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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, was 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]

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