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Mitochondria as biological targets for stem cell and organismal senescence

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ABSTRACT

Organismal aging is impacted by the deterioration of tissue turnover mechanisms due, in part, to the decline in stem cell function. This decline can be related to mitochondrial dysfunction and underlying energetic defects that, in concert, help drive biological aging. Thus, mitochondria have been described as a potential interventional target to hinder the loss of stem cell robustness, and subsequently, decrease tissue turnover decline and age-associated pathologies. In this review, we focused our analysis on the most recent literature on mitochondria and stem cell aging and discuss the potential benefits of targeting mitochondria in preventing stem cell dysfunction and thus influencing aging.

1. Introduction

1.1. Stem cells and aging

Aging is a natural, dynamic, and multi-factorial process characterized by a progressive decline in tissue integrity and function that, ultimately, enhances vulnerability toward diseases (i.e., cardiovascular and neurodegenerative diseases, diabetes, cancer, etc.). At the cellular and molecular levels, genomic instability, telomere shortening, epigenetic changes, nuclear and mitochondrial DNA damage, mitochondrial dysfunction, and cellular senescence, are some of the most well-described and intricately correlated factors involved in the biological process of cellular aging (Belsky et al., 2018; Grigoryan et al., 2018; López-Otín et al., 2013; Vizioli et al., 2020). However, chronic inflammation and stem cell exhaustion are viewed as particularly relevant in the context of senescence in aging tissues (Lee and Yu, 2020).

Adult stem cells (ASCs), or tissue-specific stem cells, constitute a rare population that resides in most organs in an undifferentiated state. Their main property is the ability to differentiate into mature cells of the native tissue and thus replace injured and aged cells throughout the lifespan of the organism, all the while perpetuating themselves through self-renewal. Small pools of ASCs have been identified in the adult human organism, including muscle (satellite cells), intestinal, skin, neural (NSCs), hematopoietic (HSCs) stem cells as well as mesenchymal stem cells (MSCs). In this review we will focus on the latter.

MSCs are adult stem cells that reside in multiple tissues, including the bone marrow, umbilical cord, adipose tissue, skin, and lungs (Meirelles et al., 2006). In addition to their already widespread distribution, these cells are also not exclusively dedicated to maintaining the homeostasis of their native tissue, on account of being able to migrate into injured tissues throughout the body and differentiate and/or provide some paracrine benefits (Jeschke et al., 2019; Liu et al., 2014). Unlike other stem cells, MSCs have been presented as useful tools in regenerative medicine, as, besides less stringent ethical concerns, they commit to several cell lineages (bone, cartilage, adipose tissue, muscle, tendon, and neuronal cells), and control disease-associated mechanisms, including apoptosis, inhibition of inflammation and activation of tissue-resident stem cells (Han et al., 2019; Wu et al., 2020; Uccelli et al., 2008).

1.2. Stem cell aging

Like most adult cells, aging ASCs undergo adverse physiological, molecular, and functional transformations, due to the accumulation of genetic and epigenetic changes which can include telomere shortening, loss of proteostasis, and growth arrest (Bonab et al., 2006; López-Otín et al., 2013; Neri and Borzì, 2020). Consequently, these cells will exhibit defective immunomodulatory and migratory capacity, changes in their secretory profile, in addition to reduced self-renewal, differentiation, and proliferation, as well as reduced DNA synthesis and repair efficiency

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(Goodell and Rando, 2015). These modifications, in turn, compromise the functional competence and dwindle stem cell niche and stem cell reserve (López-Otín et al., 2013; Neri and Borzì, 2020; Sun et al., 2021). However, cellular changes are not restricted to the nucleus, and mitochondria play a crucial role in the functional degeneration of ASCs with aging (Wan and Finkel, 2020).

1.3. Mitochondria and stem cell aging

Mitochondria are major regulators of stem cell self-renewal, multilineage differentiation, and fate determination (Ito and Ito, 2016; Correia et al., 2022). Defects in the oxidative phosphorylation system compromise the biosynthesis of intermediates for cell growth and intensify the production of oxygen-free radicals that harm nuclear and mitochondrial DNA (mtDNA) and drive mitochondrial dysfunction (Kim et al., 2018a, 2018b, 2018c; Vizioli et al., 2020). Mitochondrial dysfunction - and underlying energetic defects – in turn, mediates cellular decline that drives biological aging. As a result, senescent ASCs display malfunctioning mitochondria, with altered structures dynamics and activity (Kim et al., 2018a, 2018b, 2018c; Vizioli et al., 2020; von Zglinicki et al., 2021).

