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Mitochondria: The master regulator of aging

Pouya Sarvari* and Pourya Sarvari

Iran National Elite Foundation (INEF), Tehran, Iran

Abstract

Mitochondria are ATP-producing organelles in eukaryotic organisms that serve as the cell's power plants. Besides, mitochondria are integral to regulating cellular homeostasis and metabolism as a result of their essential roles in reactive oxygen species (ROS) production, bioenergetics, catabolism and anabolism, heme and iron-sulfur biosynthesis, iron and calcium homeostasis, apoptosis and signal transduction, as well as immunity and inflammation. It is well accepted that mitochondria are evolutionarily derived from endosymbiotic alphaproteobacteria within eukaryotic cells adapted for effective energy transduction. Although most of the mitochondrial DNA (mtDNA) is thought to have been transported to the eukaryotic nucleus during evolution, mitochondria may have preserved protein-coding genes within their own DNA. Accumulating data show that a progressive decline of mitochondria regulates aging. The present review aims to outline the role of mitochondria in various aspects of aging, including unfolded protein response, generation of ROS, and the contribution of somatic mtDNA mutations as well as inflammation in aging. Moreover, we propose mitochondria-targeted nanoparticles and mitochondrial genome editing as novel tools to modify mitochondrial genome aberrations.

Keywords: Aging; Heteroplasmy; Mitochondrial DNA; Mitochondrial genome editing; Mitochondrial quality control; Mitochondria-targeted nanoparticles; Mitochondrial unfolded protein response; Reactive oxygen species

***Corresponding author:**
Pouya Sarvari
(Pouyasarvari2008@gmail.com)

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1. Introduction

Aging is an intricate process during which continual tissue and organ function loss occurs over time, leading to increased susceptibility to death along with a decline in mitochondrial function.[1-4] This debilitation is the major cause of substantial age-related disorders, including cardiovascular diseases, cancer, neurodegenerative disease, and diabetes.[3,5] Nevertheless, aging has several common features, such as (i) impotent intercellular communication, (ii) impairment of proteostasis, (iii) mitochondrial disorder, (iv) genomic alteration, (v) epigenetic modifications, (vi) cellular senescence, (vii) nutrient-sensing misfunction, (viii) stem cell debility, and (ix) telomere shortening.[3,6] The present review, however, focuses on the regulatory role of mitochondria in the aging process.

Mitochondria, specialized organelles with two distinct membranes and a unique genome, evolved through an endosymbiotic relationship of an alphaproteobacterium with a eukaryotic cell, which is protected in exchange for a supply of energy.[7,8] Like nuclear genetic material, mitochondrial DNA (mtDNA) can undergo mutations or damage that

can be passed on to offspring. Moreover, mtDNA is more prone to stress-induced damage owing to the deficiency of protective histones, reactive oxygen species (ROS) generation in the inner membrane, and limited repair mechanisms. Thus, it possesses a mutation rate expected to be 10–20 times higher than that of nuDNA.[9,10] These mtDNA modifications can have serious effects on ATP levels and other cellular processes associated with critical and debilitating diseases, including neurodegenerative disorders (NDs) encompassing Alzheimer's disease (AD), Huntington's disease (HD), multiple sclerosis, Parkinson's disease (PD), and amyotrophic lateral sclerosis. Each of these diseases is associated with distinct regions of the brain and abnormalities involving specific proteins.[10] Nevertheless, this organelle has developed multiple stress-response strategies that contribute to the re-establishment of cellular homeostasis through mitochondria-associated ubiquitination and proteasomal degradation systems, which usually prevent mitochondrial proteotoxicity and remove damaged elements, including protein turnover.[11] It is noteworthy that mitochondria, as a complex organelle, is controlled by both nuclear DNA (nuDNA) and its own DNA (mtDNA) and that cellular homeostasis depends on the dynamic interaction between the nucleus and the mitochondria. Furthermore, mitochondria have retained some of the original bacterial genomes that coevolved with the nuclear genetic material. However, they import over a thousand proteins that are essential for diverse mitochondrial functions encoded in the nucleus. Furthermore, the coordination of nuclear and mitochondrial genomes in a cell regulates metabolism, epigenetic alterations, and a variety of activities critical for the survival and activity of mammalian cells, reflecting their close relationship.[12-14] According to several studies, mitochondrial functions decline significantly throughout aging,[4,15] followed by a reduction in cell activity, which is associated with the development of a wide range of age-related diseases. Mitochondrial dysfunction is a broad term that encompasses various biological processes, including alterations in mitochondrial protein synthesis, mitochondrial morphology and content, mitochondrial metabolism, and degradation pathways, and changes in the functionality of electron transport chain (ETC) complexes.[16] The present review outlines the role of mitochondria in the aging process that occurs through multiple distinct pathways. Moreover, we propose new areas that could facilitate mitochondrial-targeted therapies for the treatment of age-associated diseases.

2. mtDNA structure and features

Mitochondria possess circular, supercoiled, and double-stranded DNA, which accounts for 0.1 – 2% of total DNA in most mammalian cells.[17,18] Mitochondrial genes are almost exclusively inherited from the maternal parent. However, several important exceptions have been reported.[19-21] The mtDNA is structured into distinct protein-DNA complexes known as nucleoids (mt-nucleoid), and these can be observed under the microscope as punctate structures located on the matrix surface of the mitochondrial inner membrane.[22-25] The mt-nucleoid is an mtDNA transmission unit that facilitates its accurate transfer into daughter cells during cell division and also serves as a platform for mtDNA replication.[26-30] Furthermore, transcriptional factor A mitochondrial, mitochondrial single-stranded DNA-binding protein (mtSSB), and Twinkle protein have been demonstrated to colocalize with mt-nucleoid in intramitochondrial foci in living cells.[31] Mammalian mtDNA is approximately 16.5 kb in length and comprises 37 genes required for optimal mitochondrial function.[32] It is previously known that mtDNA encodes two rRNAs, twenty-two tRNAs, and thirteen polypeptides that form the core components of ETC Complexes I, III, IV, and V, which are required for the oxidative phosphorylation process (OXPHOS).[33,34] The nuDNA encodes the majority of mitochondrial proteins (about 1500), which are produced in the cytosol and transported into the mitochondria.[35,36] Furthermore, mtDNA has no introns, no gaps between genes, and no 5' or 3' non-coding regions.[37,38] The mtDNA consists of two strands: the heavy (purine-rich) strand, which encodes most of the information, and the light (pyrimidine rich) strand, which encodes the genetic information for only one polypeptide and eight tRNAs.[39] Among the 14 known DNA polymerases in humans, DNA polymerase gamma (Pol γ) is responsible for the replication and repair of mtDNA and is encoded by the POLG gene.[40-42]

3. Damage and mutations to mtDNA contribute to aging

Although both nuDNA and mtDNA are constantly exposed to external agents such as ionizing radiation, radiation, environmental toxins, and many therapeutic drugs, mtDNA is more susceptible to toxic chemicals than nuDNA due to its proximity to OXPHOS sites, lack of histone protection, and low repair activity when damaged.[43-47] ROS-induced mtDNA damage is thought to be the principal cause of mutagenesis in mitochondria, resulting in both mtDNA mutations and deletions.[48] Furthermore, mtDNA is required for the maintenance and regulation of mitochondrial functions, and its mutation rate is believed to be 10 to 20 times higher than that of nuDNA.[9] Furthermore, lipophilic cations tend to accumulate in mitochondria, particularly in the mitochondrial membranes, due to the negative charge on

the matrix side of the inner mitochondrial membrane.[49] Thus, many biologically toxic compounds and lipophilic drugs that have positive charges are imported from the cytosol and concentrated in mitochondria up to 1000-fold, which can be a threat to mitochondrial components.[50,51] mtDNA is found in large quantities (hundreds to thousands of copies per cell). However, a subset of them are affected by mutations.[52] Thus, the proportion of mtDNA mutations can profoundly affect the cellular and clinical phenotype.

