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MtDNA mutations, functional decline and turnover of mitochondria in aging

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1. ABSTRACT

Aging is a complex biological process that involves gradual function deterioration in various tissues and organs of an individual. Mitochondrial function decline can lead to cellular overproduction of reactive oxygen species (ROS) and increase in oxidative damage to biological molecules in the aging process. We have hypothesized that increased production of ROS by the mitochondria in affected tissues in patients with mitochondrial diseases and elderly subject results in increased oxidative stress and oxidative damage. Due to the similarity of human aging process to diseases related to bioenergetic function decline and mitochondrial DNA (mtDNA) alterations, aging is sometimes viewed as a "chronic" version of such diseases. Recent studies have also established that the expression profiles of several clusters of genes are altered, oxidative modification of proteins are increased and their turnover are decreased in tissues of old human subjects and animals. Accumulating evidence has suggested that mtDNA mutations, oxidative stress, defective disposal of dysfunctional proteins and a slower turnover of mitochondria are associated with aging.

2. INTRODUCTION

Mitochondria are referred to as the "power-house" of eukaryotic cells because they produce most of the adenosine 5'-triphosphate (ATP) that is required by the cells to execute various physiological functions. These double- membrane-bearing organelles harbor enzymes and coenzymes that are involved in major metabolic pathways such as citric acid cycle, β-oxidation of fatty acids, respiratory chain and oxidative phosphorylation (OXPHOS) (Figure 1).

Each human mitochondrion contains 2-10 copies of a circular double-stranded genome, mitochondrial DNA (mtDNA), which encodes 13 protein subunits of electron transport chain (ETC) (Figure 2) (1). Human mtDNA is maternally inherited and has a very high mutation rate. The 16569 bp mtDNA also encodes 2 ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs), which are essential for the translation of the RNA transcripts of the 13 genes encoded by mtDNA. More than two thousand mitochondrial proteins (including nearly 80

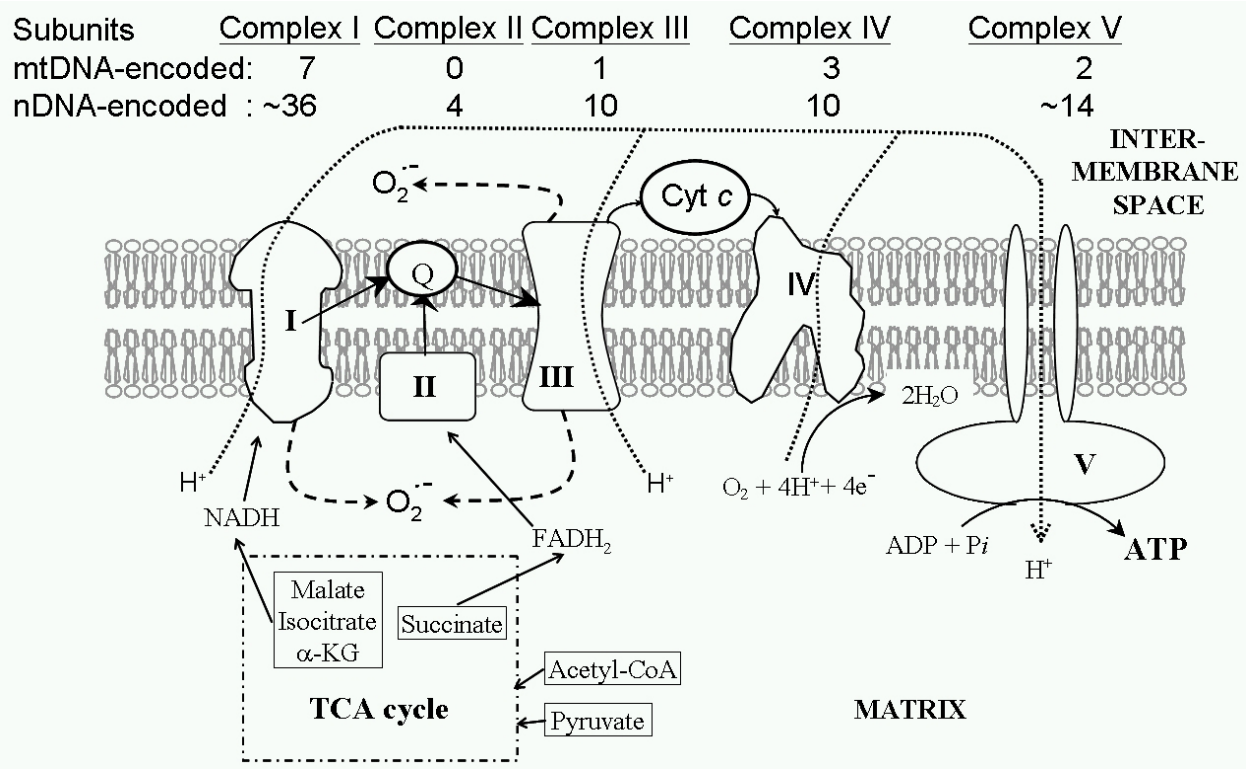

Figure 1. The energy production machinery in mitochondria. The electron transport chain (Complexes I to IV) and the ATP synthase (Complex V) are all embedded in mitochondrial inner membrane. Electrons (e^-) are transferred from Complexes I or II to Complex III and Complex IV. Cytochrome *c* (Cyt *c*) and coenzyme Q (Q) are electron carriers. Protons (H^+) are pumped from the matrix to the inter-membrane space through Complexes I, III, and IV, creating an electrochemical potential of the H^+ gradient. The potential enables Complex V to drive ATP synthase to synthesize ATP from ADP and inorganic phosphate (P*i*). Two water molecules will be produced following the reduction of an oxygen molecule (O_2).

polypeptides of the ETC) are encoded by nuclear DNA and imported into the mitochondria in a post-translational manner (Figure 1) (1).