Although some mitochondrial proteins are encoded by nuclear genes, mitochondria also possess their own genome (mtDNA), responsible for encoding 13 proteins involved in oxidative phosphorylation (OXPHOS) (Kim et al., 2018a, 2018b, 2018c; von Zglinicki et al., 2021). Due to its location, close to the electron transport chain (ETC), and lack of protective packaging or repair mechanisms, mtDNA is particularly sensitive to oxidative damage and reactive oxygen species (ROS) (Schuliga et al., 2018). Therefore, alterations in the methylation patterns and mutations in the mtDNA in aging can compromise mitochondrial membrane potential, energy metabolism, and mitochondria regulatory pathways (Kornicka et al., 2017). A study carried by Sun et al. (2022) revealed that, in aged MSCs, energy metabolism and mitochondrial genes were downregulated. Another study showed that improving mitochondrial function (through photobiomodulation) could rejuvenate these cells (Eroglu et al., 2021).

Mitochondrial dysfunction during the aging process can also occur through the DNA damage response (DDR), by means of telomere-dependent or independent DNA damage. DDR activates the complex I of the mammalian target of rapamycin (mTORC1), triggering mitochondrial biogenesis (increased mitochondrial mass) and a senescent phenotype through PGC-1β activation (Gureev et al., 2019; Kim et al., 2018a, 2018b, 2018c; Rath et al., 2021; Vizioli et al., 2020). The competitive binding, and subsequent phosphorylation, of p53 by both mTOR complexes (mTORC1 and mTORC2) have also been recently linked to the activation of senescence pathways and to the inhibition of autophagy/mitophagy (Vizioli et al., 2020; von Zglinicki et al., 2021). Loss of mitochondrial membrane potential (MMP) caused by proton leak, a decrease in oxidative phosphorylation and ATP production, as well as elevated ROS generation are also important hallmarks of mitochondrial dysfunction in senescent cells (Kim et al., 2018a, 2018b, 2018c; von Zglinicki et al., 2021). High levels of ROS accelerate telomere shortening, thus further enhancing DNA damage and the DDR-dependent activation of senescent pathways, exacerbating stem cell and tissue aging in a vicious cycle (Ogrodnik et al., 2017; Rath et al., 2021).

In sum, the accumulation of mtDNA damage and ROS in aging MSCs promotes mitochondrial dysfunction and fragmentation, that exacerbates the senescent phenotype and negatively impacts MSC differentiation potential and thus translational relevance (Han et al., 2019; Liu et al., 2020).

1.4. How aged ASCs contribute to aging

The number of stem cells gradually declines with successive replication and age and the accumulation of aged stem cells negatively impacts neighboring cells, by promoting paracrine senescence that induces inflammation (Cárdenes et al., 2018). This contributes to the deterioration of tissue turnover mechanisms that can culminate in age-related diseases (Sharpless and DePinho, 2007). Therefore, the maintenance of resilient ASC populations is a feature of healthy aging, whilst ASC senescence represents a major risk factor for the already reduced number of existing stem cells in the adult organism. In fact, some animal species, such as planarians, can regenerate their whole body, mainly due to a large population (30 %) of ASCs whose ability to regenerate greatly restricts aging (Iglesias et al., 2019).

2. Targeting aging using MSCs

An increasing number of studies, some later developed into clinical trials, emphasize the potential importance of stem cells, and MSCs in particular, as anti-aging agents, particularly in terms of reducing inflammation and maintaining mitochondrial health (Table 1). A clinical trial on frailty syndrome (mediated by increased inflammation and stem cell depletion) has revealed notable improvements in physical performance and inflammatory biomarkers after allogenic stem cell infusion (Tompkins et al., 2017). Another study showed that adipose-derived MSCs postponed the aging process of a POLG progeroid mouse model by promoting abnormal mitochondrial clearance (mitophagy) that contributed to metabolic homeostasis, and reduced ROS levels in the organism (Lv et al., 2021). Likewise, umbilical cord MSC-conditioned medium has been considered a promising tool for skin anti-photoaging (Zou et al., 2022).

The anti-aging properties of MSCs have already been extensively described in several review papers (see Zarei and Abbaszadeh, 2019; Lee and Yu, 2020; Boulestreau et al., 2020 for a few examples), and so, this review aims to 1) summarize current MSC anti-aging strategies that modulate mitochondrial function in the target tissue; and 2) describe current novel studies that target MSC mitochondria as a global anti-aging strategy (Fig. 1). Table 1 summarizes MSC anti-aging strategies through regulating mitochondrial function in the target tissue, either by directly modulating tissue mitochondria in situ or by transferring healthy MSC mitochondria to the target tissue.

