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Review

Stem cells, mitochondria and aging☆

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ABSTRACT

Decline in metabolism and regenerative potential of tissues are common characteristics of aging. Regeneration is maintained by somatic stem cells (SSCs), which require tightly controlled energy metabolism and genomic integrity for their homeostasis. Recent data indicate that mitochondrial dysfunction may compromise this homeostasis, and thereby contribute to tissue degeneration and aging. Progeroid Mutator mouse, accumulating random mtDNA point mutations in their SSCs, showed disturbed SSC homeostasis, emphasizing the importance of mtDNA integrity for stem cells. The mechanism involved changes in cellular redox-environment, including subtle increase in reactive oxygen species (H_2O_2 and superoxide anion), which did not cause oxidative damage, but disrupted SSC function. Mitochondrial metabolism appears therefore to be an important regulator of SSC fate determination, and defects in it in SSCs may underlie premature aging. Here we review the current knowledge of mitochondrial contribution to SSC dysfunction and aging. This article is part of a Special Issue entitled: Mitochondrial Dysfunction in Aging.

1. Introduction

Stem cells are characterized by two main properties: 1) ability to produce variable independent cell types, i.e. multipotency; 2) ability to self-renew, i.e. to produce an identical multipotent daughter cell. Stem cells can undergo symmetrical cell division, producing two identical stem cells, or asymmetrical division, resulting in one stem cell and one committed progenitor cell [1]. Progenitor cells have transient amplification capacity with limited lifespan, and they cannot self-renew. Stem cells are classified based on their differentiation capacity: pluripotent stem cells, such as embryonic stem cells (ES cells), can produce all the cell types of the *embryo proper* [2], and multipotent cells, such as somatic stem cells (SSCs) can give rise to the cell types of the tissue in which they reside. Nuclear reprogramming can turn somatic cells to pluripotent stem cells. These induced pluripotent stem (iPS) cells have similar characteristics as ES cells [3,4]. Multipotent SSCs have been characterized in several adult tissues where they serve an important purpose in tissue regeneration and maintenance of function throughout the lifetime of an organism. SSCs are especially essential in actively renewing cell types, such as the blood and skin, where they constantly replenish dying cells. These tissues are very sensitive for SSC dysfunction [5,6]. In post-mitotic tissues, such as the brain and muscle, SSCs are thought to be activated mainly for growth and tissue repair, but quiescent under normal physiological conditions [7,8]. In rodents, continuous flow of neural progenitors feeds the olfactory bulb, leading to net-growth of this brain region during life [9]. In humans, radioisotope-tracing studies have suggested little neurogenesis during normal human life, but specific brain areas and disease/trauma-induced neurogenesis may be exceptions to this rule [10–14]. Deficient proliferation of somatic stem and progenitor cells is deleterious for tissue maintenance, but also increased proliferation can be harmful and accelerate exhaustion of stem cell pools. Indeed, stem cell quiescence is essential for maintaining functionality and regenerative capacity of stem cell compartment.

Mitochondria are the power plants of the cell and their respiratory chain (RC) provides chemical energy for cells and tissues in the form of ATP through cellular respiration. Decreasing RC function is associated with aging [15]. According to Harman's mitochondrial free radical theory of aging, RC dysfunction is due to oxidative stress within the organelle, leading to accumulation of mitochondrial DNA (mtDNA) mutations, dysfunctional OXPHOS proteins, increased production of superoxide, and a vicious cycle of oxidative stress. This accelerates mtDNA mutagenesis and further deteriorates mitochondrial function [16]. This vicious cycle has been proposed to cause damage to biomolecules and thus disturb cellular function and lead to degenerative changes [16]. MtDNA Mutator mice, carrying a proof-reading deficient mitochondrial DNA polymerase gamma (PolG) and accumulating random mtDNA point mutations, were the first to directly test Harman's hypothesis. Indeed, these mice developed progeroid syndrome with gray hair, osteoporosis, thin skin, anemia, premature cease of fecundity and shortened

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lifespan, signs associated with advancing age [17,18]. However, surprisingly, despite mtDNA mutagenesis, these mice showed little or no evidence for increased reactive oxygen species (ROS) or the proposed vicious cycle. Accumulation of postnatal mtDNA mutations in Mutators was linear instead of exponential, and the original articles describing these mice reported no oxidative damage in their heart, liver or skeletal muscle [17,18].

