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Telomeres and Mitochondrial Metabolism: Implications for Cellular Senescence and Age-related Diseases

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Abstract

Cellular senescence is an irreversible cell arrest process, which is determined by a variety of complicated mechanisms, including telomere attrition, mitochondrial dysfunction, metabolic disorders, loss of protein homeostasis, epigenetic changes, etc. Cellular senescence is causally related to the occurrence and development of age-related disease. The elderly is liable to suffer from disorders such as neurodegenerative diseases, cancer, and diabetes. Therefore, it is increasingly imperative to explore specific countermeasures for the treatment of age-related diseases. Numerous studies on humans and mice emphasize the significance of metabolic imbalance caused by short telomeres and mitochondrial damages in the onset of age-related diseases. Although the experimental data are relatively independent, more and more evidences have shown that there is mutual crosstalk between telomeres and mitochondrial metabolism in the process of cellular senescence. This review systematically discusses the relationship between telomere length, mitochondrial metabolic disorder, as well as their underlying mechanisms for cellular senescence and age-related diseases. Future studies on telomere and mitochondrial metabolism may shed light on potential therapeutic strategies for age-related diseases.

Keywords Telomeres · Mitochondrial metabolism · Cellular senescence · Aging

Introduction

Aging is an inevitable trend of biological development, which is characterized by degeneration or loss of function at the organism, cellular and molecular levels [1]. Cellular senescence is one of the causes of individual aging [2]. Senescent cells affect surrounding cells, which gives rise to tumorigenesis by secreting multiple pro-inflammatory cytokines and chemokines, various growth factors and proteases (also known as senescence-associated secretion phenotypes, SASP) [3].With age, senescent cells will gradually accumulate in tissues. In addition, aging is a dynamic process in which the constant evolution of senescent cells impairs the removal of immune cells [4]. Anti-aging drugs are increasingly being studied [5–9]. However, the effects of aging are not all harmful, and growing evidences have shown that aging is beneficial to tissue development and repair [10].

Cellular senescence is accompanied by a series of cell divisions, which in turn leads to the loss of 25-200 bp telomere sequence and the damage of the T-loop, ultimately causing the DNA damage response (DDR) of double-strand breaks [11]. The activation of DDR mainly results in cell cycle arrest through p53-p21 and $p16^{INK4A}$-pRb tumor suppressor signaling pathways, thereby affecting cell senescence. Hence, a large number of studies have confirmed that both telomere dysfunction and the activation of downstream signaling pathways of DDR can accelerate cell senescence and stimulate the development of certain diseases, such as cardiovascular diseases, osteoporosis, neurodegenerative diseases and cognitive decline [12].

Mitochondrial dysfunction is one of the hallmarks of aging, and has been shown to be closely associated with cellular senescence, leading to the alterations in senescence-related phenotypes. Numerous studies have confirmed that mitochondrial abnormality facilitates the development of senescence-related phenotypes and inhibits cell cycle progression [13]. The depletion of mitochondrial reactive

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oxygen species (ROS) and Sirtuins can mediate aging, and mitochondrial dysfunction eventually give rise to cell senescence, the reaction known as mitochondrial dysfunction-associated senescent response (MiDAS) [14]. For a long time, energy metabolism has a regulatory effect on cell senescence. Cell energy mainly comes from the oxidative decomposition of organic substances such as carbohydrates, lipids and proteins. As one of the intermediate products, glucose participates in a variety of biosynthesis processes, which is mainly oxidized and decomposed in the body through mitochondrial oxidative phosphorylation, glycolysis, gluconeogenesis and pentose phosphate pathways [15].Therefore, it is critical to understand the mechanisms involved in these processes and how telomere, mitochondrial metabolism affect cellular senescence and its phenotypic characteristics for the research and development of anti-aging drugs and the treatment of age-related diseases.

The Role of Telomeres in Cellular Senescence

Telomeres are the repeating ends of linear chromosomes, which consist of a shelterin protein complex that protects telomeres and repeated DNA sequence TTAGGG, contributing to maintain the stability of the genome. There are two problems at the end of linear chromosomes: end protection and end replication [16]. Telomere length is regulated by three key factors: telomerase, shelterin protein complex and CTC1-STN1-TEN1 (CST) complex. CST is a trimer complex consisting of Ctc1, Stn1 and Ten1 in higher eukaryotic organisms and Cdc13, Stn1 and Ten1 in yeast Saccharomyces cerevisiae, which is mainly located in single-stranded telomere DNA and involved in the maintenance of chromosome sealing and telomere length [17]. Shelterin is composed of 6 subunits, namely telomere repeat factors 1 and 2 (TRF1, TRF2), protection of the telomere 1 (POT1), repressor/activator protein 1 (RAP1), TRF1 and TRF2 interacting nuclear protein 2 (TIN2) as well as the ACD gene (TPP1). The six components mainly play a role in protecting the ends of chromosomes and inhibiting the activation of DDR (Fig. 1). At the same time, the protein complex is essential for the formation of the T-loop. T-loop is a lasso structure formed by the telomere 3' protruding and invading the double-stranded telomere repeat array, which blocks DDR caused by double-stranded break (DSB) at the end of the chromosome, thereby inhibiting aging [18].

