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Mitochondrial DNA, mitochondrial dysfunction, and cardiac manifestations

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1. ABSTRACT

Mitochondria, the powerhouses of cells, have their own DNA (mtDNA). They regulate the transport of metabolites and ions, which determine cell physiology, survival, and death. Mitochondrial dysfunction, including impaired oxidative phosphorylation, preferentially affects heart function via imbalance of energy supply and demand. Recently, mitochondrial mutations and associated mitochondrial dysfunction were suggested as a causal factor of cardiac manifestations. Oxidative stress largely influences mtDNA stability due to oxidative modifications of mtDNA. Furthermore, the continuous replicative state of mtDNA and presence of minimal nucleoid structure render mitochondria vulnerable to oxidative damage and subsequent mutations, which impair mitochondrial functions. However, the occurrence of mtDNA heteroplasmy in the same mitochondrion or cell and presence of nuclear DNA-encoded mtDNA repair systems raise questions regarding whether oxidative stress–mediated mtDNA mutations are the major driving force in accumulation of mtDNA mutations. Here, we address the possible causes of mitochondrial DNA mutations and their involvement in cardiac manifestations. Current strategies for treatment related to mitochondrial mutations and/or dysfunction in cardiac manifestations are briefly discussed.

2. INTRODUCTION

Mitochondria are extremely specialized organelles that generate adenosine triphosphate (ATP) via the electron transport chain (ETC) and oxidative phosphorylation system (OXPHOS). They are essential for maintaining energy homeostasis in cardiomyocytes, and constitute approximately 30% of the volume of the heart (1, 2). A large amount of ATP is supplied through mitochondria daily to maintain the normal contractile function of the heart (1). In biological systems, turnover of all cellular components is a continuous process and consumes about half of the total energy provision. When the energy supply from mitochondria is limited, the infrastructure of cells gradually disintegrates and eventually cell death occurs (1). The respiratory chain system is composed of over 100 different proteins but only 13 of them are encoded by mitochondrial DNA (mtDNA), which consists of circular, double-stranded DNA (1, 3, 4). Unlike the nucleus, the cell may contain several thousand mitochondria, and each mitochondrion contains 1–10 copies of mtDNA (5, 6). Mitochondrial morphology is dynamic, and variation in the size and appearance of the cristae can be seen depending on the cell type. Mitochondria form highly dynamic networks and changes in their structure and distribution that are directed by the fusion or fission process are largely associated with metabolic function (7-10). Experimental

data suggest that further study of mitochondria is essential for understanding both healthy life and many pathological conditions since numerous biological functions, including respiration and ATP delivery, biosynthesis of amino acids and nucleotides, production of prosthetic groups such as the iron-sulfur cluster and heme, metal homeostasis, and stress signaling and defense responses, are associated with mitochondrial functions (2, 11-13). Therefore, impairment of mitochondrial functions due to primary causes such as mutations in mtDNA or secondary damage from oxidative stress results in cellular dyshomeostasis and is a prototype of mitochondrial diseases (14-19). However, the number of mitochondrial diseases may increase due to nuclear mutations affecting subunits of the ETC complex, defective mtDNA maintenance or replication, and impairment of metabolism (1, 16, 20).

It has been suggested that oxidative stress is a strong inducer of mtDNA mutations (15, 21-24). Increased production of reactive oxygen species (ROS) and their active metabolites over the threshold level induces oxidative stress, resulting in oxidation of proteins (25) and lipids (26), DNA damage (27, 28), and/or accumulation of dysfunctional organelles; these are well-established hallmarks of cardiovascular diseases, neurodegenerative diseases, and age-related complications. Oxidative stress can be practically defined as a disequilibrium between oxidants and antioxidants that favors oxidants, leading to disruption of redox signaling (29, 30). Paradoxically, short-term oxidative stress may play an important role in prevention of aging-associated complications (31), evoking protective signaling by ischemic pre- and post-conditioning (32) and participating in redox signaling to control mitochondrial function (30). Free radicals could increase the rate of mutations such as base changes and oxidative modifications of mtDNA (24, 33, 34). mtDNA oxidation also spontaneously occurs due to ROS induced as byproducts in the mitochondria during respiration (33). Tissue- or cell-damaging conditions such as hypoxia-reoxygenation could produce mtDNA oxidation in animal models as well (35, 36). The free radical theory of aging suggests a central role for the mitochondrion in normal mammalian aging, but recent works challenge the importance of mitochondrial ROS in the progression of aging (37). Recent findings showed that G→T mutations in mtDNA, which is believed to be a distinctive characteristic of oxidative damage to DNA during aging, do not significantly increase with age (38-40). Moreover, mice expressing error-prone mitochondrial DNA polymerase γ (POLγ) showed accumulation of substantial burdens of mtDNA mutations that shortened the life-span without remarkable oxidative stress (41-43). In addition, it has been suggested that the concept of mtDNA damage and oxidative stress leading to atherosclerosis may need to be revised due to low correlation between oxidative stress-mediated mtDNA damage and atherosclerosis (39, 40). In addition to mtDNA mutations (Figure 1), decrease or loss of mtDNA copy number (or content) plays a role across a range of prevalent human diseases, including cardiovascular disease and aging (44, 45). Nuclear DNA mutations can largely influence mitochondrial OXPHOS since complexes of the mitochondrial ETC are constituted by both genomes (Figure 2). Although there is strong agreement that mitochondrial energy metabolism, oxidative stress, ROS production, and cell death are highly involved in neurodegeneration or cardiovascular defects, the primary defect and the definitive cause-and-effect relationship leading to the particular pathology are unclear and require in-depth analysis.

