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Metabolic Reprogramming of Human Cells in Response to Oxidative Stress: Implications in the Pathophysiology and Therapy of Mitochondrial Diseases

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Abstract: Mitochondria are the organelles producing most of the energy and play important roles in a variety of biochemical functions in human cells. Mitochondrial defects can cause ATP deficiency and overproduction of reactive oxygen species, which are the major hallmarks of mitochondrial diseases. Abundant evidence has suggested that mitochondrial dysfunction-elicited oxidative stress can play an important role in the pathogenesis and progression of mitochondrial diseases. Mitochondria can respond to energy deficiency by the retrograde signaling to trigger a number of molecular events to help the human cells to cope with physiological or environmental changes. In this article, we first describe oxidative stress-induced cellular responses including metabolic adaptation, compensatory increase of mitochondrial biogenesis, upregulation of antioxidant enzymes, and alteration of protein acetylation in human cells with mitochondrial dysfunction. In this regard, we review recent findings to elucidate the mechanisms by which human cells motivate their mitochondria and the antioxidant defense system to respond to energy deficiency and oxidative stress, which contribute to the adaptive metabolic reprogramming in mitochondrial diseases. In addition, we emphasize the critical role of the activation of AMPK, Sirt1 and Sirt3 in the metabolic adaptation of human cells harboring mitochondrial DNA mutations. Recent studies have revealed that AMPK and sirtuins-mediated signaling pathways are involved in metabolic reprogramming, which is effected by upregulation of antioxidant defense system and mitochondrial protein acetylation, in human cells with mitochondrial dysfunction. Finally, we discuss several potential modulators of bioenergetic function such as coenzyme Q_{10}, mitochondria-targeting antioxidants, resveratrol, and L-carnitine based on recent findings from studies on human cells and animal models of mitochondrial diseases. Elucidation of the signaling pathway of this adaptive response to oxidative stress triggered by mitochondrial dysfunction may enable us to gain a deeper insight into the communication between mitochondria and the nucleus and guide us to develop novel therapeutic agents for effective treatment of mitochondrial diseases.

Keywords: AMPK, antioxidant enzyme, metabolic adaptation, mitochondrial disease, mtDNA mutation, oxidative stress, sirtuin.

1. INTRODUCTION

1.1. Overview of Mitochondrial Function in Mammalian Cells

Mitochondria are membrane-enclosed organelles in eukaryotic cells that carry out numerous biochemical reactions for the maintenance of cellular physiology and homeostasis. They generate ATP through the oxidative phosphorylation (OXPHOS) system, which is coupled with respiration *via* TCA cycle and oxidation of fatty acids. The two genetic systems in the nucleus (nuclear DNA, nDNA) and mitochondria (mitochondrial DNA, mtDNA) cooperate to maintain normal mitochondrial function, which plays a critical role in the life and death of the human cells. Human mtDNA bears 16,569 base pairs of nucleotides that encode 13 polypeptides constituting the respiratory enzyme complexes plus 2 rRNAs and 22 tRNAs. Approximately 1,500 polypeptides that build up a functional mitochondrion are encoded by nDNA and are then translocated into mitochondria in the post-translational manner, while all the mtDNA-encoded proteins are essential for the assembly of respiratory enzymes [1-3]. On the other hand, mitochondria are involved in the maintenance of Ca^{2+} homeostasis, catabolism of amino acids, and biosynthesis of heme, pyrimidine nucleotides and several steroid hormones [4]. Intriguingly, although the majority of the polypeptides constituting the respiratory enzyme complexes are encoded by the nDNA, it has been well documented that the pathogenic mutations in mtDNA have serious consequences. Abundant evidence has been accumulated to show that mitochondrial dysfunction caused by mtDNA mutation plays a pivotal role in the pathophysiology of a wide spectrum of human diseases, which include aging-related neurodegenerative diseases, metabolic syndrome, diabetes, and cancers [1, 5].

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1.2. Mitochondrial Dysfunction and Diseases

Mitochondrial diseases are a clinically and genetically heterogeneous group of disorders mostly caused by impairment of the OXPHOS system or other defects in the energy metabolism of mitochondria [1, 6]. They may result from genetic mutations in mtDNA and/or nuclear genome. Some mitochondrial diseases arise from specific mutations in nuclear genes, which are mainly involved in the assembly and function of respiratory enzyme complexes, biogenesis of mitochondria, and maintenance and replication of mtDNA. However, a large portion of mitochondrial diseases are caused by maternally inherited point mutations in the tRNA, rRNA or structural genes, and large-scale deletion or duplication of mtDNA. More than two hundreds of deletion or mutation of mtDNA have been detected in the affected tissues of patients with mitochondrial myopathy and encephalomyopathies, which has substantiated the importance of mtDNA defects in the pathogenesis of mitochondrial diseases [7]. This group of debilitating diseases is generally characterized by myopathy and muscle weakness due to structural and biochemical abnormalities of mitochondria that often appear in the skeletal muscle of patients. In addition, the majority of patients with mitochondrial diseases often display multi-system disorders and the affected tissues often harbor pathogenic mtDNA mutations, which lead to mitochondrial dysfunction. The most distinct and common mitochondrial diseases include chronic progressive external ophthalmoplegia (CPEO) and the Kearns-Sayre syndrome (KSS), myoclonic epilepsy with ragged-red fibers (MERRF), mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), Leber's hereditary optic neuropathy (LHON), the syndrome of neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP), and Leigh's syndrome. Although these genetic diseases are characterized by well-defined clinical symptoms, the correlation between the clinical phenotype and genotype is rather poor for most of the mitochondrial diseases. Besides, the

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onset of mitochondrial diseases can occur at a wide range of age and are often presented with defects in the central nervous system, cardiovascular system, the visual system, and neuromuscular system. Clinical symptoms are most predominant in the tissues with high energy demand, such as the brain, heart, and skeletal muscle. It is worthy of noting that the proportion of mutant mtDNA in affected tissues is usually correlated with the severity of the disease and clinical phenotype of the patients with mitochondrial diseases [1-3]. The heteroplasmy of mtDNA mutation and variation in the energy demand of different tissues may contribute to a broad spectrum of phenotypes in mitochondrial diseases, even within a family with the same pathogenic mutation of mtDNA. Generally, the mutation or depletion of mtDNA can cause severe defects in cellular energy metabolism that would substantially result in the depletion of ATP and over-production of ROS. In addition, the expression levels of several clusters of genes have been reported to be altered in response to mtDNA mutation-elicited oxidative stress in affected cells of the patients with mitochondrial diseases [8]. The molecular mechanism involved in the retrograde signaling from mitochondria to the nucleus, which is triggered by defects in mitochondria, has been a subject of intensive research in recent years. We have suggested that mitochondrial dysfunction-elicited ROS can confer disease phenotype and induce adaptive response to oxidative stress in the affected cells of patients with mitochondrial diseases. The in-depth elucidation of the regulatory cascades of this adaptive mechanism may lead us to a deeper understanding of the pathophysiology of mitochondrial diseases.

2. BIOCHEMICAL ABNORMALITIES AND THE PATHOPHYSIOLOGY OF MITOCHONDRIAL DISEASES

Since mitochondrial diseases are a diverse and heterogeneous group of clinical conditions, it is a great challenge to understand the biochemical and pathological consequences of mtDNA mutations. Numerous pathogenetic mtDNA mutations have been documented in a wide spectrum of mitochondrial diseases, and mtDNA mutation-caused mitochondrial dysfunction in affected cells would cause the energy demand shunt to glycolysis in an attempt to produce ATP, resulting in systemic lactic acidosis [8]. The insufficient supply of energy is thought to be the driving force of the pathological changes of most mitochondrial encephalomyopathics at the cellular level, and energy deficiency in the affected tissues can result in multi-system disorders. For example, the clinical features and symptoms of mitochondrial diseases are usually manifested as muscle weakness, ataxia, cardiomyopathy, exercise intolerance, developmental delay, dementia, unsteady gait, and poor balance [8].