During DNA replication, end replication problem is caused by RNA primer providing a 3' hydroxyl group at the 5' end of lagging strand. Upon primer removal, DNA polymerase can't continue to amplify to fill the gap, further resulting in the telomere shortening [19]. Telomere DNA is made up of a simple sequence of highly repetitive DNAs. With each replication cycle, the telomere length is shortened at each S-stage, the cells thereupon reach a replicative senescence state called the "Hayflick limit", in which cells stop dividing. Since telomere attrition is a sign of cellular senescence, telomeres are considered as a "life clock" by scientists [20]. The maintenance of telomere length is a prerequisite for the continuous division of cells, so telomerase is more active in cells that need to sustain division, such as tumor cells, stem cells, etc. And telomerase is specifically designed as a reverse transcriptase to resist telomere shortening. The two main components of human telomerase serve as templates for telomere extension: telomerase reverse transcriptase (TERT) and telomerase RNA

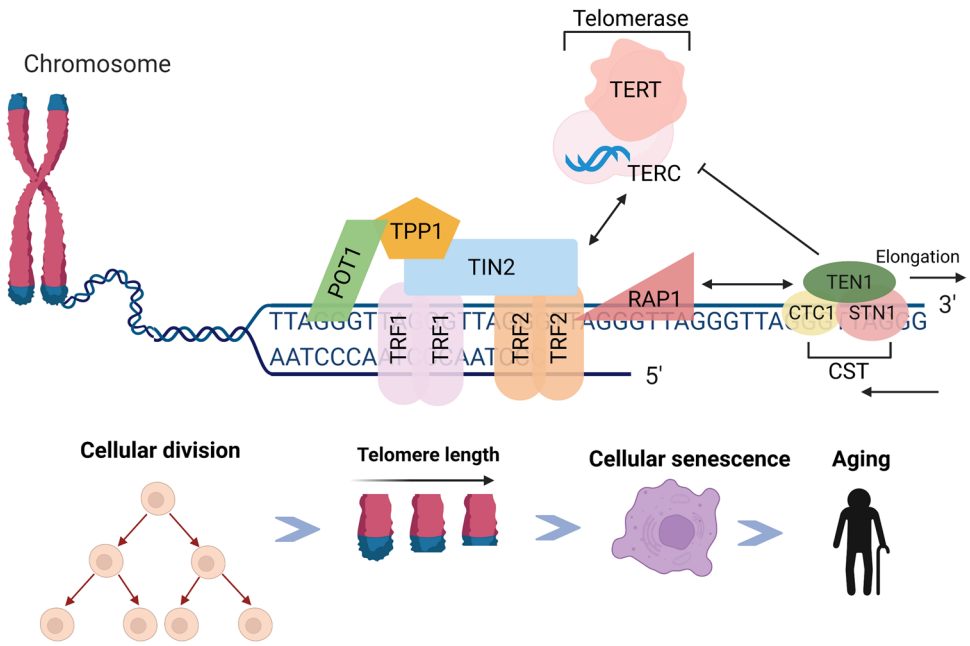

Fig. 1 Telomeres are repeating ends of linear chromosomes. The composition of the shelterin complex, CST complex and telomerase and its binding site to telomere DNA are described in the figure. Shelterin complex are primarily involved in inhibiting DDR. CST is mainly located in single-stranded telomere DNA, inhibiting telomerase activity and thus preventing telomere overstretching. Telomerase adds DNA repeat sequences at the end of telomere 3' through its RNA template. Continuous cell division leads to telomere shortening, causing cellular senescence

component (TERC), maintaining continuous cell division, which in turn slows aging. The shorter the telomere length, the higher the activity of telomerase [21]. One study found that telomeres may have four states: (1) Telomeres contain longer sequences, T-loop is intact, and DDRs are not activated. (2) The structure of the T-loop is broken, but due to the presence of the shelterin complex composition, end-to-end fusion is not caused. (3) Telomeres have been shortened to the limit, T-loop has also been completely broken, and DDR activation causes end-to-end fusion [22]. (4) DDR also occurs in the case of the presence of both the shelterin complex composition and the T-loop [23].

The current pandemic of Corona Virus Disease 2019 (COVID-19) is caused by SARS-COV-2, and worldwide data studies have confirmed that men are more susceptible. SARS-COV-2 infects various cells of the organism, such as alveolar type 2 cells (ATII). In the latest study, individuals with short telomeres have worsened pulmonary fibrosis-like lesions after infection with COVID-19. And female individuals have longer telomeres than males, which explains why the prevalence of males is higher than that of females [24]. Elderly people and people with cardiovascular diseases have shorter telomere length, so these people are also vulnerable to COVID-19 and have poor prognosis. There is growing evidence that lymphocytes are reduced in patients with COVID-19, which is actually the loss of CD4/CD8 T cells. The specific reason is related to short telomere length. Both T cells and B cells have telomerase activity, but there are individual differences in telomere length. The telomerase can only maintain the extension of B cell telomeres, rather than T cells [25]. Telomeres play a role in innate immunity after virus infection. Telomere attrition (or telomere shortening) is another hallmark of aging, which is the progressive loss in chromosome protective caps. It will accelerate the homeostatic destruction of $CD8^+$ memory T cells, and leads to cellular senescence. Therefore, telomere shortening is accompanied by innate immune disorders, which will make the symptoms of the elderly suffering from COVID-19 more pronounced and more difficult to cure [26].