Cardiac manifestations may be in the form of structural defects, functional lesions, or both (41, 46). Among the cardiac structures that can be affected, it is the myocardium that is affected the greatest (46, 47). Functional lesions such as conduction abnormalities, systolic dysfunction, arterial hypertension, and heart failure are frequently associated with structural abnormalities. Metabolic changes in the heart have emerged as key mechanisms involved in the development and progression of pathological remodeling. Recently, it has been increasingly recognized that primary and secondary mitochondrial disorders are closely associated with cardiac defects (46, 48-52). Therefore, understanding common cardiac abnormalities based on the mitochondrial background and its clinical presentation will be important to achieve better clinical outcomes (53).

In this review, we briefly summarized the impact of oxidative stress on mtDNA modifications originating intracellularly or from mitochondrial dysfunction. In addition, mitochondrial DNA mutations and the importance of notification to intervene the cardiac manifestations were briefly addressed. We presented the current view that mtDNA mutations are not merely dependent on oxidative stress but complex regulatory mechanisms are also involved in accumulation of mtDNA mutations and its final outcome of cardiac manifestations. Current strategies for treatment related to mitochondrial mutations and/or dysfunction in cardiac manifestations have been briefly discussed. However, detailed discussion of human genetic disease related to mtDNA mutations and numerous genes involved in mitochondrial disorders caused by nuclear DNA was omitted due to space limits and the scope of the current review. Aberrant activation of immune systems can influence the dynamics of mitochondria, but this issue has also not been discussed in this review.

3. MITOCHONDRIA AND THEIR MAINTENANCE

mtDNA possesses only 37 genes (Figure 1) but the mitochondrion contains at least 800–1500 proteins whose relative abundance varies between tissues (54-56). Almost all the protein components involved in transcription, replication, and protein synthesis

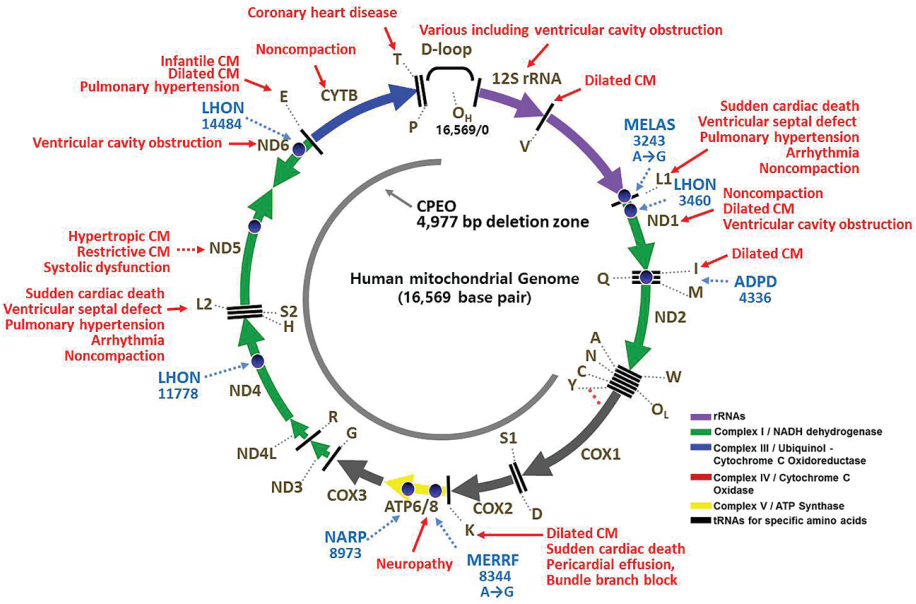

Figure 1. Human mitochondrial DNA (16,569 base pairs) and pathogenic mutations identified in mitochondrial DNA. Human mitochondrial DNA (mtDNA) codes for seven of the 43 subunits of complex I, one of the 11 subunits of complex III (cytochrome b, CYTB), three of the 13 subunits of complex IV (COX I, II, III) and two of 16 subunits of complex V (ATPases 6 and 8). It also codes for two rRNAs and 22 tRNAs. mtDNA mutations associated with known human genetic diseases and cardiac manifestations are marked as blue (broken arrow) and red (solid arrow), respectively. Mutations in tRNA genes of mitochondrial DNA are frequently found in cardiac manifestations and cause predominantly myopathic phenotypes. Deletion in a large portion of mtDNA sequences results in various forms of cardiomyopathies, ventricular septal defect, dilation of the aortic root, or pulmonary hypertension. ADPD, late-onset *alz*heimer's disease; COX, cytochrome C oxidoreductase; CPEO, chronic progressive external ophthalmoplegia; LHON, Leber's hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers; NARP, neuropathy, ataxia, and retinitis pigmentosa; ND1-6, component of rotenone-sensitive NADH-ubiquinone oxidoreductase (complex I); O_H, heavy-strand origin of replication; P, promoter of heavy and light strands.