2. Mitochondrial integrity is essential for maintaining SSC homeostasis

MtDNA Mutator mice did not present with symptoms typical for mitochondrial disease or other mouse models for mitochondrial dysfunction [19]. However, they closely resembled other mouse models with progeria, caused by defects in nuclear DNA repair and previously connected to dysfunction of SSCs [20,21]. This raised the question, whether mtDNA mutagenesis in Mutator mice could affect stem cell homeostasis. Indeed, these mice showed hematopoietic, neural and intestinal stem cell dysfunction [22–25], starting during early fetal development [23], whereas any symptoms from post-mitotic tissues manifested only after 6 months of age [17,18]. Further, the most severely affected tissues were those actively renewing and maintained by somatic stem cells.

The lifespan of Mutator mice is shortened because of severe anemia, which suggested dysfunctional hematopoietic system [22,23,25]. Mutator hematopoietic stem cells (HSC) manifested with many features resembling human HSC aging. They showed progressively decreasing repopulation activity and myeloid bias in differentiation [22,25], similar to other progerias and normally aging mammals [20,21,26]. The HSC defect was cell-intrinsic, as irradiated WT animals recapitulated the Mutator blood phenotype when transplanted with HSCs from Mutator bone marrow [25]. Reconstitution of WT bone marrow with Mutator HSCs led to severe myeloid bias in the recipients. The lineage contribution of transplanted young Mutator HSCs was similar to aged WT HSCs, whereas transplantation of HSCs from mid-aged Mutators resulted in myeloid over-representation beyond what is seen during WT aging [22]. Both erythroid and lymphoid lineages were affected already during fetal period in Mutators, and different hematopoietic progenitor cell (HPC) populations were present in aberrant proportions [23]. Owing to their HSC and HPC dysfunction, Mutators developed at 5–6 months of age progressive and ultimately fatal anemia [18,25], which shared features with human age-related anemia. Age-dependent increase in mtDNA mutation load has been shown to exist in several human tissues, and some reports have proposed increase also in HSCs, suggesting that Mutator findings might be relevant for human anemia [27–29]. Anemia is common in aging humans, and in one third of all cases the etiology remains open [30]. Unexplained anemia among the elderly is often mild and normocytic [26]. This is similar to the incipient anemia in Mutators at the age of six months, suggesting that the mechanisms may be related. In addition to SSC dysfunction, erythroid differentiation was shown to be sensitive to mtDNA mutagenesis [31,32]. During erythrocyte maturation, nucleus and organelles, including mitochondria, are sequentially removed. This removal was recently shown to be disturbed in the Mutators: mtDNA mutagenesis delayed clearance of mitochondria during erythropoiesis, and defective mitophagy was suggested to contribute to this delayed clearance [31,32]. Prolonged presence of mitochondria in erythroid cells skewed timing of iron loading, and led to increased non-protein bound iron accompanied with oxidative damage in Mutator erythroid membranes [31]. As a result of oxidative damage, the aged erythrocytes were prematurely captured and destroyed by the spleen, accompanied with depletion of iron from the bone marrow and leading to fatal anemia [31]. These findings indicated that mtDNA mutagenesis can modify stem cell signaling and function, promoting proliferation over stemness, and also affect erythroid differentiation, leading to asynchrony of mitochondrial clearance and iron loading, all contributing to development of severe Mutator anemia.

Mammalian brain manifests significant changes during aging, despite being one of the organs with the lowest regenerative potential and harboring only negligible numbers of neural stem and progenitor cells (NSCs). However, the few NSCs present in adult brain reside in specific brain regions, like the subgranular zone (SGZ) of the hippocampus, and seem to play a significant role in cognitive functions, by generating new neurons to the brain circuitry throughout life [33,34]. While NSCs are clearly not the sole factor underlying aging in brain and the extent to which age-related cognitive decline depends on NSCs is not clear, it is evident that aging reduces proliferation of NSCs [35,36]. Neurogenesis declines during aging in mice, both in the hippocampal dentate gyrus and in the subventricular zone (SVZ) [35,37], which is evidenced by decreased amount of quiescent nestin-positive neural stem cells (NSCs) in aging SVZ. NSCs were also decreased in number in old Mutators, suggesting decreased NSC quiescence as a result of mtDNA mutagenesis [23]. Mutators did not show general neurodegeneration during their shortened lifespan, but when crossed with *APP/Ld* mice, a well-established model for Alzheimer's disease, mtDNA mutagenesis was shown to exacerbate the AD pathogenesis [38]. These evidence suggest that Mutators are prone to neurodegeneration, but do not manifest it, because of their premature death due to anemia.

