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Linking telomere loss and mitochondrial dysfunction in chronic disease

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1. ABSTRACT

Telomeres and mitochondria are known to deteriorate over time. Telomere shortening is associated with aging, early senescence, and premature cell death. Mitochondrial dysfunction produces indiscriminate amounts of reactive oxygen species that may lead to oxidative damage to cellular constituents, including telomeric DNA, causing telomere shortening. In fact, patients with primary mitochondrial dysfunction (for example respiratory chain disorders) and secondary mitochondrial dysfunction (such as metabolic diseases, neurodegenerative diseases, cardiovascular diseases, and mood disorders, among others) have shorter telomeres compared to those of healthy controls. Drawing a mechanistic connection between telomere function and mitochondria biology will provide a broader perspective for understanding the pathophysiology of diseases and their relation to the aging process, and may provide opportunities for new possible treatments.

2. INTRODUCTION

Organisms are unable to live forever. Cells have a finite lifespan; as time passes cells deteriorate and decline in function, giving rise to diseases and death. This process is aging. Mitochondria are key players involved in this process; however, they are rarely in the spotlight. With time, mitochondrial function declines and contributes to the aging process. Two important forces that add to this scenario are impaired ATP production and increased production of reactive species, including oxygen species (ROS) and nitrogen species (RNS) (1-4). At physiological levels, mitochondrial ROS/RNS are important signaling molecules that can activate cellular repair functions such as the antioxidant, protein quality control, autophagy/mitophagy and anti-inflammatory systems through redox modifications of gene and protein networks. In sick cells, however, ROS production increases and the cellular repair functions are no longer able to cope with the increased levels of ROS. As a consequence, irreversible oxidative modifications are increasingly introduced and healthy redox signaling of repair functions are disturbed, leading to oxidative and redox stress (2, 5). Accordingly, many age-related chronic conditions (cardiovascular diseases, diabetes, aging, mood disorders, among others) are associated with overproduction of ROS and oxidative damage (6-9). Specifically, ROS may induce DNA damage to telomeres, the structures that cap chromosome ends (10, 11). Telomere damage may result in accelerated telomere shortening and activation of the DNA damage response (DDR). When telomere length has reached a critical point, cells arrest to halt proliferation and the tumor suppressor protein, p53, is activated in order to induce apoptosis. Also, p53 inhibits the master regulator of mitochondrial biogenesis, peroxisome proliferator-activated receptor γ (PPARγ) co-activator 1α (PGC1-α), which further decreases renewal of mitochondria and causes accumulation of damaged mitochondria (12). This draws a clear connection between mitochondrial function and telomere maintenance. This concept is of particular interest because it may shed some novel light on the pathology and mechanisms of chronic diseases. In the present review we will summarize the current knowledge on telomeres and discuss their close connection to mitochondrial function and impact on human diseases, with the hope that it may inspire