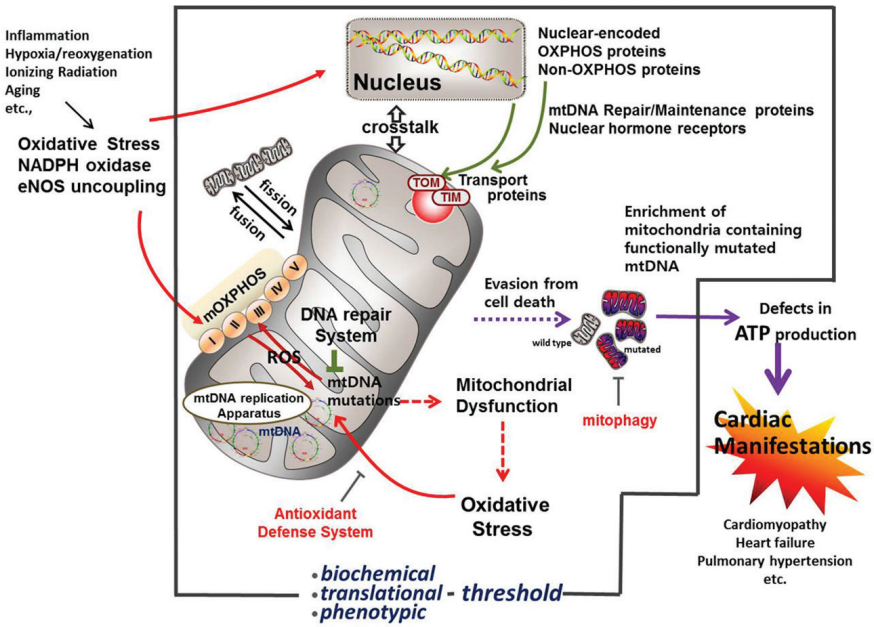

Figure 2. Mitochondrial dysfunction and cardiac manifestations. Mitochondrial DNA (mtDNA) damage can be induced by oxidative stress and/or failure in maintenance of mtDNAs. Mitochondrial dysfunction, whether caused by mutations of mtDNA or nuclear DNA, could further deteriorate mitochondrial health and result in pathological outcomes. However, the segregation of mutations and the tissue-specific thresholds, which represent biochemical, translational, or phenotypic tolerance, are important in determining the clinical features observed, but other unknown factors must also play a significant role. eNOS; endothelial nitric oxide synthase; NAPDH oxidase, nicotinamide adenine dinucleotide phosphate-oxidase; OXPHOS, oxidative phosphorylation complex.

in mitochondria are encoded in the nucleus (Figure 2) and are transported into the mitochondria subsequent to their synthesis in the cytoplasm via the transporter inner membrane (TIM)/transporter outer membrane (TOM) and other transporter proteins (1, 57, 58). It is noteworthy that the majority of mtDNA-encoded proteins are extremely hydrophobic peptides, and thus those proteins would not be easily imported by the mitochondrial translocation machinery (59). The maintenance of mitochondrial function requires a fine and subtle balance between continuous protein synthesis and degradation. A metabolic labeling study showed that, in general, most mitochondrial proteins are quite stable over prolonged periods of time, depending on tissue specificity (60). In the rat heart, it is assumed that one mitochondrion per cell is replaced every 40 min and the half-life is 14 days (60, 61); furthermore, this turnover of mitochondria may be related to the circadian cycle (62, 63).

Mitochondrial biogenesis includes mtDNA replication, transcription of mitochondrial RNA, translation of mitochondrial mRNAs, and import of both nuclear-encoded proteins and tRNAs into mitochondria (Figure 2). In addition, mitochondria contain several proteases for controlling protein turnover in the mitochondria to match the need for removal of defective peptides such as misfolded proteins and control gene expression in response to various cellular signals (1, 64). The mtDNA in the cell may be heterogenous. mtDNA in mitochondria is not naked but is packaged into bacterial nucleoid-like structures (65), and thousands of mtDNA molecules are organized in several hundred nucleoids (11, 64-66). At least 21 proteins, including mitochondrial transcription factor A (TFAM), mtSSB (single-stranded DNA-binding protein), and twinkle (a mitochondrial DNA helicase), are involved in the building of nucleoid structure (64, 65). Owing to the characteristics of mitochondrial nucleoids, the proportion of mutant to normal mtDNA can shift during the phase of segregation (67) and shows heteroplasmy, which is defined as the presence of at least two populations of mtDNA molecules such as normal (wild type) and mutated DNA, ranging from barely detectable to 100% of cells (1). In mitochondrial DNA replication, POLγ, mtSSB, and twinkle are minimal components of the mtDNA replisome (64). Unlike nuclear DNA synthesis, which is highly dependent on the cell cycle, the pattern of mtDNA synthesis displays less strict phase specificity (64, 68). During the transcription of mtDNA, mitochondrial RNAs are produced from polycistronic transcripts and undergo processing and modifications to produce mature mRNA (64). The abundance of each mRNA, rRNA, and tRNA is not equal, possibly due to further post-transcriptional modifications that affect transcript processing, maturation, stability, and degradation (69). Although there are many questions regarding the exact molecular machineries for replication and transcription of mtDNA, which are coupled events (3, 64, 69), the proper balance between mtDNA replication, mitochondrial dynamics, mitophagy, and mitochondrial biogenesis ensures continuous turnover of mtDNA. The importance of mitochondrial genetic information is stressed by the fact that mitochondria preserved very complex and unique machinery to maintain and express the content of mtDNA. Moreover, it has been speculated that proper maintenance of the mitochondrial genome warrants functional mitochondria and further contributes to nuclear genome stability (12, 18).