2.1. MSC anti-aging strategies to rejuvenate dysfunctional aged stem cells through mitochondria

New strategies have emerged with the purpose of targeting mitochondrial rejuvenating strategies as a way of deterring MSC aging and preserving tissue turnover. As previously stated, mitochondrial dysfunction influences each of the hallmarks of aging in a bidirectional way (Berry and Kaeberlein, 2021). Accordingly, each of these hallmarks have been the focus of multiple anti-aging research studies that aspire to rescue stem cell function. We will describe current studies that target MSC mitochondria as a global anti-aging treatment (Fig. 2).

2.1.1. Tackling oxygen free radicals

ROS are considered one of the main contributing factors to adult stem cell aging (Harman, 1972). Most ROS form as a by-product of mitochondrial respiration and can be ascribed to, at least, seven different mitochondrial sites, with complexes I and III being the most susceptible to electron leakage, and thus the most extensively studied. During OXPHOS highly reactive molecules, such as the superoxide anion ($O_2^{-}\bullet$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (•OH), are formed, and will cause damage to a range of cellular constituents - including lipids, proteins, and nucleic acids – contributing to accelerated aging. As such, it is pivotal to create an effective oxidative defense. One example of such defense emerges with the importance of the stem cell niche is determining MSC metabolic state. Stem cells mostly rely on anaerobic glycolysis, over mitochondrial OXPHOS, to meet energetic requirements and avoid ROS production; thus, promoting a hypoxic environment can protect the cells against oxidative damage (Moniz

Table 1
MSCs studies in anti-aging strategies focusing on mitochondria manipulation or transfer.

Purpose	Model	In vitro/in vivo	Study findings	Source
Explore the effect of menstrual blood-derived mesenchymal stem cells (MenSCs) and their mitochondria on ovarian function in aged mice	Ovaries of aged mice treated with MenSCs or their mitochondria	in vivo	"Mitochondrial-related genes were enriched in aged ovaries in both treatments; - follicular state was improved"	(Zhang et al., 2022)
Evaluate aging parameters after MSC infusion	Aged C57BL/6 mice	in vivo	"Mouse hair became shiny and dense, and the symptoms of bladder overactivity were relieved; - histopathological changes in skin, bladder, liver, and lung were apparently improved"	(Liu et al., 2022)
Evaluate the effect of MSCs co-culture in senescent cells	IMR-90 senescent cells	in vitro	"Expression of inflammatory agent IL6 was decreased; - the expression of growth factors was increased; - the number of mitochondria and the telomere length were increased with MSC treatment"	(Liu et al., 2022)
Evaluate the effect of MSCs infusion in muscle performance	Senescence-accelerated mouse prone 10 (SAMP10) mice	in vivo	"UC-MSC treatment ameliorated muscle mass loss and improved physical performance; - improved muscle mitochondrial biogenesis (mediated by AMPK-PGC1-α axis); mitigated aged muscle inflammation which was translated into an improvement in sarcopenia-related skeletal muscle atrophy and dysfunction."	(Piao et al., 2022)
Injection of MSCs to treat pelvic organ prolapse (POP)	Rat model of POP	in vivo	"MSCs downregulated Mfn2 expression and increased the expression of procollagen1A1/1A2/3A1 in the uterosacral ligament of POP rats"	(Wang et al., 2022)
Evaluate the effect of H_2O_2-primed pericyte-derived EVs in disused muscles of old mice	Aged muscle mice	in vivo	"Pericytes-derived EVs recovered skeletal muscle fiber size and extracellular matrix remodeling in aged mice after skeletal muscle disuse."	(Wu et al., 2022)
Explore ovarian function after MSC infusion	Rat model of premature ovarian insufficiency (POI)	in vivo and in vitro	"hUCMSCs restored the ovarian function; - apoptosis of theca interstitial cells was reduced through regulating NR4A1-mediated mitochondrial mechanisms"	(Luo et al., 2022)
Evaluate allogeneic stem cell therapy in a DNA polymerase gamma (POLG) knockin mice (with mitochondrial dysfunction)	Progeria animal model	in vivo	"Adipose-MSCs therapy can improve alopecia and kyphosis by promoting mitophagy; allogeneic stem cell therapy can improve aging-related symbols and phenotypes through mitochondrial quality control"	(Lv et al., 2021)
Evaluate anti-aging effect of ADSC in a co-culture system with MEFs (mouse embryonic fibroblast cells)	MSCs isolated from the adipose tissues of C57BL/6 mice	in vitro	"Replicative senescence of MEFs was postponed by promoting mitophagy, which eliminated intracellular ROS and improved the quality of mitochondria; - metabolic homeostasis was transformed from catabolism to anabolism"	(Lv et al., 2021)
Explore healthy mitochondria transfer from fetal to adult MSCs to reverse aging by using an automated optical tweezer-based micromanipulation system	Fetal and adult MSCs	in vitro	"Increase the antiaging and metabolic gene expression in the adult MSC - mitochondrial transfer from young cells could contribute to cell proliferation and metabolic rejuvenation"	(Shakoor et al., 2021)
Evaluate the effect of MSC administration in ovarian reserve	Age-related diminished ovarian reserve (AR-DOR)	in vivo	"Ovarian function, the number of follicles and the quality of oocytes were improved; - apoptosis of granulosa and stromal cells was repressed; - Ampk, FoxO3a signaling and Sod2 were increased"	(Liu et al., 2021)
Evaluate ovarian function after MSC infusion	Natural aged mice and rhesus monkeys	in vivo	"Increased follicle number, improved oocyte quality, enhanced ovarian mitochondrial function, inhibited cell apoptosis; - in non-human primates the number of follicles were higher than in control, aged, group"	(Wang et al., 2021)
Administration of ghrelin-preconditioned human MSCs or in combination with nicotinamide-mononucleotide (NMN) in aged hearts	Aged heart rats subjected to IR injury	in vivo	"Reduced infarct size and cardiotroponin release of aged myocardium, and improved cardiac function; - restored IR-induced mitochondrial reactive oxygen species and membrane potential depolarization and enhanced ATP production; - increased autophagy"	(Sun et al., 2021)
Evaluate the effect of pigallocatechin-3-gallate preconditioned adipose-derived stem cells injected into 20-month-old Wistar rats	Aging rat brain	in vivo	"Enhanced cell survival via the p-Akt pathway and improved mitochondrial biogenesis via the SIRT-1 pathway; - increased neurotrophic factor and the antioxidant activity"	(Hsieh et al., 2020)
Evaluate the skin rejuvenating effect of hMSC-conditioned media in combination with niacinamide after laser therapy	Human	randomized controlled Trial	"Reduced wrinkles and hyperpigmentation in the aging skin"	(Lee and Yu, 2020)

