, but the respiratory activity of mitochondria strongly increases in response to activation and in early progenitor cells of the hematopoietic lineage [52,55–59]. Aging-related decreases in autophagy were shown to lead to increases in mitochondrial metabolism in HSCs, driving epigenetic alterations that accelerate myeloid skewing in differentiation [60]. Of note, mitochondrial activity can influence DNA methylation in the nucleus, thereby leading to changes in the transcriptional output [61]. This is interesting since DNA methylation at specific loci in the genome can be utilized as a precise age estimator [62]. Therefore, the 'epigenetic age' could to some extent reflect the aging-associated impairment of mitochondrial function.

Studies on mitofusin-knockout mice point to another function of mitochondria in HSCs. It was shown that HSC mitochondria are required to keep cytosolic calcium levels low to avoid the induction of nuclear factor of activated T cells (NFAT)-driven differentiation pathways [63]. Together these data indicate that the maintenance of quiescent HSCs with low rates of oxidative metabolism has a protective function in minimizing epigenome alterations that lead to skewed differentiation. Nevertheless, mitochondrial mass is maintained at a surprisingly high level in quiescent HSCs [63,64], to conduct metabolism-independent functions (e.g., keep stem cell intrinsic calcium levels low). It possibly also ensures that stem cells are ready to enter the cell cycle with sufficient capacity to activate ATP synthesis on demand.

Quiescent MuSCs have been shown to preferentially use fatty acid oxidation (FAO) whereas activated MuSCs shift to glycolysis for ATP production [65]. This observation is also supported by transcriptome analyses of MuSCs showing a gene signature of FAO components in quiescent cells but induction of the glycolytic pathway in proliferating cells [54,65–67]. NSCs also exhibit high levels of FAO, which is required to maintain the quiescent state. Interestingly, modulation of FAO is sufficient to induce the exit of NSCs from quiescence [68].

Glycolysis can generate anabolic growth intermediates and at the same time is a source for rapid ATP production. Both components are required for proliferating cells. Interestingly, increased biogenesis of mitochondria occurs during the transition of the quiescent to the activated state in

MuSCs [53,65,69,70], which does not correlate with increases in oxygen consumption in activated cells [65]. It is possible that increases in mitochondrial biogenesis also fulfill additional nonmetabolic functions in activated MuSCs, as it has been described in quiescent HSCs (see above). Besides a possible role in calcium signaling, the induction of mitochondrial biogenesis in activated MuSCs may be required for the functionality of activated MuSCs during later steps of muscle regeneration. Accordingly, it has been shown that MuSCs switch back to oxidative phosphorylation during differentiation [71]. It is possible that the induction of mitochondrial biogenesis during MuSC activation is required to ensure this subsequent switch towards oxidative phosphorylation during differentiation. However, it may also involve induction of peroxisomal fatty acid metabolism, which seems to have a prominent role in activated myogenic cells [54]. Interestingly, the latter study revealed that the normally occurring switch in metabolism between quiescent and activated MuSCs is disturbed in aging mice (Figure 4). Comparing old versus young mice, quiescent MuSCs were found to rely more on glycolysis and showed impaired responsiveness to mitochondrial stimuli coinciding with reduced ATP levels [54]. Together, these data imply that impairments in mitochondrial function may contribute to the functional decline of MuSCs to exit quiescence, a concept that needs to be further investigated.

Protein Homeostasis

Aging has been associated with defects in proteostasis, like many neurodegenerative diseases including Alzheimer's and Parkinson's disease. Recently, it has been shown that quiescent and activated NSCs use different cellular mechanisms to maintain protein homeostasis [72]. The authors demonstrated that quiescent NSCs contain more insoluble aggregates and bigger

