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REVIEW

Mitochondrial DNA Mutation and Oxidative Stress

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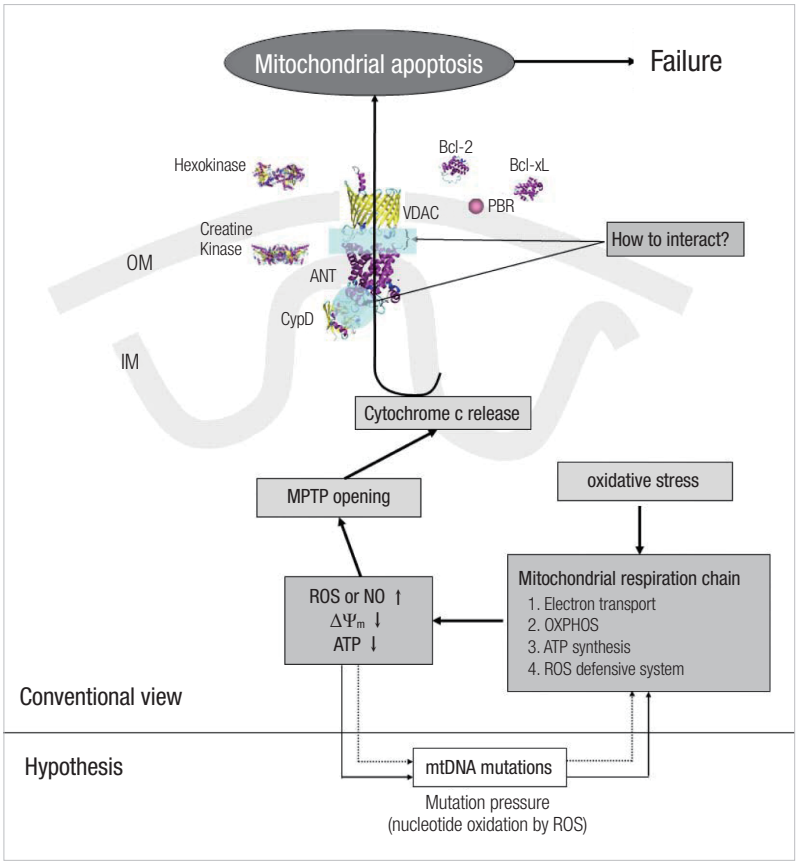
SYNOPSIS

Defects in mitochondrial DNA (mtDNA) cause many human diseases and are critical factors that contribute to aging. The mechanisms of maternally-inherited mtDNA mutations are well studied. However, the role of acquired mutations during the aging process is still poorly understood. The most plausible mechanism is that increased reactive oxygen species (ROS) may affect the opening of mitochondrial voltage dependent anion channel (VDAC) and thus results in damage to mtDNA. This review focuses on recent trends in mtDNA research and the mutations that appear to be associated with increased ROS.



Key Words: mitochondrial disease; reactive oxygen species (ROS); mitochondrial apoptosis; mitochondrial DNA mutation; mitochondrial permeability transition pore