TRF1/TRF2

TRF1 and TRF2 are double-stranded DNAs in the components of shelterin protein complex, which are directly combined with telomere double-stranded TTAGGG repeat sequence, while other components are directly combined with single-stranded repeat sequence. TRF1 and TRF2 have similar structural thresholds, each with a dimer domain and a MYB domain of C-end DNA binding. At the N-end, TRF1 contains an area rich in acidic residues, and TRF2 contains an alkaline region. TRF1 and TRF2 recruit other compound components to act as telomere end protection. Thereby, once TRF1-TRF2 is dysfunctional, it will become the direct cause of telomere DDR, leading to senescence and other senescence phenotypes [27]. Although these two proteins have similar structures, they have different functions. TRF1 is essential for telomere replication. In order to explore the protective effect of TRF1 on telomeres, some researchers studied naked mice that were resistant to hypoxia. Compared with normal mice, TRF1 in naked mice was found to improve telomere binding. And in a low-oxygen state, TRF1 protects telomeres during cell replication. The results can be speculated that TRF1 protective effect on telomeres may be positively correlated with telomere binding capacity [28]. In addition to telomere protection function, TRF1 functions primarily through other auxiliary proteins, such as polymer (ADP-ribosome) polymerase 1. The breakdown of sister telomere condensation mainly depends on TRF1-mediated polymer (ADP-ribose) polymerase 1 collection, which provides a template for DNA replication [29]. Knockouts of TRF1 and tankyrase-1 cause sister telomeres to continue to condense, proving that the absence of TRF1 protects telomeres from DNA damage and slows cellular senescence [30]. TRF2 plays a vital role in telomere protection, and the formation of T-loop depends entirely on the presence or absence of TRF2, while knockout of other components POT1, RAP1 and TRF1 has no effect on the T-loop. The activation of ataxia telangectasia mutated (ATM) and classic non-homologous end joining (c-NHEJ) signaling are the first two steps in DSB response, which can be inhibited by TRF2 [31]. It is well-known that oxidative stress is to induce premature cellular senescence. After hydrogen peroxide treatment, the quantity of TRF1 and TRF2 decreased in time, which resulted in a significant increase in the number of DNA damage markers 53BP1 and γH2AX. This suggests that oxidative stress-induced premature senescence may be caused by telomere dysfunction [32].

TIN2

TIN2 is a negative regulator of telomere length and the core component of the shelterin complex, which is essential for maintaining the function of the complex. TIN2 interacts with telomere double-stranded DNA (TRF1 and TRF2), followed directly by telomere single-stranded DNA (TPP1). Then TPP1 binds to POT1 and forms heterogenous dimer, preserving the integrity of protein complexes and chromosomes [33]. The absence of TIN2 will give rise to the activation of ATM and ataxia telangectasia and RAD3 (ATR) signaling, which further causes DDR. The results highlight the critical function of TIN2 in stabilizing the shelterin complex. In turn, the alterations in TRF1, TRF2 and POT1 can lead to loss of chromosomal stability. TIN2 and TPP1 can bind directly together to recruit telomerase to telomeres. TIN2 knockdown leads to a decline in TPP1 recruitment to telomeres, and thus gives rise to a direct reduction in telomerase

recruitment to telomeres [19]. As a single complex, TIN2 alone is not sufficient to control the telomere length, so it is necessary to play a regulatory role with TRF1 and TRF2. Dyskeratosis congenita (DC) is a hereditary form of bone marrow failure characterized by short telomeres at birth. DC is usually caused by mutations in telomerase complex TERT and TERC. However, a recent study has shown that the fifth exon of TINF2 (coded TIN2) was also mutated. TINF2 mutants lose the binding sites of TRF2 and TPP1, which can render abnormally prolonged telomeres and cancer [34, 35]. It has been proved that the loss of components in the shelterin complex can contribute to malignant tumorigenesis. Abnormal expression of TIN2 provokes telomere dysfunction and is closely related to stomach cancer [36]. Studies have confirmed that TIN2 and TRF2 are co-located in the nuclei of the cells. When the TIN2 marker was converted to the C-end, there was an overlap between TIN2 and mitochondrial marker ATP5A1 staining in the cytoplasm. It shows that TIN2 is located in mitochondria and regulates mitochondrial function, such as ATP synthesis and oxygen consumption, and this effect is not associated with the maintenance of telomeres [37]. It is well known that ROS produced by mitochondria can induce cell replicative senescence. However, how does mitochondrial ROS improve during cellular replicative senescence? RNA binding protein and human antigen R (HUR) reduce the protein expression level of TIN2 by binding specifically to TIN2. At the same time, the depletion of HUR will enhance the accumulation of TIN2 in mitochondria, followed by an elevation in ROS production and an acceleration of cell senescence [38].