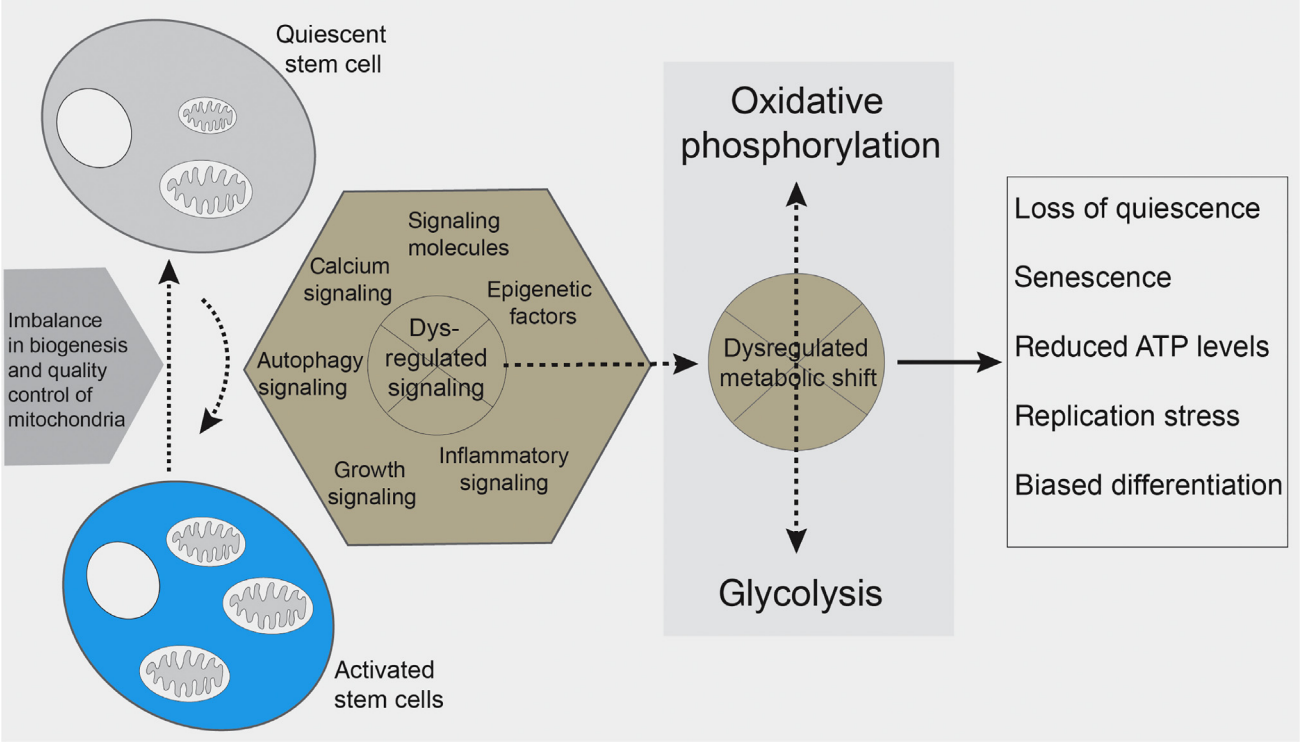

Figure 4. Disturbances of Metabolic Activity Impair the Function of Aging Stem Cells. Alterations in signaling (e.g., calcium signaling, inflammatory signaling) as well as imbalances in mitochondrial biogenesis and quality control (mitophagy) occur in aging stem cells. This leads to a dysregulated metabolic shift in quiescent versus activated stem cells. There are compartment-specific differences in the metabolic pathways that stem cells employ in the quiescent or the activated state.

lysosomes than activated NSCs. Experimental activation of lysosomal autophagy in quiescent NSCs clears the aggregates and leads to enhanced NSC activation. Interestingly, old quiescent NSCs have more protein aggregates than young NSCs and thus higher activation resistance. However, when lysosomal activity is enhanced – by systemic mTOR inhibitor treatment or by fasting – the activation potential of old quiescent NSCs is restored [72]. There is evidence that autophagy also influences the switch from quiescent to activated stem cells by regulating mitochondrial metabolism. Autophagy seems to be required for the adaptation of stem cells to distinct energetic requirements during exit from quiescence [53,65,73]. In line with this concept, the aging-associated declines in autophagy lead to alterations in mitochondrial function, which lock quiescent MuSCs in a nonactivatable stage of cellular senescence [31]. In the hematopoietic system, this aging-associated decline in autophagy results in epigenetic alterations of HSCs leading to loss of quiescence and myeloid-skewed differentiation [60].