MITOCHONDRIAL DNA MUTATIONS

Mitochondrial DNA (mtDNA) mutations are one of the major causes for genetic diseases[1-6]. So far, over 150 mtDNA mutations have been reported and major mutations result in maternally-inherited cardiomyopathy and heart failure[7-11]. There are two general types of mtDNA mutations such as large rearrangements and point mutations in the mtDNA. Large scale rearrangements of the DNA frequently involve genetic deletions and duplications, whereas point mutations usually occur in transfer RNA (tRNA), ribosomal RNA (rRNA), and some protein coding genes. The first two categories affect protein translation, causing multiple deficiencies in respiratory chain complexes, while the latter causes specific deficiencies in certain respiratory chain complexes[12]. These mutations are usually associated with multisystem disorders, lactic acidosis, encephalomyopathy and so on[13]. Gene structure and function would be dramatically affected if both types of rearrangements are present in the same individual. Deletions in mtDNA often result in the removal of multiple tRNA genes as well as some protein coding genes; sometimes, duplications are dimmers of deleted and wild type mtDNA molecule[14]. Duplications, even though not pathogenic, may cause deletions and can be transmitted maternally because most deletions or repetitive sequences are at the flank and single deletions occur in one or more tRNA genes[15]. Unlike large scale rearrangements, point mutations in protein coding genes cause specific deficiencies in some respiratory chain complexes. Point mutations in tRNA or rRNA genes also affect respiratory chain complexes. The most common type of mtDNA point mutation in human disease is tRNA gene variation. The number of different point mutations has been reported mostly in mitochondrial tRNA genes and that number continues to increase. Interestingly, mitochondrial tRNA mutations are associated with a cardiac or a neuromuscular phenotype[16]. $tRNA^{Ile}$, $tRNA^{Leu(UUR)}$ and $tRNA^{Lys}$ contain half of all known pathogenic mutations associated with respiratory muscles; the likely reason for this large number is because those three tRNA mutations are not lethal[17].

Mutations in mtDNA that hinder oxidative energy metabolism have been found to cause a wide variety of disorders in the last twenty years[18]. The mutation rate in mtDNA is about 10 to 20 times more rapid than the mutation rate in nucleic DNA (nDNA). The high mutation rate of mtDNA is due to the lack of protective histone protein or lack of DNA repair systems[19,20]. Reactive oxygen species (ROS) are also a major cause of such mutations. Furthermore, it is in the inner membrane of the mitochondria where free radicals are being generated from the electron transport chain during oxidative phosphorylation, making the mitochondria more exposed to oxidative stress[21]. To make things worse, oxidative damage by ROS is higher for mtDNA compared to nDNA because of the unique characteristics of mtDNA, which contain no introns except for the part that codes for the D loop. It is sometimes difficult to determine the precise pathogenic role of a particular mutation associated with a particular disease due to a large number of polymorphisms in mtDNA. However, the statistics resulted in that pathogenic mutations are almost met in case of the mutation that involves changes of the nucleotide position associated with special important function of tRNA, rRNA or protein molecules. Therefore, the mutation should be heteroplasmic, which is affected in various different conditions.

MITOCHONDRIAL DNA MUTATIONS LINKED TO WELL-KNOWN DISEASES

Mitochondrial diseases are caused by defects in either mtDNA or nDNA. Understanding mitochondrial gene mutations are challenging due to the complexities of mitochondrial genetics (see Table 1)[19]. mtDNA mutations have been associated with a wide variety of degenerative diseases, particularly affecting the central nervous system, heart, muscle, renal, and endocrine systems[22]. Pathogenic mitochondrial encephalomyopathics are due to defects in either one genome or intergenomic communication. MtDNA defects are mutations in mtDNA rearrangements, tRNA, rRNA or protein coding genes, and cause problems in the respiratory chain[12,15,16,19,21,23-25]. According to Marty Brandon et al, pathogenic mtDNA mutations are divided into several classes of mutations, each associated with its respective disease phenotype designation that are polypeptide mutations, protein synthesis mutations, rearrangement mutations, and control region mutations[22,26]. Mitochondrial disorders are also characterized by phenotypic pleiotropy in which the identical point mutation may lead to different disease phenotypes. A3243G MELA mutation is a good example that is found in patients with maternally inherited diabetes or deafness[27,28].

It has been more than ten years since researchers have entered into the mitochondrial genetic era and the repertoire of pathogenic mtDNA mutations is still expanding. For example, the number of mtDNA point mutations went from one in 1998 to 118 in 2000[13]. This number grew to 150 in 2002[17]. In the case of cardiomyopathies, the number of mtDNA mutations has increased from 10 to 16[15,22,26,29,30]. The major clinical entities associated with sporadic mtDNA deletions and duplication are Kearns Sayre syndrome (KSS), Pearson bone marrow/pancreas syndrome, progressive external ophthalmoplegia (PEO), and ragged red fibers (RRE)[12,15,19,29,30]. mtDNA rearrangements are much more frequently detected in post mitotic cells than in replicating cells, such as leukocytes[15]. The size and location of the deletions do not correlate with specific clinical phenotypes. The size of the deletions ranges from approximately 2 kb to 20

