Oxidative stress response elicited by mitochondrial dysfunction: Implication in the pathophysiology of aging

Chih-Hao Wang[1], Shi-Bei Wu[1], Yu-Ting Wu[1] and Yau-Huei Wei[1,2]

[1]Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei 112, Taiwan; [2]Department of Medicine, Mackay Medical College, New Taipei City 252, Taiwan

Corresponding author: Yau-Huei Wei. Emails: joeman@ym.edu.tw; joeman@mmc.edu.tw

Abstract

Under normal physiological conditions, reactive oxygen species (ROS) serve as 'redox messengers' in the regulation of intracellular signalling, whereas excess ROS may induce irreversible damage to cellular components and lead to cell death by promoting the intrinsic apoptotic pathway through mitochondria. In the aging process, accumulation of mitochondria DNA mutations, impairment of oxidative phosphorylation as well as an imbalance in the expression of antioxidant enzymes result in further overproduction of ROS. This mitochondrial dysfunction-elicited ROS production axis forms a vicious cycle, which is the basis of mitochondrial free radical theory of aging. In addition, several lines of evidence have emerged recently to demonstrate that ROS play crucial roles in the regulation of cellular metabolism, antioxidant defence and posttranslational modification of proteins. We first discuss the oxidative stress responses, including metabolites redistribution and alteration of the acetylation status of proteins, in human cells with mitochondrial dysfunction and in aging. On the other hand, autophagy and mitophagy eliminate defective mitochondria and serve as a scavenger and apoptosis defender of cells in response to oxidative stress during aging. These scenarios mediate the restoration or adaptation of cells to respond to aging and age-related disorders for survival. In the natural course of aging, the homeostasis in the network of oxidative stress responses is disturbed by a progressive increase in the intracellular level of the ROS generated by defective mitochondria. Caloric restriction, which is generally thought to promote longevity, has been reported to enhance the efficiency of this network and provide multiple benefits to tissue cells. In this review, we emphasize the positive and integrative roles of mild oxidative stress elicited by mitochondria in the regulation of adaptation, anti-aging and scavenging pathway beyond their roles in the vicious cycle of mitochondrial dysfunction in the aging process.

Keywords: Aging, autophagy, caloric restriction, mitochondrial dysfunction, mtDNA mutation, oxidative stress, vicious cycle

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Introduction

Mitochondria are double membrane-enclosed organelles that are responsible for the production of the majority of ATP through oxidative metabolism by tricarboxylic acid (TCA) cycle, β-oxidation of fatty acids and oxidative phosphorylation (OXPHOS). The NADH and $FADH_2$ generated from these metabolic pathways provide reducing equivalents to the electron transport chain consisting of a series of respiratory enzymes (Complexes I–IV), thereby establishing a proton gradient to drive the synthesis of ATP by F_o,F_1 ATPase (Complex V).[1] Notably, mitochondrion has its own genome, called mitochondrial DNA (mtDNA), which assumes a circular double-stranded DNA structure. In each human mitochondrion, there are hundreds to several thousands of copies of mtDNA that encodes 2 rRNAs, 22 tRNAs and 13 polypeptides required for the assembly of respiratory enzymes.[2] Although the majorities (>99%) of the polypeptides constituting the respiratory enzyme complexes are encoded by the nuclear DNA, it has been well documented that pathogenic point mutations in mtDNA sufficiently cause debilitating diseases.[2]

On the other hand, mitochondria are also the major source of endogenous reactive oxygen species (ROS) in human cells. The electrons leaked out from the respiratory chain can react with oxygen to produce superoxide anions. Superoxide anions can be converted to hydrogen peroxide and hydroxyl radicals by various redox reactions. Intriguingly, to prevent the oxidative damage, there is a set of scavenging systems including enzymatic and

non-enzymatic antioxidants to protect cells from the attack by ROS.[3] The superoxide anions, which can be converted to H_2O_2 by superoxide dismutase (SOD), followed by further decomposition to H_2O and O_2 by catalase (CAT) or glutathione peroxidase (GPx).[4] Nevertheless, overproduction of ROS may cause oxidative damage to proteins, lipids and DNA in cells and result in aging and several age-related diseases, including cardiovascular diseases, neurological disorders and diabetes.[5] Like each coin has two sides, ROS also play important physiological roles in human cells. It has been reported that, at lower levels, ROS may serve as a second messenger in signal transduction to regulate cellular adaptation, autophagy and cell survival.[6]

