Is Genome Instability a Significant Cause of Aging? A Review

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ABSTRACT
What is the main cause of aging has been discussed over last decades. Accumulating evidences has indicated that genome instability including mitochondrial DNA in somatic cell played an important role in human aging process. Various sources of damage such as reactive oxygen species and UV radiation can lead to the decrease of DNA robustness. In addition, the erroneous replication and failure in DNA maintenance system is also able to casus mutations and epimutations. In this review we will first summarize the relation between the accumulation of DNA damage and longevity. Then we will review the contribution made by mitochondrial free radical to the aging process. Finally, we will briefly discuss whether the telomere is an eligible biomarker of life span.

Keywords: *"aging," "genome instability," "mitochondrial DNA," "telomeres".*

1. INTRODUCTION

Aging is defined by progressive physiological decline in cellular and organismal functions that leads to a decrease in the organism's ability to adapt to metabolic stress, ultimately resulting in death. This natural and multifaceted phenomenon is influenced by many factors that are broadly classified as intrinsic (related to genes) and extrinsic (related to physiological and environmental factors) and related to disease. While the idea that aging is genetically programmed as a result of the progressive accumulation of DNA damage coupled with a decline in maintenance remains controversial, it has been acknowledged by many authors that genome instability plays a significant role.

Genome instability has long been implicated as the main causal factor in aging. It is generally referred to the tendency of genes to undergo high frequency of mutations, including point mutations, insertions/deletions, or major rearrangements. The cause of genome instability falls into 3 broad categories: (1) Chemical damage to the DNA of the genome, involving both single-strand and double strand breaks of the DNA backbone, cross-links between bases, and depurination or depyrimidination of sugars and modified bases; (2) Mutations as a result of exogenous sources such as UV radiation and environmental mutagens, or as a consequence of erroneous repair driven by the genome maintenance systems in an attempt to correct the damaged base pairs. Such genome alterations include addition, deletion, or substitution of bits of genetic code. (3) Epimutations, which is a heritable change in gene activity that is not associated with a DNA mutation but rather with the gain or loss of DNA methylation or other heritable modifications of chromatin. Somatic cells are continuously exposed to various sources of DNA damage from reactive oxygen species (ROS) to UV radiation to environmental mutagens. The ROS is an unwanted by-product that induces oxidative damage to various cellular macromolecules during the process of aerobic metabolism and plays an essential role in generating respiratory chain (RC) dysfunction and progeroid phenotypes. Similarly, UV radiation is seen responsible for the manifestation of accelerated accumulation of pathological lesions in late age. In order to cope with these free-occurring chemical lesions, a complex network of genome maintenance systems acts to remove damage and restore the correct base pair sequence. However, occasional errors may arise during DNA repair, and it is these imprecisions, as well as the occasional failure to correctly replicate the genome during cell division that constitute the foundation for mutations and epimutations. Therefore, if genome instability is a major mechanism of aging, we assume that (a) Accumulation of unrepaired DNA damage, DNA mutations, and/or epimutations increase in magnitude during aging; (b) Long-lived organisms have genome maintenance systems superior to those of short-lived

species; (c) Predominant polymorphic variants at loci of genome maintenance genes are associated with human longevity. We shall discuss these assumptions in detail later.

Along with genome instability, recent studies show that telomere length is a reliable hallmark of biological aging. In vivo and in vitro experiments illustrate that telomere length changes with age, has high inter-individual variability, is linked to basic biology, and correlates with aging and aging related disease. However, whether it is a better predictor of life span is equivocal. Only cross-sectional studies have been taken and many studies were underpowered.

In this article we first discuss concepts of how genome instability in terms of DNA damage of somatic cells, DNA mutations, epigenomic mutagenesis, and accumulation of DNA damage contribute to increasing aging phenotypes. Then, we shall discuss the significance of mitochondrial functions in DNA, and the validity of the mitochondrial free radical theory of aging (MFRTA) theory. Finally, we shall discuss whether telomere length is an eligible biomarker of aging.

1.1. DNA Damage Genome Maintenance System and DNA Mutations

DNA damage is defined as any modification in the physical and/or chemical structure of DNA that is capable of causing cellular injury and reduces viability or reproductive fitness of the organism. There are two types of cell responses to DNA damage. The first type is a burst of damage; for example, exposure to high levels of radiation can lead to various signalling pathway alterations, which result in temporary or permanent mitotic arrest in actively proliferating cells. The permanent mitotic arrest is also termed cellular senescence and has a strong relationship with the aging process. The second type is the extremity of the first one: cells can be eliminated through a spontaneous process called apoptosis. Most adult cells are not mitotically active, and during adulthood and aging the frequency of apoptosis is low compared with cell death, which occurred in normal developing animal tissues. In fact, a higher rather than lower frequency of apoptosis is associated with longevity [1].