2.1. Oxidative Stress and Oxidative Damage in Mitochondrial Diseases

The defects in the respiratory chain can lead to overproduction of ROS in mitochondria, which can further increase the oxidative damage to various biological molecules in affected tissue cells [9, 10]. A large amount of experimental results including our findings have suggested that ROS and oxidative damage elicited by mtDNA mutation play a key role in the pathophysiology and progression of mitochondrial diseases. For example, the intracellular levels of H_2O_2 and oxidative damage to DNA and lipids have been found to increase in the primary culture of skin fibroblasts of the patients with MELAS, MERRF or CPEO syndrome [11-13]. One of our previous studies revealed that as compared with the control, the content of 8-OHdG in total cellular DNA of the skeletal muscle and the intracellular concentration of H_2O_2 in muscle fibroblasts were higher in the patients with CPEO syndrome [14]. In addition, the oxidative damage to proteins and the content of superoxide anions in the muscle biopsies of patients with MELAS syndrome were significantly higher than those of age-matched normal subjects [15]. It was found that the average mitochondrial ROS level in the primary culture of skin fibroblasts from KSS patients was higher than that of age-matched normal controls [16]. On the other hand, Piccolo and colleagues [17] reported that the oxidative adducts of organic aldehyde with plasma proteins in blood of the patients with CPEO syndrome were significantly elevated as compared with those of normal subjects. Pitkänen *et al.* [18] also found an excess amount of aldehydic lipid peroxidation products in the primary culture of skin fibroblasts of the patients with Complex I deficiency. On the other hand, the elevation of oxidative stress can cause the opening of the mitochondrial permeability transition pores, which leads to the simultaneous collapse of the mitochondrial membrane potential [19]. The permeability transition pores have recently been postulated to play a role in the induction of autophagy of damaged mitochondria in the affected tissues of the patients with mitochondrial disease [20]. The mitophagy targeting at dysfunctional mitochondria was observed in the primary cultures of skin fibroblasts from MERRF and MELAS patients, respectively [21, 22]. In order to understand the molecular mechanism involved in the biochemical consequences of mitochondrial diseases caused by mtDNA mutations, cytoplasmic hybrid cells (cybrids) were established by the fusion of enucleated cytoplasts (derived from patients with a mtDNA mutation) with immortalized human cell lines that are completely depleted of endogenous mtDNA (rho zero cells, ρ^0) [23, 24]. The first transmitochondrial cytoplasmic hybrid cells were described in 1989 [25], and many lines of research have suggested that cybrids are an excellent tool for studying the biochemical and pathophysiological consequences of varying proportions of a specific mtDNA mutation [26, 27]. For example, it was found that the cybrids harboring homoplasmic mtDNA mutations of G11778A, A14484G, and G3460A, respectively, displayed defects in the respiratory chain, which were caused by not only the alterations in the steady-state levels of polypeptides constituting the respiratory enzyme complexes but also the delayed assembly of respiratory enzyme Complexes I, III, and IV [28]. Besides, in a previous study of oxidative modification to mitochondrial proteins in the cybrids harboring the A8344G mtDNA mutation from an MERRF patient by 2-D gel electrophoresis and proteomic techniques, we identified carbonylated proteins that were significantly increased in mitochondria of the MERRF cybrids as compared with the wild-type cybrids [29]. We found that the voltage-dependent anion channel (VDAC), aconitase and prohibitin (PHB) were quite susceptible to oxidative damage in the MERRF cybrids. VDAC is one of the components of the permeability transition pore complex on the outer mitochondrial membrane, which regulates the transport of ions and metabolites in and out of the mitochondria. Therefore, accumulated oxidative damage to VDAC may cause a loss of bidirectional fluxes of ions and metabolites across the mitochondrial membranes, which in turn leads to energy deficiency and elicits the pathological changes [29, 30]. As for PHB, it acts as a chaperone to stabilize proteins and prevent misfolding of mitochondrial proteins, and thus the damaged PHB protein might lose such a function and aggravate the mitochondrial dysfunction in MERRF cybrids [31]. Moreover, it was also reported that the mutant cybrids were more sensitive to oxidative stress such as UV-irradiation, which led to apoptosis as revealed by several apoptotic markers [32]. Taken these findings together, we suggest that the pathogenetic mtDNA mutation-elicited oxidative stress can cause additional damage or mutation to mtDNA, and then further impair the respiratory function. The role of ROS-driven vicious cycle could mainly contribute to the pathophysiology and progression of the mitochondrial diseases.

2.2. Apoptotic Features in Mitochondrial Diseases

Apoptotic pathway has been considered a major physiological process in triggering cell death, which is critical for morphogenesis, tissue homeostasis as well as pathogenesis of diverse diseases [33, 34]. Notably, mitochondrial dysfunction plays an important role in the initiation, execution and regulation of apoptosis since mitochondria can release specific proteins and factors to trigger the apoptotic pathway. The apoptotic proteins, including apoptosis-

inducing factor (AIF), Smac/DIABLO, and cytochrome *c*, can be released from the mitochondria through the opening of mitochondrial permeability transition pores [35]. Accordingly, many research including our previous studies substantiated the involvement of apoptosis in the pathogenesis of mitochondrial diseases including MELAS, MERRF, CPEO and KSS [34-36]. The well-characterized apoptotic features, TUNEL-positive staining as well as the immunohistochemical staining of caspase-3, p75 and Fas, respectively, were observed in the muscle from patients who carried the pathogenic mtDNA mutations. In addition, a pronounced granular appearance of cytochrome *c* accompanied by 8-OHdG and 4-HNE (one of the lipid peroxidation products) were also detected in the COX-negative, ragged-red muscle fibers of patients with mitochondrial diseases [37]. Furthermore, an *in situ* approach was employed to investigate the relationship between apoptosis, defects in respiratory chain function, and mtDNA mutation load, and the results showed that apoptosis was associated with a high proportion of mtDNA mutation [35]. Indeed, it was found that mtDNA mutations can accelerate apoptosis as evidenced by an *in vivo* study on the D257A mice with a mutant form of DNA polymerase γ, which displayed increased accumulation of mtDNA mutations in most somatic tissues and expression of apoptotic markers [38]. On the other hand, the apoptosis triggered by mitochondrial dysfunction is also involved in the seizure-induced neuronal cell death as well as in brain damage and is a cause and consequence of epileptogenesis [39]. The apoptotic features are an important determinant in the relationship between oxidative stress and the severity of the symptoms (e.g., myopathy) in patients with mitochondrial diseases.

2.3. Alteration of Ca^{2+} Homeostasis in Mitochondrial Diseases

The mitochondria also participate in the regulation of Ca^{2+} homeostasis by efficiently sequestering and releasing Ca^{2+} ions in cooperation with the endoplasmic reticulum (ER) [40]. The mitochondrial Ca^{2+} ions uptake is involved principally in inositol 1,4,5-triphosphate (IP3)-mediated Ca^{2+} release from the internal stores, which are localized in the ER. Ca^{2+} ions in the mitochondrial matrix play an important role in the regulation of intermediary metabolism of mitochondria [41]. It is well established that pyruvate, isocitrate, α-glycerophosphate and α-ketoglutarate dehydrogenase are all activated by Ca^{2+} ions, enhancing the reduction of NAD^+ and generation of the proton motive force across the mitochondrial membranes. It is noteworthy that the capacity of mitochondrial Ca^{2+} ions uptake is a determinant of the rate of production and release of ROS by mitochondria [42]. The uptake of a larger amount of Ca^{2+} ions by mitochondria can significantly increase the ROS release from mitochondria, possibly due to the interactions of Ca^{2+} ions with cardiolipin in the inner mitochondrial membrane, which leads to the structural changes in the membrane-embedded respiratory enzymes. When Ca^{2+} ions are accumulated at a high level in mitochondria, it could induce inner mitochondrial membrane permeabilization [43]. Abnormal mitochondrial Ca^{2+} ion homoeostasis indeed has been reported in various cell types with defects in mitochondrial OXPHOS function [44]. It has been shown that a perturbation of Ca^{2+} homeostasis is involved in the pathogenesis of mitochondrial diseases [45]. Human cells with mtDNA mutations associated with the MELAS and MERRF syndrome, respectively, can cause the dysregulation of mitochondrial Ca^{2+} ions resulting in an increase of the concentration of cytosolic Ca^{2+} ions [46, 47]. In addition, defects in the handling of mitochondrial Ca^{2+} ions were also observed in the primary cultures of skin fibroblasts from patients with MERRF syndrome [48]. Human cells harboring the A8344G mtDNA mutation exhibited a reduced uptake of the Ca^{2+} ions by mitochondria in response to histamine stimuli. Furthermore, dysregulation of Ca^{2+} homeostasis caused by mitochondrial dysfunction could subsequently increase the excitability due to the irregular activation of Ca^{2+}-dependent protein kinases. Thus, the cells affected by the damage caused by impairment of Ca^{2+} sequestration may lead to excitotoxicity and epilepsy [6, 8]. It warrants further investigation as to how a pathogenic mtDNA mutation affects Ca^{2+} homeostasis. It is established that mitochondrial Ca^{2+} uptake is dependent on the membrane potential of mitochondria. Thus, a decline of the membrane potential caused by mitochondrial dysfunction may play an important role in the deregulation of Ca^{2+} homeostasis. It was reported that treatment of neurons with the uncoupling agent, carbonyl cyanide chlorophenylhydrazone (CCCP), the mitochondrial uptake of Ca^{2+} was interfered and thereby altered the cytosolic Ca^{2+} level [49]. Taken the above-mentioned findings together, we suggest that an increase of cytosolic Ca^{2+} level caused by mitochondrial dysfunction may play a role in triggering epilepsy, which is often associated with an induction of hyperexcitability in the neurons of patients with mitochondrial encephalomyopathies.