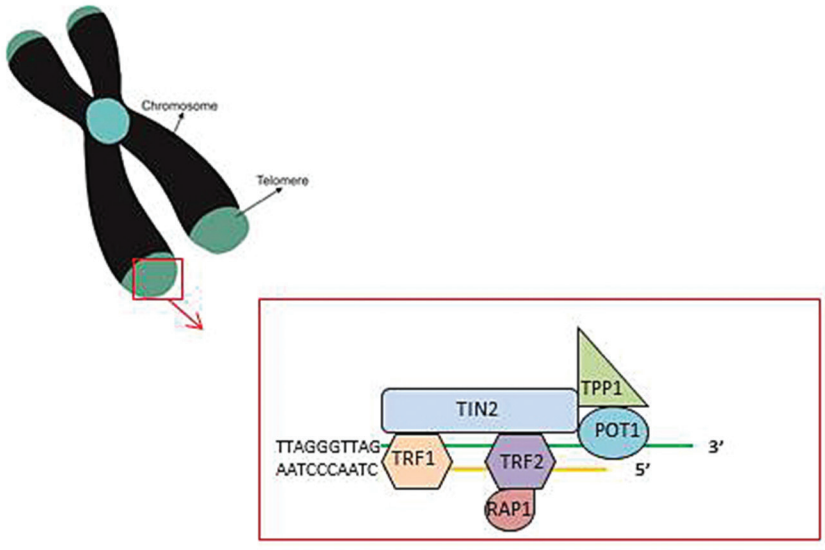

Figure 1. Shelterin complex. Telomeres provide a protective cap to chromosome ends, leaving a 3' overhang that will loop with the adjacent strand in order to secure and further stabilize the structure. The capping complex, Shelterin, is composed of human telomeric repeat factors: TRF1 and TRF2, protection of telomeres 1 (POT1), TRF1-interacting nuclear factor 2 (TIN2), tripeptidyl-peptidase 1 (TPP1), and Ras proximate 1 (Rap1). These proteins protect chromosome ends from being recognized as double-strand DNA breaks.

development of new combinations of biomarkers of mitochondrial dysfunction and new targets for treatment.

3. TELOMERES AND THEIR STRUCTURE

In 1938, Hermann Muller first identified a recurring structure located at chromosome ends, which he then named *telomere*s (13). Telomeres are nucleoprotein complexes located at the physical end of linear chromosomes, in eukaryotic cells (14) (Figure 1). These structures are composed of a guanine-rich sequence (TTAGGGG) positioned in a 5'→3' orientation (14). Telomeres' termini consist of a 3' overhang that is subsequently stabilized into a 'lasso' or loop structure by interacting proteins of the Shelterin complex (14-19). The lasso-like structure safeguards telomeric DNA, shielding it from degradative enzymes. The Shelterin complex involves the assembly of the following proteins: human telomeric repeat factors: TRF1 and TRF2, protection of telomeres 1 (POT1), TRF1-interacting nuclear factor 2 (TIN2), tripeptidyl-peptidase 1 (TPP1), and Ras proximate 1 (Rap1) (16).

Shelterin protects telomeres by changing their shape (16); when these proteins bind, they cap the telomeres preventing them from being recognized as a double-strand break and keeping the DNA molecule from entering breakage/fusion/bridge cycles (15). The exact mechanism of how Shelterin is able to modify telomeres remains unknown. However, it has been proposed that the t-loop (telomere-loop) formation is what stabilizes the structure (15, 20). By the action of TRF1 and TRF2, the 3' overhang folds back and is 'tucked in', forming a D-loop with the adjacent strand. TRF2 helps localize the D-loop and the 'tucking in' of the overhang given that it has been found to be localized at the base of the t-loop (16, 20). TRF1 might aid in the t-loop formation as it catalyzes telomeric synapsis, which leads to a coiled structure (20). POT1 can bind to the duplex end and block telomerase (16). Shorter telomeres have reduced amounts of Shelterin and therefore reduced amounts of POT1; this increases the chances for telomerase to bind and elongate telomeres.

4. TELOMERE ELONGATION AND SHORTENING

With each replication and cell division, telomeres shorten in length (14). Using human diploid fibroblasts, Leonard Hayflick established that they, in fact, had a finite lifespan. And he suggested that this was due to an internal molecular process (21). Years later, Alexei Olovnikov foresaw that the finite lifespan of cells in culture could be attributed to a shortening in their telomeres (22). It was then suggested that telomeres were drivers of replicative senescence in normal cells, and the length could perhaps be used as a 'tool' to count the number of cell divisions (23, 24). Nowadays, we know that the length of telomeres does indeed deteriorate with aging in human fibroblasts (25).