In order to maintain a healthy mitochondria, several quality control pathways function in the mitochondria (9, 62, 70, 71). As depicted in Figure 2, these quality control systems monitor mitochondrial integrity via antioxidants, DNA repair systems, and mitochondrial unfolded protein response systems, which are involved in sensing or removal of aggregated or misfolded proteins (72). Unlike the nucleus in postmitotic cells, mitochondria are dynamic, in part because they are constantly undergoing fission, which may be necessary to increase the number of mitochondria, and fusion reactions, which may be involved in the exchange of mtDNA to complement deficits in mitochondria carrying mutated genomes (73). In addition, the fission and fusion processes of mitochondria cause dynamic exchange of components, including mtDNA (70). Thus, mitochondrial fission and fusion are intimately linked and determine mitochondrial morphology. Impairment of this regulatory process in cells may contribute to age-associated accumulation of mtDNA damage. Excessive stress will induce mitophagy and ultimately lead to cell death if not recovered (70). Mitophagy is a specialized autophagic process that also involves lysosomes and leads to degradation of dysfunctional mitochondria through at least two pathways, NIX-based programmed mitophagy and PTEN-induced putative kinase 1 (PINK1)–mediated selective mitophagy (74, 75). Together, these mitochondrial quality control systems form a network that is involved in regulation at the molecular, cellular, and organelle levels (70, 74). Mechanistic insights into the fission/fusion and mitophagic processes would involve discussion of a highly complicated pathway, and a detailed description of this pathway is beyond the scope of the current review. Mitochondria are likely to be fundamental players in regulating the redox state through modulation of NAD^+/NADH or GSH/GSSG ratios, and thus histone chromatin remodeling and gene expression are influenced by mitochondrial metabolites such as NAD^+ (76, 77). This event is an example of direct crosstalk between mitochondria and the nucleus (12, 13). It has also been suggested that mitochondrial proteins could direct signals to the nucleus to regulate gene expression (12, 78); there are several nuclear transcription factors such as p53 and nuclear hormone receptors, found in both the nucleus and mitochondria, and this kind of crosstalk is necessary to cope with stress and control cell survival and growth (79). Furthermore dual mitochondrial and nuclear localization of the same

proteins represent a widespread regulatory circuit for maintaining mitochondrial homeostasis (78).

4. MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS

In mitochondria, two types of electron transfer are suggested: one is involved in metabolism (high-flux electron transfers) and the other is involved in signaling and metabolic control (low-flux pathways involving protein cysteine or methionine residues, i.e. glutathione, thioredoxin and cysteine/cysteine) (27). Dysfunction of mitochondria can induce leakage of electrons from the ETC and this further augments oxidative stress both in mitochondria and intracellular molecules. Experimentally, mitochondrial dysfunction could be characterized by inhibition of oxygen consumption, collapse of membrane potential, and reduction in ATP (80). As depicted in Figure 2, it is hypothesized that mitochondrial dysfunction can be introduced by perturbations in (a) proteins involved in the formation of respiratory chain complexes; (b) proteins involved in the transport of other proteins into mitochondria; (c) proteins involved in mitochondrial protein synthesis, proper protein folding, prosthetic group preparation, and mounting or functional complex maturation; (d) proteins involved in the delivery of necessary substrates; (e) proteins preventing macromolecule damage during cellular stress conditions; (f) and proteins involved in mitochondrial biogenesis and mtDNA maintenance (11, 19, 81, 82). Factors such as the following would also cause mitochondrial dysfunction: inflammatory mediators such as tumor necrosis factor α; dietary deficiency of vitamins, minerals, or other metabolites; toxic metals such as mercury; mitochondrial toxins such as rotenone or cardiotoxic drugs (83, 84); and disablement of the oxidant-detoxification system (80). The cause-and-effect relationship between mitochondrial dysfunction and oxidative stress has not been completely elucidated. In some cases, the lack of major benefits in intervention trials with ROS scavengers has been reported (85, 86). The final stage of mitochondrial dysfunction induces depolarization of mitochondrial membrane potential and a burst of mitochondrial oxidative/nitrative stress, eventually leading to cell death (87, 88).