Dietary Restriction (DR) and Stem Cell Function

Interestingly, the response to nutrient availability influences stem cell and organism aging. DR is the most well-documented intervention that can increase the health- and lifespan across various animal models (for review see [74]). However, in nonhuman primates the effects of DR are less clear than results in simpler organisms. Two big trials in nonhuman primates revealed different results on the lifespan-extending effects of DR that were resolved only recently in a meta-analysis of the two trials [75]. Studies in mice have shown that DR affects stem cell function, but these effects were dependent on genetic background, sex, age, and the protocol of DR treatment. Studies on skeletal muscle showed that short-term DR (3 months) improved MuSC function in culture and during muscle regeneration *in vivo* [76]. By contrast, DR had negative effects on the colony-forming capacity of MuSCs and muscle fiber size after muscle regeneration [77]. Experiments on the hematopoietic system from our group revealed that longterm DR delays early aging of HSCs when applied to 3-12 month old mice by slowing IGF-dependent HSC proliferation, thus impairing aging-associated increases in HSC numbers and concomittant decreases in the repopulation capacity compared with *ad libitum* (AL)-fed mice [78]. By contrast, lifelong DR did not ameliorate the functional decline of HSCs during advanced aging [79] suggesting that DR dependent effects on stem cell and organism aging may depend on the timing of intervention during the lifecycle of an organism. DR was also shown to have the caveat of reducing lymphopoiesis, resulting in impaired immune functions [78]. In addition, DR-induced metabolic changes can have compartment-specific effects. Studies on skin revealed that DR decreases oxidative metabolism in the epidermis but increases it in the dermis [80]. In MuSCs, DR leads to an increase of oxygen consumption and a reduction in lactate production, indicating that oxidative metabolism increases whereas glycolysis declines in response to DR [76]. Overall, DR can have positive and negative effects on stem cell maintenance and function, which appears to depend on various factors including the timing and duration of DR. Delineating aging-related mechanisms that alter the metabolic control of stem cells and how they respond to dietary interventions is of utmost importance to employ dietary interventions to improve health during aging.

Epigenetic Changes and Aberrant Induction of Developmental Pathways

It has been postulated that cell-intrinsic changes (DNA damage, defects in proteostasis, metabolic changes) and cell-extrinsic alterations (increases in inflammation) impinge on epigenetic modifications in aging stem cells (for a review see [81]). Chromatin modifications in stem cells exhibit tremendous changes during the transition of the quiescent to the activated state. In MuSCs, the chromatin is more permissive (opened) in quiescent MuSCs but becomes more repressed (closed) in response to MuSC activation [67]. In aging, quiescent stem cells, the repressive H3K27me3 mark increases in both HSCs and MuSCs [67,82]. These epigenetic alterations are