Under normal physiological conditions, mitochondria utilize ~90% of the oxygen consumed by mammalian cells for respiration. The potential danger of this oxidative process are the leakage of electrons out of respiratory enzyme complexes and subsequent reaction with oxygen, thereby producing reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\cdot}OH$). The two predominant sites of ROS production in the ETC have been located at Complexes I and III (2,3). The naked nature of mtDNA and its close proximity to the primary site of ROS production in the inner membrane of mitochondria may increase its susceptibility to oxidative damage. The content of one of the most abundant oxidized nucleosides, 8-hydroxy-2'-deoxyguanosine (8-OHdG), in mtDNA of human diaphragm or heart muscle was found to increase with age (4,5). Another study using a gene-specific DNA damage assay based on quantitative PCR revealed that damaged nucleotides could block the progression of DNA polymerase, and thereby resulted in significant decrease of amplification of the target sequence in mtDNA (6).

On the other hand, due to the multi-copy nature of mtDNA, damages on mtDNA are often preserved in living cells. The un-repaired damaged mtDNA may lead to the accumulation of mtDNA molecules with deletion, fragmentation, duplication, and point mutation, especially in the postmitotic tissues such as the brain, muscle, heart, liver, endocrine glands, kidney, and lung of the human (1,7).

The concept that mutated mtDNA can lead to diseases, and perhaps aging, was substantiated about 2 decades ago. In 1988, Holt *et al.* (8) found high levels of large-scale mtDNA deletions in patients with mitochondrial myopathies. At about the same time Wallace *et al.* (9) discovered a G→A mutation at the nucleotide position (np) 11778 of mtDNA in patients with maternally inherited Leber's hereditary optic neuropathy (LHON), respectively. To date, more than one hundred point mutations, deletions, and rearrangements of mtDNA have been documented to be associated with human diseases (Table 1) (10). The point mutations can be subdivided into mutations that affect the protein-encoding genes and those affecting the protein translation machinery (i.e., rRNA or tRNA genes) in mitochondria. The latter type of mtDNA mutations can cause global effects on mitochondrial protein synthesis.

Table 1. Biomarkers for oxidative damage and signaling molecule in aging

Genes/ genome	Common Name	Function (s)	Age-related Alteration (s)	References
Mitochondrial DNA	mtDNA	Genome of mitochondria; encoding 37 genes of electron transport chain and mitochondrial translational machinery.	Deletions, point mutations, duplications, base modifications, and depletion.	1, 4, 5, 10, 24, 28, 48
ACO2	Aconitase 2, in mitochondrial matrix	Interconversion of citrate to isocitrate via cis-aconitate in the second step of the TCA cycle.	RNA interference to knockdown ACO2 expression led to lifespan extension in roundworm. Oxidatively damage during aging in fruit flies. Preferentially degraded by the mitochondrial serine protease 15 after oxidative modification.	49, 58, 59, 72
CYP2E1	Cytochrome P450, family 2, subfamily E, polypeptide 1	Metabolism of xenobiotics.	Functional loss of CYP2E1 occurred without significant changes in the content of protein carbonyls in aging rats.	93
TP53	p53	TP53 is a tumor suppressor involved in cell cycle regulation, apoptosis, and DNA repair.	In flies, expression of a dominant-negative version of TP53 extended lifespan. Mice with activated TP53 displayed signs of premature aging.	54

For information of other genes not mentioned in this article, please refer to http://genomics.senescence.info/index.html

On the other hand, mutations in nuclear genes can also affect the stability of mtDNA and impair mitochondrial function and result in inherited diseases, which may manifest phenotypes similar to those caused by mtDNA mutations. Indeed, an increasing number of nuclear gene mutations have been identified to be responsible for or involved in the pathogenesis of some mitochondrial diseases following the Mendelian inheritance (11). Among them, human DNA polymerase gamma (POLG) has attracted most attention, since nearly a hundred mutations of *POLG* gene (15q24) have been identified (Table 2) (12). POLG is responsible for the replication of mtDNA. van Goethem *et al. (*13) first identified a missense *POLG* mutation that might lead to the occurrence of multiple mtDNA deletions in the members of an autosomal dominant progressive external ophthalmoplegia (PEO) family. Naviaux and Nguyen (14) further reported 2 *POLG* mutations in two unrelated pedigrees with autosomal recessive Alpers' syndrome. The children afflicted with the disease died at an early age and depletion of mtDNA was observed in affected tissues. Other nuclear genes that are involved in the maintenance of the stability of mtDNA include the genes encoding adenine nucleotide translocator (ANT) isoform 1 (15), thymidine phosphorylase (16), deoxyguanosine kinase (17), and thymidine kinase (18). Mutations in these nuclear genes have been reported to affect the stability or metabolism of mtDNA, causing rearrangements or depletion of mtDNA, and impair bioenergetic function of mitochondria, and result in distinct types of mitochondrial diseases (Table 2) (10,11).

3. MITOCHONDRIAL DNA MUTATIONS, RESPIRATORY FUNCTION DECLINE AND AGING

3.1. Evidence of mitochondrial DNA alterations in human aging process

It has been shown for more than a decade that the activities of mitochondrial respiratory enzymes and oxidative phosphorylation efficiency are decreased with age in human tissues (19-21). It is accompanied by an age-related increase in heart and skeletal muscle fibers with focal deficiency of cytochrome *c* oxidase (CCO) (22,23). Further analysis revealed that the activities of Complexes I and IV are most often and severely affected. We thus speculate that aging is related to mutations of mtDNA because it encodes seven and three protein subunits of these two respiratory enzyme complexes, respectively. This has been proved to be the case by extensive research in the past decade (1,5,7).