The ratio of mutant mtDNA copies to total mtDNA is sometimes referred to as the heteroplasmy level or heteroplasmy frequency.[53] The phenotypic threshold for pathogenic heteroplasmies is believed to be 60 – 90% of mitochondrial genomes inside a cell.[54-56] However, the outcome is still determined by the specific tissue in which they appear, the timing of their appearance, and the total mtDNA content of the cell.[18,57,58] The mtDNA mutations include mtDNA rearrangements such as inversions or duplications, deletions, and point mutations. Nevertheless, it is known that mitochondrial mutations contribute to multiple diseases. For example, the m.3243A > G DNA mutation in the mitochondrial *MTTL1* gene, which encodes a specific type of mitochondrial transfer RNA termed $tRNA^{Leu\ (UUR)}$, is the most frequent mtDNA point mutation and can cause a variety of symptoms depending on the extent of heteroplasmy.[59,60] This mutation was discovered in 1990 and was associated with a neurological phenotype known as mitochondrial encephalopathy lactic acidosis with stroke-like episodes.[61] Patients with this mutation typically exhibit signs of a multisystem illness, including stroke-like episodes, hyperglycemia, myopathy, intestinal immobility, deafness, headaches, and seizures. In general, mtDNA mutations increase with age and appear to differ among organs.[10,62-64] Despite mounting evidence that increased levels of mitochondrial mutations and their accumulations contribute to aging and age-related diseases,[53,65-69] other studies have questioned whether these mutations ever reach a significant level sufficient to contribute to the aging process.[65] According to studies, the production of a defective form of mtDNA polymerase γ, POLG, promotes early death in mice, as well as elevated levels of mitochondrial mutations and premature aging.[66-68] These studies vividly connect mitochondrial abnormalities to aging. However, the types and levels of mitochondrial mutations do not appear to replicate what is observed during normal aging.[69] Hence, it is uncertain whether the rise in mitochondrial mutations with age plays a key role in the aging process.

In 1956, Harman proposed the free radical theory of aging,[70] which suggests that organisms age as a result of the accumulation of oxidative damage over time, initiated by ROS produced in the mitochondria (Figure 1[iA–iB]). Furthermore, ROS can cause damage to specific macromolecules such as lipids, proteins, and, most crucially, mtDNA. The free radical theory of aging has been a popular concept in the area of aging for many years. Later, the free radical theory of aging incorporated other ideas and evolved into the mitochondrial 'vicious cycle' theory of aging.[71] It proposes that ROS formation and elevation in oxidative stress can induce damage to mtDNA, hence gradually giving rise to mtDNA mutations during life. The accumulation of mtDNA mutations, in turn, leads to increased mitochondrial ROS generation, which further increases oxidative stress and the rate of mtDNA damage and mutagenesis (Figure 1[iC]). Consequently, this "vicious cycle" of oxidative damage that is exponentially expanding causes tissue degradation and aging, which then leads to cell death.[72,73] Recently, the former free radical theory of aging has been contradicted by several studies.[74-82] Furthermore, another study found that the longevity of anaerobically grown yeast cells is shorter than that of aerobically produced yeast cells, demonstrating that aging still occurs under anaerobic conditions with little ROS.[77] These findings have prompted scientists to speculate that ROS may perform signaling activities that activate protective and adaptive responses.[83,84] Moreover, the latter 'vicious cycle' theory of aging claimed cautiously that not all mutations drive superoxide production, and in particular, mutations that block the synthesis of cytochrome b would actually abort any superoxide production at complex III that normal mitochondria may exhibit.[71,72] Although some studies endorsed the above statement,[85,86] other studies, however, did not support the idea that mtDNA mutations contribute to higher ROS generation and oxidative stress in mitochondria with age,[66,87,88] placing the mitochondrial "vicious cycle" theory of aging in question. Furthermore, several studies suggest that age-related mtDNA mutations are largely produced during mtDNA replication errors rather than unrepaired damage caused by ROS.[89,90] Nevertheless, multiple lines of evidence indicate that mtDNA point mutations and deletions can accumulate with age, which constantly increases the mutation load with metabolic consequences as well as gradual loss of cellular functions, which ultimately results in age-related phenotypes.[89-93] However, the precise role(s) mtDNA mutations play in aging and its related diseases is still being debated. In addition, the detection and exact physiological effects of mtDNA mutations, especially as they pertain to aging, have proven challenging.

Overall, the mitochondrial "vicious cycle" theory of aging has received both criticism and extensions since its introduction, and the relationship (correlative association) between ROS, mtDNA mutations, and aging seems to be