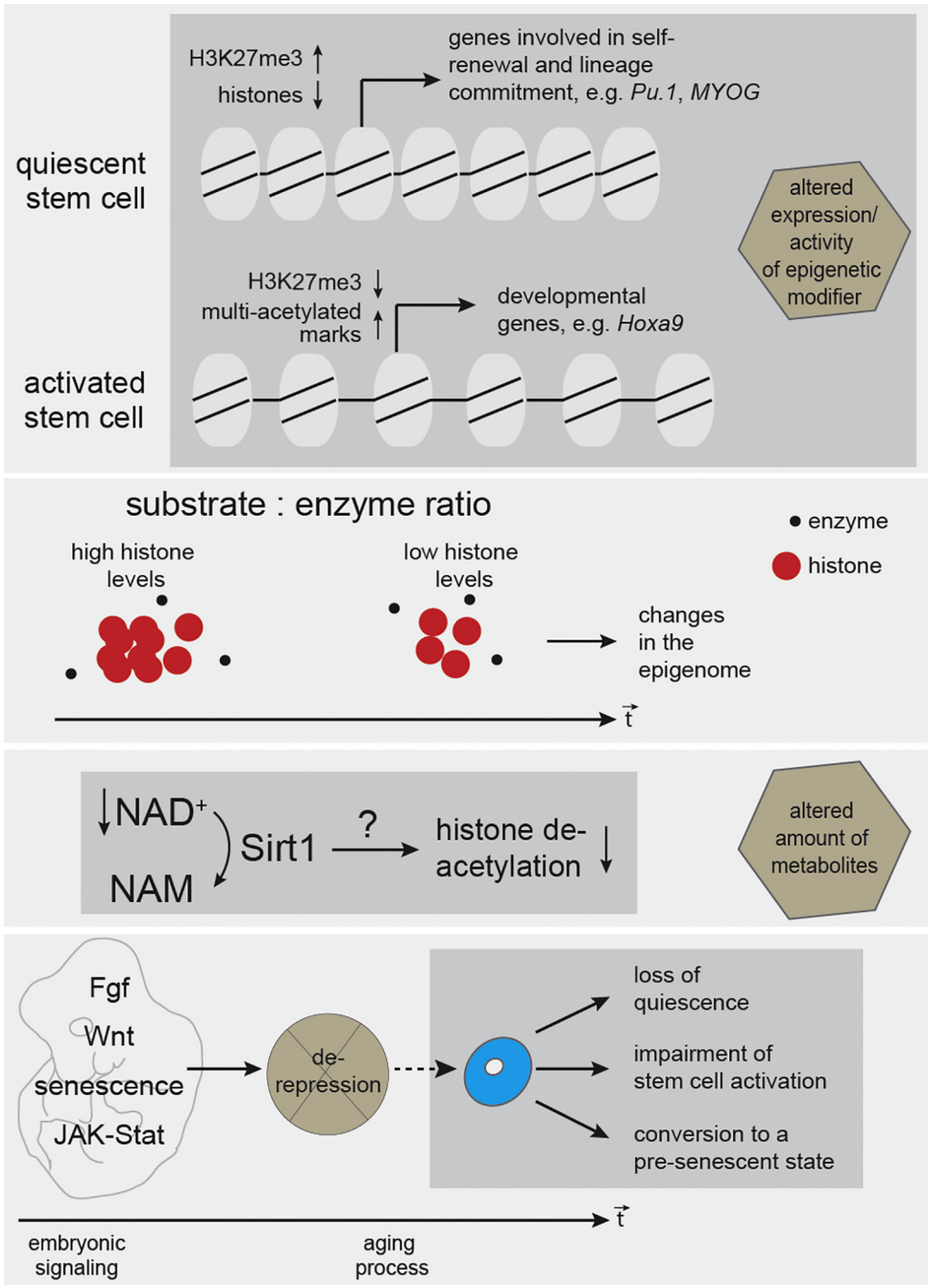

Figure 5. Changes in Epigenome Modification of Adult Stem Cells During Aging and Decay in the Regulation of Developmental Pathways Drives Stem Cell Aging by Breaking or Cementing Quiescence. Compared to young muscle stem cells (MuSCs), the chromatin of MuSCs from old mice exhibits a more permissive state (open) in the course of activation. Aging-associated alterations in the regulation of the epigenome at this transition are characterized by decreases in H3K27me3 marks and increases in multiacetylated histones. The over-induction of a permissive state of the chromatin during the activation of aged versus young MuSCs leads to the aberrant expression of developmental genes, including

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associated with altered expression of genes involved in stem cell self-renewal and lineage commitment affecting, for example, the expression of Pu.1 in HSCs and myogenin in MuSCs [67,82]. In contrast to the age-dependent increase in repressive histone marks in quiescent MuSCs, the chromatin exhibits overshooting opening (into a permissive state) in activated MuSCs from aged compared with young mice (Figure 5) resulting in aberrant induction of developmental pathways and impaired regenerative capacity of MuSCs [83]. What causes aging-associated alterations in chromatin modifications in stem cells remains unresolved. In principal, this could involve changes in the expression or in the regulation of enzymes that catalyze chromatin modifications. Alternatively, it could involve alterations in the expression levels of the substrates of chromatin-modifying enzymes. Interestingly, the expression level of several histones is strongly reduced in MuSCs from aged mice compared with young mice (Figure 5) [67]. Decreases in histone expression are also present in senescence and in response to DNA damage signaling induced by telomere dysfunction [84,85]. It is possible that the reduction of histone expression by itself leads to alterations in histone modification by disturbing the balance between substrate availability (histones) and enzyme activity. This may also explain the age-dependent increase in repressive marks (H3K27me3) in quiescent MuSCs as well as the overshooting increases in activation marks (such as polyacetylated histones) in activated MuSCs [67,83]. Decreasing histone levels in aging stem cells may lead to an amplification of modifications per histone by the prevailing enzyme activities in different stem cell states (quiescence vs activation). The causes and consequences of reduced histone expression and its impact on the regulation of stem cell quiescence and activation have yet to be explored experimentally.

In addition to an influence of histone expression levels, there is evidence that senescence and DNA damage change the activity of enzymes that regulate histone modifications [86]. It is possible that both factors, the deregulation of chromatin-modifying enzymes and the reduction in histone expression level, contribute to changes in chromatin modifications in stem cell aging. Besides the accumulation of DNA damage, aging-associated changes in metabolism may also affect chromatin modification in stem cells. For example, *Sirt1*, a member of the sirtuin family of deacetylases, has been shown to be a crucial factor for the regulation of the transition from the quiescent to the activated state in MuSCs by increasing acetylation levels and the transcription of myogenic factors [65]. NAD^+ is a cofactor for SIRT1 enzyme activity and, interestingly, the level of NAD^+ declines during aging [87].