Table 1. Mitochondrial mutations associated with diseases

Genes	Coding region	Size	Diseases	Species	References
			Point mutation		
tRNA Leu	A3251G		Cardiomyopathy	Human	[19]
	A3254G		Cardiomyopathy	Human	[13,15,24,31-35]
	A3243G		MELAS, H	Human	[13,17,24,31-34, 36-41]
	A3260G		DCM, LVH, WPW, Cardio-myopathy	Human	[15,24,31-33]
	A12308G		DCM	Human	[42]
	C3303T		CHF	Human	[15,31,34]
	T3285C		DCM	Human	[42]
	T12311C		Cardiomyopathy	Human	[34]
tRNA Lys	A8344G		MERRF	Human	[15,17,19,31,36]
	G8363A		H, MERRF	Human	[15,31-33]
	A8348G		DCM, Cardiomyopathy	Human	[33,43]
tRNA Ile	G4284A		DCM	Human	[31]
	A4269G		DCM, H	Human	[15,24,31,33,44]
	A4295G		H	Human	[15,24,31]
	A4300G		H, DCM, Cardiacmyopathy	Human	[15,24,31,33, 44,45]
	A4317G		H, FIC	Human	[15,31,32,40,44]
	C4320T		Cardiomyopathy	Human	[15,17,24]
tRNA Val	G1644A		H, MELAS	Human	[40]
	G1647A		H, MELAS	Human	[40]
tRNA Thr	A15924G		DCM, FIC	Human	[35,42]
	G15928A		DCM	Human	[42,46]
tRNA Gly	T9997C		H	Human	[15,24,26,33]
tRNA Arg	T10457C		DCM	Human	[42]
tRNA His	G12192A		Cardiomyopathy & LHON, H	Human	[41,46]
16sRNA	A1811G		DCM	Human	[42]
	A2758G		DCM	Human	[42]
	G3010A		DCM	Human	[42]
	T3197C		DCM	Human	[42]
	A2706G		Cardiomyopathy & LHON	Human	[42]
12S rRNA	A1555G		Cardiomyopathy	Human	[15,41]
	G709A		Cardiomyopathy & LHON	Human	[46]
	A750G		Cardiomyopathy & LHON	Human	[46]
	G1598A		Cardiomyopathy & LHON	Human	[46]
ATPase 6	T8993C		NARP/MILS, Cardiomyopa-thy	Human	[15,19,26]
	T9090C		DCM	Human	[42]
	G8784A		Cardiomyopathy & LHON	Human	[46]
	C8829T		Cardiomyopathy & LHON	Human	[46]
	A8860G		Cardiomyopathy & LHON	Human	[46]
Cyt b	G15243A		H	Human	[15,26,34]
	G14865A		DCM	Human	[42]
	C15068G		DCM	Human	[42]
	T15847C		DCM	Human	[42]
	T15072C		DCM	Human	[46]
	C14766T		Cardiomyopathy & LHON	Human	[46]
	A15326G		Cardiomyopathy & LHON	Human	[46]
	C15508T		Cardiomyopathy & LHON	Human	[46]
	A15662G		Cardiomyopathy & LHON	Human	[46]
	A15851G		Cardiomyopathy & LHON	Human	[46]
	G15498A		Histocytoid cardiomyopathy	Human	[15,26,41]
Unknown	T5585C		DCM	Human	[42]
D-loop	G185A		DCM	Human	[42]
	T195C		DCM	Human	[42]
	G228A		DCM	Human	[42]
	C541T		DCM	Human	[42]
	C542T		DCM	Human	[42]
	C570T		DCM	Human	[42]
	T16189C		DCM	Human	[42]
	G5585T		DCM	Human	[42,46]
	A15954G		DCM	Human	[42]
	C16003T		DCM	Human	[42]
	T16126C		DCM	Human	[42]
	G16438A		DCM	Human	[42]
	T16519C		DCM	Human	[42,46]
	T16243C		Cardiomyopathy & LHON	Human	[46]
	A16318T		Cardiomyopathy & LHON	Human	[46,47]
	G16319A		Cardiomyopathy & LHON	Human	[46]
NADH	A3360G		DCM	Human	[42]
	C3690G		DCM	Human	[42]
	G3705A		DCM	Human	[42]
	T3777C		DCM	Human	[42]
	A4079G		DCM	Human	[42]
	A4994G		DCM	Human	[42]
	A3578G		DCM	Human	[42]
	C5387T		DCM	Human	[42]
	T5426C		DCM	Human	[42]
	T10309C		DCM	Human	[42]
	A10385C		DCM	Human	[42]
	G10387C		DCM	Human	[42]
	A10825G		DCM	Human	[42]
	A12347G		DCM	Human	[42]
	C12412T		DCM	Human	[42]
	C12484G		DCM	Human	[42]
	A12674G		DCM	Human	[42]
	T13020C		DCM	Human	[42]
	G13928C		DCM	Human	[42]
	G11719A		Cardiomyopathy & LHON	Human	[46]
	G11778A		Cardiomyopathy & LHON	Human	[13,26,46]
	G11914A		Cardiomyopathy & LHON	Human	[46]
	A12361G		Cardiomyopathy & LHON	Human	[46]
	T14470C		Cardiomyopathy & LHON	Human	[46]
	A4769G		Cardiomyopathy & LHON	Human	[46]
COX	G5973A		DCM	Human	[42]
	A6047G		DCM	Human	[42]
	C6371T		DCM	Human	[42]
	T7042G		DCM	Human	[42]
	T7266C		DCM	Human	[42]
	A7768G		DCM	Human	[42]
	T9484G		DCM	Human	[42]
	T9499G		DCM	Human	[42]
	T9846C		DCM	Human	[42]
	T9862C		DCM	Human	[42]
	G3460A		LHON links cardiac conduc-tion defects	Human	[19]
	C7028T		Cardiomyopathy & LHON	Human	[46]
	T9950C		Cardiomyopathy & LHON	Human	[46]
	T9957C		H	Human	[26,48]