3. CELLULAR ADAPTATION TO MITOCHONDRIAL DYSFUNCTION-ELICITED OXIDATIVE STRESS

3.1. Alteration of Antioxidant Enzymes

Cells exhibit a broad spectrum of responses to oxidative stress, depending on the stress level [50]. Since oxidative stress plays a vital role in the pathophysiology of mitochondrial diseases, it is of clinical relevance to unravel the mechanism of oxidative stress response and to identify the protective proteins involved. One of the cellular responses to oxidative stress elicited by mitochondrial dysfunction is the induction of free radical scavengers, which are able to quickly remove ROS and damaged biological molecules [51]. The antioxidant enzymes include superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR), thioredoxin, and thioredoxin reductase together with a host of low-molecular-weight antioxidants such as ascorbic acid, α-tocopherol, retinal, folic acid, lipoic acid, and glutathione (GSH), which can dispose of free radicals to minimize their damaging effects. Upregulation of the antioxidant enzymes was first demonstrated by immunohistochemical staining on skeletal muscle biopsies of patients with the CPEO, KSS, and MELAS syndromes, respectively [52-54]. Most importantly, these findings indicate that the expression levels of Mn-SOD and, to a less extent, catalase are increased in RRFs with negative expression of cytochrome *c* oxidase. Therefore, the dramatic induction of Mn-SOD in affected tissue cells was suggested as an indicator of the onset of mitochondrial dysfunction in patients with a mitochondrial disease [55]. However, there is still a discrepancy in the observation of a reduction in the protein expression and activity levels of Mn-SOD in the affected tissues of some patients with LHON [56]. The disruption of Mn-SOD gene in the rat resulted in optic neuropathy, which was similar to the major symptom of LHON patients [56]. Moreover, it was demonstrated that enhanced ROS production led to an increase in the activities of antioxidant enzymes including Mn-SOD, GPx, and catalase in the cybrids harboring A3243G and A8344G mutation of mtDNA, respectively [57]. The selenium-dependent and -independent GPx activities were also reported to be increased in response to the deficiency of respiratory enzymes in human myeloid leukemia U937 cells after treatment with chloramphenicol (to inhibit mtDNA translation) or ethidium bromide (to deplete mtDNA) [58]. Nevertheless, it is noteworthy that a delicate balance between the expression levels of Mn-SOD, CuZn-SOD and GPx plus catalase and thioredoxin reductase would confer human cells with the ability to efficiently cope with oxidative stress. An increase in the activity of Mn-SOD or CuZn-SOD must be accompanied by a corresponding increase in the catalase and/or GPx activity to prevent excessive buildup of H_2O_2 in human cells. However, there exists an imbalanced expression of antioxidant enzymes, particularly the decrease of catalase and GPx activities in the primary cultures of skin fibroblasts from patients with the CPEO, MERRF, and MELAS syndromes [59]. The imbalanced expression of the antioxidant enzymes indicates that the production of ROS is in excess of their removal, which in turn may elicit an elevation of oxidative stress in the affected tissue cells. In this regard, there should be some unknown molecular mechanisms involved in the regulation of

other antioxidant enzymes or proteins such as CuZn-SOD, GPx, GR, catalase, thioredoxin, peroxiredoxin in the pathophysiology of mitochondrial diseases. Therefore, further studies on the mechanism of regulation of antioxidant enzymes are clinically imperative for the treatment of mitochondrial diseases such as CPEO, MERRF, and MELAS syndromes.

3.2. Upregulation of Mitochondrial Biogenesis in Mitochondrial Diseases

The aberrant mitochondrial proliferation is reported to be one of the compensatory responses frequently observed in the skeletal muscle of patients with mitochondrial myopathies. One of the observed pathological features in skeletal muscle of the patients with mitochondrial myopathy is the ragged-red fibers, which is a result of over-proliferation of the abnormal mitochondria [60]. A change in the number of mitochondria was also reported in tissue cells of some of the patients harboring a pathogenic mutation or depletion of mtDNA [61]. Actually, the alteration in the expression of nuclear genes involved in mitochondrial respiration and glycolysis was often observed in affected tissues with mitochondrial diseases [62]. In addition to the increase of mitochondrial mass, the expression levels of protein subunits constituting respiratory enzyme complexes, such as Complex V and Complex II, were found to increase in response to mitochondrial dysfunction [63]. It was proposed that the general increased expression of genes involved in ATP synthesis (mostly ATP synthase β, a subunit of Complex V) was due to a compensatory mechanism that increases the transcription of genes involved in the production of ATP. However, this change in gene expression is highly diverse and inconsistent in a variety of disease models. Several factors may affect the expression of genes in mitochondrial diseases including the type of tissues and cells, the phenotype of disease, the type of mtDNA mutation, the genetic background and age of the patients [64]. In spite of the above-mentioned factors, it has been suggested that the increased response of transcription only occurs when the deficiency in energy production has reached a certain level [65]. High levels of a pathogenic mtDNA mutation may induce an increase in the expression of mitochondrial biogenesis-related genes, nuclear DNA-encoded OXPHOS genes and certain glycolytic genes in an attempt to compensate for energy crisis in affected cells.

3.3. Metabolic Shift to Anaerobic Glycolysis in Mitochondrial Diseases

It is reasonable to observe that glycolysis is upregulated in the affected tissues of patients with mitochondrial diseases due to the energy demand [66]. The conversion of pyruvate to lactate (anaerobic glycolysis) allows regeneration of cellular NAD^+, an essential coenzyme for glycolytic flux so that it can be maintained at a higher level. In addition, the redistribution of glycolytic metabolites has been recently reported to help the target cells to produce NADPH required for the antioxidant defense through the pentose phosphate pathway (PPP) [67]. The metabolite flux through the oxidative branch of the PPP can be viewed as a part of the antioxidant defense system due to its ability in the generation of NADPH by glucose 6-phosphate dehydrogenase (G6PD). The cellular NADPH can provide the reducing equivalents for biosynthetic reactions and the oxidation-reduction involved in protecting cells from the toxicity of ROS. Besides, several NADPH-dependent antioxidant enzymes, including the thioredoxin and glutaredoxin systems, play important roles in the defense of cells against oxidative stress and in the maintenance of redox homeostasis owing to the regulation of thiol-disulfide exchange [68, 69]. Filosto *et al.* [55] demonstrated the upregulation of GSH synthesis in the biopsies of affected muscle from patients with mitochondrial diseases including CPEO, MELAS, and MERRF, respectively. They suggest that the elevation of intracellular GSH level can be considered an initial and indirect sign of respiratory chain dysfunction because it was observed at the early stages of the onset of these mitochondrial diseases. In this regard, we speculate that the increase of the GSH level may be related to an increase of glycolytic flux in the affected tissues. In a previous study, we found that the metabolism in the primary culture of skin fibroblasts from patients with MERRF syndrome was shifted to anaerobic glycolysis and this glycolytic phenotype was related to the increase of the production of NADPH in response to the oxidative stress elicited by mitochondrial dysfunction [70]. We contend that the metabolic shift is essential for the survival of cells in the affected tissues of patients with mitochondrial diseases such as MERRF syndrome, because it can provide not only an alternative supply of ATP but also a boost of antioxidant defense. However, the mechanisms by which the glycolytic and antioxidant enzymes are regulated in response to mitochondrial dysfunction have remained unclear.

4. CONSEQUENCES OF AMPK ACTIVATION IN RESPONSE TO MITOCHONDRIAL DYSFUNCTION-ELICITED OXIDATIVE STRESS

Recently, AMP-activated protein kinase (AMPK) has been shown to play an important role in the regulation of the cellular energetic status under oxidative stress [71]. To cope with the energy deficiency in human cells with mitochondrial dysfunction, AMPK can switch on other ATP-generating pathways such as glycolysis and oxidation of amino acids and switch off ATP-utilizing pathways such as biosynthesis of fatty acids and gluconeogenesis [72]. In addition, the activation of AMPK in response to the oxidative stress is also found in some mammalian cell lines and in mouse models, suggesting that AMPK is not only an energy-responsive enzyme but also a sensor for redox signals [73]. We summarize in Table **1** the findings of recent studies on the role of AMPK activation and its beneficial effects in the patients with diseases related to mitochondrial dysfunction. Intriguingly, the function of AMPK and its dysregulation in the pathophysiology of aging and neurodegenerative diseases (e.g. Alzheimer's disease and Huntington's disease) have attracted much attention [74-76]. The AMPK signaling can repress and delay the appearance of AD pathology but increase neuronal stress and trigger detrimental effects at a later stage, which may augment the pathogenesis of AD [76]. On the other hand, the decline with aging in the sensitivity and responsiveness of AMPK was reported to associate with many age-associated diseases, including cardiovascular diseases and metabolic syndrome [77]. However, the molecular mechanism and consequences of AMPK activation in mitochondrial diseases have remained mostly unclear. Recently, Mackenzie *et al.* [78] observed that AMPK was activated in response to elevated mitochondrial ROS levels in the endothelial cells from patients with type 2 diabetes. In one of our previous studies, we demonstrated that activation of AMPK was involved in the regulation of metabolic shift to glycolysis, which increased the intracellular level of NADPH that is essential for the adaptive response to oxidative stress elicited by mitochondrial dysfunction in the skin fibroblasts of patients with MERRF syndrome [70]. These findings have substantiated the notion that AMPK plays a crucial role in the defense of human cells against oxidative stress. Indeed, it was reported that AMPK can directly phosphorylate the forkhead transcription factor 3a (FOXO3a) and thereby promote the formation of subsequent transcription activation complex in the nucleus [79]. The activation of the AMPK-FOXO3a pathway can reduce oxidant-induced ROS production by upregulation of the gene expression of thioredoxin, peroxiredoxin, and catalase [79, 80]. It was reported that the induction of the AMPK-FOXO3a pathway can be regulated by oxidative stress but independent of the change in the intracellular AMP [81]. In addition, the activation of AMPK is also involved in the upregulation of the expression of mitochondrial uncoupling protein 2 (UCP2) in endothelial cells and thereby suppresses the production of superoxide anions in the mitochondria [82]. The above-mentioned responses were observed mostly in studies using endothelial cells, skeletal muscle cells, cardiomyoblasts, and neuronal cells, respectively. On the other hand,

there was an *in vivo* study of the mouse showing that deletion of the AMPK gene could cause an elevation of oxidative stress and result in a shortened life span of erythrocytes, increased RBC hemolysis, and anemia due to the down-regulation of the expression of antioxidant enzyme including catalase, Mn-SOD, and GPx-1 [83]. In light of these findings and our previous observation that the activation of AMPK was more pronounced in skin fibroblasts of MERRF patients [70], it is imperative to elucidate the mechanism by which AMPK activation contributes to the up-regulation of antioxidant enzymes in the primary cultures of skin fibroblasts from patients with mitochondrial diseases [84]. Therefore, pharmacological activation of AMPK may be beneficial for the affected tissue cells to survive under oxidative stress, which provides a new avenue for the development of effective treatment of patients with mitochondrial diseases.

