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Mitochondrial function maintenance and mitochondrial training in ageing and related diseases

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ABSTRACT

Cellular senescence driven by mitochondrial dysfunction is a key contributor to ageing and related diseases. The decline in the quality and quantity of healthy mitochondria with ageing disrupts energy production, redox homeostasis, and intracellular signaling. Mitochondrial quality control (MQC) is a cellular self-repair mechanism that protects mitochondrial function and maintains a healthy mitochondrial network. Targeted regulation of MQC is expected to moderate the development of cellular senescence and related diseases. We explored the impact of mitochondrial function on cell fate at the molecular and organelle levels, analyzed the role of mitochondria-targeted interventions for delaying cellular senescence and ameliorating age-related diseases, and pointed out the idea of increasing the critical level of healthy mitochondria to cope with internal and external stressful stimuli and to improve the ability of self-repairing by exercising and protecting mitochondria in the long term.

1. Introduction

The increase in the aged population brings more challenges than ever. The human lifespan has been extended considerably with the advancement of science and technology, especially in healthcare. Ageing has become a significant risk factor for cancer, cardiovascular disease, and neurodegenerative diseases. The World Health Organization (WHO) defines senescence as the gradual accumulation of molecular and cellular damage in the body over time. Molecular and cellular damage associated with senescence is the primary cause of ageing.[1] It is well known that ageing is closely related to various diseases. The function of the significant organs gradually deteriorates in older people, and they gradually suffer various age-related diseases.

Mitochondria are the leading energy suppliers in eukaryotic cells, and lots of studies have illustrated that their functional degradation is associated with the occurrence and development of age-related diseases.[2] The oxidative metabolism of the mitochondria provides energy for the operation of cellular metabolism; the mitochondria themselves are also constantly subject to oxidative damage. As the damage accumulates, the function of mitochondria gradually weakens, leading to more dysfunction of mitochondria, which can accelerate cell ageing. Mitochondria are essential for cellular physiological activities and signaling and may also be an initiating factor for cell damage or death.[3] Without external stressors, the cell maintains mitochondrial structure and function stability through MQC (Mitochondrial Quality Control). This process ensures mitochondria's stable and efficient function and prevents cellular dysfunction and related diseases caused by damage to mitochondria.[4] It has been demonstrated that a certain degree of enhancement of MQC can help prevent the development of various diseases, particularly those associated with ageing. However, excessive activation of the control pathway can also result in cellular damage and reduced mitochondrial function.[5] Healthy ageing can be achieved through moderate exercise, a proper diet, and targeted mitochondrial repair and functional activation (Fig. 1). Specific intervention strategies are employed to enhance cellular mitochondria quantity, quality, and functionality. This is intended to optimize cellular energy production and utilization, enhance metabolic capacity, and improve overall performance. Mitochondrial training may be defined as a process of continuous activation of mitochondrial function and quality control, with the increase of the capacity of response to cellular stress for mitochondria.

Meanwhile, it is known that mitochondrial DNA mutated more with ageing, but low levels of mutated mitochondrial DNA did not affect mitochondrial function or normal cellular energy metabolism. Usually, a

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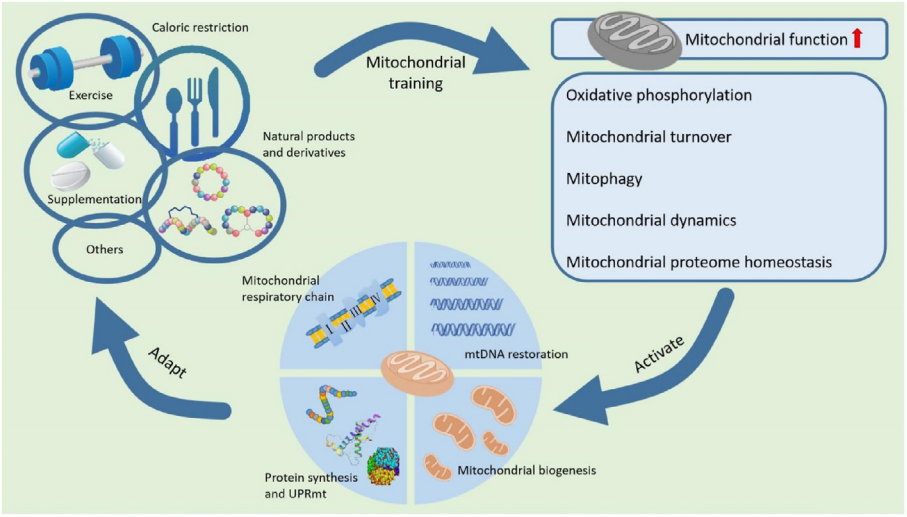

Fig. 1. Mitochondrial training. Strategies focusing on improving mitochondrial function have been paid more attention. For example, caloric restriction (CR) can reduce oxidative damage in the body. Endurance exercise training enhances the aerobic capacity of skeletal muscle by improving the quality control, content, and function of mitochondria. SIEGEL M P et al. showed that a single treatment with a mitochondrial-targeting peptide can rapidly restore mitochondrial energy to youthful levels in elderly mice, reversing declines in maximal mitochondrial ATP production, oxidative phosphorylation coupling, and cellular energy supply. These changes are more pronounced in ageing animals.[9–11] Mitochondrial training refers to an approach that involves sustained interventions such as exercise, dietary modifications, natural active products, or lifestyle modifications to achieve rapid cellular adaptation and sustained resistance to stress responses and pressures by enhancing MQC and optimizing energy production. This process would be a potential intervention to slow cellular senescence, reduce age-related dysfunction, and promote healthy ageing.

phenotypic manifestation of the genetic defect occurs only when a threshold level is exceeded.[6] Thus, within the threshold range, the cell maintains a normal mitochondrial network state by activating mitochondrial quality control. This is evidenced by the increased number of healthy mitochondria, protection of mitochondrial DNA integrity, and maintenance of proteome and active respiratory chain complex reserves.[7] In this way, the training of mitochondria may have a more vital function and tolerance against cellular ageing in the face of progressively deteriorating conditions.

The 12 hallmarks of ageing include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, disabled macroautophagy, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis.[8] The inherent fragmentation of the markers is counterbalanced by their interdependence, with the influence of one marker on the others. It is therefore recommended that each feature be considered as an entry point for future exploration of the ageing process, as well as the development of new anti-ageing drugs. Next, we will focus on the relationship between mitochondrial function and cellular senescence, illustrate the potential relationship between mitochondrial dysfunction and ageing, and provide an overview of emerging anti-ageing strategies targeting mitochondria.

2. Cellular senescence and related diseases

Cellular senescence was first discovered in human diploid cell lines; these cells go through active multiplication, gradual cessation of mitotic activity, and accumulation of cellular deprescription activation of the cyclin-dependent kinase (CDK)-inhibitor 1 (CIP1), causing cell growth to stall.[12,13] This phenomenon typically contributes to the maintenance of genetic stability. It can slow or inhibit the transformation of preneoplastic lesions into neoplastic lesions during tumorigenesis, suggesting that normal cell senescence can inhibit cancer and tumorigenesis but is reversed by irreversible functional impairment and overaccumulation. Cell senescence is often accompanied by the development of senescence-associated secretory phenotype (SASP); SASP consists of pro-inflammatory factors, growth factors, chemokines, and matrix remodelling enzymes. They can cause low-grade chronic inflammation and diseases in the body and accelerate the senescence of cells or neighbouring cells (Fig. 2).[14,15]

2.1. Cardiovascular system and cellular senescence

The heart is an essential organ of the human body. Still, with the increase of age, the gradual increase of senescent cells will harm the myocardial structure and function, which leads to the decline of myocardial contractility and diastolic function and the decline of the heart's pumping ability, eventually causing heart failure. Cardiac ageing is a heterogeneous process characterized by increased reactive oxygen species (ROS), genomic DNA damage, and telomere shortening. Supraphysiological concentrations of ROS oxidatively modify biological macromolecules, including proteins, nucleic acids, and lipids. This process generates harmful substances and oxidative damage to cellular organelles, impairing cellular function and contributing to gradual ageing.

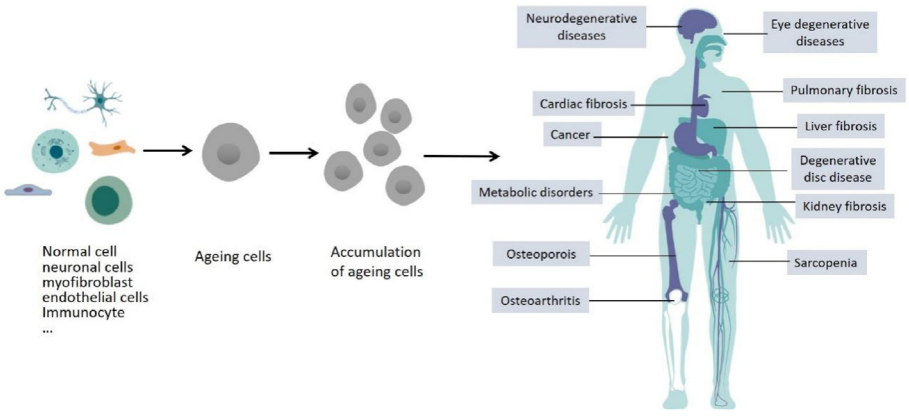

Fig. 2. Cellular senescence is associated with diseases of senescence. DNA damage, protein misfolding, mitochondrial dysfunction, and other adverse phenomena inevitably occur in cells. Normal cells activate self-protective mechanisms such as DNA repair, autophagy, antioxidative stress, and mitochondrial fusion and division to combat these adverse factors. However, cellular senescence with age is a response to this imbalance of damage and repair, which has been linked to various age-related diseases, including neurodegenerative diseases, metabolic dysfunction, and age-related diseases.