A recent study on 146 healthy individuals of ages between 18 and 89 also demonstrated that mitochondrial ATP production, mtDNA and mRNA abundance are all declined with advancing age (24). Moreover, the level of 8-OHdG in tissue cells was also increased with age. In addition, two research groups (25,26) simultaneously reported high levels of deleted mtDNA in COX-deficient substantia nigra neurons from normal elderly subjects and/or patients with Parkinson's disease. These mtDNA deletions are somatic, clonally expanded, and may account for half of the mtDNA population in individual neurons. However, the proportions of the COX-deficient substantia nigra neurons in patients with Parkinson's disease were significantly higher than those of the age-matched control subjects, which imply that mtDNA deletions play a role in the selective neuronal loss observed in patients with Parkinson's disease (25).

In fact, accumulation of various large-scale deletions in human aged tissues has been well documented (10). In early 1990s, we have reported an increase in the incidence of small tandem duplications of mtDNA in human muscle tissues with age (27,28). Münscher *et al.* (29) further documented the occurrence of A8344G in healthy people of different ages. On the other hand, Zhang *et al.* (30) reported the occurrence of A3243G in mtDNA of tissues of old individuals. Interestingly, these mtDNA mutations are originally found in the tissues from patients suffering from mitochondrial diseases. Among them, the 4977 bp and 7436 bp deletions of mtDNAs which were first found in patients with Kearns-Sayre syndrome (KSS) and chronic progressive external ophthalmoplegia syndrome (CPEO), have been recognized as the "common" deletions and as indicators of aging-associated mtDNA mutations (Figure 2). The above-mentioned findings have led to the hypothesis that accumulation of mtDNA mutations in somatic tissues is an important contributory factor to the age-related decline of respiratory function of mitochondria (Figure 3) (31-33). Indeed, we and other investigators have demonstrated that the cultured human cells harboring large-

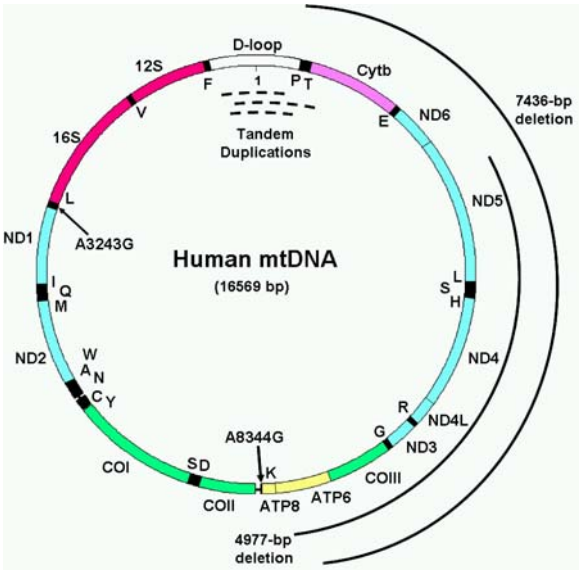

Figure 2. Aged-related mtDNA mutations and rearrangements found in aged human tissues. The A3243G and A8344G point mutations were originally detected in family members suffered from maternally inherited mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) and myoclonic epilepsy and ragged-red fibers (MERRF) syndromes, respectively. Short tandem duplications within the D-loop region and the accumulation of various large-scale deletions, especially the 4,977-bp and 7,436-bp deletions, in aged human tissues have also been well documented. These mtDNA rearrangements are most often found in the affected tissues of patients with KSS or CPEO. Two 13-bp direct repeats occur at the breaking points of the 4,977-bp deletion. Direct repeats of 6- to13-bp are frequently found at the regions flanking the duplicated/deleted sequences in mtDNA.

scale deletion of mtDNA had significantly lower respiratory chain function and higher oxidative stress probably due to enhanced production of ROS (34-36).

All these findings have led to the formulation of the so-called "mitochondrial theory of aging" (Figure 3), which contends that during mitochondrial respiration, ROS and organic free radical by-products resulted from incomplete reduction of oxygen may cause oxidative damage and mutation to the nearby mtDNA molecules. Once the mtDNA molecules with oxidative modification or pathogenic mutation are transcribed and translated, defective protein subunits may be produced and assembled to form defective respiratory chain. Defective mitochondria not only produce ATP less efficiently but also generate more ROS, which will further enhance oxidative stress and culminate in oxidative damage to various biomolecules, particularly mtDNA, in the mitochondria, and to other biomolecules in the cytosol, although with lower incidence and severity. This "vicious cycle" operates differentially in different tissues in an age-dependent manner, and results in the widely observed age-related accumulation of various oxidative damages and mutation of mtDNA, which ultimately leads to a progressive decline in the bioenergetic function of tissue cells in the aging process.

On the other hand, we found that the enzyme activities and expression levels of the free radical scavenging systems (e.g., superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) are significantly altered and result in the accumulation of hydrogen peroxide in the aging tissues and cultured cells (35). This will cause an imbalance in the amount and activity between these antioxidant enzymes, which in turn causes oxidative stress and oxidative damage to tissue cells. Moreover, DNA repair systems and mitochondrial turnover for removal of the oxidative damages by ROS and free radicals also become less efficient in the aging tissues (32-34). As a result, the mtDNA molecules with oxidative modification or mutation and defective mitochondria are accumulated and lead to the general decline of bioenergetic function in various human tissues in the aging process. The age-related decline in respiratory function may also account for the observation that many mitochondrial diseases have a delayed onset and age-related progression of the clinical phenotypes. As the somatic mutations of mtDNA accumulate, they could exacerbate mtDNA mutation-elicited respiratory chain defects until the combined defects reach a certain threshold, resulting in bioenergetic failure of the affected tissues (33,34).

Moreover, environmental insults, such as UV irradiation (37), smoking (38), betel quid chewing (39), and alcohol drinking (40) can also act as add-on factors to aggravate the mutation of mtDNA in various human tissues during aging. Thus, the combination of inherited OXPHOS defects together with the accumulation of age-related mtDNA mutations in somatic tissues provides an attractive paradigm for integrating the aging process with the delayed onset and progression of mitochondrial disorders and degenerative diseases.