The vicious cycle of mitochondrial ROS and oxidative damage in aging

In the 1950s, Dr. Harman first proposed the free radical theory of aging (FRTA), which campaigns that aging is a result of accumulation of free radicals and oxidative damage in aged tissues.[7] Later, he refined FRTA to the mitochondrial free radical theory of aging (MFRTA) because he realized that mitochondria are the major sites of ROS production in mammalian cells. The MFRTA has set a new paradigm in the study of aging by focusing on the alterations, particularly the oxidative damage, of mtDNA in the aging process.[8] The defects in mitochondrial respiratory chain induce overproduction of ROS, which may further increase the oxidative damage to various biomolecules not only in mitochondria but also in other cellular compartments. Several lines of evidence have supported the idea that aging-associated accumulation of oxidative damage and mutation to mtDNA ultimately leads to the decline in the bioenergetic function of tissue cells in elderly individuals. It has been generally accepted that a 'vicious cycle' contributes to the aging process, and a large amount of data obtained from morphological, bioenergetic, biochemical, and genetic studies of human and animal tissues has supported the MFRTA.[9–11]

Increasing the prevalence of somatic mutation of mtDNA in aging

Increase of the point mutation or deletion of mtDNA has been thought as an initiating event in the vicious cycle of MFRTA, which is based on the observations that when compared with nuclear DNA, mtDNA has a higher error rate during replication and is more susceptible to being damaged by oxidative stress. Several lines of evidence from a number of laboratories have clearly shown that mtDNA mutations are accumulated in a variety of post-mitotic tissues from old human subjects and animals.[12–15] Although some argued that the proportion of mutant mtDNAs is too low to cause a significant decline in mitochondrial function of aged tissues, the documented mutations may be just a tip of the iceberg of the aging-associated alterations in mtDNA. Moreover, the mutated mtDNA molecules may be clonally expanded and accumulated in certain tissue cells, causing a mosaic pattern of respiratory chain defects in tissues of the elderly subjects. On the other hand, studies of the mtDNA mutator mice carrying a mutation of the catalytic domain of the DNA polymerase γ (Polg) provided the causative relationship between accumulated mtDNA mutations and the pathophysiology of aging in animals.[16] Although this mouse model provided substantial support of the MFRTA, some has cast doubt about this model because the pathological changes are not caused by a natural aging process and the proportions of mtDNA mutation in various tissues of these mice are unreasonably high.[17] Moreover, Loeb and his colleagues[18] showed that the proportions of mtDNA mutation in the mutator mice were quite different for different tissues as determined by a highly sensitive method. They observed that the mice with a heterozygous mutation in the Polg gene did not reveal significant reduction in the lifespan even if the amount of mtDNA mutation was 500-fold higher than that of the age-matched wild-type mice. In addition, it was a surprise to find no increase in mitochondrial ROS production in the mutator mice.[17,19] It remains to be answered whether the mutator mice can reveal the role of mtDNA in natural aging, or it is just an artificial genetic disease model for premature aging. This also suggests that the role of mitochondria in natural aging may be different from that observed in the animals with a genetic defect leading to premature aging.

Decline in biogenetic function of mitochondria during aging

Since mtDNA mutations are accumulated in somatic tissues during aging, the expression and maturation of the mtDNA-encoded RNAs and polypeptides as well as mitochondrial respiratory function may be affected. Many studies have shown an age-dependent decrease in the bioenergetic function of mitochondria in various tissues from the human and animals, which have provided substantial support for the MFRTA.[13,15,20–22] However, it is generally accepted that mtDNA mutations are not able to cause mitochondrial dysfunction until they reach a threshold. It is conceivable that accumulation of somatic mtDNA mutations may aggravate the pre-existing defects in mitochondria until the combined defects reach a threshold and result in bioenergetic failure of the affected tissues of the elderly subjects.[23] In addition, we contend that before a pathogenic mtDNA mutation reaches a threshold, an increase in the level of ROS resulted from imbalance in the levels of antioxidant defence might have initiated the aging-related qualitative changes in the mitochondrial proteins and enzymes.[10] A number of iron-sulphur (Fe-S) cluster-containing proteins involved in the execution of OXPHOS and TCA cycle have been known to be quite labile when exposed to oxidative stress.[24] It has been well established that aconitase is a preferred target of ROS, which may cause disassembly of the Fe-S clusters in the aging tissues.[25,26] Besides, the Fe^{2+} ions released from the damaged Fe-S cluster-containing proteins may cause further elevation of ROS through the Fenton reaction.[27] Thus, it is conceivable that oxidative damage to proteins or enzymes containing Fe-S clusters may result in the functional decline of mitochondria in aging tissue cells.

Oxidative response elicited by mitochondrial dysfunction in aging

Retrograde signalling molecules including ROS, Ca^{2+}, ATP and NAD^+ or NADH released from mitochondria may act as critical messengers to trigger specific cellular responses to regulate the metabolic status.[28,29] Subsequently, these signalling molecules turn on a wide array of signalling cascades to mediate cellular physiological responses *via* genetic or metabolic regulations that ultimately lead to enforcement of the antioxidant defence system, activation of Ca^{2+}-dependent kinases, enhancement of mitochondrial biogenesis and OXPHOS and many other events. It has been contended that oxidative stress elicited by mitochondrial dysfunction plays a vital role in the aging process, which is a long-term and gradual course of deterioration. Thus, elucidation of the downstream responses to oxidative stress is essential for a better understanding of the mechanism underlying the pathophysiology of aging.