DNA mutations differ from DNA damage because while DNA damage is reversible, mutation is irremediable and can only be removed via death of the cell. Mutations emerge as a consequence of errors during the replication or repair of a damaged DNA template [2]. Such errors happen most frequently during replication, and even without damage, the innate inaccuracy of polymerases copying an undamaged template can give rise to mutations by misincorporation. Furthermore, DNA damage can dramatically accelerate mutation rates through translesion synthesis; for instance, 8-hydroxyguanine, a major lesion induced by oxygen free radicals pairs with adenine to cause transversion mutations. In addition, the mutation generated by erroneous repair can also be linked to the epigenome, which predominantly includes DNA methylation and histone modification. Epigenomic modifications are dynamic and prone to random errors which erase previously existing information as surely as DNA sequence errors do. For example, during cellular responses and in processes such as memory formation in the brain. However, the mechanism of epimutations, such as their frequency and potential impact in the aging process, is still unknown.

The DNA repair system is often referred to as a system accommodating several mechanisms to maintain the integrity of its genetic code. Along with the evolution of longer DNA fragments from the original RNA-based shorter protocell genome, DNA repair systems arose early in the history of life. DNA molecules evolved because they are more stable than RNA, in terms of their explicit DNA repair system which was able to identify and repair the simultaneous deamination of Cytosine to Uracil, preventing mispairing of the wrong base [3]. In contrast, Uracil exists as a nature base in RNA and is unable to be repaired. Furthermore, DNA molecules are more stable than RNA due to their ability to produce Thymine instead of Uracil, which is energetically less expensive [3]. Therefore, while RNA is short-lived, with an average lifespan of only 2 minutes, DNA is able to survive more than 6.8 million years.

1.2. Accumulation of DNA Damage

It has been believed that there is a possibility in which not all the DNA damage is repaired, gradually leading to a huge amount of unrepaired chemical lesions. While unrepaired DNA lesions may gradually increase with age, it is highly unlikely that a drastic age-related increase will happen. DNA damage may be in the form of oxidative lesions, such as 8-hydroxyguannine, or other highly toxic lesions which are unlikely to accumulate with age or to cause functional decline, which makes it erroneous to measure spontaneous DNA damage. This is by virtue of the highly proficient DNA repair system that is designed to recognize and remove damage quickly. If the worst were to come, severely damaged cells would undergo apoptosis, eliminating the damaged cells before they would accumulate to cause further adverse effects.

Nonetheless, most cell death occurs in normal developing tissue, and during adulthood and aging, the frequency of apoptotic cells are low. Hence, apoptotic mechanisms may become less efficient with age, at least in some tissue, and under defined stimuli, lead to accumulation of errors and phenotypic fidelity. For example, failure of lymphocytes to exert the apoptotic program can lead to a disorder that characterizes the immune system during aging. Another approach focuses

the attention to the possibility that DNA repair activity declines with age. For instance, reduced activity of various glycosylases and DNA polymerase beta are thought to contribute to the decline in base excision repair [4].

Moreover, lesions measured at a specific time are part of a steady-state situation in which new lesions are continuously induced while others are repaired. Steady-state levels of the most frequent lesions fall in the range of one lesion or fewer per million bases [5], a number that is unlikely to cause major adverse effects.

1.3. Changes in The Epigenome

The epigenome, which consists of chemical compounds that regulate gene expressions, may well be more prone to random changes than its DNA sequence counterpart. Maintenance of the epigenome, similar to that of the DNA sequence, is a challenge. For example, during S-phase of DNA replication, epigenetic marks, such as DNA methylation and histone modification, are either specifically transmitted to the daughter cell or dynamically changed. Furthermore, the epigenome that edicts the chromatin organization is taken down and reassembled [5] amid DNA replication and repair. Although the evidence for age-related DNA conformation change is possibly due to the alteration in histone modification patterns, most of the consideration has been given to DNA methylation [6]. In the course of aging, physiological responses may require systematic changes in DNA methylation. An experiment on Arabidopsis thaliana shave shows that approximately 6% of the 14 million cytosines evaluated underwent a significant change in methylation over 31 generations [7]. Therefore, the epigenome is more accessible to random changes because the epimutation rate appears to be almost 100,000 times higher than its DNA counterparts' due to the high level of epigenome fluctuation [8].

Changes in DNA methylation are observed as an epigenetic mechanism that regulates gene expression by recruiting proteins such as transcriptional regulators that regulate the activation or inhibition of a particular gene [9]. The mechanism of DNA methylation requires transferring a methyl group onto the C5 position of the cytosine forming 5-methylcytosine (5mC). While global genomic DNA methylation level decreases with age, differential methylation of specific genomic loci occurs, especially with hypermethylation of promoter associated CpG island as a function of age [10].

In addition to DNA methylation, histone post-transcriptional modifications (PTMs) determine the accessibility of DNA sequences by modifications such as acetylation, methylation, phosphorylation, and ubiquitylation [11]. Notably, perturbations in distinct histone marks are observed during aging and, in certain instances, may also affect lifespan by regulating gene expression programs associated with longevity assurance mechanisms. It is shown that the level of H3K4 trimethylation increases with advancing age, not only with promoters of highly transcribed genes, but also with sites of newly generated double-strands breaks (DSBs) [12]. Moreover, in aging somatic cells, DNA damage and homology-directed repair induces DNA methylation and chromatin remodelling, which adds an additional source of variation in gene expression.