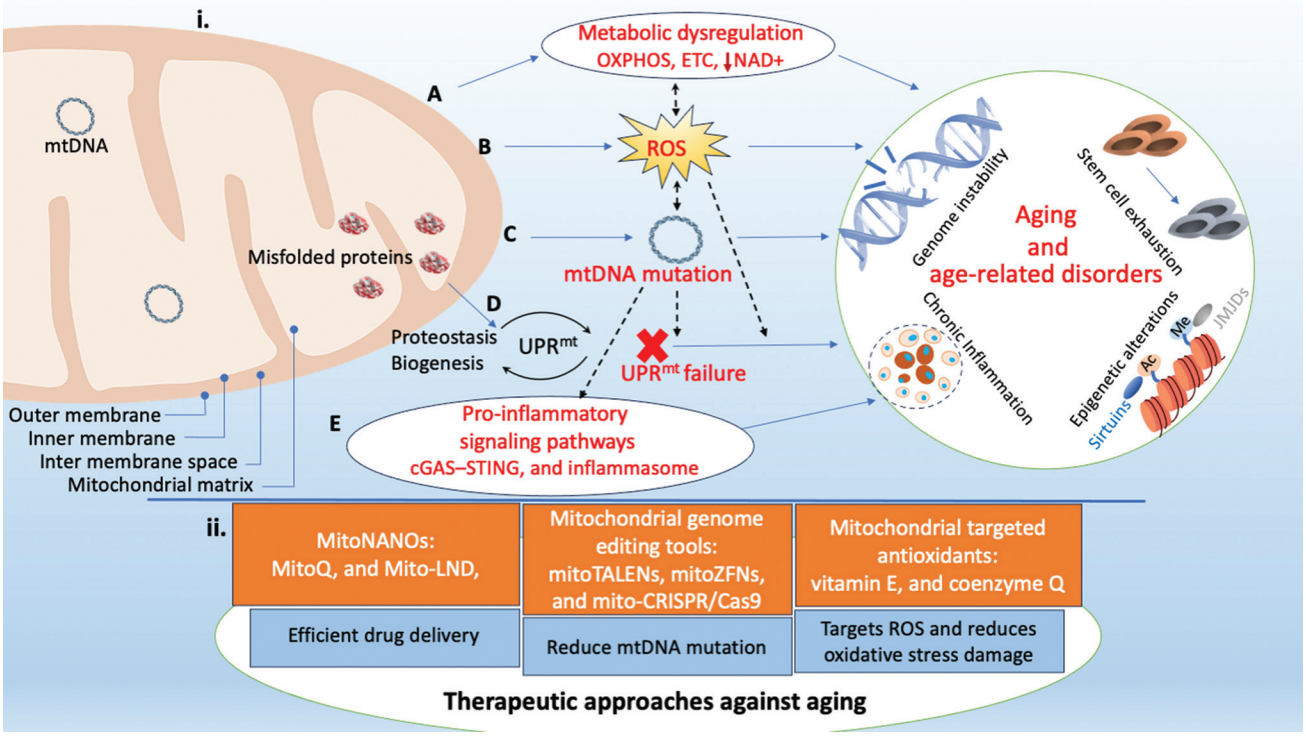


Figure 1. Mitochondria as a master regulator of aging and therapeutic approaches for aging treatment: (i) The illustration of mitochondria contribution to the aging process through distinct, but interconnected pathways—(A) oxidative stress due to ROS accumulation can cause dysregulation in mitochondrial metabolism including impairment of ETC enzyme complexes and impairment in OXPHOS which consequently promotes ROS production associated with aging and correlated with the development of age-related diseases; (B) consequently, ROS at high levels not only causes oxidative stress and damage to biological macromolecules (lipids, proteins, and nucleic acids) but also could directly damage the mtDNA, negatively affecting the nuDNA, and genome integrity which contributes to the aging phenotype; (C) mutations and accumulation of mtDNA can lead to ROS generation and elicit a coordinated alteration in the nuclear gene expression associated with aging; (D) mitochondria possess internal defense mechanisms, such as UPR^{mt}, which maintains mitochondrial proteostasis and biogenesis. However, excessive ROS generation and mtDNA damage result in the impairment or inhibition of these systems associated with aging; (E) oxidized mtDNA and its release into the cytosol can activate different PRRs and innate immune responses, including the recruitment of the cGAS-STING pathway and NLRP3 inflammasome, which can drive age-related pathologies such as neurodegenerative disorders; (ii.) therapeutic approaches for targeting aging and age-related diseases, which are discussed in the manuscript.
Abbreviations: cGAS-STING: Cyclic GMP-AMP synthase-stimulator of the interferon gene; ETC: Electron transport chain; Mito-CRISPR/Cas9: Mitochondria-targeted clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; Mito-LND: Mitochondria-targeted lonidamine; MitoNANOs: Mitochondria-targeted nanoparticles; MitoQ: mitochondria-targeted ubiquinone; mitoTALENs: Mitochondria-targeted transcription activator-like effector nucleases; mitoZFNs: Mitochondria-targeted zinc-finger nucleases; mtDNA: Mitochondrial DNA; NAD^+: nicotinamide adenine dinucleotide; NLRP3: NACHT-, LRR-, and pyrin domain-containing protein 3; nuDNA: Nuclear DNA; OXPHOS: Oxidative phosphorylation process; ROS: Reactive oxygen species; TPP^+: Triphenylphosphonium cation; UPR^{mt}: Unfolded protein response.

more complex and involves more factors (environmental, nutritional, metabolic changes, the timing of their occurrence, tissue specificity, etc.), which need further vigorous studies. Moreover, further technological advancements are required to quantify the mtDNA mutation load in numerous single cells at different time points.

4. Mitochondrial unfolded protein response (UPR^{mt}) in aging

The aggregation, misfolding, and tissue accumulation of proteins seriously threaten cellular homeostasis and are associated with various human diseases.[94] In addition, various mechanisms have evolved individually in the highly compartmentalized eukaryotic cell to guarantee the integrity of the protein-folding environments in each compartment, including the cytosol, endoplasmic reticulum (ER), and mitochondria. Since all these three compartments are exposed to unfolded polypeptides, each has developed a dedicated repertoire of specific chaperones to promote efficient folding within each cellular compartment. As a result, any unfolded/misfolded protein stress is identified at each cellular compartment and conveyed to the nucleus for stimulation of the compartment-specific chaperone gene expression.[95,96] In addition, the complex organelle architecture of mitochondria, containing two membrane barriers, the harmful effects of ROS, and the susceptibility of mtDNA to acquiring mutations make the mitochondrial environment susceptible to the accumulation of unfolded

or unassembled proteins. Nevertheless, when misfolded or unfolded proteins build up beyond the capacity of the organelle's chaperones, cells initiate a UPR^{mt} (Figure 1[iD]). The UPR^{mt} is a mitochondrial-to-nuclear signal transduction pathway that culminates in the activation of mitochondrial protection genes such as chaperones and proteases to restore protein homeostasis within the mitochondrial protein-folding environment. Molecular chaperones are found in both the mitochondrial matrix and the intermembranous space (IMS).[97,98] For instance, HSP60 chaperonin, which is localized in the mitochondrial matrix, aids in the folding of tiny, monomeric proteins. Moreover, it comprises Hsp60 and Hsp10 subunits, which together form a barrel-shaped complex.[99,100] In addition to HSP60 chaperonin, there is mitochondrial HSP70 (mtHSP70), which is located in the matrix and is known to perform various functions. The mtHSP70 forms the core subunit of the machinery, called "import motors," which provides a driving force for the proteins imported into mitochondria.[98,101] Other studies show that mtHSP70 assists in the folding of imported polypeptides in the matrix while preventing their aggregation in the matrix.[102-104] In addition, mitochondria have a number of proteases that are positioned in the inner membrane and matrix, which mediate the destruction of misfolded or incorrectly assembled proteins. Inside the mitochondrial matrix, the protein-folding environment is monitored and protected. When accumulating unfolded proteins are detected, a mitochondria-to-nucleus signal transduction pathway sends a signal from the mitochondria to the nucleus via the cytosol. This frequently results in subsequent overexpression of the relevant chaperone-encoding genes and quality control proteases, guaranteeing protein-folding homeostasis at both the protein folding and removal levels.[95]

Besides, studies show that there are other factors that could activate UPR^{mt}, including deletion of mtDNA, the increase of ROS levels (Figure 1[iD]), alteration of mitochondrial dynamics, mitochondrial chaperone or protease inhibition, and impairment of the ETC.[105-108] The transcription factor associated with Stress-1' (ATFS-1) is a major regulator of UPR^{mt} in *C. elegans*[106] and its homolog ATF5 in mammals.[109] Studies show that this transcription factor is required for the regulation of almost half of mitochondrial stress-responsive genes, such as mitochondrial chaperones and peptidases, as well as immune response genes.[106,110] ATFS-1 is known to contain a nuclear localization sequence and a mitochondrion targeting sequence (MTS), which allows it to translocate between the nucleus and mitochondria, mediating mitochondria-to-nuclear communication.[111,112] In addition, under non-stress circumstances, this protein is imported into the mitochondria and degraded by the Lon protease.[106] Conversely, when the mitochondria are stressed, mitochondrial matrix protease ClpP cleaves misfolded or unfolded polypeptides, which are subsequently exported to the cytoplasm by HAF-1.[111] Consequently, the increased accumulation of peptides in the cytosol leads to a reduced capacity for mitochondrial protein import.[106,113] As a result, ATFS-1's mitochondrial import is inhibited, resulting in its buildup in the cytoplasm, thus letting ATFS-1 travel to the nucleus, where, in cooperation with ubiquitin-like protein 5 (UBL-5) and defective proventriculus 1 (DVE-1) upregulates the expression of mitochondrial chaperones, proteases, and different metabolic and detoxification enzymes.[114,115] In such a setting, cross-compartment synchronization seems to be an essential factor in maintaining protein homeostasis during UPR^{mt}. The activation of UPR^{mt} is thought to be one of the mitochondrial processes that protect against numerous types of aging-causing damage, with complex effects on longevity.[116] Notably, studies show that the activation of UPR^{mt} mechanisms by downregulation of ETC complexes I and IV promotes longevity.[117-119] Moreover, UPR^{mt} activation has been connected to the extension of lifespan caused by various types of bacteria,[120] possibly through enhanced production of the polysaccharide colanic acid (CA), which regulates mitochondrial dynamics and UPR^{mt} in the host *C. elegans*. Another study showed that ATFS-1 enhances longevity in long-lived nuo-6 mitochondrial mutants by activating a variety of stress response pathways.[121] Furthermore, the longevity of two long-lived mitochondrial mutants, namely *clk-1 and isp-1*, was likewise demonstrated to be decreased by the knockdown of *ubl-5* and *dve*-1 which are the regulators of UPR^{mt}.[119] In addition, studies suggest that histone H3 methylation is an essential epigenetic regulator of UPR^{mt} throughout the lifespan.[122,123] Intriguingly, Merkwirth *et al.* showed that histone demethylases, namely the Jumonji C domain-containing protein family (*jmjd-1.2* and *jmjd-3.1)* are required for activation of the UPR^{mt}-mediated longevity across species. These findings indicate that the epigenetic mechanism modulates the rate of aging downstream of mitochondrial perturbations.[124] Moreover, they demonstrated that the gain of function of demethylases is sufficient to increase lifespan in a UPR^{mt}-dependent manner, whereas their loss of function greatly reduces longevity and UPR^{mt}. In addition, increasing lines of evidence support the key role of epigenetic regulators namely the sirtuin deacetylase family in the mitochondrial stress response pathways; particularly, SIRT1, SIRT3, and SIRT7 which contribute to the UPR^{mt} via different axes.[117,125-129] These findings collectively suggest that UPR^{mt} plays a key role in specific longevity pathways. However, the relationship between UPR^{mt} and longevity seems more complex and requires further studies to ascertain this relationship.