$P66^{Shc}$ mediates ROS production and mitochondrial dysfunction during aging

The 66 kDa isoform of the growth factor adapter Shc ($p66^{Shc}$) is a stress response protein that has been reported to serve as a mediator in the ROS-mitochondria-aging axis.[30] Overexpression of $p66^{Shc}$ was reported to cause mitochondrial impairment in the alterations of mitochondrial Ca^{2+} responses and fragmentation of mitochondrial network,[31] leading to cytochrome *c* release and apoptosis.[32] On the other hand, $p66^{Shc}$ knockout mice exhibited an extension of lifespan and were more resistant to oxidative stress.[33] In addition, loss of $p66^{Shc}$ protects against the age-dependent, ROS-mediated complications such as cardiovascular disorders and diabetes.[34,35] Accordingly, increase in the phosphorylation of $p66^{Shc}$ on Ser36 *via* PKC β under oxidative stress leads to its recognition by Pin1, a prolyl isomerase, and translocation into the mitochondria.[36] The mitochondrial $p66^{Shc}$ will interact with cytochrome *c*[32] or Hsp70[37] to impair mitochondrial function and thus increase the ROS production. Most importantly, it has been reported that the proportion of $p66^{Shc}$ in the mitochondrial compartment was increased in aging and mitochondrial diseases.[38,39] Besides, phosphorylation of $p66^{Shc}$ was found to target a forkhead box protein O3a (FoxO3a) and promote the phosphorylation and inactivation of FoxO3a by the recruitment of Akt.[40] Subsequently, inhibition of the nuclear translocation of FoxO3a results in down-regulation of the expression of a series of antioxidant genes. In response to the oxidative stress, particularly that in the aging process, the highly phosphorylated $p66^{Shc}$ may induce the vicious cycle that can be initiated by an increase of intracellular ROS generated from defective mitochondria and a reduction of expression of the antioxidant defence system.

Metabolic shift induced by oxidative stress in aging

Another important aspect of age-related alteration is the change in the flux of metabolism. Several recent studies demonstrated that the protein and activity levels of certain enzymes participating in glycolysis were up-regulated in senescent human skin fibroblasts.[41,42] It was observed that senescent skin fibroblasts displayed higher rates of utilization of glucose and amino acids and produced more pyruvate and lactate when compared with young skin fibroblasts.[42] Recently, we discovered that glycolytic flux was increased by an increase in the levels of pyruvate dehydrogenase (PDH) kinase (PDK) and lactate dehydrogenase (LDH) and a decrease of PDH in oxidative stress-induced senescent human skin fibroblasts.[13] It has been reported that the redistribution of metabolites from glucose metabolism not only provides energy by an alternative pathway but also increases the capacity of the antioxidant defence system *via* production of NADPH, which is produced by oxidative catabolism of metabolic intermediates.[43,44] Recently, we observed that activation of AMPK by exogenous ROS or those generated by mitochondrial dysfunction is critical for the increase of glycolytic flux and NADPH generation *via* up-regulation of PFK2 phosphorylation.[45] Increased glycolytic flux enables the affected cells to increase the carbon flux through the oxidative branch of the pentose phosphate pathway (PPP), which generates NADPH by glucose 6-phosphate dehydrogenase (G6PD).[46] NADPH is required for the regeneration of glutathione (GSH), thereby providing the reducing equivalents for detoxification of H_2O_2 and lipid hydroperoxides.[47] Besides, NADPH is also a cofactor in the thioredoxin and glutaredoxin regeneration systems for the maintenance of redox homeostasis owing to the regulation of thiol-disulfide exchange.[48,49] On the other hand, recent studies have also suggested that activation of AMPK is involved in the up-regulation of several antioxidant enzymes.[50,51] AMPK can directly phosphorylate the FoxO to promote its nuclear translocation and the formation of subsequent transcription activation complexes.[52] The activation of the AMPK-FoxO pathway can reduce oxidant-induced ROS production by up-regulating the expression of thioredoxin and peroxiredoxin.[53,54] Taken together, we contend that AMPK-mediated metabolic shift is an adaptive response by alteration of the energy resource and decline of ROS in tissue cells with mitochondrial dysfunction.