DNA damage has been implied by not only causing somatic mutations, but also triggering changes in the epigenetic landscape that associate with the DNA damage response (DDR) activation and DNA repair [13][14]. During aging, the cellular level of histones, as well as core histone density, declines considerably, suggesting the perception that during aging or other DNA damage, the loosening of chromatin structure could accelerate the inappropriate access of transcription factors on DNA [15][16]. In addition to DNA damage, it has been frequently found that nucleosome loss and histone eviction also appear in damaged DNA sites and are associated with the degradation of chromatin factors [17][18][19].

1.4. Consequence of Random Genome Alterations

When one is thinking about the possible functional consequences of somatic mutations, they often consider the change in DNA sequences as the target for functional changes, which is a controversial issue. We will explain the reason in the following section/paragraph. In another paragraph, we will introduce the consequence of random genome alterations and how they contribute to cause similar, specific phenotypes of aging.

In 1975, King & Wilson [20] published a paper which revealed that more than 99% of human polypeptide is identical to its chimpanzee counterpart's. If chimpanzees and humans share almost identical genetic code, why didn't they display analogous phenotypes? Based on previous research generated by others [21], King & Wilson concluded that the changes of genes, which are able to control and regulate the gene expression, likely explain the major organismal differences between humans and chimpanzees. In a comparative analysis of the liver transcriptomes of different primates, a set of genes with regulation under natural selection in humans was identified [22]. This not only proves King & Wilson's conclusion about the predominant role of gene regulation in differentiating organisms, but also the fact that gene regulation is to some extent inherited and could be as likely altered by mutagens as DNA sequences are.

More recently, another experiment sequenced the genomes of 21 sticklebacks and located the position that controls the creature's adaptation to different

environments, such as saltwater oceans or freshwater rivers [23]. The researchers then identified 147 regions that varied consistently in marine-freshwater evolution. Hence, while evolutionary differences tended to depend on both protein-coding and regulatory changes in the genome, regulatory changes dominated [23].

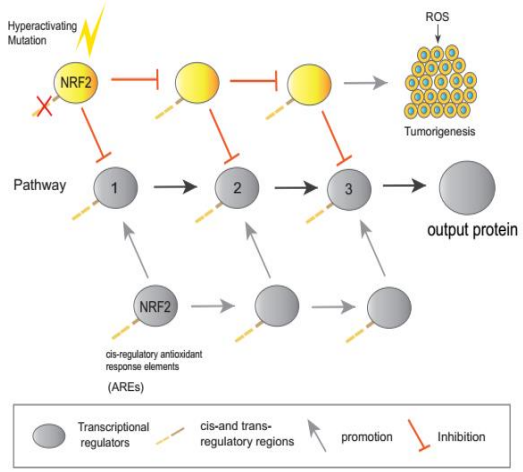

Figure 1 simplified diagram of gene-regulatory networks

Figure 1. Cell function can be affected by any mutations in the functional network that underlie the expression pathway. Genes 1, 2, and 3 and their transcriptional regulators encode the function of the output protein. The expression of each gene affects each other in a downstream cascade and can be affected by other proteins encoded by other genes within the network.

It should be noted that our cell and tissue function is provided through a gene-regulatory network, in which the effect of a random mutation on the gene or sequence can influence the physiological function dominated by any interacting cells and signalling molecules across the gene-regulatory network [24]. In fact, the output of the network can be affected by any mutation in a gene or regulatory sequence involved in this network (Figure 1). Moreover, most physiological functions are the result of cell-cell interactions, which expand the range of possible adverse effects even further. This explains why somatic mutations, both in the nuclear genome and in the mitochondrial genome, can exert adverse effects with extremely low abundance. In case of a random hit, specific functions provided by genetic networks in differentiated cells will likely deteriorate, resulting in very similar aging phenotypes across individuals of the same species and even across related species.

1.5. Genetics of Interspecies Variation in Genome Instability and Longevity

When we look at the huge differences in the phenotype of different species, the influence of genetics in longevity becomes obvious. Whereas a nematode worm can live no longer than approximately 30 days, a human can live to more than 100 years. Hence, differences in interspecies genetic makeup may well act as a critical determinant of life span. The genome maintenance system, then, would be the prime candidate for playing a role in the evolution of species-specific maximum life span, granted that the level of genome instability is affected by genetic factors that specify an organism's potential to reach an old age. Evidence has shown that long-lived species have superior genome maintenance systems compared with shorter-lived species [25][26][27][28]. This life span difference is by virtue of the more rapid rate of DNA sequence evolution in short-lived rodents compared with the corresponding evolution rate in longer-lived primates. For example, a mouse cell in culture has a much higher probability of undergoing karyotypic changes and of becoming neoplastically transformed than does a human cell, which also points towards a superior genome maintenance system in the latter.