Normal cells can resist oxidative damage caused by this ROS overload through various enzymes and signaling pathways of the antioxidant system. However, senescent cells exhibit an imbalance in the antioxidant-oxidant system, ultimately leading to the ageing or death of cells, known as oxidative stress (OS).[16] With cellular senescence, particularly of the vascular endothelium, functional changes in morphology (e.g., flattened endothelial cells) occur, which lead to endothelial dysfunction, atherosclerosis, and ultimately hypertension. Bone marrow-derived endothelial progenitor cells and klotho are reduced during endothelial cell senescence.[17] Some studies have shown that ageing increases ROS accumulation and oxidative stress in the heart.[18–20] The non-selective damage induced by oxidative stress has been strongly associated with a variety of age-related cardiovascular diseases, including atherosclerosis, myocardial infarction, and heart failure.[21,22] This is because oxidative stress increases the number of oxidized macromolecules and the level of ROS production.[23] At the same time, mitochondria are essential for endogenous ROS production. During physiological ageing, mitochondrial membrane potential (MMP) decreases, resulting in reduced energy production and increased OS due to the production of ROS.[24,25] The accumulation of dysfunctional mitochondria within cells can lead to various cardiovascular diseases associated with ageing.[26]

Consequently, both antioxidants and mitochondrial protection represent significant therapeutic targets for improving age-related cardiac phenotypes.[27–29] Another factor contributing to the deterioration of heart function is the vicious cycle of the inflammatory response of ageing cells and oxidative stress damage. Biomarkers of inflammation are consistently linked to chronic disease risk. For example, high levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are associated with an increased risk of cardiovascular disease.[30] Inflammation caused by cell death will produce excessive ROS, leading to oxidative stress damage, which induces inflammation, forming a vicious cycle in ageing cardiomyocytes.[31,32] Based on the unique features of senescent cells, current efforts are primarily invested in discovering methods to eliminate senescent cells. Pan-tyrosine kinase inhibitor Dasatinib and a natural flavonoid, quercetin (D/Q combination), have been shown to kill senescent human umbilical vein endothelial cells (HUVECs) in tissue culture selectively and to suppress the senescent cell signature under a set of pathological conditions.[33] R. Schmitt et al. designed siRNA or the small molecule ABT-737 to target and inhibit the expression of the anti-apoptotic proteins BCL-W and BCL-XL in senescent cells, specifically triggering the death of senescent cells and inhibiting the adverse cardiovascular effects of biological ageing.[34] In addition to removing senescent cells, another promising tool for targeting senescent cells is eliminating SASP without causing cell death. For example, metformin has been shown to improve cardiovascular function and prolong lifespan in diabetic patients, providing support for investigation into the ability of metformin to delay ageing in nondiabetic persons.[35] Depending on cell type, the senescence stage, and the senescence inducer type, the SASP is highly heterogeneous, and it is difficult to identify a general regulatory mechanism.[36] Therefore, there is a need to analyze individual cells by single-cell RNA sequencing to understand better the specific SASP components that drive specific functions in cardiovascular ageing. More clinical research is urgently needed. Senescent cells accumulate in the heart during ageing, resulting in decreased cardiac function, heart damage, and an increased risk of heart disease. OS and mitochondria are two key targets that require further investigation.

2.2. Nervous system and cellular senescence

Similarly, in aged people, the degeneration of the central nervous system (CNS) drives the progressive impairment of cognitive and physical abilities, the ageing brain being vulnerable to Alzheimer's disease (AD), Parkinson's disease (PD), and stroke.[37,38] One in 10 individuals aged 65 or older suffers from AD, and its prevalence continues to increase with ageing.[39] Cellular ageing increases in AD, PD, Amyotrophic Lateral Sclerosis (ALS), and Huntington's disease (HD), including microglia, astrocytes, and neuronal cell senescence. A growing body of evidence indicates that oxidative stress accelerates brain ageing and related diseases. Furthermore, it suggests that free radicals damage mitochondria and increase the production of toxic β-amyloid protein (Aβ), which is thought to be a significant cause of AD.[40] Neurons rely on the energy produced by mitochondria to maintain synapses and neurotransmission. Consequently, brain mitochondria are more active than other tissues.[41]

Nevertheless, cells within the AD brain are susceptible to oxidative damage and exhibit reduced mitochondrial respiration, reduced movement, reduced size, and disruption of the inner membrane of the cristae relative to normal brain cells.[42] Decreased endogenous antioxidant production in the ageing brain[43]; the redox environment disturbance triggers conformational changes and mitochondrial localization of the pro-apoptotic BCL-2 family (BAX, BAK, BAD, BIM). Furthermore, it has been demonstrated that increased mitochondrial membrane permeability leads to the release of pro-apoptotic proteins from the mitochondrial membrane space, including cytochrome *c*; this process activates the cysteinyl aspartate specific proteinase (Caspase-3), which initiates apoptosis by cleaving numerous essential cellular proteins through proteolytic hydrolysis.[44,45] This is why severe nigrostriatal dopamine neuron loss occurs in PD. Therefore, antioxidant supplementation and mitochondrial protection prevent ageing and ameliorate pathological changes in age-related brain diseases. In recent years, inflammation has also been identified as an essential contributor to neurodegenerative diseases. Inflammatory responses can clear toxins and unknown pathogens and promote cytotoxicity and neurodegeneration.[46] However, it is unclear whether inflammation is a consequence or a positive factor in neurodegenerative diseases. In particular, the regulatory function of mitochondria between oxidative stress and inflammatory responses in brain cells.[47] Mitochondrial dysfunction leads to excess ROS production, which brings about oxidative damage to glial cells, and normal cells activate the autophagy pathway to remove damaged mitochondria. While senescent cells are defective in autophagy, leading to the accumulation of damaged mitochondria that release their components into the cell, mtDNA, in particular, can act as a damage-associated molecular pattern (DAMP), which is sensed as a non-self molecule and triggers an innate immune-inflammatory response by binding to danger signaling receptors.[48] Microglia are significant players in neuroinflammation, and overactivated microglia release pro-inflammatory factors in response to abnormal accumulation of disease-causing proteins and invasion by pathogens.[49] Uncontrolled neuroinflammation can induce chronic inflammatory responses and accelerate the progression of neurodegenerative diseases such as AD.[50] In mouse models of neurodegenerative diseases, eliminating senescent cells improves brain function and alleviates cognitive dysfunction during ageing.[51]

2.3. Skeletal muscle system and cellular senescence

Bone and skeletal muscle are involved in a variety of physiological activities such as locomotion, protection, metabolism, and maintenance of the systemic microenvironment, and their structure and function are affected by endocrine, inflammatory, and external stresses (physical activity, drugs, pollution, and diet).[52] Disrupting the metabolic balance between anabolism and catabolism in ageing bone and muscle results in tissue damage characterized by disruption of the skeletal microenvironment and accumulation of senescent cells in muscle.[53] Skeletal muscle represents a significant site of energy production and expenditure. The presence of numerous and active mitochondria contributes to the maintenance of energy metabolic homeostasis.[54] However, the accumulation of age-related oxidative stress and DNA damage above the threshold for selective initiation of senescence in normal cells resists endogenous stresses through apoptosis. This also means it is difficult to cope with exogenous stresses that result in irreparable tissue damage.[55] Even in cellular senescence, the inability to remove these cells promptly due to a compromised immune system can also result in the accumulation of senescent cells with a reduced regenerative capacity, contributing to the

gradual ageing of the tissues.[46] Diseases of the immune system caused by senescence of immune cells will be discussed later. Sarcopenia, which reduces the quality of life in the old and causes them to be more prone to falls and fractures, is characterized by type II fibre atrophy, changes in fibre size and heterogeneity, accumulation of fat and connective tissue between fibres, decreased mitochondrial metabolism and its oxidative capacity, inflammation, and increased protein catabolism.[56] Bones have a vast reservoir of progenitor cells in the body. It has been shown that various cell types in the bone microenvironment are senescent during ageing and form heterogeneous SASPs that affect normal osteoblasts.[57] Age-related bone loss is associated with the accumulation of senescent cells, particularly osteoblasts, in the bones of older individuals. As mentioned, many senescent skeletal muscle cells are involved in age-related skeletal diseases. Removing senescent skeletal muscle cells from the skeletal system is critical for slowing skeletal ageing and alleviating disease. In INK-ATTAC or p16-3MR transgenic mice, drugs targeting *p16Ink4a* successfully remove *p16Ink4a*-positive senescent skeletal muscle cells, alleviating age-related bone loss and extending the lifespan of mice.[58,59] Targeting cellular senescence has led to the emergence of "Senolytics" (selectively eliminating senescent skeletal muscle cells) and "Senomorphics" (inhibition of SASP secretion) to neutralize and eliminate the adverse effects of senescent skeletal muscle cells, which are collectively referred to as "chemotherapeutic" strategies.[60]

Furthermore, removing senescent cells is not optimal; inhibiting cellular ageing and alleviating senescence-induced cellular damage are more beneficial. Oxidative stress and inflammation are significant factors that are closely associated with the process of ageing. Excessive ROS production in senescent cells leads to mitochondrial dysfunction and further exacerbates inflammation.[61] Mitochondria are both the primary site of ROS production and the target of ROS.[62] The synthesis and breakdown of proteins in bone and muscle require much energy, leading to the mitochondria's continuous production of loaded ROS. With the age-related decline in cellular autophagy,[63,64] damaged mitochondria cannot be removed promptly and are not replenished by healthy mitochondria, which reduces ATP synthesis. This, in turn, is associated with a reduction in protein synthesis, which ultimately causes tissue damage.[65] Furthermore, oxidative damage to the mitochondrial membrane results in the release of intra-mitochondrial components which, in turn, initiates an immune-inflammatory response and mitochondria-mediated apoptosis.[66]