Alteration of protein acetylation by sirtuins in aging

In a previous review, we have discussed the regulation of gene expression in the nucleus *via* retrograde signalling from dysfunctional mitochondria during aging.[55] Posttranslational modification of proteins is a more efficient way to regulate cellular functions than gene transcription for the human and animals to adapt to different physiological and environmental conditions. It has been proposed that sirtuins, a family of NAD^+-dependent deacetylases, play critical roles in the regulation of metabolism of fat and glucose in response to physiological changes in the energy or ROS levels.[56] In the mammals, seven sirtuins (Sirt1–Sirt7) have been discovered and are different in their tissue specificity, subcellular localization, enzymatic activity and targets. It has been proposed that Sirt1 may contribute to the anti-aging effect of caloric restriction (CR) through the modulation of mitochondrial function.[57] An activation of Sirt1 in mice by supplementation of

resveratrol in the diet resulted in an increase of the density and function of mitochondria in skeletal muscle and brown adipose tissues, thereby leading to the increase of energy expenditure and alleviation of the decline of bioenergetic function in aging.[58] A number of reports have described diverse targets of Sirt1 in the regulation of mitochondrial function (Table 1). Sirt1 can promote the biogenesis of mitochondrial by activation of PGC-1α,[59] increase of oxidative metabolism *via* repression of HIF-1α-dependent transcription of certain genes,[60] and decrease of ROS production through FoxO-dependent increase of the expression of antioxidant enzymes.[61] It has been shown that a decrease in mitochondria-elicited ROS production and increase in the efficiency of mitochondrial OXPHOS contribute to the extension of lifespan in laboratory animals.[56] Thus, the ability of Sirt1 in the regulation of above-mentioned targets provides part of the explanation of the role of Sirt1 in the anti-aging effect of CR and natural polyphenols such as resveratrol. However, there are some remarkable concerns to be addressed. Mice fed with a diet supplemented with resveratrol[71] or SRT1720, a synthetic Sirt1 activator,[72] only showed increased lifespan in the animals under metabolically stressed conditions induced by feeding them with a high-fat diet. Besides, Sirt1 transgenic mice did not show any extension in lifespan although they displayed the CR-like phenotypes and attenuation of the abnormalities in aging.[73] Moreover, it is noteworthy that there has been no evidence to substantiate a strong linkage between polymorphisms of *SIRT1* and longevity in humans.[74] These observations suggest that Sirt1 may not be a determinant in the control of natural lifespan, but it still plays a role in the adaptation to metabolic stress as well as maintenance of a healthy life and extension of stress-induced lifespan shortening of the human.

A large proportion of the mitochondrial proteins are regulated by reversible acetylation and deacetylation, and the level of acetylation is sensitive to metabolic states and dietary conditions such as high-fat diet,[75] fasting[76] and CR.[77] Three sirtuins, Sirt3, Sirt4 and Sirt5, are located in mammalian mitochondria.[78] Among them, Sirt3 is the major one that regulates the global acetylation of proteins in mitochondria. Sirt3-deficient mice displayed a global increase in the level of protein acetylation in a variety of tissues.[79] It has been shown that the Sirt3 levels are increased in liver, adipose tissues and skeletal muscle in the mice under CR,[80–82] suggesting its role in CR-induced longevity. Besides, Sirt3-dependent deacetylation regulates the activity of acetyl coenzyme A synthetase 2 (AceCS2),[63] which can catalyse the conversion of acetate to acetyl-CoA. Acetyl-CoA is an important intermediate in a wide spectrum of cellular metabolisms, and the signalling pathway involved in conferring longevity has attracted much attention.[83,84] Furthermore, the sequence variability in the enhancer of human *SIRT3* gene has been associated with the survival of the elderly subjects.[85] It is worthy to note that some studies also indicated the regulatory role of Sirt3 in the function of mitochondria (Table 1). It has been demonstrated that Sirt3 plays an essential role in the reduction of ROS and amelioration of oxidative damages and age-related phenotypes in mice with CR.[64,65] Sirt3 targets and activates isocitrate dehydrogenase 2 (IDH2), an enzyme in the TCA cycle, to enhance the antioxidant defence to replenish NADPH pool in mitochondria.[64] Besides, the deacetylation of MnSOD by Sirt3 promotes its enzymatic activity to scavenge superoxide anions (Figure 1).[65,66] Moreover, Sirt3 has been shown to regulate mitochondrial OXPHOS by activation of Complex I,[67] II[68] and V[69] in response to greater energy demand and prevent the opening of mitochondrial permeability transition pore by deacetylation of cycophilin D (CypD) on Lys166.[70] Most interestingly, sirtuins are redox-sensitive deacetylases. A recent study showed that posttranslational modification of Sirt1 by oxidants may lead to its degradation *via* proteasome.[86] Clinically, the protein level of Sirt3 was dramatically decreased in tissues in aging,[87] cardiac hypertrophy[88] and type 2 diabetes.[89] These diseases are all associated with an increase of intracellular ROS. Recently, we demonstrated that the expression of Sirt3 was significantly decreased in human cells harbouring mtDNA with the 4977 bp deletion or in the cells treated with ROS or a prooxidant.[69] These findings support the notion that overproduction of ROS in pathological states could regulate mitochondrial protein acetylation through

