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The mitochondria regulation of stem cell aging

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Abstract

Mitochondrial dysfunction and stem cell exhaustion are among the nine separate hallmarks of aging. Emerging evidence however suggests that mitochondrial activity can have a profound influence on the self-renewal and function of stem cells, thus mechanistically linking mitochondrial function and stem cell decline. In this review, we discuss how accumulation of mtDNA mutations or alterations in mitochondrial dynamics, turnover, and signaling can modulate age-dependent stem cell function. Finally, we also describe how mitochondrial substrate utilization influences stem and progenitor activity. Together, this growing body of evidence suggests that modulation of mitochondrial activity might provide a strategy to slow or reverse age-dependent stem cell decline, and potentially, slow or reverse human aging.

Introduction

The molecular basis of human aging remains obscure and no clear theoretical framework or agreed upon hypothesis currently exists. Perhaps the most well established construct is to define the molecular basis of aging through a series of distinct and seemingly universal molecular hallmarks of the process (Lopez-Otin et al., 2013). In the original formulation, this involved the delineation of nine separate hallmarks of aging that appeared to play a causal role in the process. These factors included molecular events such as telomere shortening and cellular senescence. Interestingly, two other delineated hallmarks were mitochondrial dysfunction and stem cell exhaustion. While initially portrayed as separate hallmarks, increasingly, it appears that the ability of the mitochondria to modulate aging might, in part, be driven by a particularly critical role of mitochondrial function in stem cell biology. Emerging evidence suggests that there are multiple aspects of mitochondrial biology that can impact the number and function of stem cells in various tissues. Here, we briefly review how mitochondria can modulate stem cells and how these two previously separate hallmarks of aging might in fact work in concert.

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1. Mitochondria mutation and stem cell aging

Mitochondria possess their own genome (mtDNA), which is present in variable copies per cell. What regulates the mitochondrial copy number in a given cell is not well understood. Of note, while energetic demand likely determines that a beating cardiac myocyte will tend to have more mitochondria per cell than a quiescent endothelial cell, within similar cell types, such as a stem cell and its committed progenitors, mitochondrial mass and respiratory capacity are not particularly well correlated (de Almeida et al., 2017). mtDNA encodes 13 polypeptides of the respiratory chain, 2 ribosomal RNAs and 22 tRNAs that provide the machinery for mitochondria protein translation (Anderson et al., 1981; DiMauro and Schon, 2003). In comparison to nuclear DNA, mtDNA is believed to be more susceptible to DNA damage. The basis for this increased susceptibility has often been ascribed to the notion that the mitochondrial genome is subject to high levels of oxidative damage and lacks histone protection and the full range of DNA repair mechanisms (Alexeyev et al., 2013; Su et al., 2018). However, a significant role for oxidative damage driving mitochondrial mutations is not supported by more recent in vivo evidence. For instance, in *Drosophila* and in mice, deletion of mitochondrial superoxide dismutase (SOD2) or impairing the capacity to repair oxidative-damaged mitochondrial DNA (Itsara et al., 2014; Kauppila et al., 2018a) did not appear to significantly increase mitochondrial mutations. Similarly, while mitochondria lack histones, the transcription factor TFAM may play a similarly protective role (Kaufman et al., 2007; Kukat et al., 2015).