2.4. Other body systems and cellular senescence

2.4.1. Liver, lungs and kidney

The liver is the most significant metabolic organ. Almost all the substances absorbed from the gastrointestinal tract are transported to the liver, where they undergo synthesis, degradation, and storage transformation. Therefore, age-related changes in liver function increase the body's susceptibility to age-related diseases.[67] Therefore, age-related changes in liver function increase the body's susceptibility to age-related diseases.[68] Hepatic parenchymal cells are the liver's primary cells; with ageing, they accumulate lipids, have mitochondrial dysfunction, and increase ROS production.[69] This is the main reason for the high prevalence of fatty liver in older people.[70] Kupffer cells are resident hepatic macrophages located in the liver sinusoids and involved in the innate immune response to liver infection.[71] The effects of ageing on macrophages are a decrease in phagocytosis and autophagy, as well as an increase in the production of cytokines that contribute to the inflammatory phenotype ('inflammageing') of old age.[71] Examples include TNF-α and IL-6. Reduced oxidative phosphorylation capacity, decreased membrane potential, increased mtDNA mutations, OS, and morphological changes have been reported in the mitochondria of ageing livers compared to that of the young state.[72] This is associated with the production of supraphysiological concentrations of ROS, while mitochondrial dysfunction promotes an increase in ROS, which also drives cellular senescence.[73] Liver fibrosis progresses to cirrhosis, an advanced form in which damaged tissues and cells cannot be repaired due to large amounts of extracellular matrix and the restoration of normal liver structure, marking the end of liver function and complete liver failure, and ultimately death. The most important factors are ROS and cellular senescence. The presence of senescent cells disrupts tissue structure and function and increases the production of senescent cells in liver tissue, contributing to fibre formation.[74] It is worth mentioning that fibrosis is a typical ageing change and is common in the tissues of the ageing organism. For example, prostate fibrosis in older men leads to urinary tract dysfunction (UTD), in whose tissues a decrease in mitochondrial function and an increase in cellular senescence are observed.[75]

Young normal lungs in which a small number of senescent cells are rapidly cleared by immune cells and normal cells maintain a baseline of telomere length and mitochondrial homeostasis. As fibrosis increases and the number of senescent cells increases, the immune cell response to senescent cell clearance becomes slow. Senescent cells are characterized by telomere shortening, secretion of high rates of SASP, mitochondrial dysfunction, and imbalances in mitochondrial division and fusion.[76] Chronic Obstructive Pulmonary disease (COPD) is a major healthcare problem with high morbidity and mortality. Although there is no known cure, COPD is managed through lifestyle changes and medication, but these therapies have limitations and new treatments need to be explored. Studies have shown increased expression of SA-β gal, p16, and p21 in endothelial colony-forming cells (ECFCs) derived from COPD patients, demonstrating an increase in senescent cells.[77] Pulmonary fibrosis is a significant category of end-stage pathological changes of lung diseases characterized by fibroblast proliferation and accumulation of many extracellular matrices accompanied by inflammatory damage and tissue structure destruction. For example, normal alveolar tissue is damaged, and scarring forms. It has been reported that the disease Idiopathic Pulmonary Fibrosis (IPF) is mediated, in part, by senescent cells, and cellular senescence markers are detectable within IPF lung tissue, which can be targeted to restore healthy pulmonary tissue of ageing mice.[78] For example, upregulation of senescence-related pathways in type II alveolar epithelial cells (AT II) has been noted in a mouse model of IPF where AT II Sin3a has been knocked out to induce senescence.[79]

Halloran and colleagues first discussed that the accumulation of senescent cells may be responsible for the insufficient repair capacity and functional loss in older kidneys almost 20 years ago.[80,81] During ageing, intrinsic cellular responses that repeat minor injuries continue to occur, and these injuries go virtually unnoticed for a long period until a certain threshold is reached that can impact kidney function.[58] Renal ageing and Chronic Kidney disease (CKD) share many common features, ranging from pathological and clinical manifestations to underlying mechanisms. Mechanisms common to the renal ageing process and the development of CKD include increased cellular senescence, decreased autophagy, mitochondrial dysfunction, and altered epigenetic regulation, suggesting that cellular senescence would be a potential therapeutic target applicable to both diseases.[82] Early on, for end-stage renal disease, renal replacement therapy was required, and with the development of key mechanisms of senescence such as cell cycle blockade, apoptosis inhibition, and SASPs, selective targeting of senescent cells (adopting Senolytics therapy) or systemic SASPs (adopting Senomorphics therapy) has been progressively investigated and applied in basic models of CKD and clinical trials.[83] In a study, Peter de Keizer and colleagues identify forkhead box O 4 (FOXO4) as a key regulator of senescent cell viability and demonstrate that targeting FOXO4 signaling causes apoptosis and restores renal function in rapidly ageing and naturally aged mice.[84] Meanwhile, SASP can accelerate the renal ageing process in different environments and participate in the pathological process of renal diseases, and therapeutic strategies targeting this have potential.

2.4.2. Skin and eyes

The skin consists of different cell types with different proliferative capacities. During the ageing process, senescent cells accumulate in different compartments of the human skin, leading to impaired skin

physiology. Skin ageing is characterized by slow regeneration and even permanent erosion of skin structure and function. Skin ageing is associated with impaired protection, in particular impaired wound healing and barrier function, increased inflammation, impaired water and thermal homeostasis of organisms, and susceptibility to various skin diseases, including cancer.[85] Senescent cells accumulate as the skin ages, leading to a decline in all aspects of skin function. Paracrine signaling between dermal fibroblasts and keratin-forming cells is necessary for tissue homeostasis in physiological conditions and skin diseases.[86] The crosstalk between these cell populations is altered during the ageing process.[87]

Ageing leads to a gradual decline of function in multiple organs. Cataracts, glaucoma, diabetic retinopathy, and age-related macular degeneration (AMD) are age-related ocular diseases. In the old human retina, p16INK4a is expressed in rods, ganglion cells, amacrine cells, and horizontal cells. Moreover, p16INK4a and p21Cip1 are expressed in retinal vascular vessels, elucidating the expression of canonical senescence markers in retinal cells.[88] In recent years, glaucoma has emerged as a blinding ocular disease intricately linked to cellular senescence. The principal pathways implicated are oxidative stress, mitochondrial dysfunction, DNA damage, autophagy impairment, and the secretion of various senescence-associated secretory phenotype factors.[89] AMD is a complex disease that is closely related to cellular senescence. Senescent ocular cells exhibit mitochondrial and telomere dysfunction as well as elevated oxidative stress, leading to the production of SASP and more ROS, both of which can induce and stabilise senescence in neighbouring cells.[90]

2.4.3. *Metabolic systems*

With the increase of age, the elderly group has hyperglycemia, fatty liver, hyperlipidemia, hypertension, high uric acid, and other diseases. A disease called "metabolic syndrome" disturbs the body's metabolism and damages human health. Ageing is thought to be a risk factor for metabolic syndrome, especially in obese older adults. Senescent cells accumulate in the adipose tissue of obese people and rodents. Experiments have shown that targeted elimination of these senescent cells can reduce the burden of senescent cells in obese mice, alleviate metabolic dysfunction and adipose tissue inflammation, improve glucose tolerance, enhance insulin sensitivity, and reduce the release of inflammatory mediators in obese mice. At the same time, senescent cells can cause macrophage migration into adipose tissue in obesity, and targeting senescent cells prevents and reduces the adipose tissue macrophage accumulation often associated with obesity.[91]

2.4.4. *Immune system*

As the human organism ages, immune cells undergo a process of gradual senescence, leading to a decline in immune system function and, in turn, an increased susceptibility to a variety of immune-related diseases.[92] T cells, as a core immune system component, undergo significant changes during the ageing process.[93] The initial T-cell pool becomes smaller, and the memory T-cell pool becomes larger, decreasing the number of available TCRs. The senescent T cells exhibit molecular features such as mitochondrial dysfunction, epigenetic remodelling, DNA damage, and short telomeres.[94] These senescent T cells secrete pro-inflammatory factors that trigger chronic inflammation, disrupting homeostasis in tissues and causing cardiovascular diseases, metabolic disorders, and neurodegenerative diseases.[95,96] Recently, interventions targeting senescent cells have become a hot research topic. Removing senescent cells or regulating their function is expected to slow down the ageing process and improve the function of the immune system.

The administration of Senolytics drugs, which induce apoptosis, eliminates senescent cells. For instance, the combination of Dasatinib and quercetin effectively removes a wide range of senescent cells.[97] In addition, cardiac glycosides have been shown to induce senescent cell death by altering the inner membrane potential and proton concentration of senescent cells. Modulation of the immune system enhances the body's capacity to clear senescent cells. For instance, CAR-T cell therapy can specifically recognize and remove senescent cells.[98]

Furthermore, gene editing technology has emerged as a potent tool for targeting key genes in senescent cells, impeding their senescence process. A study has shown that the accumulation of senescent cells can be reduced by inhibiting the *p16INK4a* gene.[99] Immune cell senescence has been identified as a significant factor in developing immune system diseases. Interventions that target senescent cells have emerged as a novel approach for delaying senescence and enhancing immune system function.[100] It is recommended that future studies further explore the mechanism of action and the prospect of clinical application of these interventions, which can provide a theoretical basis for the development of effective anti-ageing therapies.

3. Mitochondrial dysfunction promotes cellular senescence

Complex life is composed of eukaryotic cells, and the presence of mitochondria in eukaryotic cells is essential to evolution.[101,102] Mitochondria are organelles with a double membrane structure composed of an inner mitochondrial membrane (IMM) and an outer mitochondrial membrane (OMM). The IMM folds inward to form cristae, which provide more attachment sites for enzymes and generate energy to participate in various intracellular reactions through aerobic respiration. They are usually filamentous or rod-shaped slender bodies, and the mitochondria have a large matrix volume, making part of the gap between the IMM and the OMM very small, which is called the mitochondrial inner boundary membrane (IBM).[103,104] Despite this diversity, all mitochondria derive from a common ancestral organelle that originated from integrating an endosymbiotic alphaproteobacterium into a host cell related to Asgard archaea.[105] The essential function of mitochondria is to form an electrochemical gradient on the respiratory chain of the inner membrane through the coupled transfer of electrons to oxygen and the transport of protons from the matrix through the IMM into the intermembrane space, which provides power for the terminal complex V of the respiratory chain and catalyzes the synthesis of ATP in most cells under the action of ATP synthase. Electrochemical gradients are also used for other critical mitochondrial functions, such as maintaining homeostasis for transporting buffer signaling ions Ca^{2+}.[106,107] Reducing the electrochemical gradient signals cell activation to repair and eliminate defective mitochondrial pathways.[108]

It was earlier found in the ageing process of model animals that male houseflies enter the old age stage during the second week of adult life, as reflected in the loss of wings, and the activity of magnesium-activated adenosine triphosphatase in the giant mitochondria of the flight muscle decreases with failure in flight. The rapid decline in the activity of the α-glycerophosphate dehydrogenase dependent on nicotinamide adenine dinucleotide is a sign of wing loss in ageing male houseflies.[46] Type 2 diabetes mellitus (T2DM) is the most common chronic metabolic disease in the ageing population and is characterized by impaired glucose tolerance due to insulin-stimulated glucose metabolism dysfunction in the skeletal muscle.[105,109–111] IR in older adults is associated with increased intracellular fatty acid metabolites, possibly due to age-related decreased mitochondrial oxidation and phosphorylation activity.[112] In conclusion, the ageing process is accompanied by a decrease in mitochondria-related enzyme activities and a drop in mitochondrial quality. For example, citrate synthase activity declined, mitochondria's respiratory capacity attenuated, ROS production rose, membrane permeability increased, and damaged protein aggregation and mitophagy levels were reduced.[113] So, what is the link between mitochondrial dysfunction and cellular ageing? This is discussed next.