Table 1 Modulation of mitochondrial function through Sirt1- or Sirt3-dependent deacetylation pathways

Sirtuins	Targets	Activity	Function	Ref.
Sirt1	PGC-1α	Increase	Elevation of mitochondrial biogenesis	59
	HIF-1α	Decrease	Increase of oxidative metabolism	60
	FoxO	Increase	Up-regulation of genes expression in antioxidant enzymes	61
	ATGs	Increase	Increase of autophagy	62
Sirt3	AceCS2	Increase	Up-regulation of acetate metabolism	63
	IDH2	Increase	Up-regulation of the TCA cycle	64
	MnSOD	Increase	Reduction of ROS	65, 66
	NDUFA9	Increase	Elevation of OXPHOS	67
	SDHA	Increase	Elevation of OXPHOS	68
	ATP5O	Increase	Elevation of OXPHOS	69
	CypD	Increase	Inhibition of mPTP opening	70

ATGs: autophagy-related proteins; NDUFA9: NADH dehydrogenase 1 α subcomplex 9; SDHA: Succinate dehydrogenase, subunit A, flavoprotein; ATP5O: ATP synthase subunit O; mPTP: mitochondrial permeability transition pore.

the suppression of Sirt3 *via* oxidative stress-mediated signalling pathways and thereby impair mitochondrial function and global metabolism. These observations indicate that the timing and the level of ROS may be a crucial factor in the regulation of Sirt3 level and the acetylation status of mitochondrial proteins. Whether an increase of the ROS level can affect the transcription and/or translation of Sirt3 awaits further studies.

Autophagy in aging

Autophagy is a process by which intracellular components such as damaged organelles or aggregated proteins are engulfed and degraded within the lysosomes. Autophagy can be divided into three different forms, namely macroautophagy, microautophagy and chaperone-mediated autophagy, respectively, according to their regulatory mechanisms and the mode of delivery of cargos to the lysosome. The process of autophagy, usually referred to as macroautophagy, is initiated by the formation of phagophore, also called the isolation membrane. The targets intended to be degraded are engulfed by the phagophore to form an autophagosome. The membranes of the autophagosome then fuse with the lysosome to form an autolysosome, where the targets are digested and the components are recycled back to the cytosol for reuse.[90,91]

Reduced autophagy leads to aging and increased autophagy prolongs lifespan

Beyond the generally accepted functions in the development and adaptation of individual tissue cells, autophagy plays a pathological role in aging and age-related diseases. Recent studies showed that autophagy may play a role in the determination of lifespan in many model organisms.[92–101] Reduced autophagy has been associated with accelerated aging (Table 2), and stimulation of autophagy by pharmacological agents or genetic manipulations might have the potential to slow down the aging process and prolong the lifespan of the yeast, fruit flies, worms and rodents (Table 3). During the aging process, the expression levels of