mtDNA mutations accumulate in aged tissues derived from human subjects (Corral-Debrinski et al., 1992; Kraytsberg et al., 2006; Reeve et al., 2009; Shin et al., 2004b; Wang et al., 2001) and animal models (Kujoth et al., 2005; Trifunovic et al., 2004). Initial estimates of the mutational rate for human mitochondrial DNA came from analyzing mtDNA in intestinal crypt cells in patients undergoing surgical resection for colonic tumors (Taylor et al., 2003). This study allowed for an estimated rate of somatic mtDNA mutation rate of approximately 5×10^{-5} mutations per genome per day in humans and suggested that the mutational rate per base pair was roughly 1000-fold higher for mtDNA than it was for nuclear DNA (Taylor et al., 2003). Subsequent analysis using more advanced and sensitive sequencing methods has suggested that the true mutational frequency in humans is likely 1–2 orders of magnitude lower than the initial estimate (Hoang et al., 2016)(Kennedy et al., 2013) (Fig. 1A). In contrast, whether somatic mtDNA mutations increase in aged mice is less clear, as at least some evidence suggests that the age-dependent increase in mtDNA mutations occur predominantly through a heteroplasmic rise of germline mtDNA variants in this species (Ma et al., 2018). It should be noted that mitochondrial mutations involve both point mutations (Greaves et al., 2012; Greaves et al., 2014; Taylor et al., 2003) and large deletions (Bender et al., 2006; Reeve et al., 2009) (Bua et al., 2006; Pallotti et al., 1996). The levels of both forms of mutations appear to increase with age (Kauppila et al., 2017). Again, while deletions are relatively easy to detect, advances in sequencing methodology, including the ability to sequence individual mitochondria, has allowed for a much clearer picture of the level of the harder-to-detect point mutations. These approaches have revealed significant single-nucleotide variant (SNV) heteroplasmy between mitochondria in the same cell (Morris et al., 2017). For mouse mitochondria, this appears to be roughly four SNVs per mitochondria. The physiological importance of this pervasive micro-heteroplasmy is unclear

for either normal cell function or for stem and progenitor cell function. However, accumulation of mtDNA mutations are enriched in human induced pluripotent stem cells (iPSCs) derived from elderly patients when compared to similar stem cells derived from young adults (Kang et al., 2016). Moreover, human circulating hematopoietic stem cells (CD34+ clones) from adult bone marrow donors have significantly more mtDNA heterogeneity when compared to similar cells obtained from embryonic cord blood (Shin et al., 2004a). Interestingly, with the advent of single cell sequencing techniques, these natural mitochondrial mutations may provide a basis for barcoding cells thus providing a strategy to perform highly sensitive lineage tracing analysis in human samples (Ludwig et al., 2019).

The studies described above clearly demonstrate that stem and progenitor cell aging is accompanied by an increase in mtDNA mutations, however, they do not *per se* casually link these mutations to any functional decline in stem and progenitor number or function. This mechanistic gap has been breached by a variety of mouse models. Perhaps the best characterized of these models is the so-called mitochondrial mutator mouse (Trifunovic et al., 2004) (Kujoth et al., 2005). These mice carry a knock-in mutation in the exonuclease domain of the mtDNA polymerase (Polg), leading to severe defects in the enzyme's proofreading ability and the subsequent accumulation of widespread age-dependent mtDNA mutations. A subsequent genetic model involving double stranded mitochondrial breaks has also been described (Wang et al., 2013). The mutator mice were observed to undergo accelerated aging (Kujoth et al., 2005; Trifunovic et al., 2004) (Fig. 1A). These animals also exhibited various stem cell defects including impaired self-renewal ability of neural stem cells (NSCs) and abnormal hematopoiesis (Ahlqvist et al., 2012). Interestingly, while the accelerated aging phenotype of the mutator mice was viewed as ROS independent (Kujoth et al., 2005; Trifunovic et al., 2004), the progenitor cell defects were able to be rescued by N-acetylcysteine (NAC), a widely used antioxidant (Ahlqvist et al., 2012). It should be noted that the level of mitochondrial mutations in these animal models vastly exceeds what is observed in normal physiological aging. Moreover, while these models do develop age-dependent stem and progenitor dysfunction, these defects do not qualitatively mirror what is seen with physiological aging (Norddahl et al., 2011). Indeed, it would appear that mtDNA mutations need to accumulate to significant levels before there is any effect on either overall lifespan or stem cell function (Kauppila et al., 2018b). Except in rare instances, these levels of mitochondrial mutations do not occur with normal human aging and as such, the notion that mtDNA mutation drive stem cell aging is not clearly supported by the available data.

2. Mitochondria fission and fusion and stem cell aging

There is a growing appreciation that mitochondrial dynamics, the balance of mitochondrial fusion and fission, plays an important role in stem cell function (Chen and Chan, 2017). Most evidence points to fission predominating in self-renewing cells (Prieto et al., 2016; Wang et al., 2017), while more differentiated cells have increased levels of mitochondria fusion (Bahat et al., 2018; Forni et al., 2016; Kasahara et al., 2013). Nonetheless, examples exist where the opposite appears to hold (Khacho et al., 2016). Mitochondrial dynamics also plays an important role in maintaining cancer stem cell properties. Analyzing the stem-like cells (SLCs) derived from an immortalized mammary epithelial cell line, it was observed that mitochondrial fission was required to spatially restrict old mitochondria to the

perinuclear region of the mother cell (Katajisto et al., 2015). Furthermore, it was noted that the distribution of mitochondria between daughter cells was asymmetric, with daughter cells that got young mitochondria able to maintain their stemness. In contrast, daughter cells that received old mitochondria went on to be more differentiated (Katajisto et al., 2015). Interestingly, these insights might lead to new mitochondrial-centric strategies to treat tumors (Civenni et al., 2019).