(Continued to the next page)

Table 1. *(Continued from the previous page)* Mitochondrial mutations associated with diseases

Genes	Coding region	Size	Diseases	Species	References
Rearrangement	Rearrangement	Rearrangement	Rearrangement	Rearrangement	Rearrangement
	1067-16085, 3255-3272	3, 75 kb	Cardiomyopathy, Coronary heart disease		[49]
			Deletion		
	8316-8334, 16544-16561	7, 3 kb		Cardiac surgical patient	[50]
	8470-8482, 13447-13469	4,977 bp	Cardiovascular disease	Human, Mouse	[38,51,52]
	8637-16084	7,436 bp		Cardiac patients	[53]
	1161-1180, 6171-6190	5, 0 kb		Cardiac patients	[53]
	10004-15359	5, 355 kb	KSS, PEO	Human	[34]
	7883-15696	7,813 bp	KSS: not detail heart disease	Human	[47]
	7983-15504	7,521 bp	KSS: not detail heart disease	Human	[47]
	8281-8291	10 bp	DCM	Human	[42]
D-loop	514C	1 bp	Cardiomyopathy & LHON	Human	[46]
	515A	1 bp	Cardiomyopathy & LHON	Human	[46]

kb and may encompass the entire length of any mtDNA gene[29].

As mentioned previously, the most common class of mtDNA point mutations in humans are mutations in tRNA genes. Respiratory chain activities may be reduced when tRNA genes are affected, and neuromuscular or cardiac phenotypes are the most common examples for that. There are eleven point mutations reported in tRNA genes and two of them are $tRNA^{Leu(UUR)}$ and $tRNA^{Ile}$, which seem to be the hot spots for cardiomyopathies[15-17,19,27,36]. The best characterized syndromes caused by mutations in tRNA of mtDNA are mitochondrial encephalomyopathy, lactic acidosis, stroke (MELAS), and myoclonic epilepsy with ragged red fibers (MERRF)[21,25].