However, having superior genome maintenance does not mean that damaged cells will necessarily survive after a chemical lesion, or that the cells from less advanced genome maintenance systems will compulsorily die. In fact, cultured mouse fibroblasts can survive UV radiation as well as their human counterparts do [29]. The damaged cells that are beyond the maintenance of the genome maintenance system in mouse fibroblasts could survive and accumulate successively to form tumours. Therefore, a genome maintenance-longevity relationship is more likely to be based on repair accuracy than on cellular survival. In the aspect of telomere biology, humans tend to live longer than mice due to the lack of telomerase in charge of proliferating cells. Whereas mouse cells contain very long telomeres and abundant telomerase activity that is unrepressed, leading to both earlier and higher frequency of tumour cells. Therefore, humans have a longer life span and experience a long-lasting process of aging, due to the relatively advanced genome maintenance system and the absence of telomerase activity that slows the accumulation of damaged tissue throughout a lifetime.

Finally, differences in genome robustness may also contribute to species-specific differences in genome instability, i.e., differences in genome structure that make major phenotypic consequences more or less likely. Rather than differences in genome maintenance systems, long-lived animals may contain a more robust network that is tolerant against environmental perturbations, eluding the adverse effects of increased genome alterations, therefore benefiting its evolvability.

1.6. Genetics of Intraspecies Variation in Genome Instability and Longevity

In this last section, we will give a response and evaluate the third assumption: predominant polymorphic variants at loci of genome maintenance genes associated with human longevity. Although unsuitable as an experimental model, humans have proven to be an invaluable model of aging through the identification of natural mutants that prematurely show multiple symptoms of aging. In fact, human patients were the first models for progeroid syndromes, characterized by the premature appearance of multiple signs of normal aging and reported well before the discovery of DNA [30].

The hypothesis that genome maintenance is a major longevity assurance system in humans can be demonstrated by two different progeroid syndromes, which are remarkably both caused by a defect in the genome maintenance system. Werner's Syndrome (WS) most strongly resembles normal aging [31] and should be considered the best example. WS is caused by a dysfunction in the WRN gene, a member of the RecQ helicase family [32]. Human patients with WS show symptoms of aging, including atrophic skin, greying and loss of hair, osteoporosis, malignant neoplasms, diabetes, and shortened life span [31]. Furthermore, these patients have a greatly increased frequency of genomic rearrangements in peripheral blood lymphocytes. Another segmental progeria, Cockayne Syndrome (CS), is caused by defects in transcription and DNA repair via the nucleotide excision repair (NER) pathway. CS is an autosomal recessive disorder characterized by progressive postnatal growth failure, neurological dysfunction, and a short life span of approximately 12 years. Furthermore, premature appearance of aging symptoms resulting from inactivation or mutational alteration of genome maintenance genes in mice demonstrates that genome maintenance pathways function as major longevity assurance systems.

Based on these 2 pieces of evidence, it has been discovered that longevity has a genetic component, with an estimated heritability of average life expectancy of approximately 25% [33][34]. Hence, human segmental progeroid syndromes depend, almost without exception, on heritable defects in genome maintenance.

In conclusion, the identification of genetic factors that improve genome maintenance functions and promote longevity is likely to help us find novel strategies for the prevention and treatment of age-related disease, although currently we lack both the genetic engineering tools and critical information as to the type of changes required for improving genome maintenance function. Manipulation of loci that are involved in the coordinated regulation of critical components of genome maintenance pathways might provide an opportunity to improve genome maintenance, thereby preventing or retarding the accumulation of somatic genome alterations.

2. MITOCHONDRIAL DNA MUTATIONS

As mentioned in the above section, aging is believed to be a degenerative process caused by accumulated genome instability and damage that leads to cellular dysfunction, tissue failure, and death. Besides the nucleus genome, the mutation and damage in the mitochondrial genome also plays a role in the aging process. The majority of attention has been focused on MFRTA [35]. The theory proposes that ROS, which is considered to be an unwanted toxic by-product of aerobic metabolism, causes oxidative damage to various cellular components because of its high chemical reactivity. It is this accumulation of oxidative damage that ultimately leads to the accelerated process of aging. The mitochondria function to produce ATP through the process of oxidative phosphorylation, conducted by the four respiratory chain (RC) complexes (complexes I–IV) and the ATP synthase that is located in the inner mitochondrial membrane (Figure 2). The respiratory chain (RC) is a main source of superoxide, an abundant ROS generated in the cell. With the catalysis of SOD, the superoxide anion can be converted to hydrogen peroxide. Hydrogen peroxide itself is not a free radical; however, through the Fenton reaction it can be converted to the highly reactive hydroxyl radical (Figure 3). The hydroxyl radicals are considered to be the most damaging form of ROS, capable of causing oxidative damage to almost every molecule type in the cell, including lipids, proteins, and nucleic acids.