Mutator NSCs extracted from E12 embryos showed decreased self-renewal ability *in vitro*, indicating a severe NSC defect already during fetal life [23]. Further, Mutator fibroblasts showed compromised efficiency when reprogramming to pluripotency, and Mutator iPSCs manifested decreased clonality [39]. The dysfunction in NSC self-renewal, as well as the HPC dysfunction and the decreased reprogramming efficiency, were all rescued by treatment with n-acetyl-l-cysteine (NAC), a glutathione precursor and a direct ROS scavenger, suggesting that the stem cell phenotype in Mutators is caused by altered ROS/redox balance [23,39]. Additional evidence pointing to a role for ROS in Mutator phenotype include increased intramitochondrial H_2O_2 in Mutator iPSCs, when measured by a ratiometric MitoB/MitoP probe, as well as in old Mutator tissues; rescue of the Mutator iPSC and HPC phenotype by MitoQ treatment, and rescue of the cardiac phenotype with overexpression of catalase in mitochondria [39–41]. Small intestine of the Mutators, a tissue also dependent on active regeneration, showed morphological changes typical for aged humans and rodents [42]. These changes were consistent by disturbed SSC homeostasis and reduced intestinal stem/progenitor cell cycling [24]. Collectively, these data from Mutator studies (Table 1) strongly suggested that accumulation of random mtDNA point mutations disturbed ROS/redox signaling, leading to small changes in ROS, not high enough to cause significant oxidative damage, and led to SSC dysfunction, which explained the premature aging phenotype in these mice, and connected the cellular mechanism in Mutators to other progeria models, caused by nuclear DNA repair defects.

Different wild-type mtDNA haplotypes have recently been suggested to modify stem cell properties [44]. Mouse ES cells with identical nuclear background, but different mtDNA haplotypes, showed divergent expression profiles of nuclear genes involved in self-renewal, differentiation and mitochondrial function [44]. Further, mtDNA haplotypes also modified *in vitro* differentiation capacity of the ES cells [44]. While these findings could be partially contributed by nuclear-mtDNA mismatch and consequent subtle mitochondrial dysfunction, they

Table 1
Increased mtDNA mutagenesis affects several stem cell compartments in mice.

Cell type	Self-renewal	Proliferation	Differentiation	Reference
Neural stem cells	↓ *in vitro*	↓ *in vitro*	↔ *in vitro*	[23]
Hematopoietic stem cells	↓ *in vivo*	↓ *in vitro*	↓ *in vivo/vitro*	[23,25,31,32]
Intestinal stem cells	↓ *in vitro*	↓ *in vitro*	↔ *in vitro*	[24]
Induced pluripotent stem cells	↓ *in vitro*	↓ *in vivo/vitro*	↓ *in vitro*	[39,43]

raised an interesting question whether apparently neutral mtDNA variants could affect *in vivo* SSC maintenance and function in a genotype-specific manner.

Asymmetric cell division allows stem cells to create two daughter cells with distinct and separate cell fates. In a recent study, human mammary stem cell-like cells were shown to apportion also mitochondria, but not other organelles, asymmetrically during asymmetric cell division [45]. The cells that retained aged mitochondria differentiated, while those receiving mostly young mitochondria maintained stem cell-like properties. Further, inhibition of mitochondrial fission inhibited asymmetric segregation of mitochondria, and resulted in loss of stemness properties in the progeny [45]. These data show that stem cells rely on functional but quiescent mitochondria.