At present, much of the studies regarding mitochondrial dynamics and stem cell fate suffer from an incomplete understanding of the implications of a fused versus fragmented mitochondria. One potential explanation may be that mitochondrial dynamics alter mitochondrial-dependent signaling and can therefore exert lasting effects on cell fate. One example involving neural stem cells suggests that this might be through an ROS-dependent mechanism, where levels of fusion and fission determine ROS levels and the subsequent activation of the redox-dependent transcription factor NRF2 (Khacho et al., 2016) (Fig. 1B). Other examples in pluripotent stem cells suggest the fusion/fission balance alters calcium homeostasis, which in turn activates calcium-sensitive kinases and downstream transcription factors regulating stem cell fate (Zhong et al., 2019). Similarly, levels of the mitofusin 2 (mfn2), a gene required for mitochondrial fusion, appears to effect HSC lineage potential (i.e. the balance between lymphoid versus myeloid differentiation) by regulating calcium levels in stem cells and thereby influencing the subcellular localization and hence activity of nuclear factor of activated T cells (NFAT) (Luchsinger et al., 2016) (Fig. 1B). Interestingly, a requirement for mitofusin activity has also been recently established for the maintenance of Drosophila male germline stem cells (GSCs) (Senos Demarco et al., 2019). In this case, the absence of mitofusin impaired mitochondrial fatty acid oxidation, leading to depletion of GSCs. As such, it would appear that mitochondrial dynamics can modulate stem cell fate through multiple mechanisms including ROS levels, calcium signaling and substrate utilization.

3. Mitophagy and stem cell aging

Mitophagy is the selective degradation of damaged or dysfunctional mitochondria by the autophagy machinery. Reporter mice that allow for the detection of *in vivo* mitophagy have noted that areas in the brain enriched for stem and progenitor cells have significantly high basal rates of mitophagy (Sun et al., 2015). This is presumably because stem cells may require lower levels of ROS and therefore demand higher level of mitochondrial quality control. To what degree *in vivo* mitophagy acts as an important arm of quality control and surveillance (i.e. able to remove damaged but still functional mitochondria) versus acting solely to remove bioenergetically inert mitochondria is presently unclear. However, an intact mitophagy system is increasingly viewed as having important non-cell autonomous functions. This revolves around the ability of mitophagy to suppress the release of mitochondrial-derived damage-associated molecular patterns (DAMPs) that can stimulate the innate immune system (Sliter et al., 2018). Additional work is needed to understand whether this aspect of mitophagy represents it's most important *in vivo* function. If so, this may help to explain the known age–dependent property termed 'inflammaging', since the key regulators of mitophagy appear to decrease in aged tissues (Rubinsztein et al., 2011).

In addition to its growing importance in innate immunity, autophagy and mitophagy appears to play important cell autonomous roles in stem and progenitor cells. In the muscle stem cell, autophagic activity is impaired during aging, leading to defective satellite cell maintenance (Garcia-Prat et al., 2016) (Fig. 1C). Much of this age-dependent muscle stem cell defect was attributed to a decline in mitophagy, leading to the persistence of damaged and defective mitochondria and hence a rise in ROS levels (Fig. 1C). Indeed, treatment with the antioxidant Trolox restored the cell-intrinsic proliferative and regenerative defect seen in these autophagy-compromised aged muscle stem cells. A similar critical requirement for mitophagy has been observed in the hematopoietic stem cell. Genetic disruption of autophagy in HSCs causes accelerated aging phenotypes, including loss of stem cell quiescence and accelerated myeloid differentiation of HSCs (Ho et al., 2017). In the case of HSCs, it would appear that disrupting global autophagy leads to a mitophagic defect and a subsequent alteration in mitochondrial metabolism. These metabolic alterations, in turn, alter metabolites that can modify the epigenetic landscape and modulate stem cell function (Ho et al., 2017). Other studies have implicated mitophagy in HSC self-renewal capacity (Ito et al., 2016).