In this section we will discuss the role of mitochondria in aging, with specific emphasis on: (a) how mtDNA mutations could drive the aging process; (b) the role of mitochondrial ROS in aging; and (c) the relationship between mitochondrial function and longevity regulation signalling pathways.

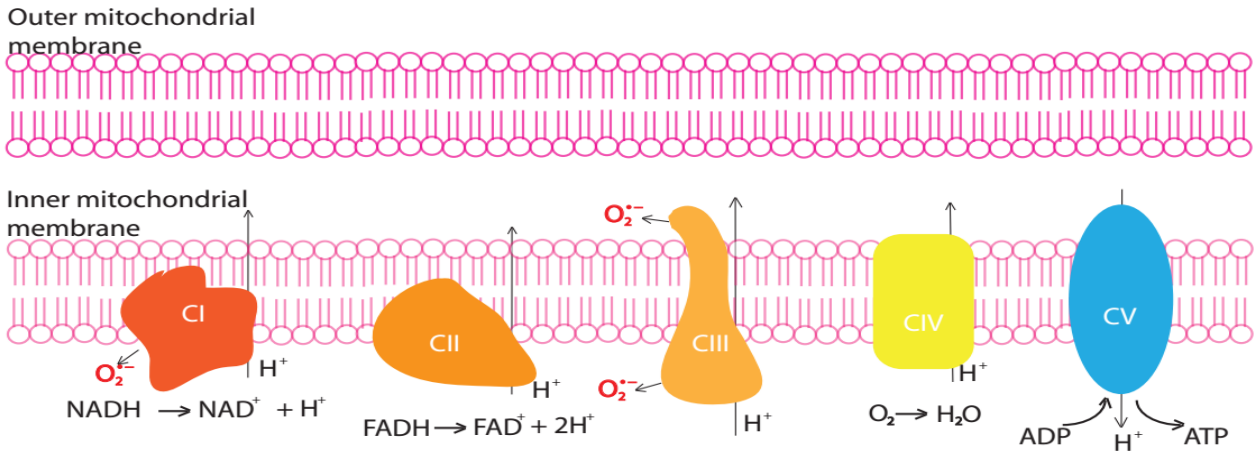

Figure 2 The electron transport chain

Figure 2. Schematic of ATP generation. ATP is generated by oxidative phosphorylation through the four RC complexes (CI–CIV) and ATP synthase (CV) located in the inner mitochondrial membrane. Protons (H+) are pumped via CI, CIII, and CIV using the energy released by the electron transfer from NADH and FADH2 to O2. This proton gradient across the inner mitochondrial membrane is the driving force of ATP synthesis.

Mitochondrial function has long been recognized to decline with aging, concurrent with the morphological alterations of it, e.g., abnormally rounded mitochondria in aged mammals [36]. The number of mitochondria, as well as the mtDNA copy number and mitochondrial protein levels, decrease with age in liver cells of mice, rats, and humans [37][38][39][40]. Additionally, compared with the young generation, rat liver mitochondria in old animals' cells has a 40% reduction of RC capacity [41]. In conjunction with general decline of mitochondrial mass and overall function, the activity of specific RC complexes and certain nuclear-encoded mitochondrial proteins also declines as mammals age [42][43][44][45]. While aging has a correlation with a decline in mitochondrial function, this observation alone does not imply causality because age-associated changes in mitochondria function might be secondary to other mechanisms. e.g., in response to hormones and physical activities [46]. Thyroid hormones, estrogens, and glucocorticoids play important roles in cell growth and differentiation. Furthermore, they are also critical regulators of mitochondrial biogenesis [47][48][49][50]. Therefore, it is assumed that age-related decline in RC function is at least partly caused by other age-related changes, such as decline in hormonal levels or peripheral insulin resistance, and an improved mitochondria function is related with the increase in caloric restriction and physical activities [51][52].

While mitochondrial function has shown to decline with age, a similar trend is suspected with mtDNA mutation. For example, in aged human central nervous systems, skeletal muscle, hepatocytes, and mtDNA deletion is observed [53][54]. In addition, in aging colonic crypts mtDNA point mutation accumulation has also been found [55]. While one might assume the cause of mtDNA mutations observed during aging is associated with accumulated, unrepaired damage, it is in fact replication errors that seems to be the more important reason [56][57][58]. This is by virtue of the unique ability of mitochondria to replicate independently of the cell cycle [59], as they contain their own genetic information that is capable of encoding 13 proteins, 22 transfer RNAs, and 2 ribosomal RNAs in mammals. Moreover, mtDNA in one cell may have more than one genotype, which is defined as Heteroplasmy. In this case, the level of mutated mtDNA relative to the wild type mtDNA can determine whether or not RC dysfunction occurs. Generally, the threshold level of mtDNA mutation that impairs RC function is high but may depend on the type of mutation. In the case of large single deletion of mtDNA, RC dysfunction only occurs if the level of mutated mtDNA exceeds approximately 60%12. Whereas certain pathogenic mitochondrial tRNA mutations only cause dysfunction if present at levels above 95% [60][61].