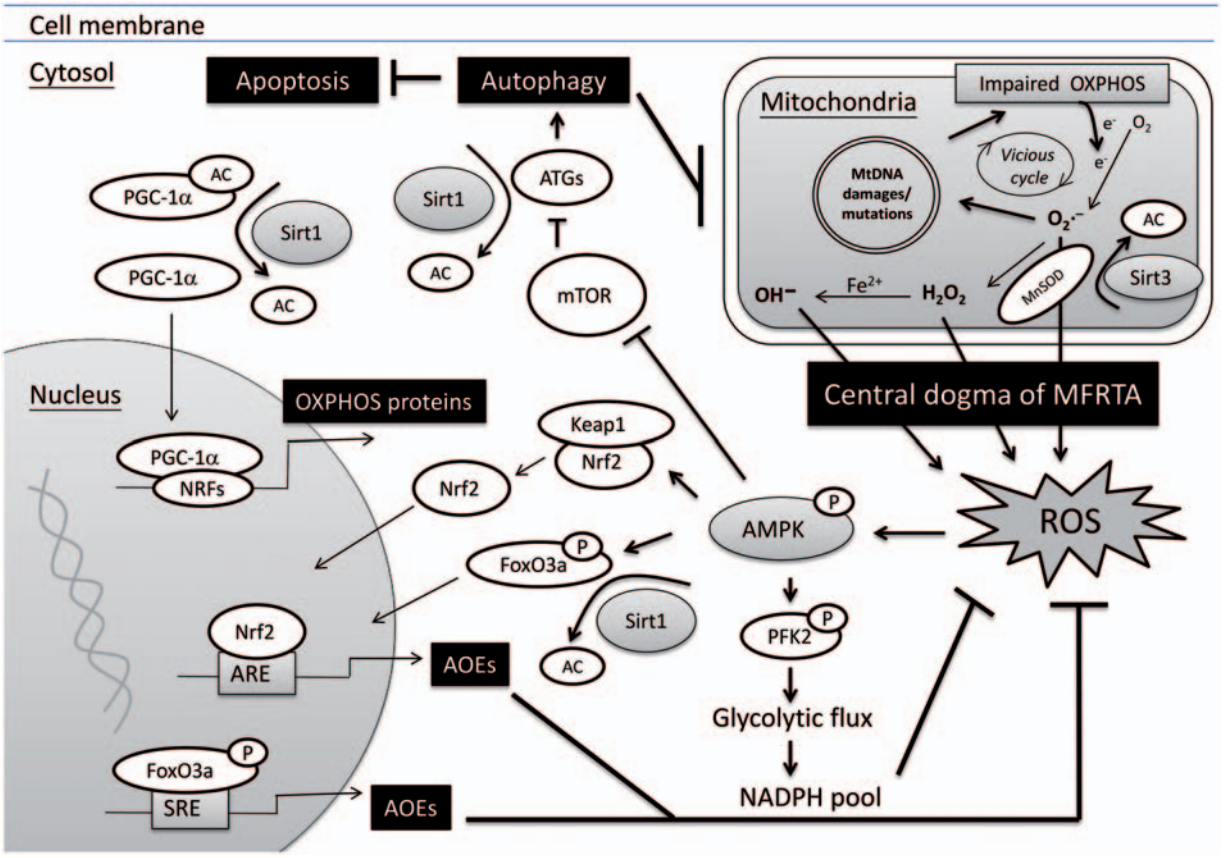

Figure 1 A scheme to illustrate the network involved in the regulation of cellular adaptation and the response to mitochondrial dysfunction-elicited ROS during the aging process. The vicious cycle of overproduction of ROS by mitochondria is the key to the MFRTA. In response to the increased production of ROS elicited by mitochondrial dysfunction, AMPK activation can increase the expression of several antioxidant enzymes (AOEs) *via* the Nrf2- and FoxO3a-dependent pathways, respectively. The activation of AMPK can also increase the glycolytic flux by activation of PFK2 and G6PD to replenish the intracellular NADPH pool. On the other hand, Sirt1 can directly deacetylate PGC-1α and FoxO3a and result in the up-regulation of the mitochondrial biogenesis and expression of AOEs, respectively, in response to excess ROS. Particularly, Sirt3 deacetylates MnSOD to enhance its enzymatic activity to remove superoxide anions produced by defective mitochondria. Moreover, activation of AMPK can efficiently inhibit the activity of mTOR, which is a negative regulator of autophagy and in turn leads to the increase of mitophagy. Importantly, an increase in the Sirt1 activity can enhance the mitophagy activity *via* deacetylation of autophagy-related proteins (ATGs). Taken together, activation of AMPK, Sirt1 and Sirt3 as well as enhanced protein expressions and activities of several antioxidant enzymes, mitochondrial biogenesis and autophagy, respectively, can prevent cell apoptosis in response to oxidative stress elicited by mitochondrial dysfunction in the aging process

Table 2 Decline of autophagic activity in aging tissues or cells

Functions	Tissues or cells	Ref.
Down-regulation of expression in autophagy-related genes	Aged human brain	92, 93
	Aged rat skeletal muscle	94
Decline of lysosomal activity	Aged rat skeletal muscle	94
	Aged rat hepatocytes	95
Decrease of expression in Fis1 and Drp1 genes	Stress-induced senescence in human liver cells	96
	Replicative senescence in HDFs	96
	Replicative senescence in HUVECs	97
Increase of mTOR signalling	Stress-induced senescent human RPECs	98

HDFs: human diploid fibroblasts; HUVECs: human endothelial cells; RPECs: retinal pigment epithelium cells.

Table 3 Extension of lifespan or healthy lifespan by activation of autophagy

Inductions	Organisms	Phenotypes	Ref.
CR	Rat	Attenuation of impaired autophagy in aged skeletal muscle	94
Spermidine	Yeast	Increase of chronological lifespan	99
	Fly	Increase of lifespan by up to 30%	99
	Worm	Increase of lifespan by up to 15%	99
	Mouse	Reduction of age-related oxidative stress	99
	Human PBMCs	Increase of survival by up to 35% after 12 days of culture	99
Rapamycin	Mouse	Increase of lifespan by up to 9% in males and 14% in females	100
Overexpression of LAMP2A in liver	Mouse	Attenuation of age-related anomalies in liver	101

PBMCs: peripheral blood mononuclear cells.