Telomeres are maintained by the telomere terminal transferase, telomerase, which consists of a ribonucleoprotein subunit (TERT) (26) and a reverse transcriptase catalytic subunit (TERC) (27). The enzyme is able to elongate chromosome ends through

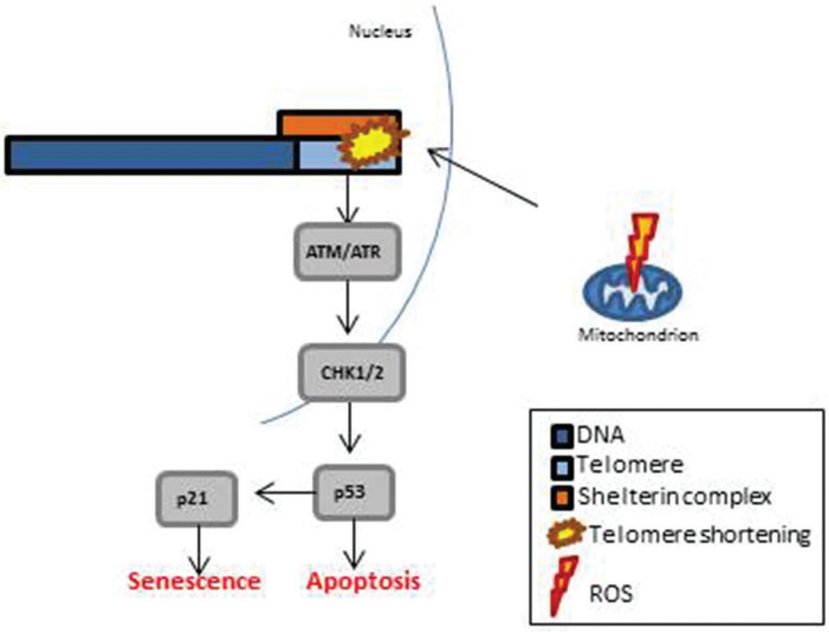

Figure 2. Telomere shortening unleashes DNA damage response cascade. Telomere shortening leads to cell death and cell arrest. When DNA damage is detected, in this case in the form of telomere shortening, the cell elicits an emergency response. The kinases ATM and ATR are activated and thus able to phosphorylate Chk1 and Chk2, which subsequently phosphorylate p53 in order to induce apoptosis. To induce senescence, p53 activates p21, an inhibitor of cell cycle. In addition, we propose that mitochondrial ROS (at pathological levels) contribute to telomere damage. Modified with permission from (90).

TERT, which provides an RNA template from where the necessary nucleotides are synthesized and added to the DNA strand (14). In the absence of telomerase, as it is the case in most mammalian somatic cells, telomeres shorten with every replication, cell division, and aging (15). When telomeres are unable to protect a chromosome, the outcome could be highly deleterious. The cell will most likely attempt to fix the situation through non-homologous end joining or homologous recombination, which can lead to end-to-end fusions, and ultimately genomic instability and possibly cancer (15, 28). Under normal conditions, telomere dysfunction is detected and the DNA damage response (DDR) is activated (29). Upstream kinases, ataxia telangiectasia (ATM) and Rad3-related protein (ATR) are activated and are able to phosphorylate checkpoint kinase 1 (CHK1) and checkpoint kinase 2 (CHK2), which consequently phosphorylate p53, a tumor suppressor that induces apoptosis and cellular senescence, through p21, to halt the growth of damaged cells (30) (Figure 2).

Three different mechanisms have been put forth on telomere shortening: *the end of replication problem*, which consists of the inability of DNA polymerase to fully replicate the lagging strand (23). *Activation of C-strand exonuclease* (31), which degrades both ends of the chromosome after DNA replication in order to allow the formation of the 3' overhang. And lastly, the main focus of this review: *oxidative stress,* which can not only cause DNA damage, but can also shorten telomeres (11).