et al., 2022). However, although antioxidants have been shown to also benefit the lifespan of small animal models and metabolically compromised animals, its effectiveness in terms of extending the lifespan of bigger organisms, such as humans, has yet to be demonstrated (Bhullar and Hubbard, 2015).

Sirt3, a mitochondrial matrix deacetylase, was shown to attenuate ROS-induced injury and inhibit MSC senescence by upregulating manganese superoxide dismutase (SOD2), the principal antioxidant molecule that scavenges mitochondrial superoxide (Ma et al., 2020). These effects were dependent on the translocation of the Forkhead box O3a (FOXO3a) to the nucleus, following Sirt3 deacetylation, and the consequent activation of catalase (CAT) and SOD2 genes. Alternatively, CAT and SOD2 inhibition was shown to block the anti-aging effect of Sirt3 transfection in human MSCs (hMSCs) collected from old donors (Zhang et al., 2020). By contrast, Sirt3 overexpression in aged hMSCs enhanced the survival rate after cell infusion into the heart (via FOXO3a-CAT and SOD2 pathway), and improved cardiac function and angiogenesis, thus decreasing infarct size, collagen content and expression levels of matrix metalloproteinase 2 (MMP2) and MMP9 upon myocardial infarction (Zhang et al., 2020). FOXO activity is positively regulated by AMPK - which is sensitive to AMP/ATP ratio and function to restore energy levels - and negatively regulated by PI3K-AKT signaling.