On top of the functional characterization of mechanisms that lead to disturbed epigenome maintenance in aging stem cells, it will be of great interest to delineate the downstream consequences on the regulation of signaling pathways that may influence stem cell aging. Transcriptional changes of several signaling components have been shown to disturb the quiescent state of adult stem cells during aging. The majority of these signaling pathways are also known to be essential for embryonic development; for example, Fgf, Wnt, p16, and JAK–Stat signaling (Figure 5). These findings support the concept that aging represents a 'developmental decay' characterized by drifted activation and increasing dysregulation of developmental pathways at post-reproductive age [88,89]. Stem cells represent the remnants of embryonic development in the

Hoxa9 as a central regulator of several developmental pathways. The expression of various histone members is reduced in quiescent MuSCs from old mice compared with young mice, which could explain the contrary epigenetic shift in old stem cells. Furthermore, levels of the cofactor NAD^+ are lower in old tissue, which could decrease Sirt1 activity leading to impaired deacetylation of target proteins including histones. As a consequence, developmental pathways such as Fgf, Wnt, and p16INK4a-dependent senescence as well as JAK–Stat signaling are de-repressed during aging, which impairs the function of adult stem cells by disturbing the maintenance of stem cell quiescence but also in the quiescence exit of activated stem cells. In turn, these defects could contribute to the switch of quiescent stem cells into senescent stem cells during aging. The aging-associated deregulation of epigenetic modifications appears to drive the derepression of developmental pathways as well as its consequences for the breakage or cementation of quiescence.

adult organism. Accordingly, stem cells express basal levels of developmental pathways. Taking these findings together, it is possible that stem cells are more sensitive to the drifted and aberrant regulation of these pathways during post-reproductive aging (for a review see [81]). Work in our laboratory revealed that aging-associated alterations in epigenome modifications in activated MuSCs result in upregulation of *Hoxa9*, which is a member of the Hox gene family of transcription factors. *Hoxa9* in turn acts as a central hub leading to the induction of several developmental pathways that impair the function of MuSCs in regenerating injured muscles in aged compared with young mice [83]. Aberrant activation of *Hoxa9* in aging MuSCs induces several developmental pathways that have previously been identified to impair the function of MuSCs in aging mice [9,30,90–94].

Impairments in stem cell function in response to the activation of developmental pathways during aging may involve direct inhibitory effects of developmental pathways on stem cell quiescence. It has been described that *Wnt5a* regulates the proliferation, regenerative capacity, and quiescence of HSCs [95–97]. Interestingly, elevated expression of Wnt5a in old HSCs impairs the regenerative potential and leads to biased differentiation in aging mice [98]. It would be important to evaluate whether changes in *Wnt5a* expression directly affect the maintenance of quiescence and/or the exit from quiescence of aged HSCs. There is evidence that Wnt5a-treated HSCs display increased p57 and p27 expression [98] (Figure 1). A recent study also demonstrated that aging-associated increases in canonical Wnt signaling (induced by niche cells) disturb the function of NSCs by locking the NSCs into quiescence thus impairing NSC activation [19]. Interestingly,

Outstanding Questions

What are the main causes of the aging-associated failure of stem cell quiescence to prevent impairments in stem cell function?

What is the impact of stem cell-extrinsic mechanisms versus stem cell-intrinsic mechanisms in driving failures in quiescence-mediated protection of stem cells during aging?

Is it possible to delay organism aging by stabilizing the maintenance of stem cell quiescence or by reducing stem cell vulnerability during exit from quiescence?

Which mechanisms of stem cell quiescence represent good targets for novel cancer therapies aiming to increase the vulnerability of tumor propagating cells?