5. OXIDATIVE STRESSS AND TELOMERES

Oxidative stress is able to cause DNA damage in the form of oxidized bases and single/double strand breaks; in fact, acute oxidative stress increases the frequency of double-strand breaks (11). Telomeres are particularly susceptible to oxidative stress because their composition is rich in guanine sequences, which are vastly vulnerable to oxidation (10). Oikawa *et al.* showed that one of the main ROS, hydroxyl radical, promotes 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) at the 5' site of telomeric 5'-GGG-3'. Telomere shortening requires DNA replication (26, 32-34). Unrepaired nucleotides in telomeres, due to oxidative damage, disrupt the replication fork and thus amplify the amount of unreplicated ends (11). Further, oxidative damage can disrupt telomere maintenance by reducing telomerase activity and accelerating telomere shortening, as it has been observed in vascular smooth muscle, leukemic, and endothelial cells (35-38). The rate of telomere shortening varies among cells, depending on ROS and antioxidant production, and telomere length shortens at a faster rate when exposed to oxidative stress, suggesting that telomeres may act as biomarkers of accumulative oxidative damage and indicators of disease development (11, 39). Chronic stress conditions such as mild hyperoxia, hydrogen peroxide, radiation, organic hydroperoxides, or transfection with oncogenes leads to premature senescence and activation of p53 (34, 40-42). Accelerated telomere shortening, however, may be slowed when oxidative stress is reduced

to levels below those of the required threshold (11, 39), using free radical scavengers, antioxidants, and low oxygen concentrations (43-45). These *in vitro* studies show a relationship between oxidative stress and telomere shortening.

Human population studies have shown a similar relationship between oxidative stress and telomere shortening. In these cases, oxidative stress and telomere shortening were associated with obesity, smoking, psychological/economical stress, and different types of cardiovascular diseases (46-48), as discussed in more details below. von Zglinicki and others have proposed that the link between these phenomena is found in mitochondria (49), the major production site of ROS (50, 51).

6. WHY THE MITOCHONDRIA?

Studies where the mitochondria specific antioxidant, MitoQ, was targeted to mitochondria, counteracted telomere shortening and lengthened the lifespan of cells by 40% under hyperoxia (52). Similar, treatment with nicotinamide has also been shown to stall telomere attrition and prolong lifespan (53). Nicotinamide is a precursor of NAD^+, which as a co-factor to a number of metabolic enzymes including the sirtuins, is essential in maintaining mitochondrial function and decreasing cellular ROS (54). Moreover, severe mitochondrial depolarization in mouse embryos, by FCCP (carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone), led to an increased production of ROS, telomere shortening, and chromosome fusion (55), suggesting a connection between telomeres and mitochondrial ROS production.

During oxidative stress, the TERT component of telomerase have been shown to translocate to the mitochondria, thus preventing telomere elongation (35, 56-58). This suggests that mitochondria protection is of higher priority than telomere maintenance. Ahmed *et al.* have shown that overexpression of TERT, in fibroblasts, protects mtDNA from acute/chronic oxidative damage by increasing mitochondrial membrane potential, and decreasing both mitochondrial and cellular ROS levels. However, the exact mechanism as to how telomerase protects mitochondria is unclear; does it protects against mtDNA oxidation, triggers repair of damaged mtDNA, elicits degradation of damaged mitochondria, or is it due to the decrease in ROS levels? This uncertainty requires further elucidation. Recent studies, however, suggest that TERT may protect mitochondrial function indirectly by regulation of mitochondrial biogenesis.

7. TELOMERES AND MITOCHONDRIAL BIOGENESIS

Mitochondrial biogenesis is the process by which healthy mitochondria divide and grow while damaged mitochondria are removed (59). It depends on coordinated transcriptional control of genes coding for proteins essential for mitochondrial function, repair and structure. Peroxisome proliferator-activated receptor γ (PPARγ) co-activator (PGC-1α) is the master regulator of mitochondria biogenesis (60) and is phosphorylated and activated by AMP-activated protein kinase (AMPK). In addition, PGC-1α enables expression of sirtuin 1 (SIRT1), which is necessary for the activation of AMPK (60).