Although the above-mentioned studies all show a positive correlation between aging and respiratory function decline, accumulation of mtDNA with mutation and oxidative damage, it is hard to conclude that these changes are causal in human aging process. Many investigators raised the question that the levels of mtDNA mutations accumulated in aging human tissues are too low to affect respiratory function. It is worth noting that studies using cell culture system and cybrids approach (36,41,42) have shown that no defect in mitochondrial respiration can be found until the specific mtDNA mutation reach a high level. However, it should be pointed out that the low levels of mtDNA mutations found in bulk tissues may be a result of the cellular heterogeneity within the tissues. Individual cells within the same tissue may carry very different proportions of mutant mtDNA, which may lead to mitochondrial dysfunction (25,26). Although it has been repeatedly shown that the overall proportion of mutant mtDNAs is low, we argue that the observed mutations may be just the tip of iceberg of the aging-associated alterations of mtDNA (34). In fact, most investigators examined the whole tissue to screen for mtDNA mutations rather than

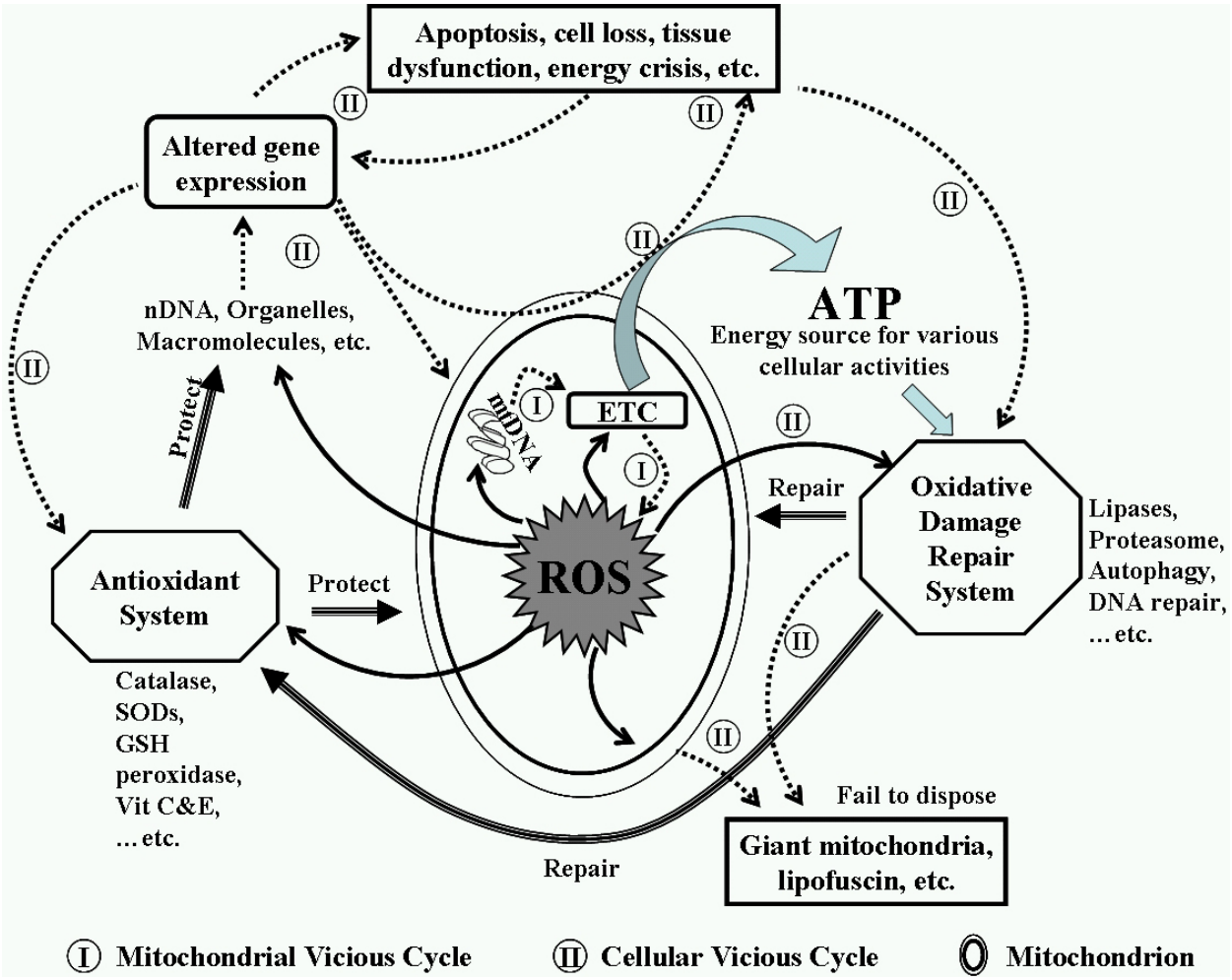

Figure 3. The double vicious cycle in aging. ROS-elicited oxidative stress, mitochondrial dysfunction, impairment of the antioxidant enzymes and DNA repair enzyme systems, and decline in the activities and capacities of the protein degradation system contribute synergistically to aging. The electron transport chain (ETC) in the mitochondrial inner membrane is actively involved in ATP synthesis. However, incomplete reduction of molecular oxygen can lead to the generation of ROS and other organic free radicals, which in turn may cause oxidative damage to both mitochondrial and cellular macromolecules (solid-line arrows). Oxidatively damaged mitochondria and mutated mtDNA-encoded defective protein subunits will impaired ETC, which results in less ATP production and more ROS outbreak (dashed-line arrows in mitochondrion)–mitochondrial vicious cycle. In the aging process, the energy depletion and enhanced oxidative stress can lead to functional deficits of various protecting systems, altered gene expression, apoptotic or necrotic cell death, tissue dysfunction, and other features of cellular aging. In addition, the damaged mitochondria and macromolecules that escape from cellular disposal systems can lead to the accumulation of cellular garbage (e.g., giant mitochondria) and the formation of lipofuscin. These cellular events may in turn worsen cell protecting systems and energy production, which lead to the occurrence of the second vicious cycle–the cellular vicious cycle.