5. OXIDATIVE STRESS, mtDNA MUTATIONS, AND mtDNA REPAIR SYSTEM

Damage to mtDNA and lack of repair of mutated DNA may lead to mitochondrial dysfunction and cell death. The mtDNA copy numbers in specific cells reflect the status of mitochondrial biogenesis, but the exact mechanism underlying the control of mtDNA copy number is still obscure. Human diseases caused by excess mtDNA copy number are rare (17), and much attention has been focused on the putative role of depletion of mtDNA and the complex status of mtDNA mutations associated with mitochondrial dysfunction. For example, large deletions of mtDNA, such as rho$^-$ or rho^0, lead to a complete lack of mtDNA and thus every necessary ETC proteins cannot be synthesized (89). Disease-based epidemiology studies estimate the population prevalence of mtDNA disease at ~1:5000; heteroplasmic mtDNA mutations are found in 1:200 of newborns (90). As depicted in Figure 1, mutation of mtDNA and the accumulation, and segregation of mutated mtDNA may be broadly associated with oxidative stress. However, oxidative stress would not be the sole cause of mitochondrial mutations and subsequent dysfunction of mitochondria since the mutations produced by oxidative modification could be repaired to some extent by the mtDNA repair system and maintenance of mtDNA is almost completely dependent on nuclear-encoded proteins. In addition, removal of malfunctioning mitochondria is influenced by the mitophagic process. Eventually necrotic or apoptotic death of affected cells containing mutated mtDNA or dysfunctional mitochondria will decrease frequency of mutated mtDNA. Clinical manifestations of mtDNA mutations are very complex due to various phenotypic presentations (18). Thus, improved understanding of the quality control in mitochondria would provide greater opportunities for preventing or overcoming mitochondrion mutation/dysfunction-associated complications in humans.

5.1. Mitochondrial DNA damage

It has been suggested that at least three factors make mtDNA particularly vulnerable to mutations (91, 92): First, the mtDNA is localized close to the inner membrane, where free radicals are produced. However, recent findings suggest that mitochondrial nucleoids are not located close to the site of ROS production as expected (93). Second, mtDNA is not extensively condensed and protected by histones but is roughly organized with nucleoid-associated proteins such as the high mobility group (HMG) box family and mitochondrial transcription factor A (TFAM) (66). Thus, tissue-specific loss of TFAM is prone to cause cardiomyopathy, muscle weakness, Parkinson-like neuronal dysfunction, and diabetes (94). Third, mtDNA repair activity is limited as compared to that in the nucleus (95). Covalent modifications of mtDNA in the nucleus are readily detected by the DNA repair system, but some types of DNA repair cannot be found in mammalian mitochondria. Because of the continuous replicative nature of mtDNA, the frequency of mutation in mtDNA can be changed by clonal expansion, even in postmitotic cells, including cardiomyocytes, during cell division or renewal (68).

Oxygen is an essential component of eukaryotic aerobic metabolism and ROS such as oxygen radicals (superoxide (O_2^-) and hydroxyl radical (OH^-), as well as certain non-radical species (hydrogen peroxide and single oxygen)) are generated as byproducts of oxygen consumption, which occurs in mitochondria under normal

physiological conditions. Oxidative stress can directly modify the sugar moiety (2'-deoxyribose residue) and DNA nucleobases of mtDNA and leading to the formation of 8-oxo-7,8-dihydroguanine (8-oxoGua), 2,6-diamino-4-hydroxy-5-formamidopyrimidine, and 8-oxo-7,8-dihydroadenine (92). Strangely, high levels of 8-oxo-2'-deoxyguanosine (8-oxodG) were found in the mtDNA of mice null for oxoguanine DNA glycosylase (OGG1), which removes 8-oxoGua from DNA, but this OGG1 deficiency does not cause mitochondrial respiratory dysfunction in the heart and liver of mice (96). This finding may be connected to the fact that a large number of base changes in mtDNA are inert because of the absence of amino acid substitutions, conservative amino acid changes, or occurrence in noncoding regions of the mitochondrial genome, with the exception of specific promoters or replication origin sequences (1, 49). A frequent mitochondrial mutation (Figure 1) is the transition from G to A (http://www.mitomap.org); mitochondrial mutations include deletions, insertions, or substitutions affecting a single gene, consistent with misincorporation by POLγ (97) or deamination of cytidine and adenosine (38) as the primary mutagenic events in mtDNA abasic sites, and single- and double-strand breaks and 8-oxoguanine DNA adducts, the major mutagenic lesions generated by ROS (33). Metabolic disturbances such as imbalance of the deoxyribonucleotide triphosphate (dNTP) pool could indirectly exacerbate some misincorporation events (92). Spontaneous decay of the nucleic acid structure of mtDNA, such as depurination, and the impact of various types of ROS and products generated as a consequence (e.g. lipid peroxides, aldehydes and *S*-adenosylmethionine) could be involved in DNA modifications (34, 98-100) and cause blockage of transcription or replication, as well as point mutations in mtDNA. Deletions in mtDNA (Figure 1) are more frequently detected in nondividing tissue, such as the cardiac muscle and brain, rather than cells with a relatively short half-life, such as blood cells, and result in a mosaic pattern, as observed in histochemical staining. Theoretically, two pathways have been suggested to be involved in the occurrence of mtDNA deletion (92): Deletions of mtDNA are generated during replication by slipped-strand homology in repetitive sequences, and deletion in mtDNA occurs during repair of double-stranded breaks (92). Irradiation (101, 102), chemical agents such as *t*-butyl hydroperoxide (103), and ultraviolet light can generate double-strand breaks which serve as critical DNA lesions that might lead to genome rearrangements (104-106). The molecular mechanism leading to deletion in mtDNA, particularly large-scale deletions varying in size from 1.3. to 8 kb, are still poorly understood (92) but these deletions are considered as a common cause of human mitochondrial diseases and are also considered to be involved in aging (1, 107-109).