5. Mitochondrial oxidative stress in aging

Mitochondria are known to generate various types of "reactive species" as side products of oxidative phosphorylation, including the reactive oxygen/nitrogen species (RONS). The increase in RONS production is linked to oxidative stress, which can cause oxidative damage to cells via apoptosis, autophagy, and inflammation if not eliminated efficiently by the cellular antioxidant defense system.[130,131] Moderate or low amounts of RONS, on the other hand, can operate as signaling molecules in the cell.[132-134] Even though mitochondrial RONS take part in numerous physiological processes, including epigenetic modifications[135] and disease progression, such as cancer,[136-141] the mechanisms of mitochondrial RONS have not been fully understood. Moreover, age-associated functional disorders can arise from RONS, which are by-products of oxygen and nitrogen originating from various sources, and their negative effects are compensated by antioxidant mechanisms.[2,142] Hence, RONS play a significant role in the development of age-associated diseases.[2,143] RONS can be derived from endogenous as well as exogenous sources. Endogenous sources consist of myeloperoxidase (MPO), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, angiotensin II, and lipoxygenase.[143,144] O_2• is another form of RONS that is produced by the reduction of molecular oxygen with supplied electrons carried by NADPH during cellular respiration. O_2• can dismutate into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD).[2,145] H_2O_2 is not considered a free radical since it does not contain unpaired electrons. However, it can produce highly reactive hydroxyl ions (OH•) through the Fenton or Haber-Weiss reactions.[2] Hydroxyl radicals are extremely reactive, particularly with phospholipids found in cell membranes and proteins.[2] On the other hand, H_2O_2, MPO, and chloride can be transformed into hypochlorous acid, a ROS-specific cellular damaging protein.[2,145] Nitric oxide (NO) originates from L-arginine via NO synthase (NOS), which forms three main isoforms: (i) Epithelial NOS involved in vascular regulation and vasodilation, (ii) neuronal NOS involved in intracellular signaling, and (iii) inducible NOS, which is released following numerous endotoxin or cytokine signaling.[2,146] Eventually, the interaction of O_2 with NO can lead to the formation of a rather reactive molecule called peroxynitrite ($ONOO^-$).[2,144,145]

The exogenous sources of RONS include alcohol, tobacco, water and air pollutants, drugs (e.g., cyclosporine, tacrolimus, gentamycin, and bleomycin), heavy or transition metals, cooking (e.g., smoked meat, waste oil, and fat), industrial solvents, and radiation, which are turned into free radicals after metabolization inside the body.[2,147] Both exogenous and endogenous forms of RONS conduct oxidative modulation of substantial macromolecules (lipids, proteins, carbohydrates, and DNA)[2,144] and can be considered as oxidative stress markers.[2,148] When there is an imbalance between the formation and clearance of RONS, oxidative stress occurs.[144] In this regard, antioxidants safeguard biological entities from deleterious free radicals comprising endogenous as well as exogenous molecules. Endogenous antioxidants consist of enzymatic and non-enzymatic forms.

The major enzymatic antioxidants are catalase (CAT), SOD, and glutathione peroxidase (GSH-Px).[2] As discussed before, SOD transforms O_2• into H_2O_2, which further breaks down to oxygen and water by CAT, inhibiting the production of hydroxyl radicals.[2] Furthermore, GSH-Px transforms hydroxyl radicals and peroxides into non-toxic compounds through the oxidation of reduced glutathione (GSH) into glutathione disulfide (GSSG), and then glutathione disulfide is reduced to the sulfhydryl form glutathione (GSH) by glutathione reductase.[2] Further antioxidant enzymes to be mentioned are glutathione-S-transferase and glucose-6-phosphate dehydrogenase.[2,149]

Non-enzymatic antioxidants are compounds that react with RONS and abate the free radical chain reactions: α-tocopherol (vitamin E), β-carotene, and bilirubin are present in the blood, whereas uric acid and albumin comprise 85% of antioxidants in plasma.[2,150] Exogenous antioxidants consist of ascorbic acid (vitamin C), which breakdown hydroxyl and superoxide radical anion, α-tocopherol (vitamin E), which plays a role in lipid peroxidation of cell membranes, phenolic antioxidants, selenium, oil lecitinas, zinc, and drugs such as acetylcysteine.[2,151] These molecules usually function as scavengers of free radicals or can even modulate the activity of enzymatic systems.[152] As already mentioned, the contribution of ROS to aging is controversial. Numerous studies have demonstrated that inhibition of oxidative stress corresponds to an increase in lifespan. On the other hand, some studies have questioned the possibility of ROS as a cause of an aged phenotype. Nevertheless, aged rats were shown to hold higher free radical levels.[153,154] On the other hand, reduced levels of antioxidants such as glutathione peroxidase (GPx), CAT, and SOD have been reported in aged rats as well as in humans.[155-161] The action of ROS is counteracted by antioxidant molecules. Studies suggest that fumarate, a metabolite of the mitochondrial TCA cycle, and its derivatives, specifically dimethyl fumarate, have antioxidant[162] and anti-inflammatory properties,[163-165] which can fight against age-related neurological disorders.[164,166-168] However, their exact molecular mechanism of action remains elusive despite their promising beneficial effects against neurological disorders.[168] The administration of antioxidants such as Vitamin E compounds, including