TPP1/POT1

POT1 is the only protein complex component that binds directly to single-stranded telomere DNA, while C-end partially binds to TPP1, and the other end of TPP1 binds to TIN2 [39]. It has been found that TPP1-POT1 complex directly increases telomerase activity and continuous synthesis ability [40]. Telomere length remains stable only when telomerase extends telomere length and telomere DNA is eroded to balance. When telomeres are long enough, they will come into a "non-extensible state" [41]. However, as a natural inhibitor of telomerase, overexpression or knockdown of POT1 can lead to excessive extension of telomeres, inducing the occurrence of DDR. Knocking out POT1 causes the DDR to be mediated by ATR and may play a role in replacing POT1 with a single-stranded binding protein - replication protein A (RPA) at the protruding end [42]. Accumulating studies have shown that TPP1 controls the nuclear localization of POT1, and TIN2 combined with TPP1 can also promote TPP1-POT1 nuclear localization [43]. Because TPP1 is very important for POT1 location, and the C-end of TPP1 interacts with TIN2, once TIN2 is knocked out, the stability of TPP1-POT1 binding ssDNA is greatly reduced, and then the accumulation of RAP leads to ATR-mediated DDR [44]. TIN2 exerts a leading effect on telomeres by binding the other five proteins to each other, forcing telomeres into a closed state. So that telomerase cannot enter into the 3' end, it will effectively act on short telomeres, no longer prolonging longer telomeres. It demonstrates that the telomerase in the body must be inhibited if this closed conformation is to be formed to protect the stability of telomeres, and that the overexpression of POT1 can undermine this structure [45]. Although TPP1-POT1 works in combination with each other, it activates ATM instead of ATRs after knocking out TPP1, which is the opposite of knocking out POT1 [46].

RAP1

RAP1 is the main component of the complex. In budding yeast, Myb domain generally binds to telomere DNA with two copies of Myb domain. However, mammalian RAP1 has only one Myb domain, so it can only combine with telomere repeat sequence by interacting with TRF2 protein at the C-terminal. In short telomeres, TRF2-RPA1 interactions can promote the formation of T-loop [47, 48]. The regulatory effect of RAP1 on telomere length is inconsistent in the current studies. Some of the results show that there is no direct correlation between RAP1 and telomere length. Other results show that RAP1 overexpression or inhibition can facilitate telomere extension [49, 50]. This is broadly similar to the POT1 effect. RAP1 deficiency in mice had no significant effect on survival rates, but it could lead to a range of metabolism-related diseases, such as obesity, reduced glucose tolerance and liver fatty degeneration. So why does RAP1 also have telomere protection in complexes? In fact, RAP1 plays an essential role in telomere maintenance and protection in the case of telomerase deficiency. And the lack of RAP1 and TERC can lead to a decline in survival rates and telomere shortening in mice, compared to the lack of telomerase alone [51, 52].

So far, cellular senescence has mostly been arisen from a reduction in telomere length. However, some studies have shown that telomere dysfunction can be independent of changes in telomere length. With age, telomere dysfunction-induced foci (TIFs) increased year by year in mice's liver and intestines, and the accumulation of this damage was independent of telomerase activity and telomere length [53]. Meanwhile, the occurrence of DDR in fibroblasts derived from the skin of the dragonfly was associated with long telomeres [54]. In fact, TRF2 and RAP1 can exist in dysfunctional telomeres, proving that shortened telomere length and decapitation (loss of protective protein components) are not the only reasons for DDR activation [55, 56].

The Role of the Mitochondrial Metabolic Alterations in Cellular Senescence

Mitochondria, as the main source of energy in cells, are involved in many cellular processes, including cell metabolism, inflammation, cell cycles, etc. Cell metabolism is usually divided into two aspects: decomposition metabolism and anabolism. Glucose oxidation is the main decomposition metabolic activity, which is closely related to mitochondrial respiration. However, mitochondrial dysfunction is also one of the key hallmarks of aging [57]. In recent years, there has been more and more research on the relationship between mitochondrial function and cell senescence. Denham Harman proposed a theory based on free radicals in 1950, in which it was speculated that the production of ROS from mitochondria could cause damaged proteins, lipids and DNA accumulation, which in turn would result in cell cycle arrest to trigger cellular senescence [58]. Although senescent cells are in a cell cycle arrest, they are accompanied by a series of metabolic changes, as well as functional decline and the occurrence of multiple diseases. Mitochondria mainly produce adenosine triphosphate (ATP) to provide energy for biochemical reactions. Normal mitochondria produce ATP mainly through oxidative phosphorylation (OXPHOS), which involves mitochondrial electron transport chain (ETC) complexes I-IV and ATP synthase (complex V). In OXPHOS process, electrons are transferred from complex I to complex IV via mitochondrial ETC, and then molecular oxygen is reduced into water. The electron transfer is coupled with translocated protons from mitochondrial matrix to the inner membrane space, and generates an electrochemical gradient, which is eventually utilized by ATP synthase to produce ATP [59, 60]. In the process of cellular senescence, dysfunctional mitochondria will produce ROS, while the accumulation of ROS will lead to the functional damage of proteins involved in OXPHOS [61]. Then mitochondrial metabolism changes, which is mainly manifested in enhanced dependence on glycolysis at the expense of reduced dependence on OXPHOS, primarily due to the increased glucose consumption and lactic acid production in senescent cells [62]. At the same time, the accumulation of Ca^{2+} in mitochondria also plays an essential role in the regulation of mitochondrial metabolism and aging. Mitochondrial Ca^{2+} overload leads to the diminished mitochondrial membrane potential and the elevated ROS production, which further causes replicative senescence [63]. Therefore, maintaining appropriate Ca^{2+} concentration to regulate mitochondrial metabolism may become a new strategy for the treatment of aging.