5. SIGNALING PATHWAYS IN THE COMPENSATORY INDUCTION OF MITOCHONDRIAL BIOGENESIS IN MITOCHONDRIAL DISEASES

It has been well documented that mitochondrial biogenesis was induced in affected tissue cells of patients with mitochondrial diseases as a compensatory adaptation to bioenergetic dysfunction due to defects in the OXPHOS system [61, 63,85]. The density of mitochondria in a human cell is tightly controlled by a number of regulatory factors involved in mitochondrial biogenesis, by which cell can cope with different physiological or environmental conditions under energy demand *via* specific signaling pathways. Recently, the term of "mitochondrial retrograde signaling", a pathway of communication from mitochondria to the nucleus was observed in affected cells with mitochondrial dysfunction under pathological conditions [86]. Most of the information regarding the regulation of the retrograde signaling has been obtained from the budding yeast. Much less is known about the downstream signaling pathways of the retrograde effectors. In mammalian cells, these signaling pathways are believed to be modulated by metabolites, Ca^{2+} ion, ROS, and the ADP/ATP and NAD^+/NADH ratios [87, 88]. Alteration of the cellular Ca^{2+}, redox and energetic homeostasis in response to mitochondrial stress may modulate the key signaling pathways, which in turn activate a host of transcriptional factors and co-regulators. Through the retrograde signaling multiple nuclear genes coding for polypeptides constituting the respiratory enzymes and other genes involved in the maintenance of mtDNA and mitochondrial biogenesis are activated to boost mitochondrial function. Among these transcriptional responses, Ca^{2+} signaling has been documented to affect a variety of transcription factors, including CREB, TORC, NFAT, NF-κB and MEF2, through activation of calcineurin and Ca^{2+}-dependent kinases under elevated concentration of cytosolic Ca^{2+} ion [89]. It has been shown that Ca^{2+}-responsive CREB and TORC can regulate mitochondrial function by activating PGC-1α (proliferator-activated receptor gamma co-activator 1α) [90]. PGC-1α is a pivotal transcriptional co-activator, which plays a key role in the regulation of the expression of Mn-SOD, polypeptides constituting respiratory enzymes, and enzymes involved in β-oxidation of fatty acids [90, 91]. The activation of PGC-1α transcription by CREB was reported to be responsible for the induction of mitochondrial biogenesis-related transcription factors including NRF1/NRF2 (nuclear respiratory factor 1 and 2), and mtTFA (mitochondrial transcription factor A) in response to mitochondrial dysfunction [91]. Abundant evidence from several lines of studies has shown that numerous stress responsive protein kinases including mTORC, PKC, MAPK and CREB are involved in the activation of a host of transcription factors, which relay mitochondrial dysfunction-elicited oxidative stress to increased mitochondrial biogenesis in the pathophysiology of mitochondrial diseases [85, 92-96]. In one of our previous studies, we showed that PKCδ was activated in the upregulation of transcription factors related to mitochondrial biogenesis to increase the mitochondrial mass of human cells in response to oxidative stress [95]. Increased expression and phosphorylation of PKCδ were observed in skin fibroblasts of the patients with MERRF and CPEO syndromes, respectively. This was accompanied by increased gene expression of transcription regulators involved in mitochondrial biogenesis such as PGC-1α, NRF-1, and mtTFA [95, 97, 98]. Besides, the regulation of PGC-1α activity is also under the control of stress signaling *via* the MAPK pathway [99]. Moreover, the cellular energy status has been demonstrated that can signal to PGC-1α through facilitating its interaction with transcription factor YY1 (Yin Yang) by mTOR-mediated signaling pathway [100]. On the other hand, PGC-1α has been shown to co-activate a number of nuclear receptors, including PPARs, ERRα, HNF4α, and GRs (glucocorticoid receptors), which contribute to the upregulation of mitochondrial biogenesis and β-oxidation of fatty acids [101]. Growing evidence has demonstrated that certain hormone receptors such steroid and thyroid, can regulate mitochondrial function by direct translocation into mitochondria [102]. It was reported that GRs could not only act as nucleus-localized receptors on nuclear OXPHOS gene transcription but also enter mitochondria and regulate mtDNA transcription [103, 104]. Moreover, GRs have been shown to possess a neuroprotective effect through the regulation of mitochondrial functions during chronic stress. In response to corticosterone, GRs can form a complex with Bcl-2 and co-translocate into mitochondria, thus modulate mitochondrial calcium and oxidation to rescue mitochondrial dysfunction and protect against cell death [103].

The competence of PGC-1α to co-activate the transcription factors involved in mitochondrial biogenesis confers PGC-1α with the ability to direct the complex program to maintain the proper function of mitochondria. Intriguingly, recent studies showed that PGC-1α activity is also controlled by a variety of post-translational modifications including phosphorylation, ubiquitination and acetylation [105-107], which can affect the function and stability of PGC-1α and even its interaction with other protein partners. Among the posttranslational modification events, the reversible acetylation and phosphorylation have been demonstrated to be the key modulators of the PGC-1α activity [105, 108]. In fact, it was shown that AMPK was able to regulate the biogenesis of mitochondria through activation of PGC-1α by protein phosphorylation [108]. Thus, the adaptive induction and activation of AMPK may contribute to the compensatory proliferation of mitochondria in the affected tissues of patients with mitochondrial diseases such as MERRF syndrome.

6. ROLES OF SIRTUINS IN THE REGULATION OF MITOCHONDRIAL FUNCTION AND METABOLIC ADAPTION IN RESPONSE TO OXIDATIVE STRESS

6.1. Role of Sirt1 in Cellular Adaption in Mitochondrial Diseases

Protein lysine acetylation has emerges as a conserved mechanism for metabolic regulation through functional modulation of target proteins [109, 110]. By these protein posttranslational modifications, cellular functions in the human and animals are more efficiently executed to adapt to physiological and environmental conditions. In mammals, protein acetylation is reversely regulated by sirtuins, a protein family of NAD^+-dependent deacetylases, which are involved in the regulation of metabolism, energy homeostasis, stress response, and longevity [111,112]. Seven sirtuins (Sirt1-Sirt7) in mammalian cells have been found to show tissue specificity in terms of subcellular localization, enzymatic activities, and targets. Among them, Sirt1 was demonstrated to play a critical role in the regulation of cell metabolism and longevity through several pathways [113]. Accumulating evidence suggests that the beneficial effect of caloric restriction (CR) is partially mediated by Sirt1, although the Sirt1-mediated pathway is not fully elucidated [114]. Many studies showed that CR can activate Sirt1 in different tissues through elevation of its transcription level or increasing its

deacetylase activity by regulation of the NAD^+/NADH ratio [115]. In addition, the activation of Sirt1 has also been proposed to contribute to the anti-aging effect of CR through the positive regulation of mitochondrial oxidative metabolism in various tissues of rodents and human skeletal muscle [116, 117]. Importantly, numerous studies have revealed that Sirt1 can modulate the activities of an array of metabolic pathways in multiple tissues *via* PPAR-γ and PGC-1α signaling cascades [118]. Sirt1 has been shown to deacetylate PGC-1α to promote its transcriptional activity, and thereby enhance mitochondrial respiration and fatty acid β-oxidation in white adipose tissues and skeletal muscle [119-121]. Moreover, abundant evidence has substantiated that the activity of Sirt1 can be regulated in response to increased intracellular levels of the ROS [122]. The JNK-mediated phosphorylation of Sirt1 is an example of the oxidative stress-induced activation of Sirt1, which subsequently deacetylates its specific targets to activate the downstream pathways to protect cells [122]. In one of our previous studies, we showed that the Sirt1 expression in human skin fibroblasts was increased upon treatment with mild stress (250 μM H_2O_2). In addition, the Sirt1 level was higher in the primary culture of skin fibroblasts from the patients with the MERRF and CPEO syndromes, respectively, as compared with those of normal controls [123]. A recent study also demonstrated that the mRNA levels of Sirt1 were elevated in the skin fibroblasts of patients with OXPHOS defect caused by a mutation in the DNA polymerase γ gene [124]. The above-mentioned findings suggest a molecular mechanism underlying the up-regulation of Sirt1, which can positively regulate PGC-1α via protein deacetylation under oxidative stress induced by mitochondrial dysfunction and energy crisis. This cascade could contribute to the compensatory induction of mitochondrial biogenesis in the affected cells of patients with mitochondrial diseases.