individual cells. Recent studies (43-46) support the idea that the mutated mtDNA molecules may be unevenly distributed and can accumulate clonally in certain cells, causing a mosaic pattern of respiratory chain deficiency in tissues during aging (22,23). The mitochondrial respiratory function of skeletal muscle was found to be severely impaired in the fiber segments harboring high proportions of mutant mtDNA (46). Moreover, a number of studies have shown that mtDNA rearrangements were abundant in COX-negative fibers in the skeletal muscle of elderly subjects, and that the proportion of mutated mtDNA was correlated with the decrease of cytochrome *c* oxidase activity (22,23,43-46).

Recent studies on the dissected substantia nigra of post-mortem human brains further revealed that very high levels of mtDNA deletions are accumulated in individual dopaminergic neurons, but similar high levels of mtDNA deletions were not found in neurons from the hippocampus of the control aged individuals (25,26). Importantly, the proportion of deleted mtDNAs increased significantly with age and neurons containing over 60% of

Table 2. Alterations of genes involved in the maintenance of mitochondrial gene stability in aging

Genes/ genome	Common Name	Function (s)	Age-related Alteration (s)	References
POLG (human) Polg (mouse)	DNA polymerase gamma	Involved in mitochondrial DNA replication and repair.	Mice with a proof-reading-deficient version of Polg displayed an increased amount of mtDNA mutations and signs of premature aging. Mutations in POLG have been associated with CNS disorders.	10, 12, 14, 47, 48
SLC25A4	Adenine nucleotide translocator isoform 1, ANT1	ADP-ATP translocase maintains a high ATP:ADP ratio in the matrix of mitochondria.	Causing autosomal dominant progressive external ophthalmoplegia with mtDNA deletions. May be involved in the maintenance of mtDNA.	15
ECGF1	Platelet-derived endothelial cell growth factor, also known as thymidine phosphorylase (TP)	Angiogenic factor which promotes angiogenesis *in vivo* and stimulates the *in vitro* growth of a variety of human endothelial cells.	Mutations in the gene encoding thymidine phosphorylase cause mitochondrial neurogastrointestinal encephalopathy (MNGIE) syndrome with multiple mtDNA deletions. Defects in TP may disturb mitochondrial nucleoside pools.	10, 16
DGUOK	Mitochondrial deoxyguanosine kinase	Phosphorylation of purine deoxyribonucleosides.	Mutation in the nuclear-encoded mitochondrial DGOUK caused hepatocerebral form of mtDNA depletion syndrome. Defects in DGUOK may disturb mitochondrial nucleoside pools.	10, 17
TK2	Thymidine kinase 2, mitochondria	Phosphorylation of pyrimidine deoxyribonucleosides.	Mutation in the nuclear DNA-encoded TK2 causes myopathic form of mtDNA depletion syndrome. Defects in TK2 may disturb mitochondrial nucleoside pools.	10, 18

For information of other genes with altered expression, please refer to http://genomics.senescence.info/index.html

deleted mtDNA molecules revealed a striking loss of COX (25,26). These findings suggest that accumulation of mtDNA mutations is correlated with the decrease of mitochondrial respiratory function observed in tissue cells of aged individuals.

3.2. Nuclear factors and free radical scavenging enzymes that affect mitochondrial aging

Recently, two groups demonstrated that in mice carrying homozygous mutation of DNA polymerase gamma gene (Polg), the catalytic subunit of the DNA polymerase exhibited defective proofreading, and that the mice showed a 3- to 8-fold increase in point mutations of mtDNA in several tissues (Table 2) (47,48). These mice had a shorter life span and manifest phenotypes (curvature of spine, osteoporosis, loss of subcutaneous fat, hair loss, etc.) of accelerated aging (47). As mentioned above, mutations of human *POLG* have been documented to be responsible for the mtDNA alterations and depletion observed in some types of mitochondrial diseases (12-14). However, in spite of mtDNA mutations, these Polg mutant mice did not show evidence of increased oxidative damage to proteins, lipids, or DNA (48), although mice over-expressing mitochondria-targeted catalase had extended life span (49). Most importantly, over-expression of catalase in the nucleus and peroxisome failed to prolong life in these transgenic animals. It is worth noting that the mitochondria-targeted catalase decreased the mean level of H_2O_2 production from heart mitochondria by 25%, and prevented the inactivation of aconitase, which is a well-documented target of oxidative damage. Interestingly, mitochondria-targeted catalase protected against age-related increases of oxidative DNA damages in skeletal muscle but not in cardiac muscle of the transgenic mice (49). Other evidence supporting that ROS contributes to aging comes from the findings that over-expression of antioxidant enzymes prolong life span in *Drosophila melanogaster*, including manganese-dependent SOD (MnSOD) and methionine sulfoxide reductase A (50,51). However, contradictory results also challenged the roles of free radicals scavenging enzymes in animal studies (52). It was reported that knockdown of SOD1 (Cu/ZnSOD) using the RNAi technology induced cell senescence and the activation of p53 (53). Activation of p53 can result in apoptosis or senescence, which depends on the intracellular level of ROS and some mitochondria-targeted Bcl-2 family proteins (Table 1) (54).