tocopherols and tocotrienols, was previously demonstrated to extend the life span and decelerate aging in different species.[169,170] Vitamin E compounds seem to extend life span in various ways, including ameliorating age-related decline in NO synthase and SOD2,[170] suppressing ROS production,[171] reducing ROS damage[172] (Figure 1[i–ii]), mitigating ethanol-induced accumulation of intracellular oxidants and counteracting the suppression of glutathione peroxidase/glutathione reductase,[173] providing protection against age-related hepatocytes polyploidization, and neuroprotection by reducing the quantity of p53-positive cells throughout the brain.[174] Furthermore, antioxidant-based therapy involving the use of natural sources of antioxidants such as Vitamin E and Coenzyme Q (CoQ, ubiquinone) appears to be effective in animal models of neurodegenerative disease, including mouse models of both PD and AD.[175] CoQ10 is naturally generated in the body and serves as an antioxidant agent, and its level declines in the body during aging.[176] CoQ10 is mostly found in the inner mitochondrial membrane of eukaryotic cells.[177] Furthermore, it acts as an electron shuttle between complexes I and II of the respiratory chain, as well as complex III in mitochondria.[178] As a result, it can exist in both oxidized (CoQ or ubiquinone) and reduced (CoQH2 or ubiquinol) forms.[179] Furthermore, CoQ10 protects against oxidative stress-induced cell death.[180] Studies show that CoQ10 protects the skin by combating free radicals, which were shown to damage collagen fibers through the activating MAPK pathway that produces matrix metalloproteinases (MMPs) such as collagenase.[181,182] Aberrations in CoQ10 biosynthesis genes cause primary CoQ10 deficiency, a mitochondrial syndrome associated with impaired OXPHOS and clinically heterogeneous diseases, including cerebellar ataxia, encephalomyopathy, infantile multisystemic form, isolated myopathy, and nephropathy.[177,178] Reportedly, some patients also developed retinopathy or optic atrophy, hypertrophic cardiomyopathy, and sensorineural hearing loss as a result of CoQ10 deficiency.[183] In addition, CoQ10 deficiency is connected with mtDNA point mutations, depletion, and deletions.[184] Nonetheless, Coenzyme Q supplementation was demonstrated to protect against age-related DNA double-strand breaks and prolong longevity in mice who consumed a polyunsaturated fatty acid (PUFA)-rich diet by attenuating oxidative alterations.[185] CoQ10 supplementation may improve human lymphocyte recovery from oxidative DNA damage due to the ordering and condensing impact of CoQ10 on cell membranes, resulting in a decrease in ROS formation and a protective benefit to DNA integrity.[186] Nonetheless, lifetime CoQ10 treatment had no effect on the lifespan of rats or mice,[187] indicating that CoQ10 might aid in preventing life span shortening as a result of cumulative oxidative insults by reducing oxidative stress damage (Figure 1[i–ii]). Paradoxically, some animal models, such as *C.elegans,* with CoQ biosynthetic deficiencies have shown an increase in life span.[188,189] This observed effect might be attributed to the maintenance of the efficiency of respiration together with an observed reduction in the production of superoxide anion in the mitochondrial electron change, which would cause lower damage to macromolecules in response to CoQ silencing. However, these data cannot be extrapolated to other biological systems, such as mammals.

6. Mitochondrial dysfunction leads to stem cell exhaustion

One of the hallmarks of aging is the reduction in stem cell numbers due to their impaired self-renewal capacity and function. Studies show that as hematopoietic stem cells (HSC) age, they exhibit increased mitochondrial OXPHOS and enhanced ROS production, indicating a direct role for mitochondria in the degenerative process. Studies show that ROS at low levels plays a positive role in stem cell biology, mainly by maintaining their stemness, quiescence, and self-renewal.[190-192] However, elevated ROS can activate stem cell differentiation, senescence, and apoptosis, resulting in their exhaustion, which is linked with the aging process and degenerative diseases.[190,193] In addition, low amounts of ROS can act as a signaling molecule to promote cardiovascular differentiation in mouse embryonic stem cells (mESCs). Meanwhile, high amounts of ROS can lead to the inhibition of cardiomyogenesis and vasculogenesis.[194] Similarly, ROS at low levels allows HSCs to keep up their normal functions, including proliferation, differentiation, and mobilization. Interestingly, several studies on POLG mtDNA mutator mice showed impairment of stem cell functions and found a wide range of defects, including impairment of neural stem cell (NSC) populations,[195] megaloblastic anemia, B-cell abnormalities,[196] and impaired reprogramming capacity into pluripotent stem cells due to increased ROS levels.[197] In addition, transplantation of HSCs from POLG mtDNA mutator mice into a normal host led to the same observed defect.[196] Another study demonstrated that the ability of POLG knockin cells to be reprogrammed into pluripotent stem cells is likewise significantly reduced; this deficiency is once again linked to an increase in the production of ROS by the mitochondria.[197] Taken together, studies on the POLG mtDNA mutator mice clearly linked mitochondria to stem cell functions, suggesting the POLG mtDNA mutator mice influence a wide range of cell types, including stem cells, their offspring, and the niche.[198]

Mitochondria can also help to maintain stem cells by regulating particular metabolites that act as secondary messengers for epigenetic regulation.[199] Numerous crucial

metabolic pathways, such as the one-carbon cycle, the tricarboxylic acid (TCA) cycle, and fatty acid oxidation (FAO), are known to be compartmentalized in the mitochondria.[200] Metabolites produced by these pathways can also serve as retrograde signals. Particularly, many of these metabolites are produced by the TCA cycle, such as acetyl-coenzyme A, succinyl-CoA, α-ketoglutarate (αKG), succinate, and nicotinamide adenine dinucleotide (NAD^+). For instance, it has been demonstrated that the ratio of αKG to succinate plays a crucial role in maintaining pluripotency in mouse mESCs through regulation of multiple chromatin modifications, including histone H3 lysin 27 tri-methylation (H3K27me3) and ten-eleven translocation (Tet)-dependent DNA demethylation.[201] In addition, NAD^+ is another important metabolite that links the mitochondria to stem cells, and its systemic decline has been reported during aging.[202,203] Furthermore, many NAD+-consuming enzymes use NAD^+ as a substrate, including the cyclic ADP-ribose synthase CD38, SARM1, poly-ADP-ribose polymerase (PARP), as well as a family of seven protein deacylases, namely sirtuins, which are present in the nucleus (SIRT1, SIRT6, and SIRT7), cytosol (SIRT2), and mitochondria (SIRT3 – SIRT5).[204-206] Alleviated age-dependent NAD^+ availability is associated with decreased activities of sirtuins, eventually disrupting the crosstalk between mitochondria and the nucleus during aging.[207,208] Reduced NAD^+ levels in NSCs were shown to recapitulate at least some of the phenotypes of stem cells during aging (Figure 1[iA]), while NAD^+ boosting through administration of precursor nicotinamide mononucleotide (NMN) could repair abnormalities induced by a decrease in NAD^+ levels in NSCs.[209] Hence, NAD^+ supplementation therapies are considered a therapeutic option to ameliorate age-related metabolic diseases.[210] Furthermore, NAD^+ boosting with the precursor NMN improved impaired glucose tolerance by restoring normal NAD^+ levels and enhancing either insulin sensitivity or insulin secretion in mice with diet- and age-induced diabetes.[211] The observed effects seem to be partially mediated by the sirtuin (SIRT) family of NAD^+-dependent histone deacetylases, which are known to regulate crucial metabolic pathways. For instance, SIRT3 was shown to regulate global mitochondrial lysine acetylation levels in an NAD^+-dependent manner.[212] Moreover, SIRT3 was shown to be abundant in HSCs, where it regulates stress responses.[213] In addition, the same study showed that SIRT3 is suppressed during aging, and its increased levels in aged HSCs improved their regenerative capacity, indicating that the plasticity of mitochondrial homeostasis controls stem cell and tissue maintenance during the aging process, and hence aging-associated degeneration can be reversed by a member of the sirtuin family.

Studies have uncovered that stem cell pool maintenance, expansion, or depletion are modulated via symmetric and asymmetric division events.[214-217] In addition, stem cells can exploit mitochondrial FAO during self-renewal along with glycolysis.[218] Inhibiting FAO in HSCs results in the loss of asymmetric division of HSC daughter cells, which is a vital process to maintain the stem cell pool during the simultaneous expansion of stem cell differentiation. Mechanistically, this process (HSC asymmetric division) is controlled by the PML-PPARδ-FAO pathway.[219] Moreover, the same study showed that PPARδ activation using PPARδ agonists increases asymmetric division and improves HSC functions. In addition, lipid metabolism is also an important player in NSC proliferation and maintenance.[220,221] Specifically, deletion of fatty acid synthase, the key enzyme of *de novo* lipogenesis, in mouse NSCs was shown to impair adult neurogenesis.[222] Taken together, mitochondria are key organelles that regulate the metabolic status of stem cells; consequently, maintaining their proper metabolic regulation is critical for lifelong health. Moreover, the modulation of pathways associated with mitochondrial metabolic dysfunction contributing to age-related stem cell exhaustion could potentially improve human health and prevent age-related diseases.