In the previous example, mitophagy was important to restrain mitochondrial metabolism in HSCs. This fact has been exploited therapeutically with the observation that the NAD^+-boosting agent nicotinamide riboside (NR) can also reduce mitochondrial activity within HSCs by increasing mitochondrial clearance (Vannini et al., 2019). Indeed, this connection between NAD levels and mitophagy may extend beyond the stem cell. In particular, there has been a number of recent studies attempting to boost NAD levels as a means of restoring mitophagy and treating age-dependent diseases. This includes rare accelerated aging syndromes such as Werner's syndrome which appears to have reduced NAD levels and impaired mitophagy (Fang et al., 2019a), as well as more common age-related neurological diseases which are also characterized by insufficient mitophagic flux (Fang et al., 2019b). Finally, another parameter that might intersect with mitophagy is the mitochondrial membrane potential. Mitochondria with low membrane potential might be specifically targeted for mitophagic clearance. Indeed, the classic stimulus to induce mitophagy involves chemical uncouplers that abrogate the membrane potential across the inner mitochondrial membrane (Georgakopoulos et al., 2017). In this context, there are a number of studies that have linked mitochondrial membrane potential to stemness in populations ranging from embryonic stem cells (Schieke et al., 2008), to HSCs (Simsek et al., 2010) to stem-like immune cell populations (Sukumar et al., 2016).

4. UPR^{mt} signaling

The mitochondrial unfolded protein response (UPR^{mt}), trigged by accumulation of misfolded proteins within the mitochondrial matrix, is an evolutionarily protective mechanism to ensure cells survive under condition of stress (Yang et al., 2016; Zhang et al., 2018). While this pathway has been elegantly dissected in *C. elegans*, its overall importance and downstream effectors are considerably less clear in mammalian cells (Tran and Van Aken, 2020). For instance, evidence suggests that certain critical elements of the UPR^{mt} identified in worms do not play a similar conserved function in mammals (Seiferling et al., 2016). With that caveat, it should be noted that several sets of observations suggest a role for

UPR^{mt} signaling in stem cell function. For instance, the transition from a largely quiescent stem cell to a dividing and activated progenitor cell is often accompanied by a burst of mitochondrial biogenesis (Folmes et al., 2012). This increase in mitochondrial number and hence mitochondrial protein synthesis represent a challenge to the mitochondrial quality control pathways, which appear to be specifically engaged when stem cells exit from quiescence (Mohrin et al., 2018). One important aspect of this quality control network involves SIRT7, a member of the sirtuin family of histone deacetylases. Evidence suggests that SIRT7 appears to mediate UPR^{mt} signaling in HSCs (Mohrin et al., 2015). This occurs because of a direct interaction between SIRT7 and nuclear respiratory factor 1 (NRF1). NRF1 represents a master mitochondrial transcription factor (Scarpulla, 2008) and evidence suggests that SIRT7 inhibits aspects of NRF1-dependent transcriptional activation, thereby reducing mitochondrial stress. In particular, in the absence of SIRT7, HSCs had increased constitutive activation of the UPR^{mt}, resulting in reduced quiescence and impaired functional activity of these stem cells (Mohrin et al., 2015). Interestingly, in the HSCs from older mice, levels of SIRT7 are reduced, and augmenting this expression, alleviates the age-dependent defects seen in these cells (Mohrin et al., 2015). Inappropriate or constitutive activation of the UPR^{mt} can also alter stem cell function in other stem cell compartments. For instance, deletion of the mitochondrial chaperone protein HSP60 within intestinal epithelial cells leads to intestinal crypts with impaired stem cell function (Berger et al., 2016).

While the above studies highlight that constitutive or inappropriate UPR^{mt} signaling can impair stem cell function, observations have also suggested that aging may result in a dampen UPR^{mt}. This impaired UPR^{mt} signaling appears to be a result of the natural age-dependent decline in NAD+ levels that occur in a wide range of organisms (Mouchiroud et al., 2013). Remarkably, increasing intracellular NAD+ levels, by treating animals with nicotinamide riboside, improved the functional properties of a wide range of mouse stem cells (Zhang et al., 2016). This effect was demonstrated to occur, at least in part, through the ability of NR to stimulate UPR^{mt} signaling (Zhang et al., 2016). As such, either too little or too much UPR^{mt} signaling appears to contribute to age-dependent stem cell defects.