MELAS syndrome is typically caused by the A to G transition in mtDNA nucleotide position 3243, located in the $tRNA^{Leu(UUR)}$ gene, which causes neurogenetic disorders[54]. Patients with A3243G mutation may present with various clinical symptoms including diabetes mellitus, occipital brain infarct, epilepsy, ataxia, and so on[12,15,17,28,36,55]. Eighty percent of the MERRF syndromes are caused by A8343G in mtDNA, which conserved nucleotide in the $tRNA^{Lys}$ gene. The clinical features of MERRF are epilepsy, myopathy with ragged red fibers, and cerebellar ataxia[15,19,36]. More than two – thirds of the mutations in mtDNA that are known to be related to human diseases are found in the tRNA genes[56]. These mutations may be due to their central function in mitochondrial protein synthesis or due to the role of protein coding genes[15,57].

A mutation in rRNA genes such as A1555G point mutation has rarely been reported in the rRNA gene associated with aminoglycoside – induced deafness or nonsyndromic deafness, which is identified in a family with maternally inherited restrictive cardiomyophathy[15].

Mutations in protein coding genes of mtDNA affect subunits of the respiratory chain complexes I, II, IV and V. The severity of the mutation mostly depends on the location of the amino acid change in the structure of the affected subunit and on the character of that particular change. Furthermore, generalizations regarding mutations in mtDNA protein coding regions have been largely concentrated in OXPHOS complexes and the major phenotypes are LHON and NARP/MILS[13,26]. Mutations in the mtDNA encoded complex I (ND1, ND2, ND3, ND4L and ND6 subunits) have been associated with LHON[26]. LHON is a maternally inherited type of bilateral blindness caused by degeneration of the optic nerve[12,19,21]. The four distinct common LHON primary mutations, which all reside in genes of complex I subunits, are T1444C, G14459A in the ND6 subunit, G3460A in the ND1 subunit, and G11778A in the ND4 subunit[13,19]. The activities of complex I have been found to be decreased in patients with Parkinson's disease. However, mutations in mtDNA encoded subunits of complex III have not often been associated with clinical phenotypes[13,26]. Those mutations were identified in patients with histiocytoid cardiomyopathy[15,26]. However, there are no known syndromes or clinical phenotypes directly associated with a particular mutation in genes encoding COX subunit[12]. NARP and MILS are known to be involved in ATPase mutations such as T8993C and T8993G in the ATP6 gene[13,19,26].

MITOCHONDRIAL COMPONENTS LINKED TO CELLULAR APOPTOSIS

Mitochondrial permeation transition pore (MPTP) is a nonselective, high-conductance channel with multiple macromolecular components. Mitochondrial permeability transition is caused by the opening of MPTP, which increases the permeability of the mitochondrial membranes to molecules with a size up to 1.5 kDa[58]. MPTP plays a major role in apoptotic cell death. The exact complex structure of MPTP including adenine nucleotide translocase (ANT) in the inner mitochondrial membrane (IMM), cyclophilin D (CypD) in the matrix, and voltage dependent anion channel (VDAC) in the outer mitochondrial membrane (OMM), remains unknown[59]. One of the major functions

of MPTP is to regulate the fluxes of adenosine 5′ triphosphate (ATP) in the matrix, the intermembrane space, and the cytosol by a process called ATP channeling. Another function of MPTP is to control mitochondrial homeostasis by a sudden increase in the IMM permeability to protons, water, and molecules of molecular mass below 1.5 kDa, therefore allowing a charge balance between the composition and the state of both matrix and cytosol[60].