Nicotinamide adenine dinucleotide (NAD^+) is a coenzyme of redox and also the center of mitochondrial energy metabolism. Several studies have shown that the decrease in the NAD^+/NADH ratio is due to mitochondrial dysfunction, a major feature of aging, known as mitochondrial dysfunction-associated senescence (MiDAS). The main reason is that reduced NAD^+/NADH ratio can activate 5'AMP-activited protein kinase (AMPK) and induces cell senescence through p53 signaling pathway [64]. The level of senescence-associated secretion phenotypes (SASP) in senescent cells is closely related to the NAD^+ content, and it is well known that supplementing NAD^+ precursor nicotinamide mononucleotide (NMN) can improve the level of NAD^+ level. Concurrently, SASP level is also elevated, promoting inflammation and cancer development [65]. Therefore, how to enhance NAD^+ level in the body without side effects deserves further investigations. Interestingly, in a recent study, the treatment with NAD^+ precursor nicotinamide riboside (NR) restored NAD^+ activity consumed by PARPs and SIRT1 in fibroblasts, reduced mitochondrial damage and mitochondrial ROS production, while delayed replicative senescence [66]. The anti-aging effect of NAD^+ precursor supplementation has been fully confirmed. At present, NMN and NR have become recognized nutritional supplements [67].

Mitochondrial function is manipulated by a number of factors. Taking in too many calories can attenuate mitochondrial function and impair stem cell function [68]. It is well known that skeletal mitochondrial function is closely related to age. In a clinical study, mitochondrial function was worse in older adults than that in young people with the same level of exercise. However, the mitochondrial function of older adults with higher exercise efficiency was significantly ameliorated when age factors were removed. The results show that certain exercise training and age can influence mitochondrial function and muscle health [69]. It is therefore shown that mitochondria are the promising therapeutic targets for muscle senescence.

The Crosstalk Between Telomere and Mitochondrial Metabolism in Cellular Senescence

Telomere damage and mitochondrial dysfunction are both fundamental features of aging. The most effective way to delay aging is calorie restriction (CR), which can improve telomere attrition, increase antioxidant capacity and reduce ROS production [70, 71]. Human leukocyte telomere length (LTL) was negatively correlated with CR, it is thus confirmed that non malnutrition CR can delay cellular senescence [72]. In line with this, CR reduced the oxidative damage to DNA and protein by inhibiting the production of ROS and promoting the clearance of antioxidants, so that the mitochondrial function will not gradually attenuate with age [73]. Some studies believe that the reduced ROS production by CR is chiefly due to the amelioration of mitochondrial proton leakage [74]. Other studies have shown that another mechanism for CR mediated ROS reduction is to facilitate ATP synthesis by balancing respiratory

movement. CR stimulates mitochondrial proliferation induced by peroxisome proliferation activated receptor coactivator 1α(PGC-1α) in mitochondria, so as to improve oxidative damage through energy generation [75].

The increase in mitochondrial ROS can lead to oxidative stress. However, because telomeres contain higher ostrich components, they are more susceptible to oxidative damage, increasing the rate of telomere shortening, and ROS can cause more DNA damage, so that cells getting into an senescence state [76]. The ROS produced by mitochondria can spread freely to the nuclei of cells, and ROS is considered an important molecule for the transmission of information between mitochondria and cell nuclei. When mitochondrial dysfunction occurs, telomere damage follows, but nuclear DNA is not injured [77]. Patients with mitochondrial diseases also have shorter telomeres compared to healthy people. This suggests a link between mitochondrial dysfunction and telomere damage [76]. Telomere damage mainly affects mitochondrial function by activating p53 and inhibiting the PGC-1α pathway. p53 has been playing the role in telomere attrition and DNA damage. PGC is a crucial regulatory factor for mitochondrial biosynthesis and metabolism, and also the major controlling factors between telomere damage and mitochondrial dysfunction. Telomere attrition will affect the expression of p53 and PGC-1α, and ultimately lead to mitochondrial damage [78]. The role of mitochondria in aging mainly includes the increase of ROS production, the decrease of ATP synthesis and the activation of cellular apoptosis [79]. Recent studies have found that telomere DNA damage occurs prior to mitochondrial metabolic disorder. When T cells are treated with KML001 (sodium metaarsenite, $NaAsO_2$, a telomere-targeting drug), cellular senescence and apoptosis will be induced. Telomere dysfunction induced by KML001 will activate p53 signaling pathway. In $CD4^+$ T cells, p53 passes through p53-PGC-1α-NRF-1 axis is closely related to telomere attrition and mitochondrial damage. Importantly, p53 regulation can influence the expression of PGC-1α and NRF-1 to improve mitochondrial function, such as mitochondrial membrane potential, OXPHOS, mitochondrial respiration, glycolysis and ATP synthesis [78].