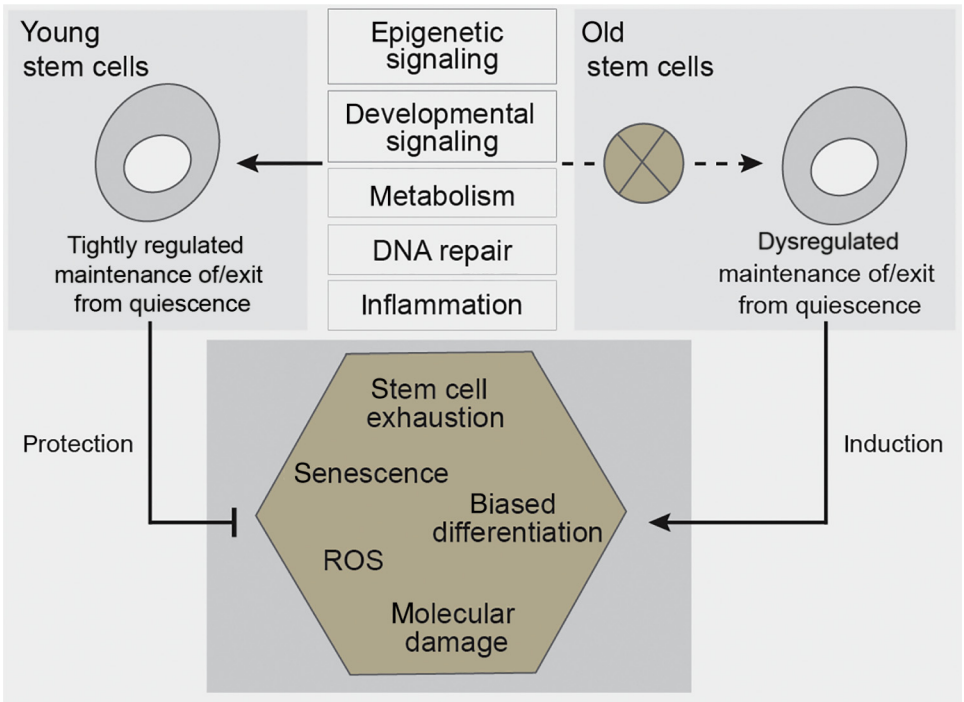

Figure 6. Model Describing the Protective State of Stem Cell Quiescence. Quiescence protects stem cells from aging by impairing the accumulation of molecular damages. However, the regulation of stem cell quiescence (both maintenance and exit) is increasingly disturbed during aging. As a consequences, the production of ROS and the accumulation of molecular damage increases and this in turn results in differentiation defects, senescence and functional exhaustion of stem cells.

the systemic modulation of Wnt signaling ameliorated the resistance of old NSCs to exit quiescence and improved proliferation in response to activation [19].

Aging-associated dysregulation of developmental pathways in niche cells also occurs in skeletal muscle and affects the quiescence of MuSCs. MuSC niche cells produce higher levels of FGF2 leading to reduced expression of *Spry1* in MuSCs [9]. *Spry1* is important for maintaining MuSC quiescence [99]. It is highly expressed in quiescent MuSCs but downregulated during MuSC activation and exit from quiescence. *Spry*1 also controls MuSC reentry into quiescence by inhibiting the ERK signaling pathway [99]. In mice, the increased expression of FGF2 by niche cells and the downregulation of *Spry1* in MuSCs result in the loss of quiescence of a subpopulation of MuSCs during aging. Interestingly, in human MuSCs, DNA hypermethylation silences the SPRY1 gene locus during aging thereby impairing their ability to return to quiescence [100]. Together these data provide evidence that deregulations of both quiescence exit and quiescence reentry can contribute to the loss of stem cell maintenance in aging muscle.

Concluding Remarks

Quiescence in principle is a protective state that is however susceptible to disturbances in aged stem cells (Figure 6). Defects in quiescence affect steady-state gene expression in quiescent stem cells, but also the failure to faithfully regulate gene network activities and metabolism changes during the activation of dormant stem cells to exit quiescence. These aging-associated failures involve cell-intrinsic processes (e.g., alterations in epigenetic stress responses, DNA damage accumulation, impairments in mitochondrial function) but also cell-extrinsic processes such as alterations in circulatory metabolites and increases in inflammation. There is increasing knowledge on the causes and consequences of defects in quiescence during stem cell aging, suggesting that failures in maintenance and exit of quiescence are important contributing factors that drive the decline of stem cell function, the loss of tissue homeostasis, and disease evolution during aging. We propose that several key questions should be addressed in future research (see Outstanding Questions) to improve our mechanistic understanding of failures in quiescence regulation during aging. In addition, this research could provide a rational basis for the development of future therapies aiming to target alterations in quiescence control to improve stem cell function and tissue maintenance during aging.

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