3. Metabolic switch is essential for stem cell function

A subset of adult stem cells remains in a dormant, quiescent state for long periods of time. This actively maintained quiescence is important for long-term functionality of stem cells. Quiescent stem cells have minimal basal metabolic activity, contain only few mitochondria and rely mainly on glycolysis for their energy production [46,47]. Even though mitochondria are few and mitochondrial respiration is low, stem cells contain a functional respiratory chain. Active down-regulation of mitochondrial oxidative phosphorylation seems to be crucial for maintaining SSC quiescence and self-renewal, probably to minimize production of ROS, an active signaling molecule and promoter of differentiation of SSCs [48]. This is consistent with the finding that some quiescent adult stem cells, e.g. HSCs, reside in hypoxic niches [49–53]. In these cells, the low oxygen tension has been reported to be sensed by hypoxia inducible factor-1α (*HIF-1α*), a transcription factor regulating cellular and systemic hypoxia response [54], which has been suggested to regulate cellular quiescence in SSCs by shifting the metabolism to glycolysis [55]. In quiescent HSCs, so called long-term (LT-) HSCs, HIF-1α upregulates pyruvate dehydrogenase kinase (Pdk) activity, leading to decreased pyruvate dehydrogenase activity and shuttling of pyruvate to anaerobic lactate dehydrogenase pathway, instead of being metabolized to acetyl coenzyme A in the mitochondria. HSCs of *HIF-1α*$^{-/-}$ mice were not able to switch to glycolytic metabolism and lost their repopulation activity, i.e. the ability to engraft the bone marrow of an irradiated recipient [46]. Overexpression of *Pdk2* and *Pdk4* in *HIF-1α*$^{-/-}$ HSCs rescued their repopulation ability [46]. Furthermore, in the hematopoietic lineage of mice with conditional knockout of PTEN-like mitochondrial phosphatase (*Ptpmt1*), HSCs could not shift from glycolytic to oxidative metabolism, which led to increased HSC pools and differentiation defects [56]. *Lkb1* kinase, an evolutionary conserved regulator of energy metabolism, was also shown to be important for maintaining HSC quiescence [57–59]. This evidence indicates a crucial role for glycolytic metabolism in stem cell quiescence, and for shift to oxidative mitochondrial metabolism upon commitment, maturation and differentiation.

Similar to SSCs, tight metabolic regulation is also important for pluripotent stem cells. Mitochondrial uncoupling protein 2 (*UCP2*), highly expressed in stem cells and down-regulated during differentiation, has been shown to dictate cell fate decisions in human pluripotent stem cells (hPSC) by influencing the metabolic switch from glycolysis to mitochondrial oxidative phosphorylation [60,61]. Despite its name, the main function of UCP2 seems not to be to uncouple mitochondria, but to regulate the respiration rate by controlling metabolite transport in the organelle [62]. Overexpression of *UCP2* during early differentiation of hPSCs blocked the metabolic shift from glycolysis to respiration and repressed differentiation [61]. UCP2 has also an important role in erythropoiesis, in proliferation of early erythroid progenitors [63]. Erythroid progenitors from *UCP2*$^{-/-}$ mice showed increased levels of mitochondrial superoxide, whereas the cytosolic ROS was decreased, leading to decreased activation of the ERK pathway and thus slow proliferation rate [63]. Induced pluripotent stem cells (iPS cells) generated from Mutator bone marrow cells, harboring increased mtDNA mutation loads, were unable to switch from glycolytic to aerobic metabolism when induced to differentiate to embryoid bodies (EB), resulting in a growth defect in the differentiating EBs [43]. Also Mutator iPS cells showed a growth defect despite WT-like ability to produce ATP [43]. Further, metabolism has been shown to regulate reprogramming of somatic cells to pluripotency. Inhibiting glycolysis reduced reprogramming efficiency and augmenting glycolysis enhanced it in mouse embryonic fibroblasts [64]. During reprogramming process, expression of glycolytic genes preceded expression of genes governing self-renewal, suggesting that the metabolic resetting is an early and active event [64]. All these data support the conclusion that glycolysis and low respiratory activity are important for maintenance of stemness of both somatic and pluripotent stem cells, and oxidative metabolism through redox signaling promotes progenitor commitment and differentiation.