In addition to the increased production of mitochondrial ROS, oxidative stress can also be elicited by a decline in the capacity of intracellular antioxidant enzyme systems (Table 3). Under normal physiological conditions, cells can cope with and dispose of ROS by antioxidant enzymes including MnSOD and Cu/ZnSOD, glutathione peroxidase and catalase. The SODs convert superoxide anion to H_2O_2, which is then transformed to water by glutathione peroxidase or catalase. These enzymes together with other small-molecular-weight antioxidants (e.g., vitamins C and E) can dispose of ROS and free radicals. When there is an increase in ROS production or exposed to mild oxidative stress, most cells can increase the expression levels or activities of some of these free radical scavenging enzymes (most notably, MnSOD) to dispose of ROS (55-57). However, a fraction of ROS may escape from the antioxidant defense system and cause oxidative damage to DNA (4-6), RNA (48), proteins (58-60) and lipids (56) in tissue cells of aged individuals. We found that the activities of Cu/ZnSOD, catalase and glutathione peroxidase in human skin fibroblasts are gradually decreased with age, but that of MnSOD was increased with age up to 65 years and decreased thereafter (35). Similar results were reported elsewhere in skeletal muscle of aged individuals (61). The decrease of antioxidant capacity and the imbalance in the mRNA and protein expression of free radical scavenging enzymes have been suggested to be a cause of increased oxidative stress and oxidative damage to tissue cells in the aging process (62).

4. OXIDATIVE MODIFICATION OF PROTEINS IN AGING

Since Denham Harman first proposed the free radical theory of aging in 1956 (63), experimental evidence have been accumulated to support the notion that oxidative

Table 3. Alterations of genes involved in cellular free radical scavenging enzymes and protection against oxidative damage in aging

Genes/ genome	Common Name	Function (s)	Age-related Alteration (s)	References
CAT (human) Cat (mouse, fruit fly)	Catalase	Catalase is an antioxidant enzyme that protects cells from oxidative damage by metabolizing hydrogen peroxide to molecular oxygen and water.	Overexpression of catalase and Cu/ZnSOD in short-lived strains of fruit flies extended their lifespan. Overexpression of human catalase targeted to mitochondria extended lifespan by about 20% in mice. Impaired expression of catalase was noted in aged individuals and several animal models.	34, 35, 49, 52, 57, 61, 62
SOD1 (human) Sod1 (yeast, fruit fly, mouse)	Superoxide dismutase 1; Cu/ZnSOD	Superoxide dismutase converts superoxide anion to hydrogen peroxide in the cytoplasm of mammalian cells.	Overexpression of SOD1 and CAT in short-lived strains of fruit flies extended their lifespan. Overexpression of Sod1 and Sod2 increased survival by 30% in yeast. Mutations in SOD1 have been associated with amyotrophic lateral sclerosis. Impaired expression of SOD1 was also noted in human aging.	34, 35, 52, 53, 56, 57, 61, 62
SOD2 (human) Sod2 (yeast, fruit fly, mouse)	Superoxide dismutase 2; MnSOD	Superoxide dismutase converts superoxide anion to hydrogen peroxide in the mitochondria of mammalian cells.	Overexpression of Sod1 and Sod2 increased survival by 30% in yeast. Mice without SOD2 were not viable and reduction of SOD2 activity increased the levels of oxidative damage to DNA. RNA interference to knockdown the expression of SOD2 resulted in increased oxidative stress and early-onset mortality in young fruit flies. Impaired expression was noted in aged individuals and several animal models.	34, 35, 50, 56, 57, 61, 62
GPX1	Glutathione peroxidase 1, cellular form	Detoxification of hydrogen peroxide in the cytoplasm of mammalian cells.	Changes in GPX1 expression have been reported in mice during aging and caloric restriction. Mice without GPX1 were more susceptibility to oxidative stress. Impaired expression was noted in aged individuals and several animal models.	34, 35, 57, 61, 62
GSR	Glutathione reductase	Reduction of glutathione disulfide (GSSG).	Overexpression of a GSR homologue in fruit flies extended lifespan in hyperoxia but not under normal conditions. There is no evidence directly linking GSR to human aging, except impaired expression was noted in aged individuals and several animal models.	34, 35, 57, 61, 62
MSRA	Methionine sulfoxide reductase A	Reduction of methionine sulfoxide to methionine after oxidative damage.	Overexpression of a MSRA homologue in fruit flies extended their lifespan. Disruption of MSRA in mice decreased their lifespan and might accelerate aging. MSRA activity was decreased in aging.	51

For information of other genes with altered expression, please refer to http://genomics.senescence.info/index.html

damages account for various changes of physiological function during aging. Age-related oxidative modifications of proteins are thought to be caused by ROS and reactive nitrogen species (RNS). Protein carbonyls are most extensively studied marker of protein oxidation and are formed either by oxidative cleavage of proteins or by direct oxidation of the side chains of lysine, proline, arginine, and threonine residues in affected proteins (64). Peroxynitrite, generated directly from the reaction of superoxide anions with nitric oxide, can nitrate aromatic amino acids to produce 3-nitrotyrosine, 3-nitrophenylalanine, and 3-nitrotryptophan (65).

The abovementioned age-related oxidative damage to proteins does not occur by a random manner (66). In the past few years, a number of proteins have been demonstrated to be very vulnerable or susceptible to oxidative damage in aging or under oxidative stress (Tables 1 and 2). Aconitase and ANT have been shown to be the preferred targets of ROS in mitochondria (58,59). It has been established that free radicals provoke cellular damage and oxidized proteins are accumulated in tissue cells during the aging process, while there is an age-dependent decrease of proteasome function in many tissues (60,67-70). Oxidative modifications to proteins usually alter their functions and modes of regulation as a result of the alterations of their structures (71), enzymatic activities (72), subcellular localization or translocation (73,74), rate of protein degradation (75) and protein-protein interactions (76). Oxidative damage to cardiolipin in the mitochondrial inner membrane may impair oxidative phosphorylation by altering the structure of multi-subunit respiratory enzymes, which is also an important factor in the decline of ATP production by mitochondria in aging (77-79). A set of age-dependent nitrated proteins in rat cardiac and skeletal muscles have been identified by proteomic approach (80,81). Those identified proteins are important enzymes responsible for energy metabolism and metabolism as well as proteins involved in the structural integrity of the cells.