genes involved in the autophagic pathway were found to be decreased *via* transcriptional regulation.[92–94] On the other hand, the activities of lysosomal enzymes are also decreased with the increase of age that leads to insufficient digestion of the products at the last step of autophagy.[94,95] It was shown that the decrease in the expression of genes that are involved in mitochondrial fission resulted in the deficiency of mitophagy in the senescent cells.[96,97] In addition, an increase in the activity of the mTOR pathway, a negative regulator of autophagy, was observed in the senescent cells.[98] These findings clearly indicate that autophagy activity is decreased in aging. Wohlgemuth et al.[94] demonstrated that CR atteuates the impairment of autophagy in skeletal muscle of the rat in the aging process. This suggests that autophagy may be one of the possible mechanisms involved in CR-induced extension of lifespan. In addition, activation of autophagy induced by pharmaceutical agents such as spermidine[99] and rapamycin[100] could significantly increase the lifespan of some organisms. It has also been demonstrated that preservation of the autophagy activity in the aging liver by introduction of the inducible lysosome-associated membrane protein 2A (LAMP2A) transgene could reduce the amounts of oxidized and aggregated proteins and the susceptibility to apoptosis, respectively.[101]

Anti-apoptotic effect of autophagy

Increased apoptosis is a hallmark of aging. Rather than displaying similar function of the self-destructive process to apoptosis, autophagy plays a predominantly cytoprotective process *via* efficient elimination of damaged or aggregated proteins and damaged organelles.[102] Therefore, autophagy can mediate protective effects in rodent models against the oxidative damage affecting the liver,[101] heart,[103] nervous system[90] and kidney.[104,105] The anti-apoptosis effect may be one of the mechanisms involved in autophagy-mediated longevity. Generally, simultaneous involvement of many proteins in both pathways is thought as one the possible reasons resulting in antagonism. Beclin 1, a Bcl-2 homology 3 (BH3) domain only protein,[106] is an essential initiator of autophagy. Beclin 1 recruits key autophagic proteins to a pre-autophagosomal structure, thereby forming the core complex consisting of Beclin 1, Vps34 and Vps15.[107] In addition, Beclin 1 is a key factor to determine whether the cells will undergo autophagy or apoptosis.[108] Beclin 1 has been shown to interact *via* its BH3 domain with a protein of the anti-apoptotic Bcl-2 family.[109] In addition to be involved in anti-apoptosis, Bcl-2 plays a role in the inhibition of autophagy. The interaction between Beclin 1 and Bcl-2 blocks the assembly of Beclin 1-mediated pre-autophagosomal structure, and thereby inhibits autophagy. Induction of autophagy promotes the liberation of Bcl-2 from the Beclin 1 complex with the potential to allow Bcl-2 to become available to block the apoptotic pathway.[110] On the other hand, release of FLIP, an inhibitor of caspase 8, from Atg3 upon induction of autophagy could exert its inhibitory effect on the extrinsic apoptotic pathway.[111] A number of recent studies have suggested that some anti-apoptotic proteins such

as Bag1[112,113] and Hsp20[114] may function to prevent cell death by activating autophagy. However, it remains to be further investigated as to whether this putative mechanism is involved in the induction of autophagy and cytoprotection.

Quality control of mitochondria

Autophagy is also a critical regulator of organellar homeostasis, particularly of mitochondria, which is termed mitophagy. This mechanism of selective elimination of damaged mitochondria might be induced by mitochondrial dysfunction.[115] Mitochondrial fission can occur in an asymmetric fashion, yielding one functional mitochondrion and a dysfunctional one with a low membrane potential, which is a target for mitophagy.[116] This preferential degradation of damaged mitochondria before inducing the vicious cycle of ROS overproduction protects the cells from apoptotsis and is believed to be a mechanism for mitochondrial quality control or a check point. Mitophagy was often induced by stress and mitochondrial damage to maintain the quality and quantity of mitochondria. Loss of this quality control has been shown in aging and aging-related disorders such as neurodegenerative diseases.[117] Most importantly, how cells select impaired mitochondria correctly to undergo mitophagy is the first mystery to unravel. Using genetic screening, Uth1, Atg11 and a mitochondria-anchored receptor, Atg32, which confer the selectivity during mitophagy, have been identified in yeast.[118–120] Surprisingly, no homologues of these proteins have been found in the mammals. Thus, a key regulator in the selectivity of mammalian mitophagy waits to be unravelled. Intriguingly, Dr. Youle and coworkers first reported that Parkin, one of the causative genes in Parkinson's disease, was selectively recruited to the mitochondria with low membrane potential in mammalian cells and triggered the autophagy-mediated degradation of mitochondria.[121] After treatment of cells with an oxidative stress inducer, paraquat[121] or a mitochondrial uncoupler, FCCP,[122] Parkin could be translocated from the cytosol to mitochondria, where specific proteins are essential for the induction of mitophagy to eliminate the defective mitochondria. Besides, the overexpression of Parkin can specifically remove mitochondria with deleterious *COXI* mutations in heteroplasmic cybrids, thereby increasing the proportion of wild-type mtDNA in cells and restoring cytochrome *c* oxidase activity.[123] Later, it was demonstrated that accumulation of Pink1 (PTEN-induced kinase 1) on the outer membrane of depolarized mitochondria mediates the recruitment of Parkin to the damaged mitochondria.[124] In addition, VDAC has also been demonstrated as an interacting protein of Parkin and serves as an essential docking site on the outer membrane of mitochondria to recruit Parkin.[125]