7. Mitophagy and age-associated diseases

Being extremely dynamic organelles, mitochondria go through various processes termed mitochondrial quality control (MQC). MQC mainly involves the coordination of multiple biological events, including constant fission and fusion, an endless transformation process occurring through biogenesis and mitophagy to affirm mitochondrial homeostasis, morphology, and inheritance.[223] Once facing challenges through oxidative or bioenergetic stress, mitochondria perform an arranged reaction containing morphological and dynamical transformation by triggering the specific molecular mechanism that synchronizes mitochondrial biogenesis, mitophagy, fusion, and fission.[224] Mitochondrial fusion and fission in mammalian cells are closely supervised by a number of proteins, including dynamin 1-like (DNM1L, recognized as Drp1), mitofusin 1 (MFN1), mitofusin 2 (MFN2), and optic atrophy protein 1 (OPA1).[225] The process of mitochondrial fission was discovered to participate in mitochondrial apoptosis and was proposed to be essential for mitophagy, while mitochondrial fusion is linked to an increase in mitochondrial metabolism.[226] The term "mitophagy," the selective mitochondrial autophagy, was first named by John Lemasters.[227] Mitophagy is a procedure during which depolarized, aged, or damaged mitochondria are selectively removed via double-membrane autophagosome for consequent lysosomal degradation.[228] The detection of this

subset of mitochondria is supported by the engagement of the mitochondrial kinase PINK1 (PTEN-induced putative kinase protein 1). When mitochondria lose membrane potential, PINK1 accumulates on the mitochondrial surface, resulting in the recruitment of the cytosolic protein Parkin, which regulates the mitochondrial protein ubiquitination and subsequently, engulfment of damaged mitochondria by membranes that then fuse with lysosomes,[229] a process known as mitophagy. Mitophagy was discovered to be a crucial process for preserving cellular health and homeostasis. Surprisingly, the BCL2 protein family was shown to take part in both mitophagy processes and mitochondrial dynamics, placing them in the spotlight of mitochondrial regulatory elements.[230] Studies reported that dysregulation of mitophagy and damage to mitochondria have been observed in several NDs correlated with aging, including PD,[231] AD, and HD.[232] Aging escalates the likelihood of the initiation of several chronic diseases, usually correlated with the build-ups in mtDNA mutations, impaired mitochondrial function, mitochondrial mass variation, enhanced cell death, and persistent immune activation[233] that are expected to happen due to compromised MQC machinery, resulting in the accretion of malfunctioned mitochondria, which consequently elevates immune activation (through ROS activation) as well as mitochondrial apoptosis (through the expression of apoptogenic factors). Several lines of the study indicate that impaired mitophagy promotes aging, while improved mitophagy, which is endorsed by reduced calorie intake and training, advocates a health-giving lifetime. For instance, the promotion of mitophagy has been shown to be connected to an increase in *C. elegans* longevity.[234] Lineage-specific expression of PGC-1 (master regulator of mitochondrial biogenesis) in *Drosophila melanogaster* results in a prolonged lifetime in this model.[235] Physical activity has been reported to trigger AMP-activated protein kinase (AMPK) activation, which in turn results in phosphorylation of the Unc-51-like autophagy activating kinase 1 (ULK1) and elevated mitophagy in skeletal muscle, which consequently stimulates mitochondrial biogenesis and enhances health conditions in murine models.[236] Besides, it has been notably reported that impaired mitophagy significantly affects PD development. Mutations in Parkin and PINK1 proteins participating in the induction of mitochondrial mitophagy were reported in PD.[237,238] Mitophagy was also reported to be linked with AD progression.[239,240] Studies have recently demonstrated that tau pathology, a hallmark of AD, disrupts mitophagy by preventing the translocation of Parkin protein to mitochondria.[241] These data underline the importance of mitophagy for proper mitochondrial function and dynamism, which in turn affect personal health conditions. In addition, a newly identified MQC mechanism, the mitochondrial-derived vesicle (MDVs) pathway, was shown to function during the early stages of cellular stress and has a key role in mitochondrial oxidative stress to maintain stable mitochondrial function.[242]

8. Dysfunctional mitochondria and inflammation during aging

As discussed, MQC maintains healthy mitochondria via the repair or selective elimination of damaged mitochondria in cells via a series of adaptive responses encompassing mitochondrial fusion and fission, mitophagy, and mitochondria-dependent cell death.[243] MQC dysfunction during aging causes an accumulation of damaged mitochondria, which contributes to aging and a variety of age-related diseases.[244] Studies reveal that when MQC is defective, mitochondrial-derived damage-associated molecular patterns (mtDAMPs) such as mtDNA and oxidized mtDNA accumulate in the cytosol, which can activate both intracellular and extracellular immune pathways affecting age-related disease progression[245,246] (Figure 1 [iA–iE]). According to studies, even in the absence of bacterial infection, mtDAMPs generated as a result of trauma might cause systemic inflammatory response syndrome.[247] Due to the lack of histone proteins, mtDNA is vulnerable to degradation and oxidation. Furthermore, mtDNA contains hypomethylated CpG patterns that are identical to those found in bacterial DNA and can be recognized as a pathogen-associated molecular pattern and hence can induce an inflammatory response.[248] Furthermore, these regions can interact with and activate membrane or cytoplasmic pattern recognition receptors (PRRs), such as the nucleotide-binding oligomerization domain-like receptor (NLR),[248] the toll-like receptor (TLR), and even the cytosolic cyclic GMP-AMP synthase (cGAS), which is a stimulator of interferon genes (STING).[249] For example, Oka *et al.* revealed that pressure-overload mtDNA release that escapes autophagic degradation causes TLR9-mediated inflammatory responses in cardiomyocytes and can cause myocarditis and dilated cardiomyopathy.[250]

In addition, the cytosolic release of oxidized mtDNA was shown to stimulate the NACHT-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome, a large multi-protein complex that controls caspase-1 activation, which results in IL-1β and IL-18 secretion and an apoptotic activation cascade.[251] In contrast, deletion of the Nlrp3 inflammasome has been found to reduce age-related activation of the innate immune system, protecting animals from a variety of age-related diseases.[252] As a result, mitophagy plays an important role in inflammation prevention by boosting mtDNA clearance from damaged mitochondria. According to research, circulating mtDNA rises with age and is linked to higher levels of serum

inflammatory markers such as tumor necrosis factor α, interleukin 6 (IL-6), regulated upon activation, normal T cells expressed and secreted (RANTES), and IL-1 receptor antagonist protein (IL-1ra).[253] However, new research indicates that not every type of cell-free mtDNA is pro-inflammatory, nor does it seem to be sufficient to consistently cause inflammation.[254] Consequently, this belief may be the result of a misunderstanding of correlational clinical investigations, and it should be tested and re-evaluated in relevant biological systems.