3.1. *Mitochondrial biogenesis*

The cells launch the mitochondrial biogenesis process in response to the energy demand triggered by developmental signals and environmental stressors. This is a self-renewal route by which new mitochondria are generated from existing ones, and the process of mitochondrial

biogenesis occurs mainly in healthy cells.[114] (Fig. 3). If mitochondrial biogenesis is attenuated, mitochondrial renewal slows down, and overall productivity decreases, resulting in an increased accumulation of damaged biomacromolecules, further exacerbating mitochondrial dysfunction in ageing cells.[115,116] In ageing, the imbalance between the production of healthy mitochondria and the clearance of abnormal mitochondria accounts for the dysfunction of mitochondrial renewal and the accumulation of damaged mitochondria in cells. Usually, cells undergo mitophagy to degrade damaged mitochondria while simultaneously initiating compensatory biosynthetic reactions to replenish their mitochondrial pool in response to a deficiency in biological energy and reduced ATP synthesis. Mitochondria are the energy basis for the activity of neurons in the human brain. Activating mitochondrial biogenesis is beneficial in delaying the onset and development of neurodegenerative diseases in the elderly.[117–119] Mitochondrial dysfunction due to the reduction of biogenesis in the blood is a potential marker to assess the risk of PD.[47] The peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) modulates mitochondrial biogenesis. More expression of PGC-1α and the adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) have been thought of as a method to extend the healthy life of the elderly.[120]

PGC-1α is a significant regulator of mitochondrial biogenesis and function.[121,122] PGC-1α regulates the expression of mitochondria-associated genes by interacting with multiple transcription factors. On the one hand, PGC-1α can activate the expression of NRF1, which further promotes the expression of mitochondrial DNA polymerase γ (POLG) and mitochondrial transcription factor A (TFAM), and TFAM acts on the nuclear genome to initiate mitochondrial DNA replication and transcription. On the other hand, PGC-1α can bind to estrogen-related receptor α (ERRα) to form a complex that enhances the transcriptional activity of ERRα. ERRα activates the expression of a range of downstream genes that promote mitochondrial biogenesis.[63] When PGC-1α is overexpressed, mitochondrial biogenesis is activated, while PGC-1α expression is inhibited, and mitochondrial biogenesis activity is decreased.[123,124] It turns out that AMPK is the upstream activator of PGC-1α, and AMPK enhances mitochondrial biogenesis not only by inducing *PGC-1α* transcription but also by activating PGC-1α through phosphorylation of threonine-177 and serine-538.[125] Simultaneously, the silent information regulator 1 (*SIRT1*) can directly act on PGC-1α to mediate its deacetylation and improve the activity of PGC-1α to enhance mitochondrial biogenesis, especially in the case of caloric restriction (CR).[126] Hydrogen sulfide is an essential regulator of cardiac mitochondrial content. Exogenous hydrogen sulfide can induce mitochondrial biogenesis through the AMPK-PGC-1α signaling cascade, improve mitochondrial respiration, increase ATP production efficiency,

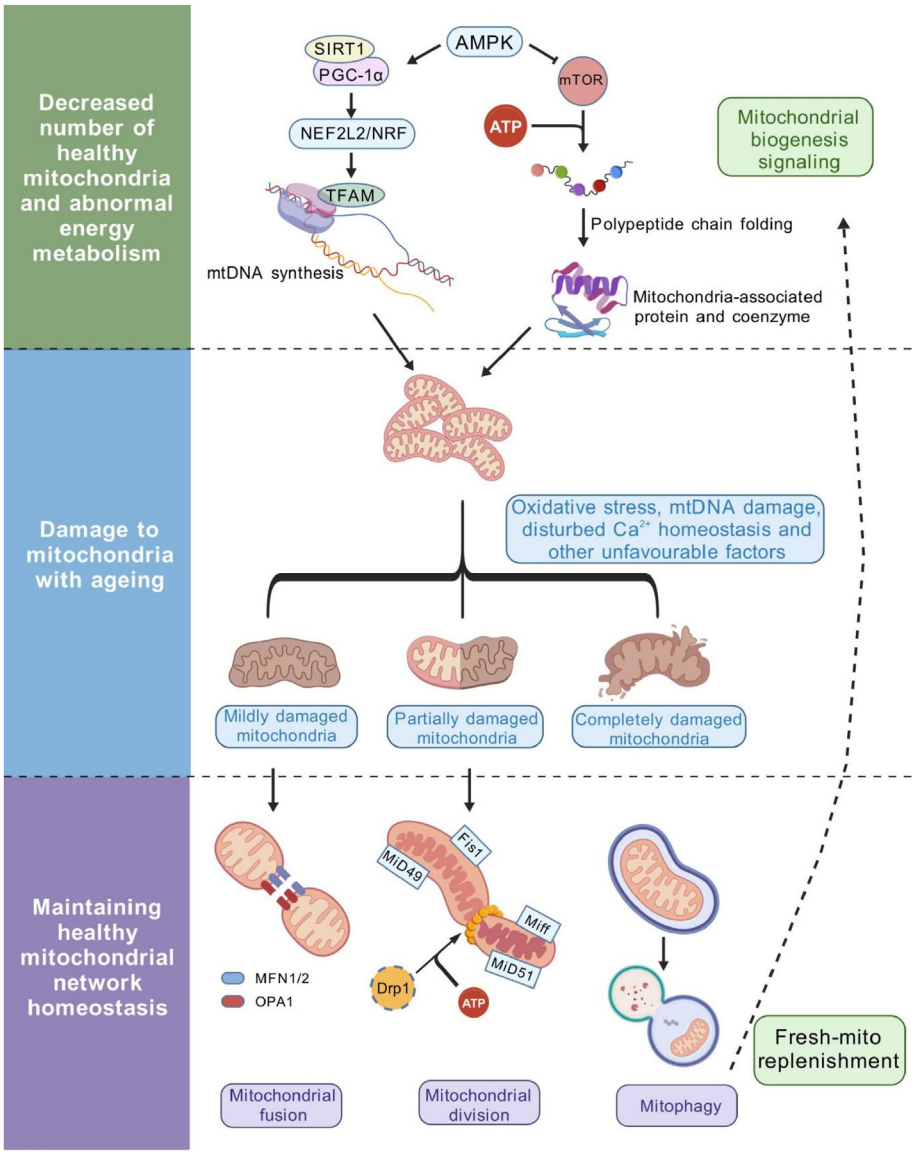

Fig. 3. Mitochondrial network homeostasis. The decrease in ATP synthesis activates the AMPK signaling pathway, then AMPK induces PGC1-α to act on the transcription factor NEF2L2. NEF2L2 acts on mitochondrial transcription factor (TFAM) to drive mtDNA transcription and translation. SIRT1 can directly mediate PGC1-α deacetylation to activate it. On the other hand, AMPK inhibits mTOR, induces the synthesis of protein, and promotes healthy mitochondrial biogenesis. Mitochondrial fusion was regulated by OPA1, MFN1, and MFN2 and finally formed a complete mitochondrial reticular state. If mitochondria are damaged, division occurs, and endoplasmic reticulum-derived membrane structures mark the division site. The OMM proteins MID49, MID51, and FIS1 recruit DRP1, which binds to MFF. The dimers and oligomers of DRP1 assemble into a helical superstructure at the division site. Shrinks hydrolysis to cut mitochondria, and the damaged mitochondria are partially cleared by the mitochondrial autophagy pathway. Then, the decline in mitochondrial numbers stimulates new mitochondrial biogenesis.

and improve cardiac function.[127] Transcription factor NF-E2 p45-related factor 2 (NRF2) allows cells to adapt and survive under stressful conditions by regulating the gene expression of various cellular protective protein networks. It activates mitochondrial biogenesis by up-regulating the nuclear respiratory factor 1 and PGC-1α.[128]