On the other hand, there is abundant evidence to support that Sirt1 is involved in the regulation of metabolism and stress tolerance in response to physiological changes under oxidative stress [119]. Numerous studies have substantiated the notion that Sirt1 plays an important role in modulating the cellular response to oxidative stress through stimulating the expression of antioxidant enzymes and proteins *via* the FOXO-mediated pathway [125-128]. Overexpression of Sirt1 has been shown to protect cells from oxidative damage by inducing the expression of catalase and accompanying attenuation of age-related cardiomyopathy involving hypertrophy in animals [126]. Under oxidative stress, Sirt1 can be activated and subsequently deacetylate and activate the proteins of FOXO family including FOXO1 and FOXO3a, which in turn increase the gene expressions of Mn-SOD, catalase and thioredoxin to decrease the intracellular levels of ROS [127, 128]. The FOXO family proteins have been considered as the potential factor for the promotion of longevity and alleviating age-related diseases in animals. One of the recent studies showed that Sirt1 can protect β-cells against oxidative stress by deacetylating and increasing the activity of FOXO1 [126]. In addition, it was found that the regulation of antioxidant genes by Sirt1 was dependent on the activation of both FOXO3a and PGC-1α in the vascular endothelial cells [129, 130]. Moreover, several research groups have shown that the increased expression of Sirt1 in response to oxidative stress may be related to the induction of antioxidant enzymes, which was mediated by the Sirt1-FOXO signaling pathway to protect the patient's cells from oxidative damage [125-128]. However, the induction of Sirt1 seems to be unable to fully cope with the increase of oxidative stress in affected tissue cells with mitochondrial dysfunction. In fact, similar to the adaptive effect of Sirt1 on stress tolerance, increased number of mitochondria in the affected tissues of patients might have little contribution to the energy need. This is why lower ATP contents and higher ROS levels have been always observed in affected tissues or skin fibroblasts from patients with mitochondrial diseases. In this regard, the discrepancy between the expression level of Sirt1 and its actual enzyme activity may be one of the reasons for inefficient adaptive response in human cells with mitochondrial dysfunction. It still needs to be further addressed as to whether the increase in the expression of Sirt1 induced by mitochondrial dysfunction-elicited oxidative stress could result in an increase of the enzyme activity of Sirt1 and bring about a benefit to the cells. It is worth noting that overexpression or increase of the activity of Sirt1 has been reported to be neuroprotective in Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD) through the upregulation of the cell survival and antioxidant defense mechanisms [131] (Table **1**). Recently, accumulating experimental evidence suggests that Sirt1 is a potential target for the design of novel therapeutic agents to treat neurodegenerative diseases (Table **2**). Taken together, these findings have led us to conjecture that the induction of Sirt1 elicited by oxidative stress may be responsible for metabolic reprogramming and antioxidant defense system in the patients of mitochondrial diseases. This also suggests that Sirt1 may be involved in the signaling pathway of the cross-talk between defective mitochondria and the nucleus to regulate cellular adaptation to mtDNA mutation-elicited oxidative stress. The pharmacological interventions that target the Sirt1 and its downstream cascades may provide a novel avenue for effective treatment of mitochondrial diseases.

6.2. Potential Role of Oxidative Stress-Induced Decline of Sirt3 in Mitochondrial Diseases

Sirt3 is the best characterized sirtuin in mitochondria and has emerged as the major regulator of deacetylation of mitochondrial proteins [132]. It is preferentially expressed in highly metabolic tissues [133, 134]. Sirt3 acts on numerous targets that are involved in many metabolic pathways, which indicates the pivotal role of Sirt3 in the regulation of mitochondrial metabolism and energy homeostasis. It has been reported that Sirt3-catalyzed deacetylation is involved in the regulation of TCA cycle, β-oxidation of fatty acids, OXPHOS, urea cycle and ketogenesis. We and other laboratories have shown that Sirt3 plays a crucial role in the maintenance of energy homeostasis through the activation of mitochondrial OXPHOS by deacetylating polypeptides constituting respiratory enzyme Complexes I, II, and V [135-137]. In addition to Sirt1, Sirt3 expression was also found to be altered in the mouse as a response to CR and other dietary interventions [138-140]. Accumulating evidence suggests that Sirt3 may contribute to the beneficial effect of CR due to the increased expression of Sirt3, which was observed in liver, skeletal muscle and brown adipose tissues of mice during fasting and CR [141-143]. On the other hand, Sirt3 is considered as a potential regulator of the detoxification of the ROS, which attenuates oxidative damage and age-related pathological changes in mice during CR [144, 145]. It has been shown that deacetylation of Mn-SOD by Sirt3 increases its enzymatic activity to scavenge superoxide anions [146, 147]. In addition, Sirt3 also activates isocitrate dehydrogenase 2 (IDH2) to replenish the NADPH pool in mitochondria to enhance the antioxidant defense [146]. A number of studies have shown a decline of Sirt3 in mice fed on the chronic ethanol assimilation or high-fat diets [138, 139, 148], which have been known to increase the production of ROS. Clinically, the expression level of mitochondrial Sirt3 was decreased in mice with age or age-related diseases such as type 2 diabetes and cardiac hypertrophy [146-148]. Moreover, a deficiency in Sirt3 has been demonstrated to be associated with the occurrence of age-related cardiac hypertrophy [149, 150]. In a recent study, it was shown that the inherited mtDNA variation can interfere with the expression of Sirt3 in human cybrids in response to oxidative stress [151]. Likewise, one of our recent studies showed that the protein level of Sirt3 was significantly decreased in the primary cultures of skin fibroblasts from patient with CPEO syndrome as well as in the cybrids harboring mtDNA with the 4,977 bp deletion [136]. By treatment of human cells with H_2O_2 or superoxide anions generated by menadione, the expression of Sirt3 was substantially suppressed and in turn

Table 1. The beneficial effects of the activation of AMPK, Sirt1 and Sirt3 in human diseases

Modulators	Diseases	Model	Effects	Reference
AMPK	MERRF	Human skin fibroblasts	Enhanced glycolysis and redox balance	[70]
	HD	Mouse cortical neuronal cells	Stimulated mitochondrial biogenesis	[74]
	AD	AD transgenic mice	Promoted Aβ peptide metabolism	[75]
	Diabetes	Human endothelial cells	Inhibited mitochondrial ROS production	[78]
	Diabetes	Human endothelial cells	Stimulated mitochondrial biogenesis	[79]
	Endothelial dysfunction	Human endothelial cells	Increased thioredoxin expression	[80]
	Cardiovascular diseases	Human endothelial cells	Enhanced Bcl-2 expression	[229]
Sirt1	Aging	Rat and human cell lines	Increased Ku70 activity	[116, 117]
	Metabolic syndrome	Mouse animal model and mouse myoblast cells	Increased PGC-1α activity	[118, 119]
	MERRF / CPEO	Human skin fibroblasts	Increased response to oxidative stress	[123]
	Endothelial dysfunction	Human endothelial cells	Stimulated formation of a FOXO3a/PGC-1α complex	[129]
	Mitochondrial disease	MtDNA mutator mouse	Stimulated mitochondrial biogenesis	[131]
Sirt3	HD	Mouse cortical neuronal cells	Stimulated mitochondrial biogenesis	[74]
	Metabolic syndrome	Mouse model	Increased oxidative metabolism of fatty acids	[133]
	OXPHOS deficiency	Mouse embryonic fibroblasts	Increased mitochondrial Complex I activity	[134]
	CPEO	Human skin fibroblasts	Increased mitochondrial Complex V activity	[136]
	Diabetes	Mouse myoblast cells	Increased response to oxidative stress	[137]

Abbreviations: MERRF, myoclonic epilepsy with ragged red fibers; HD, Huntington's disease; AD, Alzheimer's disease; CPEO, chronic progressive external ophthalmoplegia; OXPHOS, oxidative phosphorylation.

led to a decrease of the Sirt3-mediated protein deacetylation. Moreover, we demonstrated that the 4977 bp mtDNA deletion-elicited oxidative stress caused a decrease of Sirt3 expression and decline of the ATP hydrolysis activity of Complex V. Taken together, we suggest that oxidative stress is involved in the decline of Sirt3 because the above-mentioned disease models are all associated with an increase of intracellular levels of ROS.

In addition to the finding of Sirt3 deficiency in the progressive cardiac pathology, a loss of Sirt3 has also been shown to promote the progression of hearing loss in mice with aging [143, 152]. Some of the patients carrying pathogenic mtDNA mutations were found to develop cardiac hypertrophy or sensory-neural hearing loss, especially the patients with CPEO or KSS syndromes. Thus, whether the Sirt3 deficiency could conceivably contribute to the clinical phenotypes in CPEO or KSS patients is worth further investigation. On the other hand, it has been documented that a portion of the patients with mitochondrial diseases were found to be more susceptible to developing type 2 diabetes and insulin resistance [153]. Particularly, a notable portion of the MELAS patients often manifest the clinical features of diabetes mellitus. Interestingly, Sirt3 deficiency was also reported to be involved in the pathogenesis of metabolic syndrome [149, 150]. These studies showed that the high fat diet could down-regulate Sirt3 and cause hyperacetylation of hepatic mitochondrial proteins in mice, which accelerate the development of metabolic syndrome-related phenotype such as insulin resistance and obesity. Therefore, whether Sirt3 deficiency caused by mtDNA mutation-elicited oxidative stress is involved in the onset of diabetes in patients with mitochondrial diseases warrants further investigation.