The absence of a fair correlation between the ability of antioxidant defences and longevity casts doubts on the causal role of oxidative damage played in the aging process. If as stated in MFRTA, life span is determined by the level of free radical damage, and the cell's response to damage by secreting a variety of antioxidant defences that transfer ROS into less harmful by-products, then the lowering of ROS concentration by increasing cellular antioxidant production which in return should slow the process of aging and prolong life span. However, while some reports tend to show that dietary supplementation with antioxidants may improve cellular function [60][61], numerous human intervention studies have shown no beneficial effect with antioxidant nutrients such as Vitamin E, beta carotene, or Vitamin A.

in contrast, they may even increase the mortality rate [62]. Furthermore, instead of extending life span, the overexpression of mitochondrial antioxidants has been associated with shortening of the life span. In a comparison study of naked-mole rats, the longest-living rodent species with maximum life span of 25-30, and mice whose maximum life span is 3-4 years, it is found that both rodents have similar mitochondrial ROS production level despite a huge difference in life span. Thus, this study suggests that there is no clear correlation between ROS production and species-specific lifespan, and that ROS are not simply unwanted by-products of metabolism, but can also act as important signalling molecules to promote longevity.

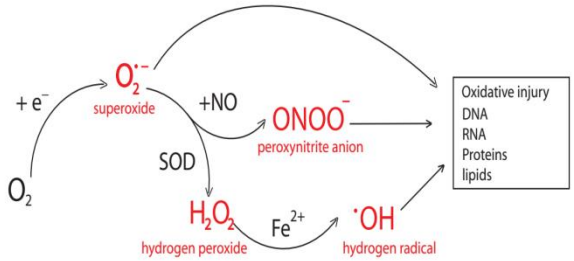

Figure 3 Superoxides causing oxidative injuries

Figure 3. Superoxide is an abundant ROS in the cell, which is a side product of electrons transferring in the respiration chain. Superoxide anion is converted to hydrogen peroxide by manganese SOD. Even though hydrogen peroxide is not substantially harmful for the cell, it can be converted to the highly reactive hydroxyl radical (OH•) in the presence of transition metals via the Fenton reaction.

Similarly, a number of recent studies proposed that ROS is involved in different cell activities, such as cell cycle progression, cell signalling, apoptosis [50][63][64] It has been found that the exercise-induced increase in ROS production and oxidative stress in human skeletal muscle ameliorate insulin resistance and improve glucose metabolism [65]. Hence, it is important to maintain a physiological level of ROS, an increase in ROS production above a certain level has a detrimental effect on cellular physiology. In conclusion, the subtle increase in ROS production with age may not explain the rather severe physiological alterations occurring during aging, and the absence of correlation between oxidative stress and longevity suggest that oxidative damage does not play such an important role in age-related disease. The fact that mtDNA mutator mice express a progeroid phenotype with embryonic-onset dysfunction of somatic stem cells suggests a more predominant role for somatic stem cells in aging than the expected ROS. Importantly, an equilibrium between antioxidant and level of ROS should be established to prevent cellular functional impairment.

Signalling pathways have been reported to regulate longevity by promoting secondary changes in mitochondrial energy production or mitochondrial biogenesis. It is believed that aging-associated phenotypes have been linked not only to mitochondrial dysfunction, but also to aberrant mitochondrial biogenesis caused by impaired retrograde signalling regulated by nuclear genes and factors dependent on mitochondrial metabolism [66]. The role of nutrient-sensing pathways and dietary restrictions is becoming more crucial in terms of mediating longevity by mitochondrial metabolism [67][68][69][70]. It has been shown that impaired Insulin/IGF-1 signaling IIS and inhibition of TOR activity extend the life span in worms, flies, and mammals, and reduced nutrients availability (CR) extends the life span in species ranging from yeast to mammals [71][72][73][74]. The effects of CR on longevity are very complex and include many organs and different pathways; for instance, CR decreases the incidence of cardiovascular diseases in animals through a reduction of metabolic rate and oxidative damage, which consequently inhibits signalling pathways regulated by mitochondria-derived ROS, the exact mechanism remain unknown [75].

3. IS TELOMERE A BIOMARKER OF AGING?

According to the American Federation of Aging Research, a well-defined biomarker of aging criteria involves four aspects as follows [76]:

1. It must predict the rate of aging. In other words, it should tell exactly where a person is in their total life span. It must be a better predictor of life span than chronological age.

2. It must monitor a basic process that underlies the aging process, not the effects of disease.

3. It must be able to be tested repeatedly without harming the person, like, a blood test or an imaging test.

4. It must be something that works in humans and in laboratory animals, such as mice. This criteria is so that aging can be tested in lab animals before being validated in humans.