In an aging model, p53 activation in response to the DNA damage response, leads to suppression of AMPK and mitochondrial dysfunction (61). Similarly, studies of mice with TERT deficiency and telomere dysfunction showed compromised mitochondrial biogenesis and function in various tissues such as liver and heart (12). These observations were shown to be caused by suppression of PGC-1α and PGC-1β and their downstream targets such as genes that are required for oxidative phosphorylation, β-oxidation of fatty acids, gluconeogenesis, and ROS defense; it resulted in disturbed oxidative phosphorylation, diminished ATP production, compromised gluconeogenic capacity, and increased ROS, signifying a clear connection between mitochondria and telomere function (12). Sahin *et al.* suggested that telomere dysfunction leads to DDR activation, and consequent activation of p53, which binds to PGC-1α/ PGC-1β promoters and represses expression of PGC-1α/PGC-1β and their downstream targets. As a consequence, mitochondria biogenesis declines and ROS levels increase (12, 62). In another study, removal of PGC-1α in apolipoprotein knockout mice, by Xiong *et al.*, showed oxidative stress, telomere dysfunction and shortening, DNA damage, accelerated aging, and atherosclerosis (63). This further emphasizes the key role of PGC-1α in the system and suggests a vicious cycle of mitochondrial and telomere dysfunction (Figure 3).

Although these studies show that p53 decreases mitochondrial biogenesis controlled by the AMPK/PGC-1α,β axis, other studies have shown that p53 activates the AMPK/PGC-1α,β axis and increases mitochondrial function and oxidative defense mechanisms. The exact mechanisms that control different outcomes of p53 activation still need to be elucidated. However, it has been suggested that p53 may switch from a pro-survival to a pro-aging or pro-apoptotic protein depending on its activity level, which may be controlled by the severity of oxidative damage (62). These mechanistic links between mitochondrial biogenesis and telomere function seem relevant in order to understand the contribution of mitochondrial and telomere dysfunction to the aging process (3,62). PGC-1α and PGC-1β are known to decrease in chronic diseases, including aging, which results in mitochondrial dysfunction and increased production of ROS, which can cause mtDNA mutations and further ROS production. Under mild stress levels,

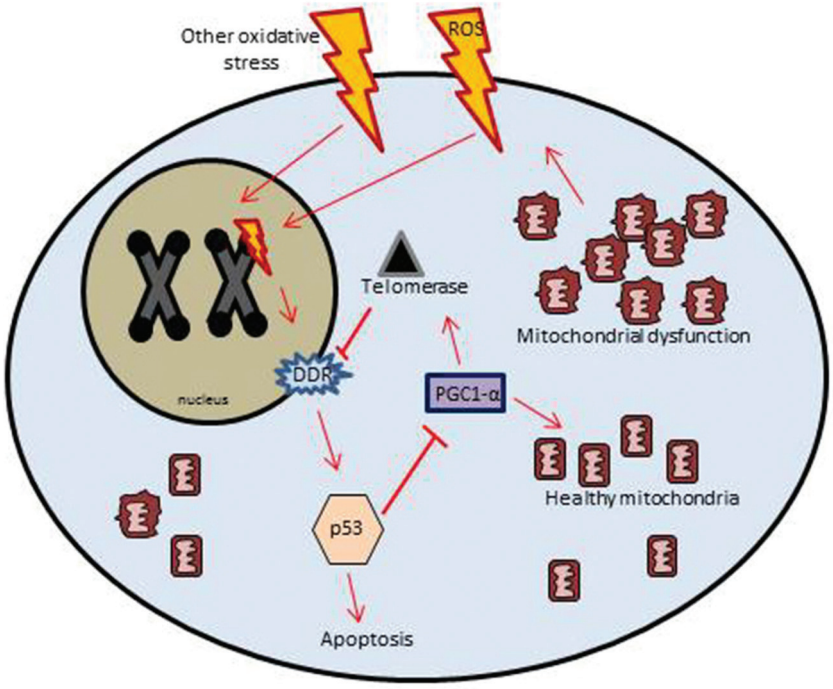

Figure 3. Vicious cycle between oxidative stress and mitochondrial dysfunction. In the presence of DNA damage response (DDR), p53 activation causes the suppression of PGC1α/β, which leads to mitochondrial dysfunction and further production of reactive oxygen species, which results in oxidative stress (ROS). This situation, on the other hand, can inflict damage to telomeres and cause its shortening which triggers DDR, involving p53 activation, and thus amplifying the dysfunctional system. In addition, PGC1-α may control the expression of TERT, which may lead to inhibition of the DDR. Modified with permission from (3).