On the other hand, in addition to OXPHOS deficiency, several metabolic defects are also frequently observed in some of the patients with mitochondrial diseases. For example, the lipid storage myopathy is commonly found in patients caused by a decrease in β-oxidation of fatty acids [154]. Besides, the defects in TCA cycle have also been observed in some patients with mitochondrial disease, which may lead to an increase of intermediates of TCA cycle such as α-ketoglutarate, succinate, fumarate, and malate [155]. Most importantly, Hirschey *et al.* [156] demonstrated that a loss of Sirt3 could lead to a decline in the enzymatic activity of long-chain acyl-CoA dehydrogenase (LCAD) and thereby decreasing β-oxidation of fatty acids, which then culminates in obesity, insulin resistance, and hyperlipidemia [133]. Therefore, Sirt3 deficiency may be associated with the above-mentioned metabolic defects in the affected tissues of patients with mitochondrial diseases. Additionally, the defects in pyruvate dehydrogenase complex (PDHC) have been observed in several patients with Complex I deficiency. The increased acetylation level of PDH was recently observed in a rodent model of hypertensive heart failure as compared with those of controls [157]. PDH catalyzes the conversion of pyruvate to acetyl-CoA, thereby promoting the entry of pyruvate into TCA

Table 2. Clinical trials of drugs for the treatment of human diseases with mitochondrial dysfunction.

Drug	Disease	Clinical trial (phase)	Dosage (daily)	Identifier	Sponsor
Resveratrol	Type 2 diabetes	I	500 mg	NCT01677611	Kroc Teckphat Hospital, Singapore
	Friedreich ataxia	II	500 mg	NCT01339884	Murdoch Childrens Research Institute, Australia
	Cardiovascular disease	II	350 mg	NCT01449110	National Research Council, Spain
	Obesitas/ insulin resistance	III	120mg/500mg	NCT01158417	Kalendar Health, USA
	Alzheimer's disease	II	500 mg	NCT01504854	Alzheimer's disease Cooperative Study, USA
	Metabolic syndrome	II	N.A.	NCT00654667	University of California, USA
SRT501	Type 2 diabetes	I	N.A.	NCT00920803	GlaxoSmithKline, UK
SRT2104	Skeletal muscle atrophy	I	N.A.	NCT01039909	GlaxoSmithKline, UK
	Type 2 diabetes	II	N.A.	NCT01018017	GlaxoSmithKline, UK
	Type 2 diabetes	II	20 mg	NCT00937326	GlaxoSmithKline, UK
Bezafibrate	Fatty acids oxidation defect	I	N.A.	NCT00983788	Rigshospitalt, Denmark
Ketogenic diet	Epilepsy	IV	N.A.	NCT00552526	Oslo University Hospital, Denmark
	Refractory status epilepticus	II	N.A.	NCT01796574	Johns Hopkins University, USA
	ALS	III	N.A.	NCT01016522	Institute of Child Health, USA
L-carnitine	Heart failure	II	1 g	NCT01580553	Lee's Pharmaceutical Limited, China
	Skeletal muscular atrophy	II	1 g	NCT00227266	University of Utah, USA
CoQ_{10}	Type 2 diabetes	II	N.A.	NCT00703482	Solvay Pharmace, Australia
	ALS	II	2.7 g	NCT00243932	Columbus University, USA
	Mitochondrial disease	III	400 mg	NCT00432744	University of Florida, USA
	Metabolic syndrome	IV	N.A.	NCT01087632	Federico II University, Italy
	Huntington's disease	II	2.4 g	NCT00608881	Massachusetts General Hospital, USA
	Parkinson's disease	II	2.4 g	NCT00180037	Dresden University of Technology, Germany
$MitoQ_{10}$	Parkinson's disease	II	40-80 mg	NCT00329056	Antipodean Pharmaceuticals, Inc., New Zealand and Australia

N.A. indicates not available.

cycle. PDH deficiency was reported to affect the aerobic metabolism due to reduced pyruvate utilization [158]. Therefore, it needs to be further investigated as to whether increased acetylation of PDH impairs its enzyme activity and thereby promotes the pyruvate accumulation in patient cells with Sirt3 deficiency. Taken together, the above-mentioned findings support the notion that overproduction of ROS by cells in the pathological state could lead to dysregulation of the acetylation of specific mitochondrial proteins and impairment of the global metabolism, which may be an ultimate consequence of the suppression of Sirt3 mediated by oxidative stress-induced signaling pathways (Table **1**).

7. THERAPEUTIC TARGETS FOR METABOLIC MODULATORS IN MITOCHONDRIAL DISEASES

Current treatment of mitochondrial diseases varies considerably. Clinical goals of mitochondrial disease therapy are to enhance the energy yield and reduce ROS production and thus improve or delay the symptoms of the disease. Compromised energy metabo-

lism due to defects in the OXPHOS system plays a critical role in the pathogenesis of mitochondrial diseases and neurodegenerative disorders. The major therapies have focused on the way to increase mitochondrial biogenesis and/or energy production, with an attempt to halt disease progression and improve the function of affected tissue cells of patients. Accumulating experimental evidence supports the strategy to treat mitochondrial diseases by enhancement of mitochondrial biogenesis and metabolic activity through pharmaceutical modulation of PGC-1α. Besides, compounds acting on the Sirt1 pathways may hold great promise for the development of novel therapies for treatment of mitochondrial diseases. These therapies involve the manipulation of the intake of nutrients and the administration of compounds (e.g., resveratrol) that stimulate mitochondrial biogenesis and/or increase the expression of Sirt1 and PGC-1α, respectively, in the targeted tissues (Table **2**).

7.1. Pharmaceutical Activators of PGC-1α

Upregulation of PGC-1α stimulates the function of the OXPHOS system in cells harboring mtDNA mutations and may thus provide therapeutic benefits to the patients with mitochondrial diseases. Activation of PPARγ/PGC-1α-mediated pathways has been shown to improve the clinical phenotype of mitochondrial myopathy in mice with OXPHOS deficiency [131, 159]. Overexpression of PGC-1α was shown to be able to prevent bioenergetic deficiency and delay the progression of early-onset COX deficiency in skeletal muscle of the transgenic mice [159]. The potential in the design of compounds targeting PGC-1α may pave a new avenue for the development of therapeutic agents for treatment of patients with diseases caused by mitochondrial dysfunction. Bezafibrate, a well-known PPAR-panagonist, was found to ameliorate the muscle pathology by increasing the ATP level and mitochondrial biogenesis in the skeletal muscle of mitochondrial *COX10* knockout mice [160]. A recent study showed that long-term treatment of bezafibrate could improve some of the aging phenotypes in the mice harboring a POLG gene mutation and exhibited mitochondrial dysfunction [131]. Bezafibrate has also been shown to increase the expression and activity levels of mitochondrial respiratory enzymes in human skin fibroblasts with respiratory chain deficiencies [161]. Treatment of patients with bezafibrate has shown beneficial effects on tissues undergoing chronic degeneration or mitochondrial dysfunction, which has underscored the potential of using bezafibrate as a promising pharmaceutical agent for treatment of diseases caused by mitochondrial dysfunction.

7.2. Resveratrol as a Mitochondrial Nutrient and Activator

Resveratrol is a natural polyphenol primarily found in red wine, which has been proved to have antioxidant activity and has emerged as a probable Sirt1 activator. This compound has attracted increasing attention due to its health benefits through diverse physiological and biochemical actions, especially its effect in the protection against oxidative stress. Resveratrol was firstly linked to the cardioprotective effect of red wine and was subsequently shown to prevent or slow down the progression of several aging-related diseases, especially neurodegenerative disorders, cardiovascular diseases and type 2 diabetes [162-164]. The pharmacological effect of resveratrol on the extension of life span and delay of the age-related phenotype were demonstrated in several animal studies [165-167]. Accumulated evidence has suggested that the beneficial effect of resveratrol depends on the activation of Sirt1-mediated pathway and AMPK activation, respectively [168, 169]. On the other hand, a number of animal studies suggested that resveratrol possesses anti-diabetic activity and that its administration to the rats and mice can protect against metabolic diseases [170-172]. Indeed, by the treatment of mice with resveratrol, the increase of aerobic metabolism and mitochondrial biogenesis are related to the Sirt1-regulated deacetylation and activation of PGC-1α. These effects could also improve the diet-induced obesity, insulin resistance, and diabetic phenotype [171, 172]. Since it is well known that mitochondrial dysfunction is often a causative factor in insulin resistance and type 2 diabetes, there have been intensive studies on the potential use of resveratrol for the treatment of metabolic diseases and aging-related diseases (Table **2**). On the other hand, in the field of neurodegenerative diseases, resveratrol has been shown to exert beneficial effects in an animal model of Alzheimer's disease (AD) [163]. Results from epidemiological studies indicate that mild consumption of red wine can protect the human subjects from AD. In cell-based assays, resveratrol has been validated as a neuroprotective agent that can shield mammalian cells from diverse toxic stimuli via its antioxidant effect. It has been shown that AD mice given resveratrol displayed a high level of deacetylated PGC-1α, diminished hippocampus neurodegeneration and prevented learning impairments [173]. Besides, the protective effect of resveratrol was also observed in patients with Parkinson's disease (PD) [174]. In addition, administration of resveratrol to the PD mice could prevent the animal from neurotoxicity and attenuated the loss of midbrain dopaminergic neurons, which suggests possible therapeutical effect in the treatment of PD patients with resveratrol [175]. Likewise, a similar neuroprotective benefit of resveratrol was recently shown in Huntington's disease (HD) transgenic mice [176, 177]. Moreover, rodents fed with a diet supplemented with resveratrol were found to exhibit PGC-1α activation and prevention against the diseases frequently associated with mitochondrial dysfunction. In fact, a clinical trial to test the benefits of resveratrol in AD has been registered [178]. Although no clinical trials have been completed on patients with primary mitochondrial disorders, the above-mentioned studies of the effects of resveratrol on OXPHOS defect-related diseases have revealed a therapeutic potential of the supplementation of resveratrol in the diet for the patients with mitochondrial diseases.