9. Mitochondria-targeted drug delivery approaches in age-related diseases

Mitochondrial-targeted therapeutics are recognized as a revolutionary tool for diagnosing, preventing, and treating a wide range of age-related human diseases, including cardiovascular disease, metabolic disorders, cancer, and neurodegenerative diseases. Nonetheless, the mitochondria are made up of at least six compartments: the outer membrane, inner border membrane, IMS, cristal membranes, intracristal space, and matrix.[255] Hence, effective mitochondria-targeted drug delivery is challenging owing to the mitochondrial double-membrane and its complex structure, as well as the highly negative potential nature of the membrane. Therapeutic and tiny compounds, on the other hand, may diffuse through the outer membrane through passive diffusion and phospholipid cardiolipin.[256] It is noteworthy to mention that the pore in the outer membrane is wider, and hence, therapeutic molecules can easily traverse through this pore. The limited, highly folded inner mitochondrial membrane, on the other hand, has narrower transition slits that separate the mitochondrial matrix and IMS, making it difficult for many therapeutic compounds to cross the mitochondrial matrix. Nanotechnology has brought new hope over the years by bringing innovative compounds, such as nanoparticles (NPs), that can be employed for the clinical diagnosis and prognosis of a variety of illnesses, including numerous cancer kinds.[257,258] Furthermore, because of their deep tissue penetration capabilities, NPs not only increase drug half-life and increase drug accumulation in tumor tissues,[257-259] but they also provide a platform for weakly soluble medicines to be encapsulated and delivered more efficiently into circulation.[260] In addition, they can be rationally engineered to target certain intracellular organelles such as mitochondria,[261] ER,[262] Golgi apparatus,[262] and lysosomes.[263] Recently, multiple organic and inorganic NPs combined with conventional chemotherapeutic medicines to produce biocompatible, multifunctional mitochondria-targeted nanoplatforms.

Nevertheless, mitochondria-targeted NPs (mitoNANO) evolved to enhance the therapeutic targeting of mitochondria as well as overcome drug resistance and reduce the unwanted effects of the delivered drugs.[264] Mitolonidamine[265] and cisplatin[266] are two typical instances of mitochondria-targeted anticancer medications that promote programmed cell death and thereby prevent cancer cell survival, progression, and metastasis.[267] Interestingly, mitoNANOs loaded together with lonidamine showed 10-fold higher antitumor properties in comparison to pure lonidamine.[264] Furthermore, research indicates that mitochondria-targeted NPs have antiproliferative and cytotoxic impacts in tumor cells but not in healthy cells.[268] Moreover, mitoNANO-based therapies can accelerate mitochondrial blockades in cancer cells through multiple ways, including respiratory inhibition, modulation of the mitochondrial permeability transition pore, inhibition of the ETC, inhibition of anti-apoptotic protein family members, suppression of phenotypes linked to mutated DNA, and the promotion of mitochondrial-regulated cancer cell death.[264] In general, intracellular uptake is the first step for NPs to transport the drugs to the mitochondria. To complete this process, the cell membrane's negatively charged phospholipids must adhere to the positively charged, minute NPs, which leads to drug endocytosis and the formation of endolysosomes. Then, the endolysosomal membrane rupture occurs inside the cytoplasms, which results in the release of its contents, and the mitochondria to be targeted intracellularly.

There are various NP-based drug delivery methods used to treat mitochondrial disorders that aid in protecting drug payloads, including hydrophobic and hydrophilic compounds, from their elimination and degradation. NPs are classified into different groups according to their chemical and physical characteristics, surface area, shapes, or sizes, including liposomes, liposome-like vesicles (DQAsomes), MITO-porters, micelles, polymeric NPs, dendrimers, metal NPs such as gold NPs (AuNPs), quantum dots, or nanoscale semiconductor crystals, and each of them possesses specific features.[256] In addition, they can be accumulated in tumor cells utilizing either active or passive targeting techniques. The latter technique uses the enhanced permeability and retention phenomena and particular features of solid tumors, such as leaky vasculature and disrupted lymphatic drainage, which results in NP extravasation throughout the leaky blood arteries. On the other hand, active targeting is based on the ligand-receptor interaction system, which means that ligands attached to NPs can recognize molecules that are selectively overexpressed in tumor cells. The passive target technique for synthesizing mitoNANO provides some merits, such as simplicity and cost-effective synthetic procedure. However, the aggregation behavior of passively targeted NPs provides a reason for concern because it can result in their rapid clearance from the biological system. The active targeting of the mitoNANO approach requires

mitochondria-targeting ligands to be linked either to drug-loaded or free NPs. There are different ligands that can be linked to NPs depending on the prospective strategies and features connected with these ligands, including prices, synthesis complexity, off-target toxicity, immunogenicity, lengthy blood circulation time, and delayed clearance from the biological systems. Some of these ligands that are conjugated to therapeutic agents to improve their efficacy include tetramethylrhodamine-5-isothiocyanate,[269] triphenylphosphonium (TPP^+),[270,271] Vitamin E analogs,[272] dequalinium (DQA),[273] cationic N-heterocyclic carbenes,[274] and coumarin conjugate.[275] Among them, TPP^+ is probably one of the most extensively investigated ligands in targeting mitochondria for drug delivery. When TPP^+ is conjugated with NPs loaded with drugs, it increases their lipophilicity and enables them to escape from lysosomal compartments and easily penetrate the mitochondrial membrane bilayers. In addition, cancer cells facilitate the transfer of the TPP^+-labeled NPs from the cytoplasm into mitochondria, which can be up to a hundred times faster than non-functionalized NPs due to the mitochondria's higher negative plasma membrane potential in cancer cells compared to healthy cells.[264,276,277] For this reason, the mitochondria-targeted agents were developed by installing a triphenylphosphonium cation (TPP^+) joined to diverse bioactive molecules such as Mito-Met (metformin conjugated with TPP^+) and MitoQ (ubiquinone attached to TPP^+),[278] which exhibit enhanced lipophilicity and increased cellular uptake. A recent study shows the application of mitochondria-targeted lonidamine (Mito-LND) by coupling LND to TPP^+ through a connector aliphatic chain could potently inhibit primary lung cancer and suppress brain metastasis from primary lung cancer in an orthotopic animal model, which was more effective than LND in both cases.[267] Moreover, Mito-LND could induce autophagic cell death by inhibiting mitochondrial complexes I and II and stimulating ROS formation. In addition, curcumin-loaded nanostructured lipid carriers (NLCs) hold great potential to treat neurodegenerative diseases like AD.[279] NLCs are made up of a mix of solid and liquid lipids that can easily cross the blood–brain barrier (BBB),[280] one of the most challenging physiological barriers that exist between the central nervous system and peripheral circulation.[281] Curcumin is an herbal extract that has antioxidant as well as anti-inflammatory characteristics. When included with NLCs for targeted administration, curcumin can also reduce the formation of amyloid beta (Aβ) plaque by reducing the aggregation of amyloid β peptides and decreasing neurodegeneration.[279] In addition, silica NPs (SiNPs) were also utilized for BBB targeting due to their capacity for cellular absorption and accumulation in the intracellular amyloid cells (Aβ1-42), which showed decreased Aβ1-42 plaque formation and hyperphosphorylation.[282] Besides, conjugating the polyamidoamine dendrimers with an anti-inflammatory and antioxidant agent known as NAC (N-acetyl cysteine) was shown to target the activated microglial and macrophage (Mi/Ma) and astrocytes at the site of brain injury by penetrating the BBB. Thus, its application is a promising tool for treating mitochondrial dysfunction associated with neurological disorders.[283] Overall, the field of mitoNANO has sparked tremendous interest, and significant efforts are being made to develop mitochondrial-targeted nanomedicine. Preclinical and clinical investigations, on the other hand, are required to understand the safety of these drug delivery systems as well as their potential for a wide range of disorders.