Another recent approach to tackle free radicals in this context is the combination of resveratrol (a Sirtuin 1 activator, see below) and 5-

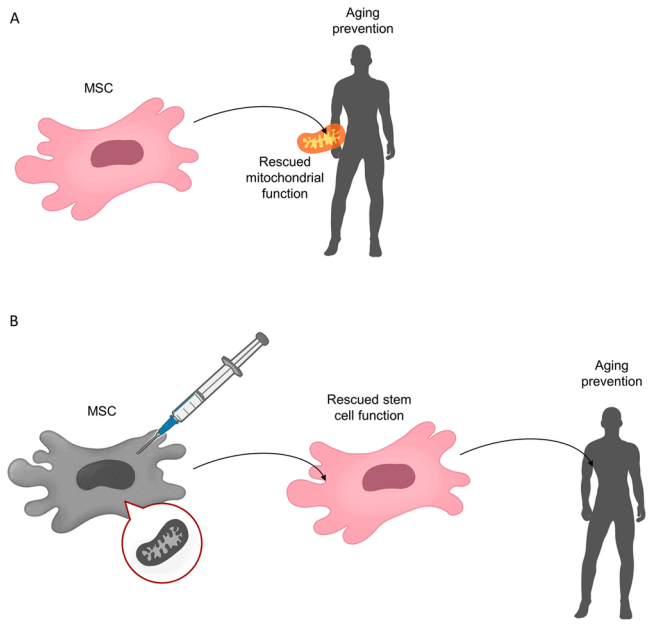

Fig. 1. Targeting aging through MSCs. A) Anti-aging strategies that modulate mitochondrial function in the target tissue; B) Therapies targeting dysfunctional mitochondria of aged MSCs as a global anti-aging treatment.

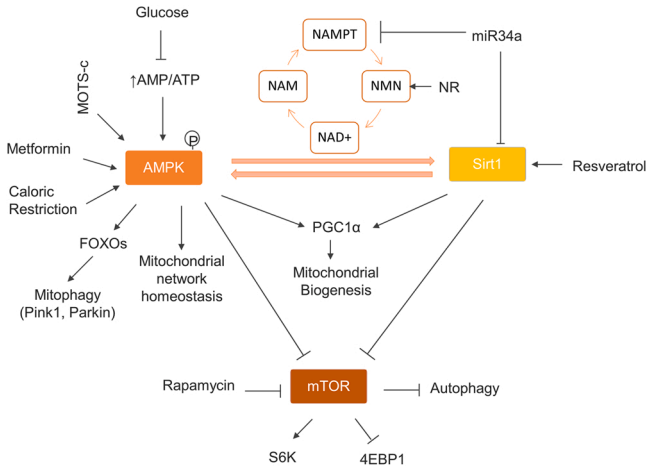

Fig. 2. Signaling pathways targeted by anti-aging strategies through mitochondria. The activation of AMPK and Sirt1 as well as the inhibition of mTOR signaling influence mitochondrial dynamics and promote mitochondrial biogenesis (through PGC1α) and homeostasis. The activation of AMPK by NAD+, increased AMP/ATP ratio, molecules such as MOTS-c and metformin as well as nutritional based interventions, improve mitochondrial homeostasis and inhibit mTOR. Sirtuin 1 (Sirt1) activation, by molecules such as resveratrol or by the inhibition of miR34a, can activate AMPK and inhibit mTOR. The inhibition of mTOR, by rapamycin for example, will promote dysfunctional mitochondrial clearing, activate autophagic processes, impede the activation of senescence pathways, etc. Abbreviations: AMP-activated protein kinase (AMPK), Sirtuin 1 (Sirt1), mammalian target of rapamycin (mTOR), Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha, Nicotinamide adenine dinucleotide (NAD+), Adenosine monophosphate/Adenosine triphosphate (AMP/ATP).

azacitidine, that were described to significantly reverse senescence in MSCs, by increasing mitophagy through upregulation of proteins implicated in mitochondrial quality control, namely Pink and Parkin (Kornicka et al., 2019). While mitophagy – the process by which damaged mitochondria are recycled – is reported to be pro-longevity, mitochondrial fragmentation – that also involves fission – has been linked to aging (Amartuvshin et al., 2020). It remains to be determined in which context fission represents a pro-aging or pro-longevity process. For instance, in aged Drosophila ovarian germline stem cells a shift of mitochondrial dynamics toward fusion, by Drp-1 deletion, prevented cell loss (Amartuvshin et al., 2020). Alternatively, a study carried out in C. elegans, revealed that a specific balance in mitochondrial dynamics (driven by both fusion and fission processes), and network remodeling coordination that are required to the pro-longevity action promoted by AMPK and dietary restriction (Weir et al., 2017). Moreover, authors reported that aged flies fed with rapamycin, known to stimulate the clearing of dysfunctional mitochondria (via inhibition of mTORC1), also prevented cell loss (Amartuvshin et al., 2020). In accordance, mitochondrial-derived peptide MOTS-c, an AMPK activator, enhanced the quality of aged MSCs, reduced the aged phenotype by activating AMPK and inhibiting mTORC1, and enhanced mitochondrial homeostasis by decreasing oxygen consumption rates, ROS levels and lipid synthesis (Yu et al., 2021).