7.3. Sirt1 Agonists

In light of the beneficial effect of resveratrol on laboratory animals, there has been increasing interest in the development of small Sirt1 activators as the potential therapeutical agents for treatment of aging-related disease and metabolic diseases. Sirt1 agonists contain the natural polyphenolic compounds such as resveratrol and quercetin, and the synthetic small molecules. Since natural compounds cannot have a high induction of Sirt1 activity [179], some synthesized molecules are among the most represented compounds selected for the activation of Sirt1. Currently, several molecular agonists of Sirt1 have been undergoing clinical trials, including SRT2104, SRT501, and SRT1720 [180-182]. The therapeutic efficacy of SRT1720 has been verified in the treatment of metabolic disorders in animal models, which showed that the diabetic phenotype was improved [182]. The two other agonists, SRT501 and SRT2104, have also been demonstrated to be effective in the treatment of type 2 diabetes [180, 183]. SRT501 has entered phase II clinical trials, and the oral supplemented doses of 1.25 or 2.5 mg per day for the human was safe and well tolerated [181]. Besides, the phase II clinical trial of SRT2104 in adult diabetic subjects has been finished. Moreover, the newly synthesized dihydropyridine derivatives were demonstrated to be able to prevent human cells from senescence and improve mitochondrial function of C2C12 myoblasts through the activation of PGC-1α [184]. Therefore, current pharmacological development of active agonists of Sirt1 may lead to the development of new drugs for treatment of diseases related to mitochondrial dysfunction.

7.4. L-Carnitine

L-Carnitine plays an important role in the metabolism involving β-oxidation and esterification of fatty acids. It can efficiently transfer long-chin fatty acids across the mitochondrial membrane as acylcarnitine esters, which are oxidized to acetyl-CoA used by the TCA cycle to generate ATP. L-Carnitine homeostasis in the human body is maintained by the acquisition of carnitine from dietary sources, a modest rate of endogenous carnitine biosynthesis, and

efficient renal reabsorption. In the skeletal and cardiac muscles, carnitine plays a pivotal role in the translocation of long-chain fatty acids into the mitochondrial matrix for subsequent β-oxidation. However, plasma levels of carnitine are frequently decreased in patients with the primary defects of the OXPHOS system. This may partially reflect an impairment of β-oxidation of fatty acids in some of the patients with mitochondrial diseases [185, 186]. Therefore, oral supplementation of carnitine for patients with mitochondrial diseases has been a common treatment [186, 187]. Carnitine administration has been shown to improve the bioenergetic function and oxidative capacity of mitochondria in patients with OXPHOS deficiency [188]. It is noteworthy that combinations of carnitine, vitamins, and some cofactors (e.g., α-lipoic acid) have been used to treat mitochondrial diseases [189-191]. A combination of acetyl-L-carnitine and α-lipoid acid as dietary supplements for rats has been proved to efficiently prevent defects of the oxidative function of mitochondria [189].

7.5. Effects of the Ketogenic Diet

The ketogenic diet has been used for years in treating children with seizures who are resistant to conventional antiepileptic drugs [192, 193]. It has also been reported to be successful in the adoption of the ketogenic diet for the control of epilepsy in the children with mitochondrial diseases [194, 195]. This diet has also been shown to have beneficial effect on some patients with mitochondrial disorders by optimizing the mitochondrial function [196, 197]. A previous study demonstrated that rats fed on a ketogenic diet can induce mitochondrial biogenesis in the muscle through increasing multiple respiratory enzymes and mtDNA replication and activate β-oxidation of fatty acids. Intriguingly, these effects were found to be associated with the upregulation of PGC-1α[198]. Accumulated evidence revealed that the increased energy metabolism *via* upregulation of mitochondrial biogenesis and induction of the antioxidant defense are involved in the neuroprotective effect of the ketogenic diet in mice with a large-scale deletion of mtDNA [199-202]. In mice with late-onset progressive mitochondrial myopathy, ketogenic diet has been shown to improve mitochondrial function in muscle fibers and halt disease progression [203]. Since some of the patients with mitochondrial diseases present secondary impairment of β-oxidation of fatty acids, it warrants further investigation to determine whether this dietary therapy is suitable for the treatment of this group of patients.

7.6. Supplementation of Coenzyme Q_{10} (CoQ_{10})

Oxidative stress plays an important role in the pathophysiology of most of the mitochondrial diseases. Thus, a reduction of the deleterious effects of ROS may have therapeutic effects for the ROS-related mitochondrial disorders. A large body of evidence supports the use of antioxidant supplements to dispose of the excess ROS in the affected tissues of patients with mitochondrial diseases [204]. Coenzyme Q_{10} (CoQ_{10}) is endogenously synthesized in mammalian cells, which is an integral component of mitochondrial electron transport chain. It shuttles electrons to Complex III from Complexes I and II as a mobile carrier of reducing equivalents. In addition to its role in the OXPHOS system, CoQ_{10} can participate in redox shuttling as a potent scavenger of free radicals [204]. Since the safety of CoQ_{10} has been well documented, it is one of the most commonly recommended metabolic treatments for patients with mitochondrial diseases. The main source of CoQ_{10} is intracellular biosynthesis *via* mevalonate pathway, although a small portion is usually acquired from the diet. However, the defects in CoQ_{10} biosynthesis were observed in some of mitochondrial diseases such as Leigh syndrome [205]. It has been reported that treatment of patients with CoQ_{10} could reduce the ROS-induced DNA damage and apoptotic cell death in the biopsies of patients with mitochondrial diseases, as well as restoring the function of the OXPHOS system [206, 207]. Our previous studies also showed that pre-treatment with CoQ_{10} can reduce the ROS production and UV- or H_2O_2-induced apoptosis in human cells harboring a pathogenic point mutation or large-scale deletions of mtDNA [33, 34]. The administration of CoQ_{10} in patients with sporadic CPEO or KSS syndrome and with mitochondrial encephalomyopathies has also been demonstrated to be beneficial [208, 209]. In addition, the clinical trial of CoQ_{10} in patients with neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease, showed that CoQ_{10} was rather effective in restoring the mitochondrial function and slowing down the progression of these diseases [210]. In clinical treatment of mitochondrial diseases, CoQ_{10} was used at a dose as high as 2,000 mg per day, and more than 2,400 mg was suggested for the adults who have low gastrointestinal absorption [211, 212]. On the other hand, a newly developed $MitoQ_{10}$ has been used as a mitochondrial target-antioxidant to specifically remove the ROS generated by mitochondria. By conjugating with triphenylphosphonium (TPP^+), the $MitoQ_{10}$ molecule has been used in a wide range of mitochondrial disease models to protect against oxidative damage [213]. TPP is the lipophilic cation moiety used to conjugate antioxidants, which can selectively target to mitochondria [214]. It has been shown that $MitoQ_{10}$ is able to target to mitochondria and improve mitochondrial function by efficiently scavenging the ROS. Dietary supplement of $MitoQ_{10}$ in animals could protect mitochondria from ischemia/reperfusion injury of the heart [215]. $MitoQ_{10}$ was demonstrated to protect against the development of hypertension and to reduce cardiac hypertrophy in a mouse model [216]. Recent studies also showed that $MitoQ_{10}$ can block the development of multiple features of the metabolic syndrome [217]. In a mouse model of AD, $MitoQ_{10}$ was shown to prevent the emergence of neuropathology [218]. Several lines of evidence have supported the use of mitochondria-targeting antioxidants as a potential therapeutic approach for the treatment of neurodegenerative diseases, including AD, PD and HD [219-221]. Although $MitoQ_{10}$ is now under clinical development, orally administered MitoQ can be useful for the treatment of a wide spectrum of human diseases that are associated with oxidative damage of mitochondria such as mtDNA mutation-elicited mitochondrial diseases. $MitoQ_{10}$ is a novel compound to attenuate mitochondria-specific oxidative damage and has the potential to become a new therapeutic agent for the treatment of mitochondrial diseases. Notably, there has been active research and development in the design of the Mito-vitamin E, Mito-TEMPOL, and Mito-NAC for the treatment of human diseases caused by mitochondrial dysfunction [222]. In light of the promising progress, the development of antioxidants targeting to mitochondria has been expected to yield productive outcome.