4. Reactive oxygen species as determinants of SSC fate

Cellular ROS production is reflected by the oxidative activity of the cell, the major ROS producer being the mitochondrial respiratory chain. Superoxide, which is readily converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD), is a byproduct of oxidative phosphorylation [65,66]. Unlike superoxide, H_2O_2 is able to cross mitochondrial membranes making it an important signaling molecule [66–68]. ROS levels have been shown to modulate somatic stem cell fate. Increase in ROS production upon aging in human mesenchymal stem cells [69], with concomitant decrease of their regenerative potential and mitochondrial function suggests that mitochondrial metabolism may contribute to SSC aging. When hematopoietic stem cells were extracted from bone marrow and sorted based on their intracellular ROS activity, the ROS^{low} cells had higher self-renewal potential than the ROS^{high} cells, which showed early HSC exhaustion after serial transplantation. The self-renewal of the ROS^{high} cells was rescued by antioxidant supplementation [70]. ROS activity also affected the *in vitro* differentiation capacity of HSCs: ROS^{high} cells showed myeloid bias similarly to aged HSCs [70]. Indeed, ROS are established as differentiation factors for HSCs [48]. In the epidermis ROS has been shown to promote differentiation and certain level of ROS is essential for proper skin function [71]. Conditional knock-out of mitochondrial transcription factor A (TFAM) from the basal layer of epidermis resulted in ablation of respiratory chain, reducing oxygen consumption to minimal and thereby decreasing ROS, which led to enhanced proliferation and severely disrupted differentiation of epidermal stem cells [71,72]. These data show that ROS is a rheostat for stemness and proliferation—low ROS boosts stem cell pool, and increased ROS – even if subtle – promotes progenitor commitment and differentiation.

Functional decline of HSCs during aging has been linked to accumulation of DNA damage in mouse models with defective DNA repair or damage recognition, like the *Atm*$^{-/-}$ or *FoxO1/3/4*$^{L/L}$ mice [20,21,73]. These models show decreased HSC quiescence manifesting as decreased repopulation capacity and a shift towards myeloid differentiation, both being features of aged HSCs [20,73]. Interestingly, also *Atm*$^{-/-}$ and *FoxO1/3/4*$^{L/L}$ mouse models showed increased ROS in their HSCs, and antioxidant (NAC) treatment reversed the HSC phenotype and rescued the repopulation defect [20,73]. In *Atm*$^{-/-}$ mice increased ROS activated p38 MAPK, a member of the mitogen-activated protein kinase family, leading to upregulation of *p16*^{Ink4a} and *p19*^{Arf} [74]. *p16*^{Ink4a} and *p19*^{Arf}, which are transcribed from the same genetic locus, were originally characterized as tumor suppressors. *p16*^{Ink4a} and *p19*^{Arf} are regularly upregulated in senescent cells, where they restrict cells from entering the cell cycle. In a recent GWAS meta-study *INK4a*/ARF was identified as the locus genetically linked to the highest number of different age-associated pathologies [75]. The role of *INK4a/ARF* transcripts in stem cells is suggested to differ from that in committed cells [76]. Increased expression of *p16*^{Ink4a} and *p19*^{Arf} associated with defective self-renewal of HSCs has also been shown in *Bmi-1*$^{-/-}$ mice [77], as well as in WT HSCs with high ROS levels upon aging [70,78,79]. Disrupting

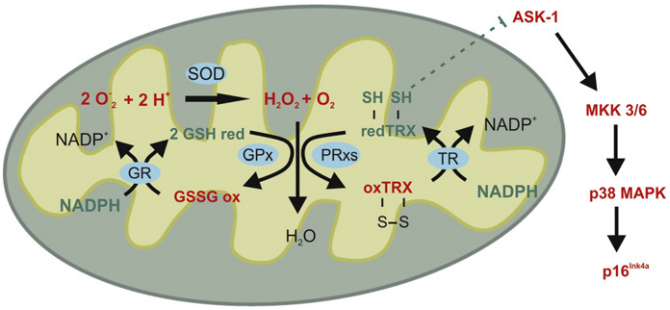

Fig. 1. Schematic representation of mitochondrial ROS defense mechanisms and hypothetical route of p38 MAPK–p16^{Ink4a} pathway activation by increased superoxide production from the respiratory chain. Mitochondrial ROS defense pathways showing decreased molecules in response to ROS in green and increased or activated molecules/proteins in red. Enzymes are highlighted with blue. GR = glutathione reductase, GSH red = reduced glutathione, GSSG ox = oxidized glutathione, GPx = glutathione peroxidase, SOD = superoxide dismutase, PRxs = peroxiredoxins, redTRX = reduced thioredoxin, oxTRX = oxidized thioredoxin, ASK-1 = apoptosis signal-regulating kinase 1, MKK3/6 = dual specificity mitogen-activated protein kinase kinase 3 and 6, p38 MAPK = p38 mitogen-activated protein kinases, p16^{Ink4a} = cyclin-dependent kinase inhibitor 2A.