The rate of both protein synthesis and protein degradation declines with age, however, the inability to eliminate damaged macromolecules may be more catastrophic than the synthesis of new defective ones. The four major classes of cellular proteolytic enzymes are caspases, calpains, cathepsins, and proteasomes. Alteration of caspase activity has been reported in a variety of tissues in aging mammals, but how apoptosis affects aging needs to be further investigated (82-84). A recent report demonstrated that disruption of a key apoptotic gene, caspase-2, had a significant impact on aging in the mice (85). Calpain activity has been implicated in various aging phenotypes, including cataract formation, erythrocyte senescence, diabetes mellitus type 2, hypertension, arthritis,

and neurodegenerative disorders (86). In fact, defective proteins resulted from oxidative damage and various stresses are usually disposed of by the barrel-like proteasomes (87) and autophagy (88) in mammalian cells. It has been widely accepted that proteins with short half-lives tend to be eliminated by the proteasomes, whereas proteins with longer half-lives and organelles are degraded by autophagy (89). Although the decline of proteasomal activity in aging has been well documented (60,67-70), the molecular mechanism involved remains unclear (90). Ferrington *et al.* (91) demonstrated that the content of 20S proteasome in muscle of the rat was increased with age. However, the decrease with age of proteasomal regulatory components (PA700 and PA28) caused an overall decrease of proteasomal activity. In addition, oxidation state of the proteasome subunits and accumulation of oxidized and cross-linked proteins during aging can inhibit the proteasome function (92). Thus, it is hard to determine whether functional decline of proteasome is a cause or result of aging. Carrard *et al.* (92) proposed that a decrease in the expression of the regulatory components of proteasome, alterations and/or replacement of proteasome subunits, and formation of inhibitory cross-linked proteins may all contribute to the functional decline of proteasomal activity in aging tissue cells.

Age-dependent increase in protein levels but decline in activities of enzymes has also been reported in senescent rats. Wauthier *et al.* (93) reported that the functional loss of CYP2E1, an isoform of cytochrome P450s, can occur without significant change in the content of protein carbonyls, a marker of protein oxidation in aging and age-related diseases (Table 1). This may also imply the importance of oxidative stress-elicited post-translational modifications. Recently, lower content of protein carbonyls in histones of hepatocytes of the old rat was proposed to have physiological implications in the alteration of the structure and function of chromatin in aging by influencing transcription, replication, and/or DNA repair activities (94). Thus, a better understanding of the qualitative and quantitative changes of proteins in the aging process may help us gain a deeper understanding of the biology of aging and age-related diseases.

5. ALTERATIONS OF GENE EXPRESSION IN THE AGING PROCESS

By using cDNA microarray, the expression profiles of genes affected in aging have been analyzed in several human tissues, and the results have been very fruitful (95-99). These age-related alterations in mRNA levels may reflect changes in gene expression, in mRNA stability or both. Elevation in the expression of genes corresponding to stress response and decrease in the expression of metabolic and biosynthetic genes in skeletal muscle were observed in human aging. Zahn *et al.* (99) reported the results obtained from 81 muscle samples from donors of different ages, and compared their results with those obtained from human kidney or brain tissues. They concluded that a common aging signature consists of six genetic pathways. The genes in the following four pathways displayed increase of expression with age: genes in the extracellular matrix, genes involved in cell growth, genes encoding factors involved in complement activation, and genes encoding components of the cytosolic ribosomes. By contrast, genes involved in chloride transport and genes encoding subunits constituting the mitochondrial respiratory enzymes showed decrease of expression with age. Further analysis showed that the expression levels of proteins essential for electron transport chain are decreased with age in the human, mouse and fly, suggesting that this may be a general biomarker for aging in a broad range of species. These findings have further substantiated the notion that mitochondrial function decline is one of the results of the accumulation of oxidative damage and mutation of mtDNA in tissue cells in the aging process.

6. MITOCHONDRIAL TURNOVER DEFECT IN AGING

In addition to proteasomal protein disposal system, the degradation of damaged mitochondria and other organelles is carried out by another cellular disposal system, autophagy (88). Autophagy is a common degradation pathway for long-lived proteins, organelles, and the plasma membrane (100), resulting in cellular integrity of post-mitotic cells. It is also responsible for cell survival at times of limited nutrients and embryonic development. It has been shown that mitochondria are continuously generated and degraded, with an average half-life of 9 to 24 days in various tissues of the rat (101).

Autophagy can be further classified into macroautophagy, microautophagy, and chaperone-mediated autophagy, according to the mechanisms by which the substrates are delivered to the lysosome. Autophagy also plays important roles in immunity: xenoantigens are degraded and then presented along with major histocompatibility complex class II on cell membrane–an important process of antigen presentation (102). Autophagy selective for degradation of mitochondria is sometimes referred to as mitophagy (103). During mitophagy, a portion of the cytosol and mitochondria of the target cell are sequestered into a double membrane vesicle, and an autophagosome is formed. Autophagosome subsequently migrates to the lysosome and fuses with the lysosomal membrane to form an autophagolysosome. Lysosomes of post-mitotic cells become bloated with aggregates of oxidized, glycated, or cross-linked proteins which are resistant to enzymatic degradation in somatic tissues of aged individuals. These substances found in aged tissues are lipofuscin, a hallmark of cellular aging (104). It has been argued that due to the rapid turnover of mitochondria in tissue cells, oxidative damage to mitochondrial membranes and proteins is normally less of a concern than oxidative damage to mtDNA. However, lysosomes can become less efficient in removing worn-out mitochondria in aging tissue cells. Since degradation of damaged macromolecules through proteasome and autophagy is an energy-consuming process, the functional decline of mitochondria during aging may have synergistic effect on the overall cellular disposal system, resulting in a “double vicious cycle” (Figure 3).