5. Mitochondrial Fatty acid oxidation (FAO)

Mitochondrial substrate utilization is emerging as an important aspect through which stem cell fate and function can be modified. Perhaps the best-studied example involves the role that mitochondrial fatty acid oxidation (FAO) plays in modulating stem cell activity. Mitochondrial metabolism of long chain fatty acids requires the sequential activity of the mitochondrial outer membrane protein carnitine palmitoyltransferase-1 (CPT 1) and the activity of the mitochondrial inner membrane protein carnitine palmitoyltransferase-2 (CPT 2) to allow fatty acid transport across the double mitochondrial membrane and subsequent β-oxidation and ATP generation (Lundsgaard et al., 2018). It has been observed that maintenance of neural stem/progenitor cells (NSPCs) quiescence requires high levels of FAO with high expression of CPT1a. Treating NSPCs with the CPT1 inhibitor Etomoxir inhibits FAO leading NSPCs to exit the quiescence state and undergo apoptosis (Knobloch et al., 2017). Genetic inactivation of CPT1a in adult NSPCs also lead to decreased NSPCs expansion and enhanced NSPCs cell death. Moreover, mice administrated malonyl-CoA, an

endogenous inhibitor of Cpt1a, promoted NSPCs to exit quiescence (Knobloch et al., 2017) (Fig. 2A). This combination of genetic and pharmacological approaches are important, as the use of etomoxir alone to disrupt FAO has led to potentially misleading conclusions (Divakaruni et al., 2018; Nomura et al., 2016; Raud et al., 2018).

In the context of the HSC, FAO appears to play an important role in stem cell maintenance as well as stem cell function. The ligand-activated transcription factor peroxisome proliferator-activated receptor δ (PPARδ) is the predominant mediator of FAO signaling in HSCs. Evidence suggests that genetic deletion of PPARδ, or treatment with etomoxir, leads to a reduction in FAO and a decrease in HSC repopulating ability (Ito et al., 2012). In contrast, activation of PPARδ with synthetic ligands that presumably mimic endogenous fatty acids, was shown to result in improved HSC function (Ito et al., 2012). Interesting, blocking FAO using etomoxir or PPARδ deletion also modulated HSC function, particularly the decision for an HSC to undergo symmetric (i.e. divide into two committed daughter cells) or asymmetric (give rise to one stem cell and one committed cell) division (Fig. 2B). Using single cell assays, it was demonstrated that FAO is necessary to maintain the requisite percentage of asymmetric divisions. These asymmetric division are, in turn, needed to regenerate and maintain the HSC pool and hence maintain bone marrow repopulation ability (Ito et al., 2012).

The role of FAO also extends to intestinal stem cells (ISCs). Recent evidence suggests that short-term acute fasting augments the organoid-forming capacity of ISCs in both young and aged mice, in part, by inducing a fatty acid oxidation (FAO) program. Interesting, in ISCs, fasting strongly induced expression of CPT1a, the rate-limiting enzyme in FAO. Inactivating CPT1a in the intestine led to reduced primary and secondary organoid formation of crypts, as well as decreased ISC number and long-term ISCs maintenance (Mihaylova et al., 2018). Moreover, the diminished regenerative capacity and number of aged ISCs appears to be responsive to manipulation of the FAO pathway. For instance, when aged mice were treated with GW501516, a potent transcriptional PPARδ activator, there was an induction in FAO and an increase in crypt Lgr5+ and Olfm4+ ISC/early progenitors (Mihaylova et al., 2018) (Fig. 2C).