2.1.2. *Targeting sirtuins*

The Sirtuin protein family consists of seven members allocated to distinct subcellular sites. Each sirtuin (NAD^+-dependent class III histone deacetylases) plays a key role in stem cell function particularly due to its importance in metabolism and due to its deacetylase activity. In addition, sirtuins interact with some of the most important molecules in aging: AMPK, mTOR, FOXO and insulin/IGF-1 signaling (Zhao et al., 2020). Complementarily, some authors consider Sirt1 and Sirt3 as key contributors for the health benefits associated with caloric restriction.

NAD^+, a sirtuin activator, is a relatively dynamic and abundant element in most cells, implicated in more than 500 metabolic reaction and its levels have been shown to decline with age. In accordance boosting NAD^+ improves mitochondrial function and decreases DNA damage (Rajman et al., 2018). During low cellular energy conditions, NAD^+ acts by activating sirtuins to induce the re-establishment of energy homeostasis. As Nampt is involved in NAD^+ biosynthesis, it also indirectly modulates Sirt1 deacetylase activity. The decrease in the abundance of acetylated (inhibited) PGC-1α, a regulator of mitochondrial biogenesis, is one of the most relevant targets of Sirt1. Its reactivation through the PGC-1α/AMPK signaling pathway is responsible for maintaining metabolic homeostasis by preventing oxidative stress and for abolishing senescence-induced MSC dysfunction (Li et al., 2018). For these reasons, compounds that activate sirtuins and improve mitochondrial biogenesis have been valued as promising anti-aging factors. In this context, it has been shown that miR-34a expression levels positively correlate with aging in several human and rodent aged organs (including the lung, heart, liver, kidney, and brain), and its pro-senescent role has been linked to mitochondrial dysfunction and Sirt1 repression (Zhang et al., 2015). Overexpression of miR-34a in young MSCs induced a senescent-like phenotype that could be rescued by Nampt restoration (Pi et al., 2021). Inhibition of miR-34a, in turn, improved proliferation in aged MSCs, suppressed cellular senescence markers (P53, P21, and P16) and enhanced anti-senescence markers (SIRT1, HTERT and CD44) (Mokhberian et al., 2020). Nampt was also identified as a direct target gene of miR-34a, as miR-34a led to a drop in Nampt expression levels, lowered the NAD^+/NADH ratio, and decreased Sirt1 activity in naturally aged and senescent MSCs (Pi et al., 2021). Another Sirt1 activator, the SRT1720, was found to protect aged MSCs from apoptosis, to influence their engraftment into an infarcted nonhuman primate heart, and elevate mitochondrial respiratory capacity, mitochondrial membrane potential and mitochondrial biogenesis (Zeng et al., 2021).

Sirt3 primarily localizes in the mitochondria, where it influences ^{mt}ROS scavenging and ^{mt}ROS homeostasis, via SOD2 deacetylation. Sirt3 is downregulated in in vitro aged MSCs, and a wound-healing assay carried out in mice revealed that its activation, triggered by EphB2/Nrf-2 signaling, delayed the progression of MSC senescence while enhancing its therapeutic function (Jung et al., 2017). Additionally, Sirt3 was

found to target SOD2, IDH2, SDHA, NDUFA9, and GLUD1 (Ahn et al., 2008; Finley et al., 2011; Someya et al., 2010); mitochondrial metabolism and stress-controlling genes involved in mitochondrial quality control processes, which include restoration/degradation of misfolded proteins and regulation of mitochondrial dynamics (mitophagy and mitochondrial biogenesis). Sirt3 overexpression also discontinued senescence signals, restored mitochondrial oxidative stress, and enabled differentiation, in other stem cell models, after treatment with the pro-aging molecule tert-butyl hydroperoxide (tBHP) (Santos et al., 2022).