To verify whether the telomere is a biomarker of aging, several research groups did different experiments to support the idea that there is an inverse relationship between telomere length and age-sensitive observation. However, the results are equivocal. For example, in Cawthon's study [77], a threefold higher mortality rate from heart disease and more than eightfold higher mortality rate from infectious disease was observed among a study of 143 individuals aged 60 years or older with the shortest telomeres. However, Bischoff and colleagues [77] found no correlation between telomere length and survival in a cohort of Danish twins and

singletons (mean age= 81), with chronological age being a better predictor of survival. Furthermore, longitudinal studies of telomere length change were performed by Martin-Ruiz and colleagues [78], which showed that there was no obvious association between mortality and rates of change nor any difference in mortality between individuals who showed a decrease versus an increase in telomere length.

Critically, shortened telomeres are one of the triggers of cellular replicative senescence [79][80][81][82][83], which may contribute to organismal aging. There are also several inherited diseases of premature aging (e.g., dyskeratosis congenita) [84] that were found to have mutation genes that are responsible for telomere maintenance which caused premature aging. Hence, telomere length can predict the disease that underlies the effect of aging. To be more specific, telomere length would show significant shortening in diseases of premature aging, but this shortening did not reflect the basic process of aging. Instead, organs that show functional decline during normal aging might be a better biomarker of aging, such as lung function declines with age and predicts cognitive performances and mortality [85][86][87]. Moreover, Yaffe and colleagues' study [88] also indicated that markers of biological aging may change over the life span and that a single biomarker may not be sufficient to reflect aging across a variety of biological systems. Therefore, different panels of biomarkers may be necessary at different ages to assess biological age [89]. This study raises the possibility that telomere length may be related to different aging-related measures at different ages. To conclude, telomere length is not a universal biomarker of aging and hence does not reflect the general underlying aging process.

The most prevalent way of collecting samples of telomere length estimation is from peripheral blood. Studies have shown that the cell from peripheral blood is a surrogate of somatic cells and can represent telomere length in other tissues where aging-related changes may be occurring [90]. Nonetheless, assessing the relationship between leukocyte telomere length and tissues, where there is little cell turnover, such as in a neuron, will be more problematic. Hence, using samples under relevant investigation domain, such as lung tissues for respiratory phenotypes, may be more applicable than using telomere length estimated in leukocytes.

Animal models have been employed to investigate the role of telomere in aging. Mice are widely used models for both aging and senescence because of their similarity to humans. However, there are fundamental differences in telomere biology in humans and mice [91]. Even so, mouse telomere length in a number of tissues have shown significant shortening with increasing chronological age. In addition, mice that overexpress telomerase have an extended life span [92]. These findings suggest that telomeres do play an indispensable role in aging and aging-related conditions and diseases. Hence, while using mice as a sample might not be a good inference to the relationship between telomeres and aging, nonhuman primates have been proposed as an alternate animal method since they are genetically more closely related to humans than to other mammals [93].

In conclusion, whether telomere length is an eligible biomarker of aging remained equivocal. The telomere length, to some extent, does not conform to the first and second criteria. It is still unclear whether telomere length is an indicator of normal aging processes or is a marker of prodromal aging related disease, such as cardiovascular disease. Additionally, mortality studies are few, and outcomes may be biased in older cohorts because of survivor effects. Furthermore, single measurements of a particular parameter may not indicate the burden of its accumulated effects over the life span. For example, a sole measure of inflammation is unlikely to provide an indication of the cumulative burden of inflammation over the lifetime. Hence, in the future, stronger findings may emerge from studies that have measured baseline telomere length earlier in life, such as middle age. Longitudinal studies on intra-individual rates of change may provide more precise investigation of telomere length dynamics, whereas cross-sectional studies may be easily influenced by inter-individual variability caused by inherited and environmental differences. It should also be made clear which telomere length measurement is the most informative and useful marker.

4. CONCLUSION AND DISCUSSION

It is generally acknowledged that genome instability caused by the accumulation of DNA damage, mutation, and epimutation plays a significant role in the aging process. In addition, a number of studies suggested that there is a link between ROS-induced mitochondrial dysfunction and aging. However, the real mechanism of ROS in the aging process and the question of whether there is a biomarker that predicts the life span better than telomeres remain unknown. Thus, an important future discussion should be focused on the following aspects.

4.1. The Role of ROS in Mitochondrial Aging

While a significant number of studies has advanced our understanding of the role of mitochondria in disease and aging, further experiments are still needed to investigate the extent of RC dysfunction and embryonic-onset dysfunction of somatic stem cells' role in premature aging. As age-associated changes in mitochondrial function might be secondary to other mechanisms, e.g., in response to hormones and physical activities, more attention should be focused on the significance of caloric restriction and physical activity in affecting longevity. It is shown that CR extends life span

in species ranging from yeast to mammals by decreasing the incidence of cardiovascular diseases and participating in various signalling pathways that aim to increase mitochondrial biogenesis and respiration. However, the fact that CR is able to inhibit signalling regulated by mitochondria-derived ROS poses a problem. Although ROS are well known for its tendency to cause oxidative damage to virtually every molecule type in the cell, previous studies have shown that ROS might be a beneficial substance in extending one's life span to regulate cell cycle progression, cell signalling, and apoptosis. Surprisingly, all these events take place during development. Therefore, the beneficial effect of CR might depend on an individual's progression of life. While taking CR during adulthood would slow down the process of aging and increase the life span, individuals who take CR regularly during development may be at risk of expressing symptoms associated with growth and hypoplasia. Similarly, it is difficult to predict the side effects of CR being taken by pregnant women.