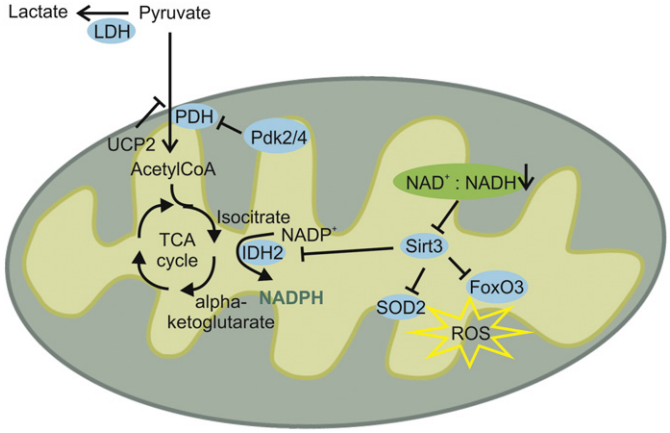

Fig. 2. Possible mechanism by which decreased NAD$^+$/NADH ratio could affect mitochondrial ROS/redox status via Sirt3. Upon decreased NAD$^+$ pool, Sirt3 is unable to deacetylate its targets, leading to weakened ROS defense and decreased NADPH levels. LDH = lactate dehydrogenase, UCP2 = uncoupling protein 2, PDH = pyruvate dehydrogenase, pdk2/4 = pyruvate dehydrogenase kinases 2/4, IDH2 = isocitrate dehydrogenase 2, SOD2 = superoxide dismutase, FoxO3 = forkhead box O3, Sirt3 = sirtuin 3.

negative regulation of *p16^{Ink4a}* and *p19^{Arf}*expression in *Hmga2*$^{-/-}$ mice led to decreased NSC self-renewal, which was partially rescued by simultaneous knock-out of *INK4a/ARF* locus [80]. The importance of the p38 MAPK–p16^{Ink4a} pathway in SSC fate determination has also been shown in NSCs with constitutively active apoptosis signal-regulating kinase 1 (*Ask1*), a serine/threonine mitogen-activated protein kinase kinase kinase (MAP3K5), which is upstream from p38 MAPK [81]. Ask1 is shown to be inhibited by reduced thioredoxin. It is also shown that upon increased ROS, thioredoxin is oxidized leading to autophosphorylation and activation of Ask1 [82,83]. The SSC phenotype in Mutator mice may well be related to induction of Ask1–p38 MAPK pathway by mtDNA mutagenesis.

The sensitivity of the stem cell pool to subtle changes in ROS levels makes SSCs also sensitive to antioxidants. While n-acetyl-l-cysteine treatment rescued both the NSC and HPC phenotypes in mtDNA Mutator embryos *in vivo* [23], treatment with mitochondria-targeted ubiquinone (MitoQ) had contradictory effects on SSCs, and rescued the Mutator HPC phenotype but was harmful to NSCs, both Mutator and wild-type, in the same embryos [39]. MitoQ, a strong antioxidant that accumulates several hundred-fold within mitochondria, was more potent than NAC in ameliorating self-renewal of Mutator stem cells *in vitro* but showed dose-dependent toxicity to both NSCs and iPSCs also *in vitro*, with NSCs being most vulnerable [39]. These data indicate sensitivity of SSCs to ROS, and support the conclusion that redox-linked mechanism is relevant both for SSC dysfunction in mtDNA Mutators and in progeroid mice with genomic DNA repair defects. Mitochondrial ROS defense mechanisms and pathways discussed in this chapter are shown in Fig. 1.