The main difference between chromosomes and mitochondrial DNA is whether there is a telomere structure or not. But mitochondrial DNA and telomere DNA share the same characteristics as replication pressure: D-ring, epigenetic regulation, G-quadrangle and DNA heterogeneous double-stranded, supercoiled [80].

The key role of telomerase is to cancel out telomeres shortening and maintain telomere length. In addition, telomeres also have the function of sustaining cell survival. It has been found that telomerase catalyzes the removal of subunit TERT, stimulated by oxidative stress, from the nuclei and locates in mitochondria. This co-localization of TERT and mitochondria protects mitochondrial function and ameliorates nuclear DNA damage and apoptosis by reducing mitochondrial ROS production [81, 82] (Fig. 2). Intriguingly, cells that completely remove telomerase have no or very little DNA damage. In contrast, cellular DNA damage that has not been totally removed telomerase from cells increases. The authors believe that telomerase may

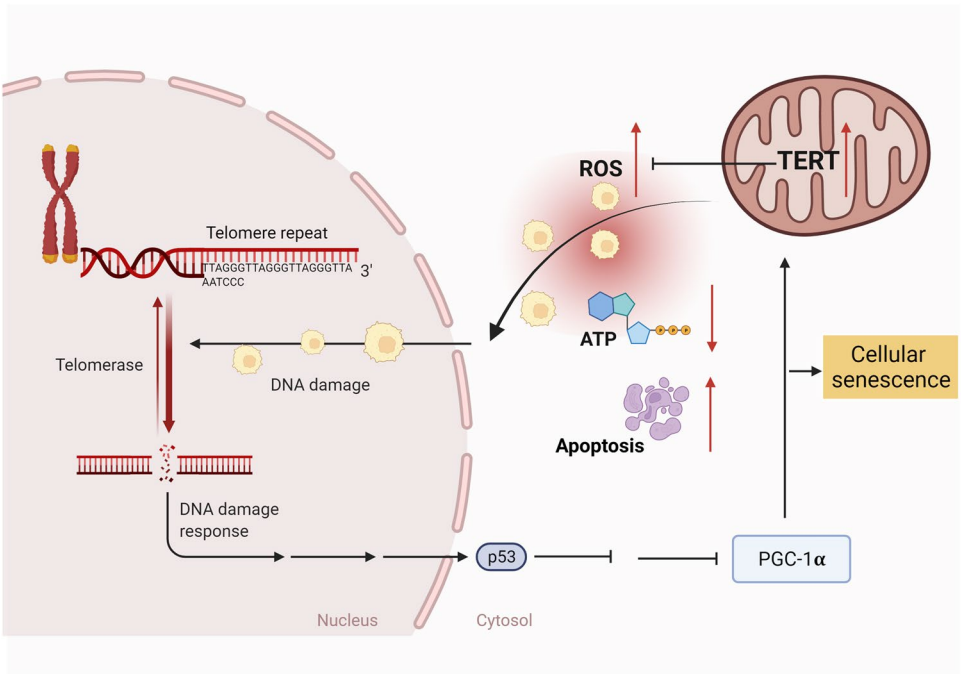

Fig. 2 ROS produced by mitochondria enters the cell nucleus and causes the telomere DDR, activates p53, inhibits PGC-1α signaling pathway and affects mitochondrial function, eventually induces cellular senescence. Telomerase catalyzes the removal of subunit TERT stimulated by oxidative stress from the nuclei and locates in mitochondria. This co-localization of TERT and mitochondria protects mitochondrial function by reducing mitochondrial ROS production.↑:increase;↓:reduce;⊢,—:inhibition

have negative effects on repair enzymes in the nuclei of cells, possibly in the event of increased DNA damage [83]. This also explains why telomerase cannot continue to play its role after DNA damage to a certain extent. In further studies, it has been shown that the expression of TERT in human fibroblasts can reduce damage to mitochondrial DNA after oxidative stress, which is mainly mediated by base excision repair (BER), and the presence of hTERT does not interfere with BER repair [84] (Table 1).

Telomere and Mitochondrial Metabolism in Age-related Diseases

Aging is a risk factor for many chronic diseases, such as type 2 diabetes, osteoporosis, Alzheimer's disease, etc. Using aging models, studies have found that there are many mechanisms involved in these age-related diseases, including telomere length and function, as well as mitochondrial dysfunction.

Type 2 Diabetes

Type 2 diabetes (T2DM) is an epidemic characterized by insulin resistance and abnormal insulin secretion, and an aging society drives the progression of T2DM. Chinese people are most likely to develop T2DM. Leukocyte telomere length (LTL) and mitochondrial DNA copy number (mtDNAcn) are both markers of senescence, and it has been confirmed that leukocyte mtDNAcn in older women are positively correlated with leukocyte telomere length [85]. It has been mentioned that there is a vicious cycle between mitochondrial dysfunction and telomere attrition, which may be resulted from hyperglycemia [86]. A latest research has found, a protein-E4orf1, from human adenovirus-AD6, can improve blood glucose in older mice and reduce the progression of adipose degeneration to prevent non-alcoholic fatty liver disease (NAFLD). And the expression of E4orf1 maintains mitochondrial integrity and alleviates telomere attrition to delay age-related cellular senescence [87].