3.2. Mitophagy

Autophagy refers to the ability of cells to provide nutrients and energy by degrading non-essential components in response to the transient stress of survival, thus sustaining life. Correspondingly, autophagy may degrade potentially toxic proteins to prevent cell damage or the process of apoptosis. Autophagy degrades components in the cytoplasm of yeast by placing them in nutrient-deficient media, and autophagy is essential for yeast survival.[129] This autophagy mechanism also exists in human cells and is closely related to cellular senescence.[130,131] Mitophagy is a specific autophagic process that removes damaged or excess mitochondria from cells. Much evidence has shown that the level of defective mitochondria in the cell increases during ageing, the integrity of mitochondria is damaged, and faulty mitochondria cannot be cleared, accelerating the cell ageing process.[132,133] Cells generally clear damaged mitochondria through the mechanism of mitochondrial autophagy,[120] which can be divided into three pathways: (I) Ubiquitin-dependent PINK1/Parkin-mediated mitochondrial phagocytosis. When mitochondria are damaged, PINK1 stabilizes in the OMM, activating Parkin for subsequent mitochondrial protein ubiquitination. Finally, autophagy receptors such as p62 were recruited to mediate the phagocytosis of mitochondria with the autophagosome membrane interacting with microtubule-associated protein 1 light chain 3 (MAP1LC3/LC3). The endoplasmic reticulum provides a possible source of autophagosome membranes, in which the autophagic core complexes vacuolar protein sorting 34 (VPS34) and unc-51-like kinase 1 (ULK1) initiate membrane formation. Membrane formation is further mediated by WD repeat domain, phosphoinositide interacting 1 (WIPI1) and WD repeat domain, phosphoinositide interacting 2 (WIPI2), then recombinant autophagy related protein 16 like protein 1 (ATG16L1) and LC3 are recruited, which promotes the formation of autophagosomes. The autophagosome fuses with the acidic lysosome to remove the damaged mitochondria, a step regulated by the synergistic action of the autophagosome and lysosome proteins.[134,135] (II) Ubiquitin-independent receptor-mediated mitochondrial phagocytosis. Autophagy receptor proteins such as recombinant BCL2/Adenovirus E1B 19 kDa interacting protein 3 (BNIP3), BNIP3-like (NIX), and FUN14 domain containing 1 (FUNDC1) are recruited to the OMM. The receptor proteins interact directly with LC3 to mediate mitochondrial elimination. BNIP3 is an induction of BCL2-interacting protein 3, a mitochondrial outer membrane protein that induces mitochondrial phagocytosis in the adult nervous system and prevents the accumulation of dysfunctional mitochondria in the elderly brain.[136] (III) Alternative degradation pathway. Cellular pathway of local mitochondrial degradation through partial mitochondrial phagocytosis and mitochondria-derived vesicles to degrade damaged mitochondria and macromolecules.[137] (Fig. 4). The age-related weakening of mitochondrial autophagy affects removing damaged cellular components and accumulating waste products, including dysfunctional mitochondria in cells. This promotes cell ageing. The mtDNA replication defects, errors, and/or repair failures in senescent cells are the leading causes of

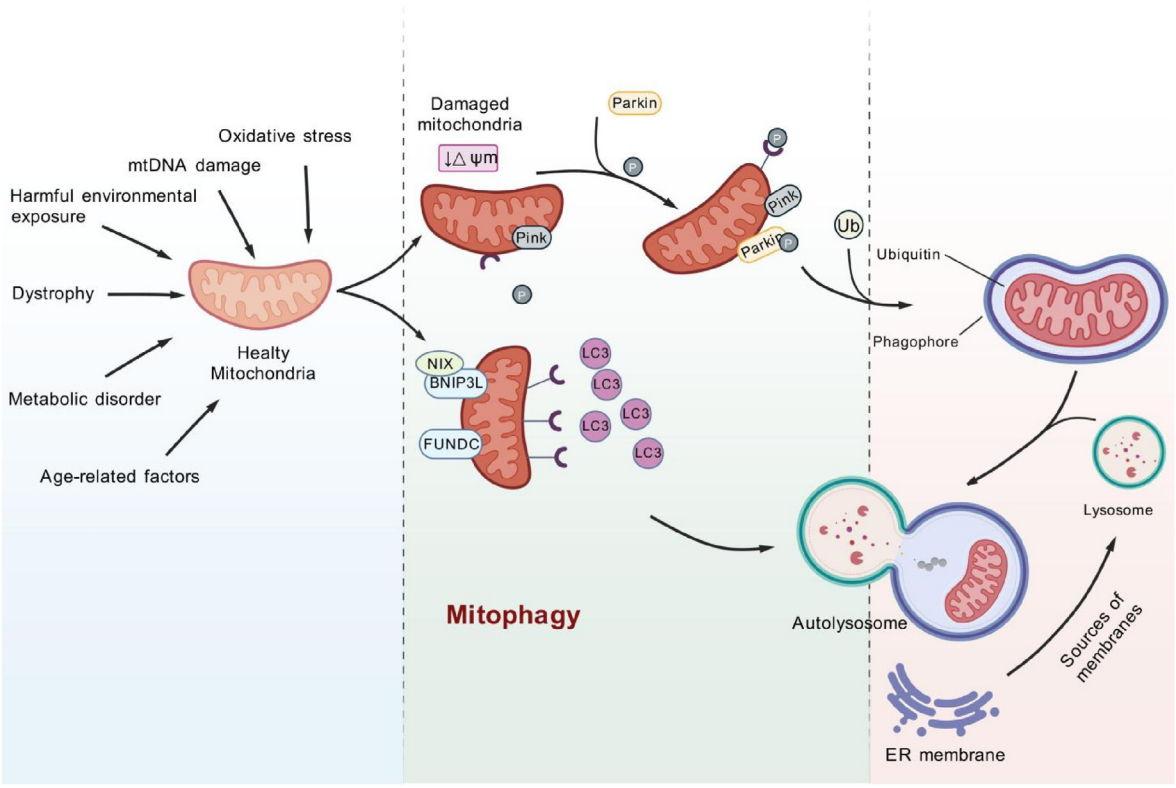

Fig. 4. Mitophagy. Ubiquitin-dependent: PINK/Parkin-mediated mitochondrial phagocytosis: PINK is localized to the outer mitochondrial membrane, recruiting Parkin activation and subsequent ubiquitination of mitochondrial proteins. As an autophagy signal, the autophagy receptor p62 and others are recruited to interact with LC3 and mediate the formation of autophagosomes. Ubiquitin-independent receptor-mediated mitochondrial phagocytosis: BNIP3L and NIX autophagy receptors are recruited in the outer mitochondrial membrane to form autophagosomes by interacting with LC3. In addition, in the ATP supply and hypoxia stress cases, fully activated AMPK α, through phosphorylation of ULK1, promotes VPS34 complex formation and further phosphorylation of the mitochondrial membrane protein FUNDC, mediated by interaction with LC3, facilitating mitochondria and autophagosome integration to complete mitophagy. The endoplasmic reticulum provides a source of autophagosome membranes. We confirm that all figures/images in this article are original and were created by the BioGDP.[139,140]

mitochondrial biological damage, mitochondrial autophagy decline, and cell bioenergy failure, which promote the permanent cell cycle arrest of cells and accelerate ageing and tissue lesions.[138] To sum up, mitochondrial homeostasis is necessary to maintain the normal state of cells; that is, the balance of mitochondrial biogenesis and mitochondrial autophagy is conducive to the extension of a healthy life. The failure of damaged mitochondria to be effectively cleared can bring about a series of cellular stress responses that aggravate ageing and age-related diseases, especially neurodegenerative diseases.

3.3. Mitochondrial dynamics

Mitochondria are highly dynamic organelles continuously dividing and fusing to form a reticular state within cells.[141] Mitochondria achieve intracellular energy distribution, transfer proton flow, and control steady-state Ca^{2+} concentration through the reticular state, facilitating efficient ATP production and distribution.[142–144] The inner membrane area of mitochondria in senile cardiomyocytes decreased significantly with ageing, the respiratory capacity decreased, and mitochondria deteriorated gradually.[145] Mitochondrial DNA mutation and accumulation of unfolded or damaged mitochondrial proteins induced by excess ROS exceed a certain threshold to perform mitochondrial dysfunction in aged cells. Mitochondrial Quality Control, a pathway of gene repair and degradation of damaged proteins, mitigates this deterioration at the molecular level. Normal cells had a mitochondrial antioxidant defence system against oxidative damage caused by excess ROS. In addition, mitochondria complete mitochondrial quality control through fission and fusion pathways.[146] The heart is the organ that needs the most energy and is rich in healthy mitochondria, which rarely fuse or fission and remain static in heart muscle cells.[147] The key to cellular mitochondrial quality control is maintaining its network structure's dynamic balance. Two neighbouring mitochondria fuse, and one fissions into two. If this dynamic mitochondrial plasticity is unbalanced, a nutrient-rich environment accompanies a fragmented mitochondrial network, whereas starvation leads to elongated and fused mitochondria.[148]

Interestingly, the mitochondrial division is unequal; upon an increase in the impairment within the mitochondria, damaged compartments are segregated through fission and cleared by the mitochondrial autophagy pathway.[148] The primary function of mitochondrial dynamin is to mediate mitochondrial fusion and division. When mitochondrial dynamin cannot function normally, the damaged and dysfunctional mitochondria accumulate and accelerate the occurrence or development of neurodegenerative diseases, muscular dystrophy, sarcopenia, senescence, and metabolic diseases.[149,150] Regulating mitochondrial fission and fusion to maintain the number of healthy mitochondria in cells is an effective method to treat cardiovascular diseases and promote the function of an ageing heart.[115] The abundant OMM protein provides a target for regulating mitochondrial dynamics. OMM protein coupled with continuous fission and fusion. Mitochondrial division separates the damaged mitochondria and eliminates them through mitochondrial autophagy. Mitochondrial fusion mixes slightly damaged mitochondria with healthy ones to repair damage through functional complementarity and to inhibit the abnormal increase in mitochondrial autophagy arising from the accumulation of damaged mitochondria.[151] Deletion of some OMM proteins, such as FUNDC1, Mitofusin 1 (MFN1), and Metaxin 2 (MTX2), induces mitochondrial dysfunction, including network fragmentation and oxidative phosphorylation, resulting in cell senescence and decreased proliferation.[152–154]