Autophagy is regulated by ROS and CR

It has been suggested that ROS elicited by mitochondrial dysfunction is a major signal leading to the induction of autophagy.[126] Accumulating evidence suggests that the outcome of autophagy induction by mild oxidative stress is beneficial to cell survival. However, excess damage caused by high level of ROS would not allow the cells to maintain the autophagy response and may result in cell death.[127] Oxidation of Atg4 by H_2O_2 under starvation condition led to inhibition of its deconjugation activity, which allowed the formation of autophagosome membrane while treatment of cells with antioxidants abolished the induction of autophagy.[128] It was demonstrated that CR can induce autophagy through the inhibition of the mTOR signalling by insulin/insulin-like growth (IGF) factor, which blocks the mTOR pathway by Akt activation.[129] It was reported that the activation of AMPK and Sirt1 play an important role in regulating the CR-induced lifespan extension.[52,57] Recently, emerging evidence has also substantiated their importance in the induction of autophagy under energy crisis or oxidative stress. AMPK was found to increase the activity of autophagy by direct activation of Ulk-1, an initiator of autophagy, under nutrient starvation conditions.[130,131] Besides, activation of AMPK by FoxO3a-mediated increase of AMP/ATP ratio was linked to inhibition of the mTOR and induction of autophagy in senescent cells induced by oxidative stress.[132] In addition to the activation of AMPK and autophagy by nutrients deprivation, we have demonstrated that AMPK can be activated by oxidative stress.[45] These findings suggest that the activation of AMPK plays an important role in the autophagy induced by ROS. Moreover, it has been shown that Sirt1 may deacetylate several Atg proteins including Atg5, Atg7, Atg8 and LC3 and thus lead to the enhancement of autophagy through the increase of their activities.[62] Taken these findings together, we emphasize that nutrient deprivation and energy deficiency-induced AMPK and Sirt1 may trigger the autophagy, which thereby contributes to the increase of lifespan.

Concluding remarks

There are many aging models established from genetic manipulation or drug induction in laboratory animals and cultured cells, which have been employed to investigate the pathophysiology of aging. In the past two decades, a number of genes has been identified to cause premature aging or to extend the lifespan of the yeast or animals.[129,133] These findings indicate that no single gene or a unique factor can serve as the master control of the aging process. In contrast to artificial aging, the natural aging is a slow, gradual and long-term process associated with the accumulation of oxidative damage and metabolic dysregulation. Moreover, most of the age-related disorders including neurodegenerative diseases, metabolic syndrome or arthritis usually display a defect in maintaining homeostasis of the redox status and cellular metabolism. In this review, we contend that the network of oxidative stress responses involved in the pathophysiology of aging is complicated and interconnected. Mitochondria play the central role in the scheme due to the fact that the great majority of ROS are produced in these organelles in mammalian cells (Figure 1). Oxidative stress responses elicited by mitochondrial dysfunction including the up-regulation of antioxidant enzymes, the quality control of mitophagy and metabolic switch are beneficial for the cells to maintain metabolic

homeostasis and against intracellular ROS level for adaptation or survival of tissue cells in the aging process. Sirtuins may sense the alteration of cellular redox or metabolic status (NAD^+ and NADH ratio) to regulate the function of many regulators such as PGC-1α, FoxO3a, MnSOD and autophagy-related proteins. Losing a part of this regulatory feedback network in response to oxidative stress may initiate the vicious cycle of mitochondrial ROS overproduction and result in aging and aging-related diseases. Thus, insensitivity or impairment in this biochemical process by an excess and long-term presence of ROS may be an important contributor to the pathophysiology of aging. Most importantly, to enhance the efficiency of this connective network may be a novel approach to slow down the aging process of the living organisms. It is worthy to note that changes in life style and eating habits, such as CR and exercise, are efficient for the improvement of many parts of this network to promote longer lifespan and maintain better healthy life than genetic manipulation used to establish animal models.[64,65,94] In addition to rodents, CR improves health conditions by promotion of mitochondrial function, attenuation of the aging-associated metabolic dysregulation and reduction of the incidence of age-related diseases in the rhesus monkeys, although there are still controversies regarding the effect of CR on the extension of lifespan.[134,135] More extensive and in-depth studies on primates are warranted to resolve all the inconsistent observations. It is our opinions that examination of the cellular response to oxidative stress induced by mitochondrial dysfunction can provide useful information to unravel the molecular basis of the pathophysiology of the aging process. In addition, a better understanding of the oxidative stress response of human cells is of clinical importance in the management of aging and age-related disorders.

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