Voltage dependent anion channel (VDAC) constitutes the major pore-forming protein with a single 30–35 kDa polypeptide[61]. VDAC plays a major role not only in energy production by control of metabolite transfer, but also in mitochondria-mediated apoptosis. VDAC has several functions that are related to its nature as a barrier such as involvement in protein import and compartmentalization. VDAC is also believed to be the main pathway for diffusion of polar solutes, ions, metabolites such as ADP/ATP, succinate, and citrate through the OMM[62,63]. In the high-conducting or open state, there is a strong preference for anions. However, this preference is reversed upon channel closure, which allows the conversion to a low-conducting state. Channel permeability to charged species is strongly dependent on the sign of the charge[64]. Gating of VDAC has been shown to be capable of controlling the flow of metabolites. Closure of VDAC is involved in the initiation of apoptosis, which is a closely regulated form of programmed cell death. Furthermore, it is during apoptosis when cytochrome c is released from mitochondria into the cytoplasm.

Various factors increase the probability of MPTP opening. In certain mitochondria, such as those in the central nervous system, high levels of Ca^{2+} within the mitochondria can result in opening of the MPTP[65,66]. This is favored because Ca^{2+} binds to and activates Ca^{2+} binding sites on the matrix side of the MPTP[67,68]. MPTP induction is also due to the dissipation of the difference in voltage between the inside and outside of the mitochondrial membrane potential ($\delta\psi$)[69,70]. The presence of free radicals and another result of excessive intracellular calcium concentrations can also cause the MPTP to open[71].

The pore is formed from a complex of the VDAC, ANT, and CypD at contact sites between the mitochondrial outer and inner membranes[72]. The other soluble and membrane proteins of MPTP are peripheral benzodiazepine receptor (PBR), creatine kinase (CK), hexokinase (HK), and Bcl family proteins (Figure 1)[60,73]. However, the exact molecular identity of MPTP has not been established completely. The complex structure of VDAC,