Conclusion:

While initially viewed as separate hallmarks, the growing intersection between mitochondrial activity and stem cell function has clearly contributed to our overall understanding of aging. To what degree either a decline in mitochondrial function, or an impairment in stem cell function, actually contributes to human aging, awaits further validation. For many years, studies of mitochondrial dysfunction in human aging were confined largely to post-mitotic tissues such as skeletal muscle (Short et al., 2005). However, as highlighted here, the molecular impact of mitochondria on the aging process has focused increasingly on the stem and progenitor cell population. Our understanding of these processes remains woefully incomplete and questions remain as to which of the various properties of the mitochondria are the most critical for maintaining stem cell function in the aged organism. While we reviewed aspects of mtDNA mutations, dynamics, turnover, signaling and substrate utilization, other properties of mitochondria including mitochondrial

biogenesis, calcium handling and ROS generation are also clearly relevant to stem cells. Nonetheless, an emerging picture has evolved in which the properties of stem cells, including their age-dependent maintenance and function appears to be actively modulated by the quality and function of intracellular mitochondria. Further refinement of these insights will undoubtedly lead to novel approaches to improve age-dependent stem cell decline and potentially, to slow or reverse human aging.

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Highlights

- Mitochondrial function modulates stem cell function
- Age-dependent decline in mitochondria might contribute to stem cell dysfunction
- Mitochondrial dynamics, signaling, quality and substrate utilization effect stem cells