Mitochondrial fusion is completed by a two-step process involving simultaneous fusion of OMM and IMM. Currently, it is known that three kinetic protein-related GTPases are involved: MFN1, MFN2, and OPA1 mitochondrial dynamin-like GTPase (OPA1).[155] MFN1 works in mitochondrial tethering, whereas MFN2 operates in a later fusion reaction process.[156] OPA1 is a crucial regulator of mitochondrial intima fusion and crest remodelling.[157] Mitochondria fuse to form tubular or elongated mitochondria to create a network of interconnected mitochondria. Mitochondrial fission involves three steps: (I) Mark the site of division, and mitochondrial fission factor (MFF) is anchored to OMM as a binding site for dynamin-related protein 1 (DRP1), which is recruited around the mitochondria. (II) The DRP1 dimer and oligomer assemble into a helical superstructure around the labelled fission site. (III) The helical superstructure continuously shrinks and severs OMM and IMM by GTP hydrolysis. Mitochondrial dynamic proteins of 49 (MID49), mitochondrial dynamic proteins of 51 (MID51), and mitochondrial fission 1 protein (FIS1) mediate the aggregation of DRP1 in OMM.[155] DRP1, as the primary regulator driving mitochondrial fission, is regulated by mitochondrial phosphatase PGAM family member 5 (*PGAM5*) and clustered mitochondria homolog (CLUH). PGAM5 activates DRP1 to participate in mitochondrial division through dephosphorylation. CLUH binds to the mRNA encoding DRP1 receptors MID49 and MFF to up-regulate their expression. Promoting recruitment of DRP1 from cytosol to OMM.[158,159] Therefore, the dynamic processes of mitochondrial fission and fusion are strictly regulated to maintain the homeostasis and dynamic balance of the mitochondrial network, which is essential for normal cells.[160] Mitochondrial network disruption is an essential manifestation of mitochondrial dysfunction and promotes cell ageing. Therefore, activating mitochondrial division and fusion to restore the integrity of the mitochondrial network is a promising treatment for age-related diseases.[161] To target MFN1 fusion activity, a small molecule agonist was developed. Termed S89, it rescued mitochondrial fragmentation and swelling following ischemia/reperfusion injury by interacting with the GTPase domain of MFN1,[162] thus delaying ageing-derived senescence resulting from mitochondrial DNA mutations. Another study identified a peptidomimetic small molecule, MASM7. MASM7 activates MFN2 pro-tethering conformation and enables mitochondrial fusion, resulting in increased membrane potential, mitochondrial respiration, and subsequent ATP production, promising to reduce age-related degenerative metabolic disease.[163] Targeting mitochondrial dynamics for the prevention and treatment of cellular senescence and age-related diseases is strong.

3.4. Mitochondrial protein quality control

More than 40 proteases in different compartments of mitochondria are collectively known as mitoproteases, which limit the accumulation of short-lived, regulatory proteins within mitochondria, modulate the activity of mitochondrial proteins, and mediate the degradation of damaged proteins. The loss of mitoproteases seriously damages the functional integrity of mitochondria, promotes senescence, and leads to various diseases.[134,164] Impaired or dysfunctional mitochondrial protease function is associated with ageing and a lot of pathologic conditions, such as neurodegenerative diseases, metabolic syndrome, and cancer. According to their function and location, mitochondrial proteases can be divided into three main categories, including: (I) "Intrinsic mitochondrial proteases", which function mainly in the mitochondrion; (II) "Pseudo--mitotic proteases", which have structural similarity to proteases but lack some critical residues for catalysis, and these can regulate the activity of homologous proteases. (III) "Transient mitotic proteases" are mainly related to apoptosis or autophagy and translocate to mitochondria to perform additional proteolytic activities. The above three groups can be further divided into three subgroups according to the catalytic category: Cys, metalloproteinases, and Ser proteinases.[165] Mitochondrial protein generation requires a complex folding and assembly process to obtain the active state of enzymes. Therefore, endogenous enzymatic components, including multiple chaperones and proteases as interconnected functional networks, are the basis for maintaining mitochondrial protein homeostasis.[166] Moreover, the loss of membrane homeostasis is the leading cause of the degradation of mitochondrial protein quality. Studies have shown that mitochondria-associated endoplasmic reticulum membranes (MAMs) in rats' hearts and skeletal muscles are damaged during ageing. The decrease of highly interconnected proteins with ageing in an ER-mitochondria organization network, including

voltage-dependent anion channel 1 (VDAC1), SAMM50, MIC60, and MTX1, has been identified as a potential factor for age-related MAMs dysfunction.[167] FUNDC1, containing the FUN14 domain, is an OMM protein that mediates the formation of MAMs, whereas increasing MAMs promote angiogenesis and delay the occurrence of age-related neurodegenerative diseases, inflammation, and metabolic dysfunction.[168]

Lon proteases degrade misfolded proteins as a significant component of the energy-dependent proteolytic enzyme system in the mitochondrial matrix compartment. In addition, mitochondria also activate the mitochondrial unfolded protein response (UPRmt), which helps misfolded proteins return to regular protein conformation and assists the correct folding of newly synthesized proteins. HDA-1, the *Caenorhabditis elegans* ortholog of mammalian histone deacetylase (HDAC) is required for mitochondrial stress-mediated activation of UPRmt. *C. elegans* responds to mitochondrial stress by activating UPRmt to buffer the mitochondrial folding environment, rewire the metabolic state, and promote innate immunity and lifespan extension.[114] Lon protease homolog 1 (LONP1) is a principal mitochondrial protease resident in the matrix. The gradual loss of mammalian LONP1 level with ageing promotes the abnormal accumulation of mitochondrial retention proteins in muscle, inducing the activation of the autophagic lysosomal degradation program of muscle loss and causing muscle loss and weakness.[169] Sustained mitochondrial health depends on coordinated biogenesis and clearance, both regulated by continuously targeting proteins to organelles.[170] This requires a constant supply of mitochondrial protein input. In the mammalian PD model, it was found that the downregulation of the mitochondrial translocase of outer mitochondrial membrane 20 (TOM20) and translocase of inner mitochondrial membrane 23 (TIM23) leads to the inhibition of complex I, which damages the mitochondrial protein input, disrupts the mitochondrial protein balance, and further deteriorates the development of age-related diseases.[171] Lon protease deficiency reduces energy production of oxidative phosphorylation and decreases mitochondrial gene expression.[172] ER is a place for protein processing in cells. ER membrane is associated with almost all cell compartments, and the mitochondria-ER contact site (MERCS) may be crucial in developing ageing and age-related diseases. Mitochondrial or ER dysfunction results from oxidative stress, mtDNA mutations, misfolded protein accumulation, and organelle turnover defects.[173] The ER inositol 1,4,5-trisphosphate receptor, type 2 (ITPR2) calcium-release channel, and calcium fluxes from the ER to the mitochondria are drivers of senescence in human cells. ITPR2 knockout mice showed extended lifespans and reduced age-related phenotypes, possibly due to ITPR2 loss that weakened MERCS and increased MERCS sufficient to trigger premature cell ageing.[174]

3.5. Oxidative damage reaction and oxidative phosphorylation activity decreased in senescent cells

Stem cells or progenitor cells are generally considered candidates for promising regenerative applications because of their high proliferation capacity and the potential to differentiate into other cell types[175,176]; however, stem cells in senescent tissues exhibit the characteristics of cellular senescence and the accumulation of senescent cells with decreased regeneration and differentiation potential is one of the significant factors in the tissue lesions associated with senescent diseases.[177] Mesenchymal stem cells (MSCs) are an emerging strategy for treating many degenerative diseases. However, there is evidence that the function of MSC decreases with age, manifested by an oxidative stress damage state of increased mitochondrial ROS and decreased mitochondrial membrane potential. Compared with the young sample, NADH dehydrogenase (ubiquinone) iron-sulfur protein 6 (NDUFS6) is depressed in aged MSCs.[178] Mitochondrial activation is critical to the differentiation and proliferation of stem cells, and they are also essential cell cycle regulators.[179] Two critical cyclically regulated signaling pathways, the *p53* and *p38* signaling pathways, control the increase of mitochondrial mass, membrane potential, and cell ROS production. The effects of ROS on human endometrial-derived MSCs were tested by exposing them to non-lethal doses of hydrogen peroxide, and hydrogen peroxide was found to cause cell senescence through the *p53/p21/pRb* and *p38/MAPKAPK2* pathways. Increasing ROS levels cause most cells to enter irreversible cell cycle arrest.[180]

Werner syndrome (WS) is a premature ageing disorder caused by mutations in a RecQ-like DNA helicase. Mice lacking the helicase domain of the Wrn homolog exhibit many phenotypic features of WS, including oxidative stress and metabolic dysfunction, compared to wild-type (WT) animals. Compared with WT mice, the levels of antioxidant proteins such as glutathione (GSH) were reduced. ROS levels in liver tissues were increased, and the difference was more significant in the elderly stage.[181] The depletion of NAD^+ in WS mice showed reduced total ATP synthesis and impaired mitochondrial autophagy. NAD^+ repletion can effectively prolong the lifespan of WS and delay accelerated ageing by restoring NAD^+ metabolic profile and improving mitochondrial quality through cell cycle-related factors Cdt1 and ULK1-dependent mitophagy.[182] With the increase of age, the activity of the two central proteolytic systems involved in protein quality control in cells, namely, the autophagy-lysosome system and the ubiquitin-proteasome system (UPS), decreases with ageing. The efficiency of the DNA repair pathway and protein balance pathway is reduced, resulting in the accumulation of ROS, damage to mitochondrial integrity and cell homeostasis, and promotion of cell ageing.[67] Ageing muscle shows significantly reduced regeneration, and the expression of the "anti-ageing" protein α-Klotho decreases with age, resulting in loss of mitochondrial crest integrity and decreased mitochondrial productivity, which inhibits functional regeneration of aged muscle in *vivo*.[183] An age-dependent deterioration in mitochondrial morphology was also shown in PD, induced by the loss of Nato3, a major developmental transcription factor in progressive degeneration of dopaminergic neurons.[184] Therefore, mitochondrial integrity destruction and oxidative stress induce cell senescence, and senescent cells promote ROS production and damage accumulation, further promoting cell senescence. Notably, although free radicals are generally associated with cell damage and inflammation at high levels, they may also protect organisms from more significant subsequent stress at lower levels through an adaptive response called "Mitohormesis".[185]