5.2. mtDNA damage–repair system

Combined action of mitochondrial fission and mitophagy could provide an elegant, self-purifying mechanism for removal of defective mtDNA molecules from the cellular pool (95, 110). So far, there is no evidence that mutated mtDNA could be selectively degraded by any of the mitochondrial nucleases. When the extent of mtDNA damage exceeds the repair and buffering capacities of mitochondria leading to respiratory chain deficiency and mitochondrial dysfunction, mutated mtDNA should be deleted from the pool. Due to the highly oxidizing environment of the mitochondria even under normal conditions, the lagging strand of mtDNA is kept in the single-stranded condition for a prolonged time during mtDNA replication, and thus spontaneous mutations will easily develop. In mtDNA replication, there are increasing possibilities that misincorporation of bases or uncorrected mutations will be caused by POLγ due to its low fidelity or other factors. Several DNA repair systems were identified: direct reversal (111), base excision repair (92, 95), mismatch repair, nucleotide excision repair, and DNA double-stranded break repair (95). The major DNA repair pathway in mitochondria is excision repair, which includes the following: (a) Base excision repair (BER), which occurs in both the nucleus and mitochondria. This BER pathway incudes recognition and incision by DNA glycosylase glycosylases (OGG1; 8-oxoguanine DNA glycosylase (UNG); uracil DNA glycosylase (NTH1); endonuclease III-like 1 (NEILI1/2); Nei endonuclease VIII-like 1 and 2 (MUTYH); mammalian homolog of *Escherichia coli* MutY (MTH1); an 8-oxoGTPase present both in the nucleus and mitochondria), abasic-site processing by apurinic/apyrimidinic endonucleases, DNA synthesis, and then final ligation of single-strand nicks, conducted by POLγ, which resynthesizes missing DNA patches, and DNA ligase (LIG3), which seals the DNA fragments (73). (b) Post-replicative mismatch repair in base mismatches and 1–4 nucleotides insertion/deletion loops. (c) Recombinational repair system in double-strand breaks (92, 95). Therefore, the accumulation of mtDNA mutation is not only determined by the higher mtROS formation rates but also by the status of the mitochondrial DNA repair system (112). However, it is unclear whether oxidative stress could directly or indirectly affect the mtDNA repair system.

5.3. Inheritance of mitochondrial mutations

The mutation frequency of mtDNA is higher than that of nuclear DNA by several orders of magnitude (113, 114). Although this difference was initially attributed to increased mtDNA damage due to elevated concentrations of endogenous ROS produced as byproducts of oxidative phosphorylation (21, 41), many recent studies argue against any significant contribution of oxidative damage in accumulation of mtDNA mutations (34, 37). In line with this, dynamin related protein 1 (Drp1)–mediated mitochondrial fission and mitofusin (MFN) 1, MFN2 and optic atrophy factor 1 (OPA1) directed fusion process will contribute to the segregation of affected mtDNA (7, 8). Inheritance of mitochondrial DNA mutations is dependent on the

mutation type, such as only blockage of replication or possible propagation of a novel mutation (24, 115), and phenotypic manifestations take months or years (1). The distinction between variants with point mutations and variants with large deletions will be an important factor in inheritance of mitochondrial mutations (Figure 1). It is conceivable that mtDNAs with some deletions are more rapidly replicated and thus have a selective advantage in propagation compared to normal counterparts (92). This might be related to the fact that the fraction of deleted mtDNAs in muscle also increases with age and is also possibly higher in the brain (116).

6. MITOCHONDRIAL DISORDERS AND CARDIAC MANIFESTATIONS

The constantly beating heart is the organ with the greatest oxygen consumption in a resting body (117). Although mitochondrial disorders are extraordinarily diverse from the view of phenotypical presentation and genetic background (74), mitochondrial dysfunction associated with mtDNA mutation appears to play a role in the development of heart failure (41, 118-121). Severe exercise limitation is typical of mitochondrial cardiomyopathies with associated skeletal myopathy, and further investigation frequently reveals premature lactate acidosis during exercise (41). Various mtDNA mutations have been detected in heart tissue (Figure 1). The cardiac manifestations in mitochondrial disorders is gradually being recognized but their frequency, sex distribution, age at onset, affection of other organs, or affected cardiac tissue is diverse (41, 46, 49, 90). In protein-coding genes of mtDNA, the most frequently identified biochemical abnormalities are related to deficiencies of complexes I and IV (122). A large number of mutations in mtDNA genes have been mapped to mt-tRNA loci possessing a high frequency of point mutations, despite accounting for just 5–10% of the mtDNA (Figure 1). Furthermore, mischarging of mt-tRNAs occurs more frequently as a consequence of certain mutations and leads to a gain or loss of function of the protein involved (123, 124). Besides mtDNA mutations, impaired autophagic clearance of protein aggregates or deteriorating mitochondria will have multiple consequences, including increased arrhythmia risk, decreased contractile function, reduced tolerance to ischemic stress, and increased inflammation; thus autophagy represents a potentially important therapeutic target to mitigate the cardiac consequences of aging (125).