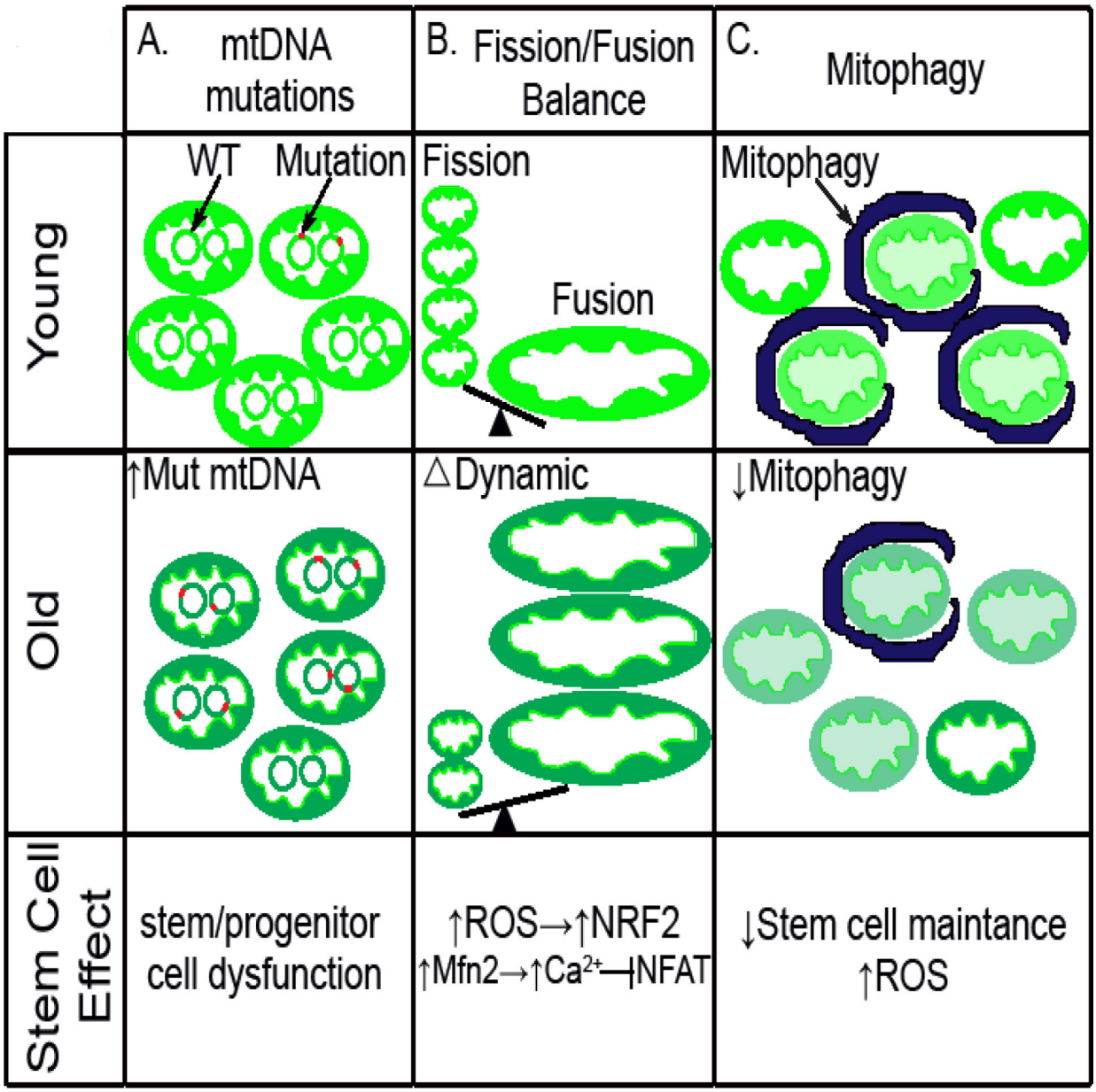

Figure 1.

Mitochondrial DNA mutation and dynamics have a broad effect in stem cell aging. (A) mtDNA mutations that accumulate as stem cell age may partially contribute to dysfunctional stem/progenitor cells. (B) The mitochondrial fission/fusion balance go to the side of fusion as stem cell aged, leading to increased ROS mediated by NRF2 as well as altered Mfn2/Ca^{2+}/Nfat signal cascade. (C) Mitochondrial quality is less controlled due to decreased mitophagy in old stem cells, leading to decreased stem cell maintenance, increased ROS levels and potential immune activation.

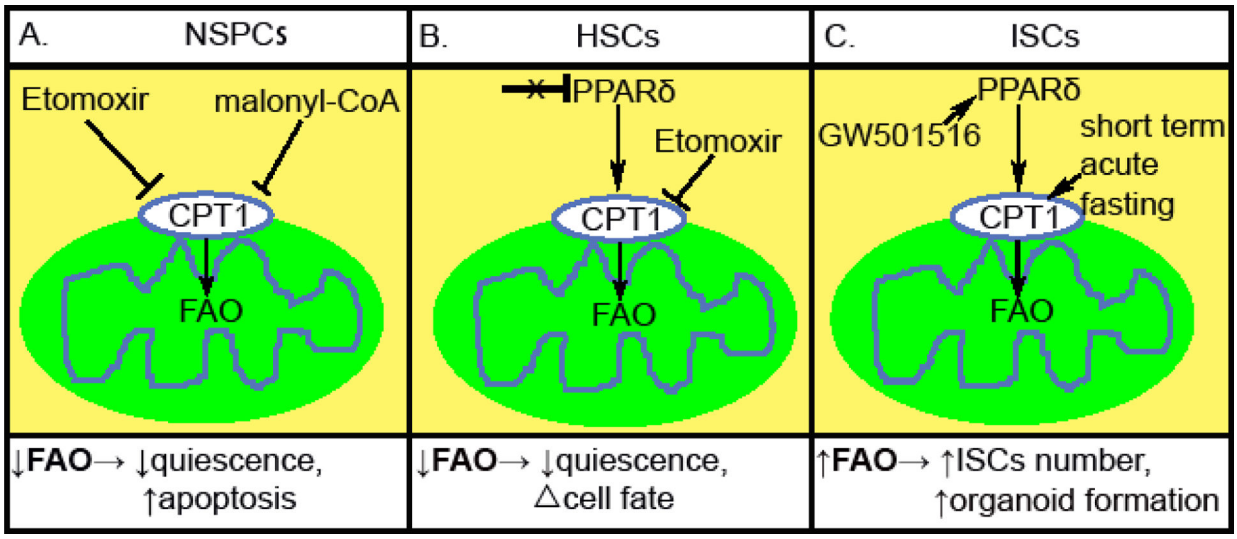

Figure 2.

Modulating fatty acid oxidation (FAO) impacts tissue-specific stem cell function. (A) Inhibiting CPT1 by either Etomoxir or malonyl-CoA lead to decreased FAO in NSPCs, leading to reduced quiescence and increased apoptosis. (B) Inactivation of PPARδ or inhibiting CPT1 by Etomoxir lead to reduction of FAO in HSCs. This triggers an exit from quiescence and an alteration in symmetric versus asymmetric commitment. (C) Short term acute fasting or GW501516 treatment leads to activation of FAO in ISCs, and a subsequent increase in crypt Lgr5+ and Olfm4+ ISC/early progenitors, as well as crypt organoid-forming capacity.