2.1.3. Targeting the UPR^{mt} system

The accumulation of misfolded proteins within mitochondria, as a result of a lack of coordination between mitochondria and nucleus-encoded genes, triggers the activation of a stress pathway, known as the mitochondrial unfolded protein response (UPR^{mt}), that ensures correct mitochondrial proteostasis (Shpilka et al., 2018). As a result, the activation of UPR^{mt}, at least to some extent, has also emerged as a key player in the aging process. The knockdown of the mitochondrial ribosomal protein S5 (Mrps5) in nematode worms and mice was shown to induce a dyscoordination between mitochondria and nuclear encoded mitochondrial proteins, resulting in a decrease in mitochondrial respiration, activation of the UPR^{mt}, and increased longevity (Houtkooper et al., 2013). A similar molecular mechanism, based on mitonuclear imbalance and UPR^{mt} activation, was triggered by rapamycin and resveratrol (Houtkooper et al., 2013). In aged mouse muscle stem cells (MuSC) the UPR^{mt} pathway was confirmed to be restored by raising the concentration of NAD^+ (Zhang et al., 2016). As previously stated, the content of NAD^+ is critical for mitochondrial function and Nampt overexpression, a key protein in the NAD^+-Sirt1 axis, was able to attenuate senescence-associated phenotype in aged rats and late-passaged MSCs (Ma et al., 2017), whereas its absence enhanced senescence by depleting NAD^+ levels and attenuating Sirt1 activity (Wang and Finkel, 2020). In addition, multiple precursors of NAD^+ have been confirmed to delay the onset of senescence. Zhang et al., showed that the NAD^+ precursor nicotinamide riboside (NR – a form of vitamin B3 naturally found in milk) was able to rejuvenate aged mice MuSCs, prevent MuSC senescence in a mouse model of muscular dystrophy, and delay senescence in NSCs and melanocyte SCs, thereby increasing mouse longevity (Zhang et al., 2016). Those studies, also corroborated in several other stem cell lineages (Mohrin et al., 2015; Rajman et al., 2018; Schöndorf et al., 2018), have established the importance of UPR^{mt}-mediated mitochondrial checkpoints in aging, but also the importance of nutritional based interventions (Lin et al., 2021). In this context, there is growing evidence that there is a link between aging and the mild stimulation of pathways that perceive adversity (such as the UPR^{mt} system), thereby creating an emerging interest in fasting/caloric restriction diets (Colman et al., 2009; Hegab et al., 2019).

2.1.4. Mitochondrial transfer in aging

MSCs are known to spontaneously donate healthy mitochondria to neighboring cells that exhibit mitochondrial dysfunction, as a means of improving OXPHOS and ATP production. The process behind organelle biogenesis and donation can be attributed to the uptake of dysfunctional mitochondria released by the damaged cells, or to environmental stressors such as ROS and inflammatory mediators, whereas its transport has been ascribed either to the formation of tunneling nanotubes (TNTs) or by microvesicle transport (Gomzikova et al., 2021; Liu et al., 2014). Alternatively, artificial delivery can be accomplished by transplanting mitochondria-rich MSCs to a damaged site (passive horizontal transfer), or by supplementing the cell with previously isolated mitochondria (Chang et al., 2019; Gomzikova et al., 2021; Kim et al., 2018a, 2018b, 2018c; Kitani et al., 2014; Shakoor et al., 2021). Mitochondrial delivery systems can vary greatly between studies and target cells: the organelle can be microinjected into single cells (Shakoor et al., 2021), centrifuged or incubated with the recipient cell (Kim et al., 2018a, 2018b, 2018c; Kitani et al., 2014), transferred via peptide-mediated delivery or introduced with the help of magnetic particles (Macheiner et al., 2016), and even relocated using an automated optical tweezer-based micromanipulation system (Shakoor et al., 2021).

Direct and indirect mitochondrial transplantation procedures have been at the center of recent studies focusing on the repair of damaged tissues, from the treatment of acute lung injury (Islam et al., 2012; Morrison et al., 2017), ischemia/reperfusion lesions in ischemic heart disease (Han et al., 2016), to spinal cord injury (Gollihue et al., 2018). Some authors even report the possibility of increasing oocyte competence through mitochondrial transfer (Ferreira et al., 2021). Concomitantly, this procedure has been considered a plausible anti-aging approach, with the potential of decreasing age-related mitochondrial dysfunction in MSCs and promoting tissue turnover. In fact, a recent study by Guo et al. has revealed that autologous mitochondrial transfer could prevent the loss of therapeutic properties of bone marrow MSCs (BM-MSCs) with age, by upregulating OXPHOS and ATP production and subsequently, increasing proliferation, osteogenesis, and bone healing (Guo et al., 2020). Shakoor and colleagues have also demonstrated that, by transferring healthy mitochondria from fetal MSCs, they could restore the bioenergetic function of aged adult MSCs to a fetal-like state, thereby rejuvenating the aged phenotype (Shakoor et al., 2021). Accordingly, future research into this phenomenon might improve the success rate and efficiency of stem cell-based therapies.