In addition, maintaining regular physical activity has shown to decrease the risk of cardiovascular mortality, prevent the development of some cancers, lower the risk of osteoporosis, and increase the endurance of cells and tissues to oxidative stress, vascularization, and energy metabolism. A reported reduction of mortality of 31% to 35% in people who participate in regular leisure-time or daily physical exercise is seen in contrast with inactive persons. Furthermore, with consideration of confounding factors that could affect mortality, a 0.43 to 4.21 years higher life expectancy in physically active compared to inactive persons are predicted by high-quality studies. However, when calculating the net gain in life expectancy involving confounding factors, positive influence of physical activity on major risk factors for mortality should be considered. Thus, the actual gain in life expectancy should be greater in terms of favourable effect on risk factors such as arterial hypertension, coronary heart disease, and stroke. Importantly, the amount of exercise may differ between individuals, as the initial health may differ by a great range.

Furthermore, future investigation on the correlation between antioxidants and life span needs to be conducted and should focus on increasing the upper threshold of equilibrium between oxidants and antioxidants by experimenting with physical activities and CR. Hence, the mechanisms for dealing with changes in ROS levels could be strengthened, increasing the body's ability to respond to higher levels of ROS without causing fundamental oxidative damage, thereby expanding one's life span.

4.2. Predict Life Span in a Better Way

While there is clear evidence that telomeres are involved in cellular aging, since they are identified as one of the triggers of cellular replicative senescence, many authors have suggested specific functional decline, e.g., lung function declines as potential biomarker of aging. Therefore, future experiments should investigate telomere length in tissues of organs which exhibit functional decline during normal aging in longitudinal study. It should be noted that prediction of where a person is in their total life span should be considered as the combination of total mortality rate, in terms of certain biological systems that are not necessarily involved in normal aging, and telomere length in organs involved in the basic aging process. Methods of using telomere length and general health to predict one's life span could be designed in the future: first, taking a sample from individuals during their early age and analysing their average telomere length of organsand then re-checking every 5 years to speculate on to what extent the individual is aging. Nevertheless, this method would require advanced science and technology to resolve the length of specific telomeres and defining the speed of aging might also be critical. Furthermore, other confounding factors, e.g., intake of drugs, smoking or excessive exposure to UV radiation might lead to dysfunction of organs or tissues that is not involved in normal aging, and thus leading to a higher mortality rate than expected by the skim.

While collecting samples of telomere length under a relevant domain, such as lung tissues, remains applicable, similar methods involving collection of telomeres from peripheral blood are more prevalent. However, studies have shown that the peripheral blood is composed of a heterogenous mixture of cell types of all ages, and the proportion of cell types within a sample may change with age, and with other processes such as infection. Hence, it is believed that leukocyte telomere length may be more relevant to the biological systems in which leukocytes are associated, such as the immune and cardiovascular systems.

To our knowledge, telomerase is the enzyme responsible for maintaining the length of telomeres by adding guanine-rich repetitive sequences. In human somatic cells proliferation potential is strictly limited to 60-70 cell divisions, followed by cellular senescence, contrary to most tumour cells, which have unlimited replication potential. While the key role in this process of the system of the telomere length maintenance with involvement if telomerase is not yet understood, it is proposed that the decreasing level of telomerase activity during aging might be partly due to alteration in epigenome. The telomerase enzyme is composed of 2 major components that work together. The component produced from the TERT gene is known as hTERT, and the other component is produced from a gene called TERC, known as hTR. In addition, telomerase associated proteins or shelterin complex, is crucial for both the maintenance of telomere structure and its signalling function. The reduced level of shelterin complex activity and down-regulation of TERT and TERC genes results in

a decreased expression of telomerase and telomere shortening with advancing age. During aging, the loss of barrier genes and increased gene transversion could facilitate the spreading of heterochromatin, leading to silencing of adjacent TERT and TERC genes. Furthermore, increased DNA methylation of promoter regions of TERT and TERC genes and histone modifications could result in histone compaction reduces the accessibility of DNA sequence and thus affect transcription of telomerase-associated genes. Moreover, telomerase-associated genes could be lost by DSB during cell division, with histone loss accompanied by release of fragmented chromosomes into the cytoplasm when the nuclear envelope breaks. Interestingly, as the immune system's ability to detect and correct cell defects also declines during aging, proinflammatory signals caused by changes in histone PTM are not effectively detected, leading to accumulation of senescence cells, fever, inflammation, and tissue destruction.

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