Cardiac manifestations in mitochondrial disorders includes cardiomyopathy, arrhythmia, heart failure, pulmonary hypertension, dilation of the aortic root, pericardial effusion, coronary heart disease, autonomous nervous system dysfunction, congenital heart defects, or sudden cardiac death with the syndromic or non-syndromic phenotype (41, 46, 48-50, 126, 127). Unlike mitochondrial disorders associated with neurodegeneration or neurological disorders that are largely noticeable (16, 111, 128), cardiac manifestations in mitochondrial disorders are currently underestimated (41, 46, 48) but these mitochondria-associated complications serve as an important predictor of morbidity and mortality (48). It should be stressed that tolerable mutations in mtDNA do not eventually lead to malfunction of mitochondria (Figure 2) because of the existence of normal mitochondria and the threshold in the context of mitochondrial deficit for the expression of the disease depending on tissue specificity (15). For example, in certain cases, the presence of approximately 10% of wild-type mtDNA is sufficient to maintain a normal respiratory rate and at least 80–90% mtDNA deletion is required for compromised complex IV activity (23, 128). In addition, it is recognized that the energy threshold of mitochondria initiating pathological changes is different from that in the original tissue (128). These complex behaviors of mtDNA mutations may create another hurdle in identifying mitochondrion-associated cardiac manifestations. In addition, the mitochondrial dysfunction noted in connection with cardiac manifestations is not limited to mtDNA mutations but is also related to nuclear DNA mutations and impairment of mitochondrial quality control system (Figure 2).

7. THERAPEUTIC APPROACHES FOR MITOCHONDRIAL DISORDERS

Mitochondrial disorders-associated cardiac manifestations are categorized as hereditary or sporadic disorders (129). Although interesting and often imaginative therapeutic strategies have been successful *in vitro*, therapeutic regimens for mitochondrial disorders with cardiac manifestations need more extensive development based on mitochondrial genetics and quality control system. As briefly outlined in Figure 3, treatment guidelines for mitochondrial disorders are generally based on supportive or complementary management of the symptoms rather than their genetic correction (41, 71, 81, 130, 131). First, mitochondrial biogenesis is considered as a set of molecular events by which cells replace or increase their mitochondria through the proliferation of pre-existing organelles, based on the activation of peroxisome proliferator activated receptor-g coactivator-1a (PGC-1α) and AMP-dependent kinase (41, 76, 132, 133). In addition, it is not yet clear but there is another attempt to regulate mitochondrial biogenesis through putative estrogen receptor (133, 134), glucocorticoid receptor (135), and thyroid receptor (71) binding sites located in mtDNA. It should also be considered that mitochondrial biogenesis can be engineered by exercise programs (136) and has been shown to be safe and beneficial for patients with mitochondrial disease (137). Second, interventional efforts for modulation of respiratory chain function have been extensively investigated in an experimental setting, and currently significant efforts are being

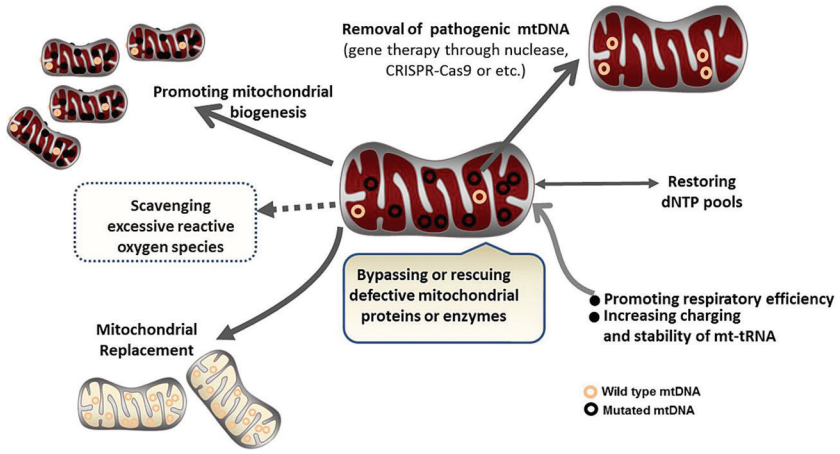

Figure 3. Therapeutic approaches for dysfunctional mitochondria. Replacement of aberrant mitochondria with healthy mitochondria is a promising strategy but is limited by the available techniques and poor understating of the complex behavior of mitochondrial genetics, which creates a hurdle for clinical development. Additional strategies include the following: (a) relieving metabolic stress and increasing mitochondrial biogenesis, (b) improving the efficiency of oxidative phosphorylation or the mitochondrial protein synthesis system, (c) restoring mitochondrial DNA (mtDNA) homeostasis or shifting mtDNA heteroplasmy, (d) rescuing defects of mitochondrially encoded proteins by recoding for nuclear expression and mitochondrial targeting. dNTP, deoxyribonucleotide triphosphate.