Mitochondrial diabetes is a type of diabetes characterized by mitochondrial monogene mutations, and it is often misdiagnosed as type 1/2 diabetes. In fact, mitochondrial diabetes and T2DM have common features of shorter telomeres than healthy people [88]. At the same time, short telomeres make diabetes worse by promoting β cell apoptosis to repress insulin production [89].

Table 1 Telomeres and mitochondrial metabolism interact to influence cellular senescence

Factor	Telomere functional change	Mitochondrial metabolism change	Cellular senescence	Reference
TRF1-TRF2	telomere replication↑ telomere binding↑	\	delay	[28]
	53BP1↓、γH2AX↓			[32]
TRF1-polymer (ADP-ribose) polymerase 1	DDR↑	\	accelerate	[29, 30]
TRF2	T-loop formation↑	\	delay	[31]
TIN2-TPP1	telomerase collection to telomeres↑	\	delay	[19]
TIN2	\	ROS↑、ATP↓	accelerate	[37, 38]
POT1	telomerase activity↑↓ telomere length↑ DDR↑	\	accelerate	[42]
RAP1-TRF2	T-loop formation↑	\	delay	[47, 48]
RAP1	telomere length↑	\	accelerate	[49, 50]
	\	ATP↑ 、fatty acid metabolism↑	delay	[51, 52]
Ca^{2+}	\	ROS↑ mitochondrial membrane potential↓	accelerate	[63]
NR	\	ROS↓	delay	[66]
CR	LTL↓	ROS↓	delay	[72, 73]
	DDR↓	ATP↑、mitochondrial proliferation↑	delay	[75]
KML001	DDR↑	ATP↓、glycolysis↓	accelerate	[78]
TERT	DDR↓	ATP↑、ROS↓	delay	[81, 82]

TRF1: telomere repeat factors 1; TRF2: telomere repeat factors 2; TIN2: TRF1 and TRF2 interacting nuclear protein 2; POT1: protection of the telomere 1; TPP1: the ACD gene; RAP1: repressor/activator protein 1; DSB: double-stranded break; DDR: DNA damage response; 53BP1 and γH2AX: DNA damage markers; ROS: reactive oxygen species; ATP: adenosine triphosphate; NR: nicotinamide riboside; CR: calorie restriction; LTL: leukocyte telomere length; KML001: sodium meta-arsenite, $NaAsO_2$, a telomere-targeting drug; TERT: telomerase reverse transcriptase; ↑:increase;↓:reduce;\:unchanged

Osteoporosis

Osteoporosis is a complex and common disease in the elderly population, and the number of patients is increasing year by year. Osteoporosis is caused by a variety of factors, including bone strength damage and changes in bone microstructures [90]. People over the age of 60 are more likely to develop osteoporosis. Due to the aging of the elderly physical condition, patients are often accompanied by spinal malformation, lumbago, etc. And the mortality rate and disability rate are significantly elevated [91]. With age, the balance between osteogenic differentiation and adipogenic differentiation is broken, that is, osteogenic differentiation decreases, while adipose differentiation increases. Adipose tissue accumulates in bone tissue, eventually leading to diseases such as osteoporosis or fractures [92]. In recent years, more and more research has been made on the relationship between telomeres, aging and bone formation. In a study, the authors used hydrogen peroxide to treat cells, causing cell replicative senescence. And telomere length was shortened with the passages. It was found that the bone differentiation ability of cells was also reduced, accompanied by declined cell proliferation [93]. Telomerase, as a protective component of telomeres, can not only prolong telomeres but also prevent cell replicative senescence. And studies have confirmed that the heterogeneous expression of telomerase can promote bone differentiation of bone marrow stromal stem cells (BMSSC) [94]. However, after the subcutaneous transplantation of BMSSC expressing telomerase (BMSSC-Ts) into mice, it has been found that bone-forming ability was improved. The mechanism is to facilitate the expression of bone-related genes CBFA1, osterix and osteocalcin, thus accelerating the progression of the cell cycle from G1 to S [95]. In the mean time, defects in telomere maintenance molecules can also give rise to a decline in bone-forming capacity. Osteoporosis is common in Werner syndrome (WS) and DC, featured by telomere dysfunction. Correspondingly, the TERC mutation is a common form of DC mutation: autosomal dominant. And WS is produced by Wrn mutation. As a result, in mice with $Terc^{-/-}Wrn^{-/-}$ double-mutation defects, their bone mass decreased and bone differentiation was impaired [96]. Mitochondrial dysfunction has a serious detrimental effect on bone formation. So far, mitochondrial DNA polymerase γ (Plog) is the only DNA polymerase found in mitochondria, responsible for DNA replication and mitochondrial repair in all cell types. Compared to wild mice, mitochondrial mutation mouse models ($PlogA^{mut-mut}$) accumulate mitochondrial DNA point mutations at a rate of 3–5 times higher, so it is an excellent early-aging mouse model. The $PlogA^{mut-mut}$ genotype impairs bone differentiation, accelerates bone loss, and promotes osteoclast formation [97]. Mitochondrial transmembrane protein OPA plays an important role in mitochondrial function, so it is possible to further explore the relationship between OPA and radiation-induced osteoporosis. In the osteoporosis mouse model, the expression of OPA mRNA and protein increased, the mechanism of which is to activate the p38 signaling pathway, decrease mitochondrial ATP production decreased, and facilitate cell apoptosis [98]. Long-term glucocorticoid therapy can induce osteoporosis, and excessive glucocorticoids can also cause an increase in ROS production, leading to mitochondrial dysfunction. In osteoblasts, H_2S avoids damage caused by oxidative stress via inhibiting the AMPK signaling pathway. Further studies have found that H_2S reduces the bone cell injury caused by dexamethasone through protecting mitochondrial function [99].

Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disease that is more common in people from 40 to 90 years old and has a genetic component in 2% of cases. The typical manifestations are cognitive disorder, short-term memory loss and other problems. The major pathological characteristics are neurotic plaques (NPs) and neurofibrillary tangles (NFTs) [100]. Neurofibrillary tangles are formed by the accumulation of the tau protein with excessive phosphorylation in the brain and play an important role in the development of AD. Excessive phosphorylated Tau destroys the cytoskeleton, leading to synaptic dysfunction and neuronal death [101]. Toxic protein tau can be degraded in the normal human brain. However, in the aging process and AD, tau degradation ability is weakened, the presence of pathological tau can accelerate neuronal ROS production and aggravate oxidative damage. In this case, TERT is expressed in adult brain tissue and located in mitochondria to reduce ROS production and protect neurons from pathological tau [102]. Therefore, it is shown that the activation of TERT may play a preventive role in neurodegenerative diseases. However, two telomerase activators, TA-65 (a high-purity jaundice extract) and GRN510 (a small molecule based on cycloastragenol) can be used to up-regulate TERT expression levels to increase telomerase activity, reduce the expression of toxic protein tau, and improve symptoms associated with neurodegenerative diseases [103]. A new finding is that when dietary restriction (DR) and rapamycin were treated, the amount of TERT protein in the mitochondria in the brains of mice increased significantly. But in other tissues, such as the liver, there is no same tendency [104]. Rapamycin is an inhibitor of mTOR signal, and it can drive more TERT to mitochondria in the brain of mice by reducing mTOR signal. It is strongly illustrated that the mTOR signal participates in TERT location and reduces ROS generation [105].

Since AD has a higher incidence in the elderly, it is assumed that TERT protein levels will be down-regulated when the disease occurs. However, a team of researchers

found there was no decline in TERT protein levels at any one time during the development of AD [106]. In contrast, the levels of TERT in neuronal mitochondria in Braak stage were higher than those in healthy older adults [107]. However, whether TERT locating in mitochondria can actually protect neurons from oxidative stress remains to be investigated. TERC, the RNA component of telomerase, is used to be a template to prolong telomere length. TERC gene knockout mice show the characteristics of short telomeres, which is considered to be a model of premature aging. Another TERC gene named alternative TERC (alTERC) was found in mice, which can interact with human TERT protein to form active enzyme. Overexpression of alTERC in mouse brain can up-regulate telomerase activity and protect neurons from oxidative stress. This protective effect is not related to the expression of TERT [108].

Conclusion and Future Prospects

Mitochondria and telomeres are mostly the direction of two independent research. However, they can be linked together through aging. Characteristics of cellular senescence include mitochondrial dysfunction and telomere shortening. As the two main characteristics of cell senescence, there may be some crosstalk between mitochondria and telomeres, and the ROS produced by mitochondria directly affects the rate of telomere shortening, further accelerating the cell senescence. Telomere damage influences mitochondrial function through p53-PGC-1α signaling pathways and is also closely related to cell senescence. Age-associated diseases such as T2DM, osteoporosis and AD are concerned with telomere and mitochondrial function. Therefore, telomere function and mitochondrial function can be considered as targets for the treatment of age-related diseases. Although aging is inevitable, increased chronic disease-related risk factors with age can potentially affect lifespan.

The effects of telomeres and mitochondria on aging are more extensive, while the effect of telomere related proteins on cellular senescence and its mechanism are rare in the current research. It is well known that mitochondrial dysfunction causes metabolic disorders, and there is also a strong correlation between metabolism and aging. However, it is still unclear whether there is a closed-loop relationship among telomeres, mitochondria, metabolism and aging, and whether there are key genes that can link them to affect age-associated diseases. These issues are worthwhile exploring thoroughly in the future. What is more, after establishing the age-related disease models, the effects of telomere and mitochondrial metabolism on aging can be probed, meanwhile in-depth mechanisms and new drug development will also be investigated. Clinical trials for the novel drug candidates should then be carried out to determine their ability to treat age- related disease.

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declarations

Conflict of Interest The authors declare that they have no competing interest.

Ethical Approval Not applicable.

Consent to Participate Not applicable.

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