Mitochondria are considered to be the main sites of ROS production. In the process of electron transfer in mitochondrial respiration, the leaking electron and molecular oxygen generate a lot of superoxide anion ($O^{2\bullet-}$) and hydrogen peroxide (H_2O_2), and some mitochondria are damaged.[186] Excessive ROS can also destroy mitochondrial membrane permeability, leading to the disorder of Ca^{2+} balance inside and outside mitochondria, and Ca^{2+} efflux acts as a second messenger to initiate multiple signaling pathways and promote apoptosis. Mitochondrial calcium uptake family member 3 (MICU3), a regulator of MCU, might maintain normal levels of mitochondrial Ca^{2+}, attenuate oxidative stress and apoptosis, and restore age-related loss of skeletal muscle mass and function.[66] Prolonged exposure to high levels of ROS results in injury to cellular macromolecules (including proteins essential for oxidative phosphorylation) and mtDNA.[187] Even the mitochondrial dysfunction in peripheral blood mononuclear cells (PBMCs) that shows reduced mitochondrial ATP production, increased proton leakage, and oxidative stress can also give rise to mild cognitive impairment (MCI) in the elderly population.[188] Mitochondria are driven by the electrochemical gradient along the respiratory chain to generate ATP through oxidative phosphorylation (OXPHOS) in an oxygen-dependent manner.[179] The activity of OXPHOS gradually declines during ageing, as demonstrated in fibroblasts from patients with Hutchinson-Guilford progeria syndrome (HGPS).[189] The expression of cytochrome *c*, complex IV component cytochrome *c* oxidase subunit I (COXI), and β-ATPase of complex V protein was significantly lower than that of normal cells, resulting in severe damage of OXPHOS. Therefore, ageing is closely related to the reduction of mitochondrial energy production, involving the inhibition of the mitochondrial tricarboxylic acid (TCA) cycle, the inhibition of the glycolytic pathway, and the significant decrease in the activity level of

mitochondrial respiratory complex protein.[150,190,191] OXPHOS, as the primary production mode of cells, has decreased activity in ageing.

Meanwhile, abnormal mitochondrial function produces excess ROS, which promotes cell ageing.[116] Instead, mitochondria have higher activity in cells at earlier developmental stages, and the OXPHOS activity is higher. Mitochondria have efficient ATP production capacity and self-repair ability.[192] Due to oxidative stress damage, excessive ROS cannot be cleared effectively. Ageing can produce large ROS production, destroy protein balance, change genomic stability, and promote cell ageing.[193] NRF2 is a key transcription factor for the antioxidant response, and its activity decreases gradually with the increase of age.[194] However, the total content of NRF2 in the heart of old and young mice is similar at the non-stress basal level.[195] Therefore, the activity of NRF2 is the key to delaying cell senescence. But Kelch-like ECH-associated protein-1 (Keap1), Glycogen synthase kinase-3β (GSK-3β), essential leucine zipper transcription factor 1 (Bach1), and p53 participate in the negative regulatory mechanism of NRF2 to reduce its activity with ageing.[196] Meanwhile, it has been shown that maintaining NRF2 activity can protect cells from the effects of oxidative stress and ageing.[197,198]

4. Mitochondrial function maintenance and mitochondrial training to delay cellular senescence and treat related diseases

4.1. Supplementation

A variety of natural compounds have been found to enhance mitochondrial function and protect healthy mitochondrial networks, which is expected to slow down the ageing process. Caffeine is gradually favoured by the vast majority of people in daily life. Studies have found that after long-term caffeine consumption, mitochondrial function is improved, autophagy level is increased, antioxidant protein expression is up-regulated, and intestinal atrophy is inhibited in intestinal ageing.[199] Coenzyme Q10 is a highly effective antioxidant that has been shown to have anti-ageing effects. Studies have reported that coenzyme Q10 reversed age-dependent loss of mitochondrial function in reversed epithelial tissues by improving epithelial cells' electron transport chain capacity and antioxidant capacity and increasing ATP production.[200] Sulforaphane is a well-known activator of *NRF2*. By increasing the expression and activity of NRF2 in the renal cortex and decreasing the protein expression of NRF2 repressor Keap1, it can significantly enhance the antioxidant capacity of kidney mitochondria in aged mice and improve kidney injury but does not affect the expression of NRF2 in young mice.[201] Some peptides and natural antioxidants targeting mitochondria, polyphenols, can prevent retinal nerve ageing and delay vision loss, mainly by protecting mitochondrial function and inhibiting Akt/mTOR activity, to prevent age-related eye diseases and delay ageing.[202–204] Ageing is characterized by failure to maintain protein balance and mitochondrial homeostasis, accumulation of starch deposits, and mitochondrial dysfunction in skeletal muscle. NAD^+ supplement nicotinamide riboside (NR) and high-intensity interval training (HIIT) were found to effectively stimulate the mitochondrial imbalance, and UPRmt to restore mitochondrial homeostasis and reduce amyloid accumulation.[205,206] Lipoic acid is a compound that eliminates free radicals that contribute to accelerated ageing and disease. It has been found that lipoic acid supplementation may be an adjuvant to the treatment of mitochondrial damage and cognitive decline associated with ageing and neurodegenerative diseases.[207] Spermidine (SPD), a major mammalian polyamine, can delay cardiac ageing by activating mitochondrial biogenesis. Studies have shown that SPD activates mitochondrial biogenesis through *SIRT1*-mediated PGC-1α deacetylation to alleviate cardiac ageing.[208] It has been reported that alginate oligosaccharides (AOS) have the potential anti-ageing activity of improving mitochondrial biogenesis, maintaining mitochondrial integrity, and enhancing the adequate clearance of damaged mitochondria. In addition, AOS can also reduce ROS production and oxidative stress state, which further inhibits the destruction of heart mitochondria.[209]

Endothelial cell senescence is a significant risk factor in cardiovascular diseases. Metformin is an activator of the *AMPK* pathway, which retards endothelial cell senescence through the upregulation of DOT1, which is a highly conserved methyltransferase of lysine 79 (H3K79) of histone H3 mediated by the deacetylase *SIRT1*. Increased trimethylation of H3K79 can promote mitochondrial biogenesis and function. Furthermore, a chronic low-dose administration of metformin significantly attenuated vascular ageing and inhibited age-associated atherosclerotic plaque formation in $ApoE^{-/-}$ mice.[210] Eramitide (SS-31) and Nicotinamide Mononucleotide (NMN) were able to reverse age-related changes in the hearts of mice. In addition, both were effective in upregulating the abundance of electron-transport chain-associated proteins that are depleted by ageing and in deacetylating highly acetylated mitochondrial proteins such as pyruvate dehydrogenase, carnitine palmitoyltransferase 1, and ATPase 50.[211] PGC-1 proteins are master regulators of energy production and expenditure in the cell; senescence of retinal pigment epithelial (RPE) cells may be associated with changes in PGC-1α function. It was shown that PGC-1α was involved in antioxidant defence through interaction with Nfe212 (nuclear factor erythroid 2-related factor 2), a transcription factor important in protection against oxidative stress resulting from damage and inflammation.[212] That study also showed that superoxide dismutase 2 (SOD2), an enzyme clearing an excess of mitochondrial ROS, was activated by PGC-1α.[90] Table S1 lists additional anti-ageing strategies, focusing on mitochondrial research.

Testosterone supplementation improved exploratory behaviour, attenuated neuronal degeneration and neuronal loss, and ameliorated mitochondrial dysfunction by enhancing both antioxidative capacity and biogenesis of mitochondria in the aged male rats. Besides, testosterone enhances the function of the mitochondrial complex V in the substantia nigra. It upregulates ATP levels, to some extent, maintains dopaminergic function in the striatum nigra in aged male rats.[213,214] Substantia nigra provides dopamine for the brain, but there is mitochondrial dysfunction of the substantia nigra in the early stage of ageing and Parkinson's disease. Melatonin is an endogenous free radical scavenger synthesized by neuronal mitochondria and decreased with ageing and neurodegeneration. Inadequate melatonin levels disrupt mitochondrial homeostasis, releasing mtDNA and activating cellular soluble DNA-mediated neuronal inflammatory responses.[215] (Table S1).

4.2. Lifestyle

In recent years, the function of CR in humans, which can delay ageing and promote health, has been widely discussed. CR has been proven to promote mitochondrial generation in liver tissue and protect mtDNA from damage with the increase of age in mammals, but CR has a weakened effect on improving ageing in older mice (over 32 months of age). However, this only partially limits CR's anti-ageing and improvement of mitochondrial dysfunction.[216] Ageing-like changes in skeletal muscle can also be ameliorated by proper exercise and intermittent eating throughout life.[217,218] Diet-related anti-ageing methods have been utilized for anti-ageing, such as CR and intermittent fasting, which have been proven to have sound effects on prolonging ageing, and dietary changes and additives have shown beneficial effects on improving mitochondrial quality and ageing.[219,220] Table S1 lists additional anti-ageing strategies: focusing on mitochondrial research.

4.3. Exercise

The mechanisms by which endurance exercise improves mitochondrial quality, quantity, and function focus on the following: activation of the PGC-1α and AMPK signaling pathways to increase healthy mitochondrial biosynthesis and clearance of damaged mitochondria; enhancement of the efficiency of oxidative phosphorylation and antioxidant capacity; and regulation of the expression of mitochondrial fusion and fission proteins to maintain the dynamic balance of the mitochondrial network in response to different intra-/external cellular stresses.

Taking HIIT as an example, HIIT modulates a series of cellular adaptive responses related to mitochondrial biogenesis and mitochondrial autophagy in skeletal muscle by activating the mitogen-activated protein kinases (MAPK) signaling pathway and PGC-1α and by compensating for the increase in the production of ROS.[221] Whereas endurance training increased the expression levels of proteins and mRNAs of mitochondrial growth transcriptional regulators, it also increased the content of mtDNA in skeletal muscle, such as *cAMP, PKA, AMPK, PGC-1α, CREB, SIRT1/3, TFAM,* and *NRF1/2*.[222] Moreover, prolonged exercise has also been shown to stimulate mitochondrial dilation, resulting in increased mitochondrial ATP production and tolerance to environmental and climatic changes after exercise, but the mechanism of this response remains to be elucidated.[223] Effective types of exercise and protective measures include aerobic exercise (e.g., brisk walking, swimming, running, etc.), strength training (exercise using equipment or one's body weight), balance and coordination training (e.g., tai chi, yoga), and flexibility training (stretching). For protective measures, warm up and stretch before exercise, avoid over-exercise, arrange exercise time reasonably, use suitable exercise equipment, and reduce sedentary time.[224,225] (Table S1).