10. Mitochondrial genome editing tool against aging

Genome editing is a powerful method for eliminating or replacing genetic abnormalities at specific loci to reprogram or modify their expression. Genome editing tools such as zinc-finger nucleases, transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 have already been utilized in a variety of scientific research to generate targeted changes across multiple species.[284-289] Nevertheless, mitochondrial genome editing studies began with the application of restriction endonuclease enzymes, including SmaI, ApaLI, and PstI, designed to be specifically utilized in mitochondria.[290-293] According to a number of studies, the use of mitochondria-targeted restriction endonucleases (mtREs) can change the ratio of mutant to wild-type mtDNA heteroplasmy in human somatic cells and mice models. The reports signified that mtREs notably reduce the amount of mutated mtDNA.[291,292] Nevertheless, the mtRE strategy has its limitations and challenges. For example, a single restriction site (XmaI) has been identified in around 200 mtDNA variants.[294] Further revolutionary strategies have been established to prevail over the restrictions and boundaries in the application of mtREs. The application of mitoZFNs or mitoTALENs, comprising a MTS, a specific DNA recognition site, and a FokI nuclease, has effectively changed the mitochondrial heteroplasmy ratio in earlier studies.[295-300] Zinc finger domains are the recognition motifs of the mitoZFN that can identify a 12-bp sequence. In contrast to mitoZFN, mitoTALEN takes advantage of TAL effector proteins as recognition motifs that can identify around 17 nucleotides.[301] As well as the established mitoTALEN strategy, a newer method called the mitoTev-TALE approach has been efficiently utilized to modulate the mutant mtDNA in cybrid cells.[302] In this method, instead of the FokI nuclease, the I-TevI nuclease is attached to TAL effector proteins. Nonetheless,

a few evident disadvantages have been reported for this approach, including demanding a CNNNG cleavage site for I-TevI nuclease.[290] Lately, a substantially unique method has been described for mtDNA editing, termed double-stranded DNA deaminase (DddA)-derived cytosine base editors (DdCBEs).[303-306] The DdCBE is comprised of mitoTALE proteins, the interbacterial toxin DddA, and also a uracil glycosylase inhibitor (UGI), which is designed to specifically generate C•G-to-T•A alterations in human mtDNA with extreme target accuracy.[301] Surprisingly, studies have confirmed the successful application of DdCBEs for mtDNA base editing in various species that can be mentioned as human embryos, mice, rats, zebrafish, and plants.[304,306-312] Regardless of the bright outcomes of applying mitoZFNs and mitoTALENs to mtDNA editing, these approaches have restrictions and boundaries. Either mitoZFN or mitoTALEN techniques should be meticulously aimed at and devised to identify a certain range of mtDNA sequences, which require a vast amount of effort and a costly assembly procedure.[301] Furthermore, the present viral-based delivery technology (adeno-associated virus, AAV) is insufficient for the size of the coding nuclease sequences.[313] The newly emerged programmable clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) genome engineering approach has facilitated the above issues as it benefits from a single guide RNA (sgRNA) to distinguish and target a specific 20 bp DNA sequence and a Cas9 nuclease to cleave this particular DNA sequence.[314-316] Notably, the Mito-CRISPR/Cas9 system has been published to easily cleave-targeted mtDNA in HEK293T cells[317] and zebrafish.[318] Despite the above, the crucial hurdle to this approach is introducing the exogenous sgRNA into mitochondria. While various studies have revealed substantial efforts to overcome this challenge, there are still unsolved drawbacks that demonstrate the ineffectiveness of this approach so far.[301,317,319] A visual summary of the mitochondrial contribution to the aging phenotype, alongside its therapeutic approaches targeting age-related disorders, is depicted in Figure 1.

11. Conclusion

Taken together, aging is a time-dependent deterioration in cell performance that is associated with the loss of cellular homeostasis. Mitochondria are central to various pathways in homeostasis because of their essential contribution to generating ROS, bioenergetics, apoptosis, catabolic and anabolic metabolism, and signal transduction. Accumulating evidence suggests that progressive mitochondrial impairment is associated with numerous aspects of aging, such as mtDNA mutation buildup, increased oxidative damage, and impaired respiratory capacity. However, the exact biological cause remains undetermined. Nevertheless, mitochondria contribute to the aging process through multiple distinct pathways that are responsive to environmental variations. However, the functional crosstalk between these pathways and their effects on the aging process remains largely uninvestigated. Moreover, the importance of nuclear and mitochondrial genes and their intricate cross-talk in regulating aging-related mitochondrial dysfunction adds more complexity to our understanding of the aging process. mtDNA is susceptible to accumulating mutations during the lifetime of the cell, resulting in increased heteroplasmy. Beyond a certain threshold, heteroplasmy of mtDNA mutations leads to the disruption of cellular homeostatic mechanisms, which can be translated into deleterious physiological consequences driving age-associated diseases.[320,321]

Increased ROS production has been associated with aging. Moreover, mitochondria are considered both producers and targets of ROS, which participate in cell homeostasis by acting as signaling molecules. However, ROS overproduction has harmful effects on cell homeostasis, resulting in age-related oxidative stress that can disrupt cell function, mtDNA mutations, the development of various pathologies, or even premature death. Regardless of the unanswered questions about the role of ROS in oxidative stress or as signaling molecules and their relationship with mitochondrial dysfunction in aging, antioxidant therapy, such as the administration of Vitamin E and CoQ10 compounds, seems quite promising for slowing down the aging process. However, further validation is required before their application in clinical trials against age-related diseases.

Furthermore, a growing number of studies have linked UPR^{mt} activation, mediated by epigenetic modifying enzymes, to the aging process and age-related disorders. However, it is important to highlight that the UPR^{mt} pathway has yet to be thoroughly described. Besides, mitochondrial perturbation can lead to complex cellular responses that can take various forms determined by the type of stressor and target tissue, going beyond the currently conceived model of UPR^{mt}. Hence, progress toward the complete characterization of UPR^{mt} is of great importance. As discussed, mitophagy is a highly evolutionarily conserved cellular process that specifically degrades damaged mitochondria in response to damage or stress to maintain a healthy mitochondrial population and, therefore, contributes to MQC. Nonetheless, mitophagy declines during aging and is linked to age-associated diseases, including cardiac dysfunction and neurological disorders. Thus, interventions that modulate mitophagy emerge as a promising therapeutic approach to counteract age-related disease development or progression. However,

the exact regulatory mechanisms of mitophagy and its role in age-related diseases remain elusive. Over the past decades, significant endeavors have been made to unravel the role of mitochondria in aging, along with targeted interventions. Yet, preventing mitochondrial dysfunction in age-related disorders is a major challenge.

Nevertheless, NPs (NPs) are capable of targeting particular cells and localizing within mitochondria, which facilitates treatments associated with age-related diseases and mitochondrial dysfunction disorders. Hence, NPs conjugated with mitochondriotropic ligands provide a promising tool for efficient mitochondrial delivery with proper nanoformulations. Although these nanopreparations have been demonstrated to efficiently transport a wide range of payloads to the mitochondria in both *in vivo* and *in vitro* systems, various clinical and pre-clinical investigations are needed to fully understand the safety of these drug delivery approaches. Recently, therapeutic gene editing techniques to correct DNA abnormalities, including mtDNA mutations and deletions, have offered the promise of curing human age-related diseases. Nevertheless, these techniques can help lower the mutation rate within impaired mitochondria, leading to a shift in mtDNA heteroplasmy, reversing mitochondrial defects, and hence improving mitochondrial function. However, the mitochondrial bilayer membrane is a significant obstacle to effective genetic manipulation. Furthermore, most heteroplasmic mtDNA mutations differ from the wild-type by a single nucleotide, and the ability of gene editing tools to detect single-nucleotide mutations is restricted, which can result in off-target editing or catastrophic mtDNA loss. Besides, efficient tissue-specific *in vivo* delivery of therapeutic genes that mostly rely on AAV as the delivery vehicle has proven challenging. Finally, a better comprehension of the role of mitochondria in aging remains a prerequisite for combating aging and age-related disorders.

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