3. Concluding remarks

Stem cells enable the adult organism to regenerate in a daily basis and are essential to safeguard us from the aging process. Therefore, anti-aging treatments are required to increase stem cells in number, quality, and regenerative potential. Moreover, obtaining a deeper understanding of the drivers of aging will also allow us to treat multiple diseases correlated with the aging process (cardiovascular and neurodegenerative diseases, diabetes, cancer, osteoporosis, etc).

Collectively, available findings suggest that mild cellular stress, (e.g., prompted by slight ROS production and lack of proteostasis) could be beneficial towards promoting healthy aging, by mobilizing mitochondrial quality control mechanisms and stimulating mitonuclear communication. Restoring the vitality and function of cells through mitochondria modulation have had considerable interest, and recent findings in animal models have prompted a notable enthusiasm in the field. Nonetheless, mitochondria as disease driver must be explored in the human organism to tackle organismal specificities that could help to devise new therapeutic approaches (Fig. 3).

3.1. Future perspectives

More than 1000 MSC-focused clinical trials have been registered. Since these cells physiologically support the regeneration of multiple tissues, the development of promising cellular therapies can be envisioned, although the applicability of this approach in different circumstances remains to be fully established. Despite their availability throughout the organism, however, MSCs have a limited expansion capacity and suffer premature aging in vitro, hindering their therapeutic benefit. This is a particular point of interest that could increase applicability and is worthy of pursuit.

Reversing MSC aging by protecting against DNA damage can be a challenging affair, but chemical manipulation of DNA through epigenetics, which is directly influenced by the metabolic state of the cell, appear to be a promising solution to this dilemma and might serve as novel pharmacological target. Multiple molecules targeting mitochondria are currently being evaluated under clinical trials and are expected to provide a beneficial effect on human aging. Joining these two approaches (MSCs and mitochondrial function) might provide a stimulus for future studies on the identification and safety-testing of molecules in a broader sample population, and we anticipate that the development of

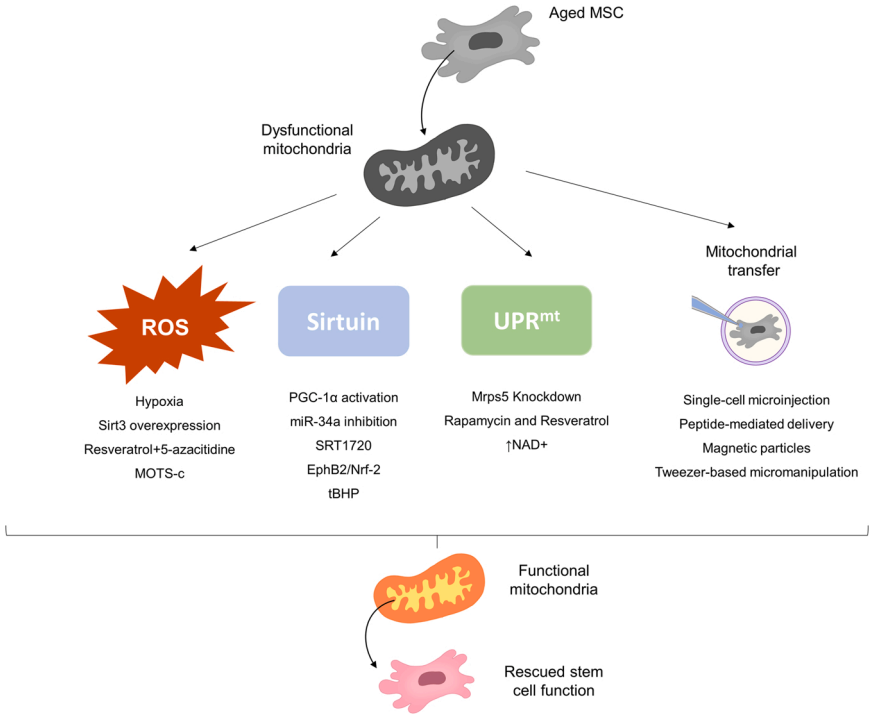

Fig. 3. Schematic representation of key mitochondrial regulators that influence aging of MSCs. Reactive oxygen species, sirtuins, the mitochondrial unfolded protein response (^{mt}UPR) and mitochondrial transfer have been highlighted as mitochondrial anti-aging approaches to tackle MSC aging.

therapies focused either on the recovery of mitochondrial function or on the transfer of healthy mitochondria into damaged aged tissues, may be an important strategy in increasing tissue resilience and MSC function in aging.

CRediT authorship contribution statement

Ana Branco: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Inês Moniz:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **João Ramalho-Santos:** Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

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