p53 activation will allow activation of AMPK/PGC-1α,β to repair and maintain cellular functions. At higher levels of ROS, or when telomeric damage accumulates to a level required to activate the DDR, TERT exits the nucleus and p53 is activated to inhibit mitochondrial biogenesis, and initiate pro-aging senescence. This may be an adaptive mechanism to prevent further ROS production from a damaged mitochondrial network and necrotic cell death. At even more excessive stress levels, p53 seems to by-pass the AMPK/PGC-1α,β axis and activates apoptosis to eliminate cells that are too damaged to survive. Functional consequences of p53 may also be tissue dependent. In tissues with a higher turnover demand, for example blood cells, the system can afford to lose cells by apoptosis; and in tissues with a lower turnover demand, for example in the brain, it is more affordable for the system to inhibit mitochondrial function rather than lose cells (3). In our opinion, both scenarios are a defense mechanism very much dependent on the tissue's needs.

One of the previous aging models suggests that mitochondrial dysfunction, in the form of oxidative stress and inflammation, may accelerate telomere shortening in post-mitotic tissue, and therefore individuals carrying cells with excessive oxidative damage may experience cellular senescence and premature aging/disease (64). On the other hand, studies by Sahin *et al*, which revealed that telomere dysfunction in itself can induce mitochondrial dysfunction and oxidative damage (12, 62), have promoted the idea that telomere length to some extent may be the preceding factor, so that individuals, who were born with short telomeres are already predisposed to developing mitochondrial dysfunction, diseases, and early aging during adulthood (65). Although this telomere-mitochondrial model supports the idea that telomere shortening may influence mitochondrial dysfunction and vice versa, further studies are required to fully understand how these pathways are connected and activated in different aging and chronic cell stress models. Moreover, the studies that closely associate mitochondria and telomeres (12, 62) have been done in genetically modified mice. Even though animal models have provided understanding in biology, one cannot assume that this scenario will be applicable to humans. However, a recent study by Tyrka *et al* showed a relationship between telomere function and mitochondrial biology. This study showed that in human whole blood samples, longer telomeres related to a higher mitochondrial DNA copy number. This suggests the possibility that similar mechanistic links between mitochondrial function and telomere biology may also exist in humans (65). Below, we will briefly discuss telomere dysfunction in some chronic diseases, where mitochondrial dysfunction and oxidative stress are known to be important players in initiation or progression of disease development.

8. RELEVANCE TO HUMAN DISEASES

Patients with Friedreich's Ataxia (FRDA) suffer from a hereditary central nervous system condition, which consists of decreased frataxin, a mitochondrial protein involved in the formation of iron-sulfur clusters (66). This leads to mitochondrial dysfunction and overproduction of ROS, making the cell more vulnerable to oxidative stress. Oxidative stress and inflammation are thought to contribute to telomere shortening in leukocytes of FRDA patients (67). In fact, telomere loss in patients with FRDA was significantly higher when compared to healthy controls (67). Patients that have suffered from the disease for a longer period of time have been reported to possess notably shorter telomeres, suggesting that telomere length could be used as a biomarker of disease progression (67). Patients with other primary mitochondrial dysfunction, as it is the case for respiratory chain disorders, such as mitochondrial myopathy, encephalopathy, lactic acid, and stroke (MELAS) and leber hereditary optic neuropathy (LHON) have also been shown to have shorter telomeres in their white blood cells, when compared to controls (68). In addition, patients with mitochondrial diabetes caused by mitochondrial DNA mutation (m.3243A>G), have also been shown to have shorter leukocyte telomere length compared to controls, suggesting a connection between telomere length and disease development (69). Mitochondrial dysfunction with increased mitochondrial ROS production is a hallmark of these disorders (5,70). The exact mechanism by which ROS production is initiated and propagated in human diseases are not known. However, disturbances in the integrity of the respiratory chain seem to play a principal role in both primary and secondary mitochondrial diseases, as discussed in (2). Primary mitochondrial disorders have been shown to produce ROS by increased electron leakage from the affected mitochondrial respiratory chain. Mitochondrial DNA is, because of its proximity to the site of ROS production, prone to oxidative damage, which can cause further respiratory chain damage and electron leakage initiating a vicious cycle of ROS production (71). This connection between telomere and mitochondrial dysfunction seems not to be exclusive to mitochondrial or nuclear gene defects that directly affect mitochondrial components, as telomere shortening has been observed in other chronic diseases, where mitochondrial dysfunction is induced by some external and yet less defined mechanisms.