7.7. Nutrition Supplementation of other Redox Agents

Besides CoQ_{10}, there are other antioxidants under development for the therapy of mitochondrial disorders. Ascorbic acid is another important antioxidant used for the treatment of patients with mitochondrial diseases due to the fact that it can directly enter mitochondria in its oxidized form *via* glucose transporter 1 (Glut1) and thus protects mitochondria from oxidative injury [223]. Treatment of patients with MERRF or MELAS syndrome with ascorbic acid (1 g/twice per day) was reported to improve the medical complications and survive longer with less functional disability [224]. *N*-acetylcysteine (NAC) is another interesting therapeutic option. It is an *N*-acetyl derivative of cysteine and can increase the glutathione pool and subsequent antioxidant defense. In addition, pyruvate is one of the antioxidants that not only reduces the intracellular ROS level, but also boosts mitochondrial function through the activation of the pyruvate dehydrogenase complex (PDHC) by inhibiting the PDH kinase (PDK). It was recently reported that long-term administration of sodium pyruvate could effectively improve the exercise intolerance and restore mitochondrial function in the affected tissues of several patients with Leigh's syndrome [225]. Furthermore, other antioxidants including vitamin E, vitamin A, and α-lipoic acid were also shown to be effective in improving the bioenergetic function in various animal models and cultured cells from patients of

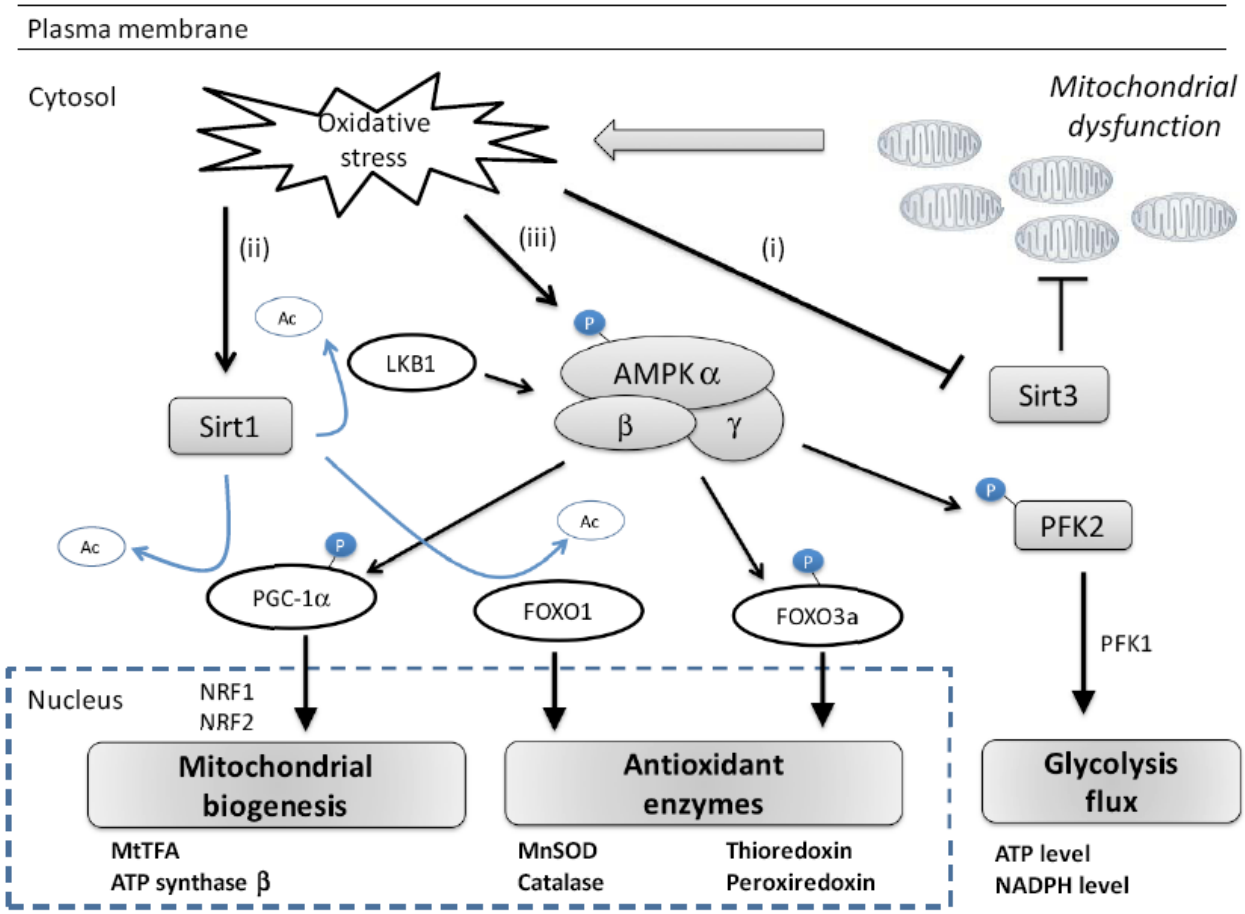

Fig. (1). Illustration of the molecular network involved in the regulation of cellular adaptation and response to mitochondrial dysfunction-elicited oxidative stress. The intracellular oxidative stress is increased in cells with mitochondrial dysfunction. (i) The increased oxidative stress can cause the decline of Sirt3 protein expression, a major regulator of mitochondrial protein deacetylation, which may further exert interference and inhibition of the mitochondrial functions including TCA cycle, β-oxidation of fatty acids, urea cycle, ketogenesis, and the OXPHOS system. (ii) However, the up-regulation of Sirt1 expression in cells is one of the responses to enhanced oxidative stress. By deacetylation and activation of FOXO1 and PGC-1α, Sirt1 can substantially boost the mitochondrial biogenesis (e.g., up-regulation of mtTFA and ATP synthase β, a subunit of mitochondrial Complex V) and increase the expression of antioxidant enzymes (e.g., up-regulation of Mn-SOD and catalase), respectively. In addition, the activated Sirt1 can deacetylate LKB1 to facilitate its intracellular translocation and ability to activate AMPK. The activated AMPK can phosphorylate PGC-1α and FOXO3a, which in turn upregulate the mitochondrial biogenesis and antioxidant defense system (e.g., up-regulation of the biosynthesis of thioredoxin and peroxiredoxin, respectively) in response to elevated oxidative stress. (iii) The activated AMPK can also increase the glycolytic flux by activation of PFK2 so that the affected cells with mitochondrial dysfunction can not only obtain ATP, but also replenish the intracellular NADPH pool via pentose phosphate pathway (PPP) to cope with the oxidative stress. Taken together, we suggest that the activation of Sirt1 and AMPK plays a crucial role in the upregulation of mitochondrial biogenesis, antioxidant enzymes and glycolytic flux, respectively, in the cellular response to the oxidative stress elicited by mitochondrial dysfunction.

mitochondrial diseases. Vitamin A could be a beneficial therapeutic option in mitochondrial disorders due to the recent finding that retinol, the most common dietary form of the vitamin, was a key regulator of mitochondrial function *in vitro* [226]. However, the clinical benefits of these micronutrients with antioxidant activity need to be substantiated by well-designed clinical trials. Recently, a supplemental cocktail containing vitamins, cofactors, ascorbic acid, and CoQ_{10} has been developed for treatment of mitochondrial diseases [227]. Clinical trials of CoQ_{10} with other antioxidants have been conducted to treat some of the patients with mitochondrial diseases and neurodegenerative diseases, respectively. A recent study showed that the capacity of ATP synthesis in the lymphocytes from patients with diverse OXPHOS defects was significantly increased after treatment for 12 months with a mixture of CoQ_{10} (350 mg daily), vitamin C, L-carnitine, vitamin B complex, and vitamin K_1 [228].

8. CONCLUDING REMARKS

Although compromised energy metabolism and accumulation of ROS play critical roles in the pathophysiology of mitochondrial diseases, the molecular mechanisms underlying the onset and progression of pathological changes in affected tissues cells caused by the pathogenic mtDNA mutation have not been fully understood. The mitochondrial dysfunction-elicited ROS production axis forms a vicious cycle, which not only impairs the bioenergetic function of mitochondria but also disturbs Ca^{2+} homeostasis and redox status in the affected cells harboring a pathogenic mtDNA mutation. Many lines of evidence have supported the crucial role of mitochondrial ROS in the regulation of adaptative cellular response to changes of physiological and environmental conditions. In this review, we report that oxidative stress elicited by mitochondrial dysfunction can trigger an array of adaptive responses, which include the upregulation of antioxidant enzymes, metabolic reprogramming,

and abnormal mitochondrial proliferation to confer advantages for cells to survive (Fig. **1**). In addition, we summarize the recent findings that AMPK and Sirt1 are involved in the modulation of the response of human cells to oxidative stress. Although human cells can undergo metabolic switch to obtain ATP through glycolysis when aerobic metabolism is compromised, the mitochondrial biogenesis is usually up-regulated as a compensatory response but the increase in the number of mitochondria might make only little contribution to the bioenergetic outcome. On the other hand, in contrast to the response of Sirt1, Sirt3 has been shown to be down-regulated in response to oxidative stress and consequently leads to an impairment of the OXPHOS system. This decline in the expression of Sirt3 can influence the global acetylation status of mitochondrial enzymes, which may lead to defects of β-oxidation of fatty acids and accumulation of some metabolic intermediates in the affected tissues of patients with mitochondrial diseases. The above-mentioned observations suggest that a defect in this regulatory network of response to oxidative stress may initiate the vicious cycle of mitochondrial damage and contribute to the disease-related pathologies in patients with mitochondrial diseases (Table **1**). One of the important advances in studies of mitochondrial diseases is the development of natural and synthetic compounds that have been proved to be able to improve the biogenesis or respiratory function of mitochondria in affected cells. Most of these compounds have antioxidant activities, and some of them have entered clinical trials, and some trials have yielded promising results. Therefore, the findings of recent studies have substantiated the idea that increasing the efficiency of the oxidative stress response and supplementation of mitochondria-targeting antioxidants may provide a novel strategy to slow down the progression of diseases and to alleviate the symptoms of the patients (Table **2**). A better understanding of the molecular mechanism underlying the oxidative response in the reprogramming of cellular metabolism may guide us to develop novel therapeutic strategies using metabolic modulators for future treatment of human diseases caused by mitochondrial dysfunction.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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