5. NAD$^+$ levels regulate SSC function via mitochondrial sirtuin Sirt3

NAD$^+$/NADH ratio is a major regulator of cellular nutrition status, by activation of sirtuins and mitochondrial biogenesis upon restricted nutrition [84]. This ratio is regulated by metabolic activities. Respiration produces NAD$^+$, when NADH is oxidized by respiratory chain Complex I. Thus the metabolic shift during differentiation, from glycolytic to oxidative mode, is expected to change the NAD$^+$/NADH ratio. In order to maintain the glycolytic flux in stem cells, NAD$^+$ has to be constantly regenerated by conversion of pyruvate to lactate. This reaction uses NADH as a coenzyme and converts it back to NAD$^+$ [85]. NAD$^+$ levels have been shown to modulate both differentiation and self-renewal of neural stem cells. This has been shown by genetically or pharmacologically inactivating nicotinamide phosphoribosyltransferase (*Nampt*), the rate-limiting enzyme in NAD$^+$ salvage pathway. Ablation of *Nampt* in adult mice reduced the NSC pool and proliferation *in vivo* [86]. Both NAD$^+$ and Nampt levels decreased during aging in mouse hippocampus, especially in nestin-positive quiescent neural stem cells [86], as did neurogenesis [87], while supplementation with nicotinamide mononucleotide (NMN), the substrate of Nampt, restored the decrease in nestin-positive neural progenitor cells [86]. The decreasing NAD$^+$/NADH ratio could therefore contribute to stem cell dysfunction during aging [88,89]. Increasing NAD$^+$/NADH ratio is sensed by sirtuins, which are a family of lysine-modifying acylases utilizing NAD$^+$ as a co-factor, while controlling organisms' response to nutrients [90]. Three out of seven known mammalian sirtuins are mitochondrial, including Sirt3, which deacetylates and thus activates several proteins involved in ROS defense [91–93]. Sirt3 is essential in HSC maintenance during aging, and has been reported to enhance superoxide dismutase (SOD2) activity and reduce mitochondrial superoxide [94]. Young *Sirt3*$^{-/-}$ mice had normal HSC function, however during aging the HSC repopulation activity decreased progressively and prematurely, similarly to Mutators [94]. Upregulation of *Sirt3* in aged HSCs, as well as NAC supplementation for *Sirt3*$^{-/-}$ HSCs improved their functionality [94]. Furthermore, defects in Sirt3 target proteins resulted in SSC dysfunction. For example transcription factor forkhead box O3 (FoxO3) contributed to HSC self-renewal and quiescence, as well as for neural stem cell self-renewal and lineage determination [95–97]. In ovaries, FoxO3 regulated primordial follicle activation and *FoxO3*$^{-/-}$ mice showed premature ovarian failure and infertility, whereas overexpression of constitutively active *FoxO3* led to enhanced fertility and postponed onset of menopause in mice [98,99]. Sirt3 deacetylates and thus activates isocitrate dehydrogenase 2 (*IDH2*), a mitochondrial enzyme that converts NADP$^+$ to NADPH [100,101]. IDH2-mediated reduction of NADP$^+$ accounts for one fourth of the mitochondrial NADPH pool suggesting that failure to activate IDH2 would also decrease reduced glutathione and thioredoxin, and therefore weaken the ROS defense system [100]. Sirt3 has been reported to interact with IDH2 in aging-related hearing loss, but no role has yet been reported for it in somatic stem cells [100]. Fig. 2 illustrates pathways by which NAD$^+$/NADH ratio could affect mitochondrial ROS/redox status via Sirt3.

6. Conclusions

Somatic stem cells are highly sensitive to changes in metabolic environment, and metabolic cues guide cellular transitions from quiescence to activation and from self-renewal to differentiation.

Mitochondria are in the center of energy metabolism, but they are also important contributors to sensing and signaling of cellular nutrient and energy status. Mitochondrial theory of aging suggested mitochondrial dysfunction to lead to increased ROS, causing cellular damage and further aggravating the aging process. Recent knowledge has modified this hypothesis. Reactive oxygen species, especially H_2O_2 produced by the mitochondria, as well as NAD+/NADH ratio, are crucial signals for stem cell function. The contribution of mitochondria to aging-related symptoms may therefore be mediated by subtle changes in redox-mediated signaling in SSCs, instead of oxidative damage on tissues. Sensitivity to redox signaling is consistent with pluripotent and somatic stem cells relying heavily on non-oxidative glycolysis, requiring mitochondrial quiescence for maintaining SSC quiescence. This makes SSCs sensitive to antioxidants as well as small changes in OXPHOS activity, rendering mitochondria key organelles to modulate stem cell functions. Thus, despite their highly glycolytic nature, SSCs rely heavily on the integrity of mitochondria and mtDNA to maintain their normal function, explaining why mitochondrial defects in SSCs lead to premature aging-like symptoms.

Author contributions

K.J.A, A.S and R.H.H wrote the manuscript.

Conflict of interest

The authors of "Stem cells, mitochondria and aging" declare no conflict of interest.

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