In 2002, Tolkovsky *et al.* (105) first raised the possibility that mitochondria-specific autophagy might be helpful in the maintenance and regeneration of mitochondria in cells during development. In addition, since mitochondria play a central role in many apoptotic events, selective and careful removal of damaged or "activated" mitochondria from the rest of healthy mitochondria and other organelles can prevent cells from total cell destruction initiated by apoptotic stimuli. Indeed, down-regulation of Bcl-2 could induce autophagy in a caspase-independent manner in leukemic HL60 cells (106). Tolkovsky and coworkers also reported that overexpression of Bcl-2 could prevent complete removal of mitochondria (probably through mitophagy) in sympathetic neurons treated with Boc.Aspartyl (O-methyl)CH2F (107). Boc.Aspartyl (O-methyl)CH2F is a compound that can partially block apoptosis in neurons under deprivation of growth factors. When growth factors were added back to the cell culture after initiation of apoptosis, the neurons could survive for more than 6 days before undergoing secondary cell necrosis. Interestingly, mitochondria gradually disappeared on day 3, without any noticeable effect on other components of the target cells. The clearance of damaged mitochondria in neurons was prevented by overexpression of Bcl-2, which indicates that there was a cross- talk between mitochondrial apoptosis and mitophagy.

More recently, a mitochondrial outer membrane protein, Uth1p, was found to involve in selective degradation of mitochondria in the yeast (107). The absence of Uth1p can render yeast cells more resistant to rapamycin- and starvation-induced autophagy. On the other hand, Bermamini and coworkers demonstrated selective degradation of mitochondria harboring a higher level of 8-OHdG in mtDNA in rats that had been treated with an antilipolytic agent, 3,5-dimethylpyrazole (108,109). Due to these important discoveries of the role of autophagy in the regulation of mitochondrial turnover, it is not surprising to find that a decline in autophagic activity can lead to the accumulation of damaged macromolecules, especially the ROS-generating organelles, mitochondria (110). It has been also known for decades that appearance of giant mitochondria is another characteristic of cellular aging (111). Terman *et al.* (112) demonstrated that these giant mitochondria are actually poorly autophagocytosed damaged mitochondria. Inhibition of autophagy in neonatal rat cardiac myocytes with 3-methyladenine also resulted in the irreversible accumulation of giant mitochondria.

7. CONCLUSION AND PERSPECTIVES

Aging is a natural, gradual, and multifactorial biological process. Many of the studies conducted on cultured human cells and animals have revealed that aging is characterized by decline of bioenergetic functions, elevation of oxidative stress due to increased production of ROS by mitochondria in the cells. Most of these characteristics or phenomena gradually occur in advancing age in organs and tissue cells, which are usually correlated with accumulation of mutations and oxidative damage of mtDNA, aberrant proteins, and defective mitochondria in somatic tissues. These findings suggest that the endogenous and exogenous factors that result in oxidative stress, mitochondrial dysfunction, impairment of the antioxidant enzymes and DNA repair enzyme systems, and decline in activities and capacities of protein degradation may contribute, in a synergistic manner, to the aging-associated phenotypes (Figure 3).

On the other hand, abundant evidence have been accumulated to support the notion that mitochondrial function decline, oxidative stress, and increase of oxidative damage play important roles in the pathogenesis of neurodegenerative diseases including Parkinson's disease, Alzheimer disease, Huntington's disease, amyotrophic lateral sclerosis, and dementia (112). PTEN-induced putative kinase 1 (PINK1), DJ-1, and parkin, are encoded by genes in nuclear DNA but are translocated to or functioning within mitochondria. Mutations in genes encoding these proteins have been correlated with the familial type of Parkinson's disease (113). It has been demonstrated by many investigators that over-expression of these proteins can increase the capability of mitochondria to cope with apoptotic stimuli (114,115) and oxidative stress (116-119) in the fruit fly. Moreover, mitochondrial toxins or respiratory chain inhibitors such as 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP) and rotenone have long been utilized to generate animal models for the study of Parkinson's disease. Recent studies have revealed the protective effects of the PINK1, DJ-1, and parkin genes towards these respiratory toxins in mice and human cells (120-122). Furthermore, a mutant α-synuclein, which is implicated in the pathogenesis of Parkinson's disease was found to impair the chaperone-mediated autophagy (123). α-synuclein can be found in Lewy bodies, which are abnormal protein aggregates commonly found in the brain tissues of patients afflicted with Parkinson's disease.

Taken together, the above recent findings further strengthen the notion that mtDNA mutations, respiratory chain dysfunction and oxidative stress are contributory factors to aging and that treatment with agents that improve mitochondrial respiratory function or those that increase the antioxidant capacity of tissue cells may be effective for the deceleration and better management of aging and age-related neurodegenerative diseases.

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Abbreviations: ROS: reactive oxygen species, mtDNA: mitochondrial DNA, ATP: adenosine 5'-triphosphate, OXPHOS: oxidative phosphorylation, ETC: electron transport chain, rRNA: ribosomal RNA, tRNA: transfer RNA, 8-OHdG: 8-hydroxy-2'-deoxyguanosine, CPEO: chronic progressive external ophthalmoplegia, KSS: Kearns-Sayre syndrome, MERRF: myoclonic epilepsy and ragged-red fibers, MELAS: mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, LHON: Leber's hereditary optic neuropathy, POLG: DNA polymerase gamma, PEO: progressive external ophthalmoplegia, CCO: cytochrome *c* oxidase, MnSOD: manganese-dependent SOD, Cu/ZnSOD: copper/zinc-superoxide dismutase, RNS: reactive nitrogen species,

ANT: adenine nucleotide translocase, CYPs: cytochrome P450s, MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

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