4.4. Transplantation and other

Unlike classical drug therapy, mitochondria of healthy ovarian cells are transferred to abnormal ovarian cells through micromanipulation, which can improve the fertility of low-quality oocytes for age-related infertility in female germ cells.[226] Moreover, the mitochondrial transfer restores mitochondrial function, reduces ROS levels, and activates mitochondrial autophagy to clear damaged mitochondria and improve cell function in different ageing cells.[227–229] Similarly, healthy mitochondria can also affect damaged mitochondria in the ageing brain, restoring mitochondrial homeostasis and improving cognitive impairment.[230] Mitochondrial density is greatest in photoreceptors, particularly cones that have high energy demands and mediate colour vision. Hence, the retina ages faster than other organs, with a 70% ATP reduction over life and a significant decline in photoreceptor function. Mitochondria have specific light absorbance characteristics influencing their performance. Shinhmar uses 670 nm light to improve photoreceptor performance and measures this psychophysically in those aged 28–72 years. Rod and cone performance declined significantly after approximately 40 years of age. 670 nm light had no impact on younger individuals, but in those around 40 years and older, significant improvements were obtained in colour contrast sensitivity for the blue visual axis (tritan) known to display mitochondrial vulnerability.[231] More interventions are listed in Table S1.

5. Summary and outlook

Cell senescence, characterized by permanent stagnation of proliferation, is a response to both endogenous and exogenous stress. As the number of senescent cells increases, different tissues and organs will show various degrees of ageing, and some stem cells will also enter the senescence stage. During ageing, some decreases are observed in various physiological functions such as immune function, hormone levels, brain cells' number and connections with each other, and myocardial contractility. These get worse in neurodegenerative diseases, myocardial infarction, sarcopenia, and metabolic syndrome. SASP secretion amplifies the effects of endogenous cell proliferation stagnation and impairs tissue regeneration in chronic ageing-related diseases. Cellular senescence is a double-edged sword. The beneficial role of senescent cells in embryonic development is prominent. Cellular senescence is helpful for certain vital activities, such as normal development and morphogenesis of embryos, tissue repair, wound healing, and inhibition of tumor growth. However, the accumulation of senescent cells, beyond the body's ability to clear and repair, can induce chronic inflammation and a decline in tissue and organ function, contributing to age-related disease and even a soaring likelihood of cancer.

First of all, with the increase of age, the number of healthy mitochondria decreases in the cell, and some defective mitochondria accumulate in the cell and damage the mitochondrial network, reducing the efficiency of energy generation and destroying intracellular and extracellular signal transmission, which accelerates cell senescence. Secondly, the dynamic changes of mitochondria are controlled by mitochondrial fusion and division for the regular operation of the whole mitochondrial network in the cell. Mitochondria enhance communication between each other and maintain cell homeostasis through the fusion mechanism; damaged mitochondrial parts are eliminated by division, and healthy mitochondrial parts are preserved for efficient cell metabolism. If damaged mitochondria could not be repaired as above, the accumulation of damaged proteins and the loss of critical proteases in mitochondria would aggravate mitochondrial dysfunction, further inhibiting mitochondrial turnover, ineffective fusion, and division, and boosting cell senescence. At the same time, mitochondrial dysfunction would produce excessive ROS in oxidative phosphorylation to produce ATP, damage ageing mitochondrial mtDNA by oxidation, and reduce the quality of mitochondria and energy production. Lots of ROS would also damage other cellular macromolecules and destroy the typical morphology of cells. Thus, maintaining the mitochondrial proteome and homeostasis is highly important in cellular survival and organismal health.[232]

A small amount of mitochondrial dysfunction does not necessarily hurt the cell, as within a specific range, the mitochondria clear these dysfunctional mitochondria through autophagy.[185] What is the threshold level for accumulation of mitochondrial dysfunction, and how does accumulation of mitochondrial DNA replication damage reach that threshold level remain to be investigated. It remains to be studied how a certain degree of mitochondrial DNA replication damage can be controlled at a threshold level. The function of mitochondria is greatly affected by external pressure, which makes mitochondria an excellent model against the odds.

Healthy mitochondria are vital for maintaining the normal function of cells, so it is interesting to find appropriate methods to improve mitochondrial quality to reduce cell senescence or improve the function of senescent cells. Research on mitochondria as a target for anti-cellular senescence has focused on two main areas: to improve and/or restore mitochondrial function and to improve the number of mitochondria. Some substances help improve mitochondrial function by promoting mitochondrial autophagy, increasing mitochondrial biogenesis, regulating mitochondrial fusion and division, restoring mitochondrial protein homeostasis, improving mitochondrial energy production efficiency, and improving the oxidative defense system. Mitochondria are considered highly plastic organelles. This plasticity enables the mitochondria to undergo morphological and functional changes in response to cellular demands.[233] Mitochondria exhibit different morphologies, such as enlargement, elongation, fragmentation, or aggregation of the mitochondrial network to regulate energy metabolism in the cell and adapt to internal damage and external environmental stressors.[234,235]

Cellular senescence is triggered by a variety of factors, including mitochondrial dysfunction. However, there is no clear causal relationship between cellular senescence and mitochondrial dysfunction in the biological ageing process, and together they characterize senescence. With age, the mitochondrial antioxidant defence system gradually weakens, leading to excessive accumulation of ROS. These ROS damage the mitochondria's DNA, proteins, and lipids and affect other components of the cell, ultimately leading to cellular dysfunction and senescence. Although the removal of senescent cells is simple and effective, the issue of specificity is more challenging to achieve, whereas the protection and activation of mitochondria can occur at all stages of life and can be achieved in a variety of ways, so targeting mitochondria is considered to be more effective in improving the function of senescent cells and in mitigating the onset and progression of age-related diseases. Given the highly dynamic and plastic nature of the mitochondrial network, is it possible to continuously train mitochondria through chronically low levels of stimulation, such as exercise, dietary changes, nutrient intake, or active

substance and drug interventions? More research is needed to make mitochondria more powerful, more efficient at self-healing and waste removal, and with higher critical levels of resistance to environmental degradation. Mitochondrial function and regulatory mechanisms are complex, and there are many unknowns and challenges in the research of anti-ageing and ageing-related diseases related to restoring damaged mitochondrial function.

CRediT authorship contribution statement

Gan Gao: Writing – original draft, Software, Methodology, Investigation. **Zhihui Xie:** Resources. **Hongliang Huang:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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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.

Appendix A. Supplementary data

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Abbreviations

MQC	Mitochondrial Quality Control	CIP1	cyclin-dependent kinase (CDK)-inhibitor 1
SASP	senescence-associated secretory phenotype	ROS	reactive oxygen species
OS	oxidative stress	MMP	mitochondrial membrane potential
IL-6	Interleukin-6	TNF-α	tumor necrosis factor-α
HUVECs	human umbilical vein endothelial cells	CNS	central nervous system
AD	Alzheimer's disease	PD	Parkinson's disease
ALS	Amyotrophic Lateral Sclerosis	HD	Huntington's disease
Aβ	β-amyloid protein	Caspase-3	cysteinyl aspartate specific proteinase
DAMP	damage-associated molecular pattern	UTD	Urinary Tract Dysfunction
IPF	Idiopathic Pulmonary Fibrosis	CKD	Chronic Kidney disease
FOXO4	forkhead box O 4	COPD	Chronic Obstructive Pulmonary disease
ECFCs	endothelial colony-forming cells	AT II	type II alveolar epithelial cells
AMD	age-related macular degeneration	IMM	inner mitochondrial membrane
OMM	outer mitochondrial membrane	IBM	inner boundary membrane
T2DM	Type 2 diabetes mellitus	PGC-1α	peroxisome proliferator-activated receptor gamma coactivator-1α
AMPK	adenosine 5′-monophosphate (AMP)-activated protein kinase	POLG	mitochondrial DNA polymerase γ
TFAM	mitochondrial transcription factor A	ERRα	estrogen-related receptor α
SIRT1	silent information regulator 1	CR	caloric restriction
NRF2	NF-E2 p45-related factor 2	LC3	microtubule-associated protein 1 light chain 3
VPS34	vacuolar protein sorting 34	ULK1	unc-51-like kinase 1
WIPI1	WD repeat domain, phosphoinositide interacting 1	ATG16L1	autophagy related protein 16 like protein 1
BNIP3	recombinant BCL2/Adenovirus E1B 19 kDa interacting protein 3	MFN1	Mitofusin1
MTX2	Metaxin 2	DRP1	dynamin-related protein 1
MID49	Mitochondrial dynamic proteins of 49	FIS1	mitochondrial fission 1 protein
PGAM5	mitochondrial phosphatase PGAM family member 5	CLUH	clustered mitochondria homolog
VDAC1	voltage dependent anion channel 1	UPRmt	mitochondrial unfolded protein response
LONP1	Lon protease homolog 1	TOM20	outer mitochondrial membrane 20
TIM23	inner mitochondrial membrane 23	MERCS	mitochondria-ER contact site
ITPR2	ER inositol 1,4,5-trisphosphate receptor, type 2	MSCs	Mesenchymal stem cells
NDUFS6	NADH dehydrogenase (ubiquinone) iron-sulfur protein 6	WS	Werner syndrome
UPS	ubiquitin-proteasome system	H_2O_2	hydrogen peroxide
MICU3	Mitochondrial calcium uptake family member 3	PBMCs	peripheral blood mononuclear cells
MCI	mild cognitive impairment	OXPHOS	oxidative phosphorylation
HGPS	Hutchinson-Guilford progeria syndrome	TCA	tricarboxylic acid cycle
Keap1	Kelch-like ECH-associated protein-1	GSK-3β	Glycogen synthase kinase-3β
Bach1	essential leucine zipper transcription factor 1	NR	nicotinamide riboside
HIIT	high-intensity interval training	SPD	Spermidine
AOS	alginate oligosaccharides	H3K79	highly conserved methyltransferase of lysine 79
NMN	Nicotinamide Mononucleotide	SOD2	superoxide dismutase 2
MAPK	mitogen-activated protein kinases		

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