undertaken to translate these results to applications in humans (81, 133). The literature on the efficacy of these putative proliferators (e.g., bezafibrate; the polyphenols resveratrol, epicatechin, and ribonucleotide ribonucleotide 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR); coenzyme Q10 and its analogs) in ameliorating mitochondrial dysfunction is complex and often contradictory (133), and this topic requires further study. Bypassing the pathogenic mechanism through removal of noxious metabolites has also been attempted for correction of biochemical defects in mitochondria. Fox example, administration of dichloroacetate (DCA), which is a pyruvate dehydrogenase complex (PDH) kinase inhibitor, reduced lactate production in primary mitochondrial disorder and improved brain function (138). Third, many mitochondrial disorders are due to mutations in genes encoding mt-tRNAs (132), leading to a deficiency in mitochondrial protein synthesis and subsequent abnormality of oxidative phosphorylation (137). Therefore, screening of small molecules to identify compounds that can stabilize mt-tRNAs is worth investigating (19). Finally, gene therapy and strategies aimed to correct, bypass, and avoid the transmission of the molecular defect of mitochondria are gaining attention in recent years and the results are promising (121, 133). For the treatment of mtDNA-oriented disorders, the removal and/or prevention of continuous propagation of the mutated genome will be favorable to obtaining a therapeutic outcome (Figure 3). Trials are being performed on selective degradation of mutated mtDNA by using mitochondrial-targeted zinc finger nucleases (137, 139-141). These trials are focused on switching heteroplasmy through increasing the amount of wild-type mtDNA and thereby correcting the biochemical deficiency. In addition, rescue of the mitochondrial phenotype after lentiviral adeno-associated virus delivery of a wild-type copy of the candidate gene has been under investigation (19, 142). Recently, there has been considerable interest in the CRISPR-Cas9–mediated genome editing process in mitochondrial disorders (137), or carrier-free targeting of the mitochondrially importable RNA into living human cells was designed (143, 144). However, mitochondrial gene therapy is not simple since DNA or RNA molecules do not easily penetrate mitochondria *in vivo*. In clinical applications, there are great limitations regarding safe delivery of the therapeutic agent into mitochondria in an organ-specific manner (145). In addition, therapeutic interventions for mitochondrial diseases in cardiac manifestations should prevent death of cardiomyocytes and at the same time be efficient with respect to their effects on mutated mtDNA or malfunctioning mitochondria before reaching its limit in the affected cell.

8. CONCLUSION

Relatively less data are available on the pathogenesis of cardiac manifestations in relation to mitochondrial involvement, which may be related with mitochondrial dysfunction at various levels depending on the outcome of the mutated gene (46) and current limitations of diagnostic tools (Figure 1). Abnormalities in mitochondrial function that contribute or lead to human disease, especially in the heart, can be derived from several sources such as innate mitochondrial defects, inflammation, hypoxia-reoxygenation, ionizing radiation, or aging. Oxidative mtDNA damage would be a cause but not the sole determinant in producing mtDNA mutations. When the amount of mutated mtDNA exceeds the recovery potential of mitochondrial repair, mutated mtDNA accumulates in mitochondria. Moreover,

the majority of proteins involved in mitochondrial biogenesis and maintenance and mtDNA replication are controlled by nuclear DNA and are transported into mitochondria (Figure 2). Therefore, the rate of mitochondrial mutations can also be affected the by status of nuclear DNA and mitochondrial transport apparatus. The extent of involvement of oxidative stress in accumulation of mtDNA mutations and the cardiac phenotypic manifestations will need further validations through experiments. Currently, cardiac manifestations in mitochondria disorders are underestimated and causes of mitochondrial disorders are not clearly demonstrated. Therefore, exact identification of mitochondria-mediated cardiac manifestations is necessary. In addition, developments of clinically applicable strategies for controlling mitochondrial functions, correcting mtDNA mutations, controlling the biogenesis, and quality control systems will be promising in treating heart dysfunction or failure (Figure 3).

9. PERSPECTIVES

Both the nuclear and mitochondrial genome may be strongly connected to susceptibility to heart failure, the speed of disease progression, or the response to pharmacologic therapy (131). In clinical situations, identification of mitochondrial dysfunction associated with cardiac manifestations, with a syndromic or non-syndromic phenotype, is not easy, since sampling of the myocardium for detecting mitochondrial dysfunction and mtDNA mutations is not a routine operative procedure (46). Complex genetic variations in mtDNA in the same population is another hurdle for diagnosis and for genetic counseling, which is dependent on accurate molecular genetic diagnosis and current possible regimens for intervention (16). It is believed that mitochondrial mutations and the subsequent dysfunction of mitochondria are not directly associated with oxidative stress, and therefore, greater caution is required to interpret the etiologies of mitochondrial dysfunction associated with cardiac manifestations. In addition, it is clear that the understanding of mitochondrial genetics will lead to improved diagnosis as well as novel ways to treat severe mitochondrial dysfunction associated with cardiac manifestations.

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