Telomere shortening has been shown in a variety of psychological disorders (6). Peripheral blood mononuclear cells of highly stressed women (46) and leukocytes of individuals with major depressive disorder, bipolar disorder, anxiety, and post-traumatic stress disorder (6,72) showed an accelerated telomere shortening, which may explain the increased mortality and morbidity seen in these conditions. These psychological disorders are also associated with mitochondrial dysfunction and oxidative stress that may cause DNA damage (73-75).

Moreover, patients suffering from diabetes type 1 or 2, cardiovascular diseases and atherosclerosis have also been reported to have shorter leukocyte telomere length when compared to controls (76-81). Similar, Parkinson's and Alzheimer's disease have been shown to have accelerated shortening of telomeres in leukocytes or peripheral blood mononuclear cells (82-84). Decline in mitochondrial function (for example, downregulation of oxidative phosphorylation) and increase in mitochondrial DNA damage as well as oxidative stress also contribute to the development of these diseases (85-89), suggesting a linkage between telomere shortening and mitochondrial dysfunction also in these diseases. Thus, telomere shortening and mitochondrial dysfunction seem to be a general finding in many age-associated diseases, such as neurodegenerative diseases, atherosclerosis, and cardiovascular disease, supporting the importance of the telomere-mitochondria axis. However, for most of these conditions the cellular mediators are still unknown, and future work may show if telomere shortening in all cases relate to decreased PGC-1α/β and mitochondrial biogenesis.

9. PERSPECTIVES

As discussed in the present review, emerging data suggest a connection between telomeres and mitochondria. They seem to exist in a dependent cycle, where the dysfunction of one of the systems worsens the condition of the other, adding further strain to the cells' overall health. There are indeed many aspects of the mitochondria-telomere axis that need to be unraveled, and further human studies are required to determine the applicability of the discussed model to humans. Nonetheless, a deeper understanding of the intricate pathways and mechanisms, which link mitochondrial dysfunction and telomere shortening, may provide a broader perspective for understanding the contribution of mitochondrial dysfunction to age-related diseases, not to mention that it could open the door to discovering new treatments.

Moreover, the fact that telomere length may be an indicator of accumulative oxidative damage potentially makes it an ideal biomarker of the extent of mitochondrial dysfunction and chronic cell stress. To this comes that the measuring of telomeres can be performed in blood, which is a stable and easy-to-access sample. Implementation of telomere length as biomarker for chronic cell stress may promote clearer identification of patients for future research studies, and in the long run improve diagnostics, prognostics, and treatment of chronic diseases.

10. ACKNOWLEDGEMENTS

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Abbreviations: ROS: reactive oxygen species; RNS: reactive nitrogen species; DDR: DNA damage response; PGC1-α: peroxisome proliferator-activated receptor γ (PPARγ) co-activator 1α; TRF1: telomeric repeat factor 1; TRF2: telomeric repeat factor 2; POT1: protection of telomeres 1; TIN2: TRF1-interacting nuclear factor 2, TPP1: tripeptidyl-peptidase 1; Rap1: Ras proximate 1; FCCP: carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone; TERT: telomerase reverse transcriptase; TERC: telomerase RNA; ATM: ataxia telangiectasia; ATR: ataxia telangiectasia and Rad3-related protein; CHK1: checkpoint kinase 1; CHK2: checkpoint kinase 2; FRDA: Friedreich's Ataxia; MELAS: mitochondrial myopathy, encephalopathy, lactic acid, and stroke; LHON: Leber's hereditary optic neuropathy

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