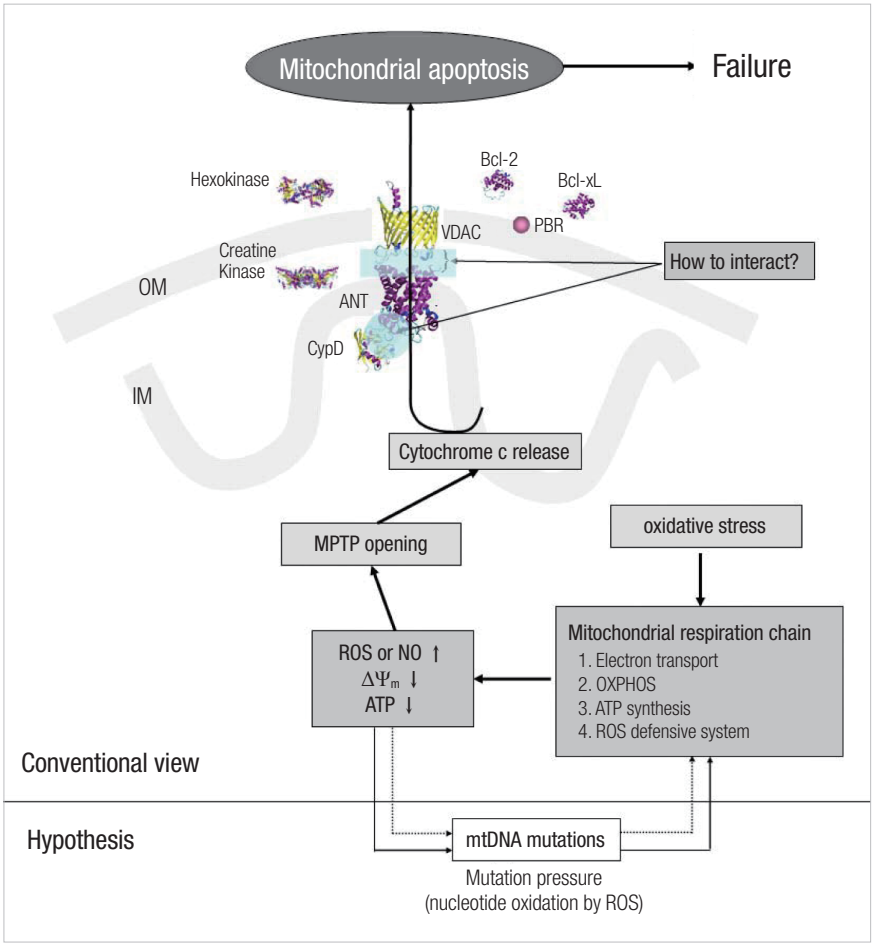


Figure 1. Schematic representation of a proposed pathway map between respiratory chain molecules, ROS generation, mitochondrial DNA mutation (or damage), and MPTP opening. Oxidative stress (or strong sympathetic activation in the present study) may lead to a defect in mitochondrial respiratory chain function, increased ROS generation, and mtDNA damage or mutations. The increased ROS level causes depolarization of mitochondrial membrane potential ($\Delta\Psi_m$) and subsequent opening of the MPTP and cytochrome c release. Moreover, the increased ROS may serve as a strong mutagenic pressure of mtDNA (i.e., nucleotide oxidation).

ANT, and CypD is important to identify modulation of MPTP. CypD belongs to a cyclophilin family of peptidyl prolyl-*cis*, *trans*-isomerases (PPIases). CypD plays a major role in protein folding[74]. CypD binds to the IMM in a cyclosporin A (CsA) sensitive manner[75,76]. The 3D crystal structure of human CypD at a resolution of 1.71 Å (PDB ID: 2bit) from X-ray crystallography was identified as a drug target for the treatment of cardiac disorders[77]. CypD has an active site associated with ANT or CsA.

ROS ARE TRIGGERING FACTOR FOR mtDNA MUTATION?

Apoptosis occurs when the cell is damaged and infected with a virus or when cells go through stressful condition such as ROS enrichment or lost function. This is to prevent malfunction of cells such as cancer and to main cell. Apoptosis can be triggered by various stimuli such as DNA damage, ROS, oxidative stress, and ionizing radiation. Oxidative stress due to an increased ROS is one of the stimuli for apoptosis involved in mtDNA mutations and MPTP opening which are the triggering factors in mitochondrial apoptosis[78,79]. Oxidative stress is related to most processes and has a major effect on hypertrophy, ion flux, and calcium controlling. The increased ROS level brings depolarization of mitochondrial membrane potential ($\Delta\Psi_m$), subsequent opening of MPTP, and the release of cytochrome c (Figure 1). ANT, an inducer of mitochondrial permeabilization transition, produces conformational changes by nitric oxide. Ultimately the increased ROS level in mitochondria results in apoptosis. The conformational changes of MPTP play a major role in modulation of mitochondrial apoptosis. Further investigations of mtDNA related to oxidative stress and detailed conformational changes of MPTP structure related to variation of the ROS level in mitochondria will clarify the precise modulation of mitochondrial apoptosis.

It has been considered that mitochondrial DNA (mtDNA) mutation is quite irrelevant to mitochondrial permeability transition pore (MPTP) opening in the mitochondrial apoptotic pathway. However, recent results show that mitochondria-generated reactive oxygen species (ROS) are important as the critical triggering factors in mitochondrial apoptosis to explain the relationship between mtDNA mutation and MPTP gating[78,80]. Conformational changes of MPTP increase mitochondrial transition permeability (MPT), which is one of the reasons for apoptosis. Mitochondrial apoptosis pathway is controlled by MPTP in both outer and inner mitochondrial membranes. Oxidative stress due to the increase of ROS affects the opening of MPTP to release cytochrome c, which is an important molecule in apoptosis. Of course, mtDNA mutations are rested in ROS or mtDNA replication errors.

The mitochondrial free radical theory of aging (mFRTA) implicates reactive oxygen species (ROS)-induced mutations of mitochondrial DNA (mtDNA) as the major cause for aging. Although there is reasonable evidence for age-dependent increase in mtDNA mutations, the dynamics by which these ROS-induced mutations accumulate are still largely unclear. Still there are severe argumentations, although Kim et al.[9] observed that the acquired mutations were quickly adapted in rabbit heart. Con-current analysis of the mtDNA mutation and ROS maintenance should be prepared in the future. Understanding the precise mechanism of the accumulation of mtDNA mutations and how they damage mtDNA is possible by accurate quantification of the oxidative stress and mutational burden with significant experimental challenges.

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