Review

Mitochondrial Genome (mtDNA) and Human Diseases

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DOI: https://doi.org/10.15354/si.21.re201
The author declares no competing interest.

Mitochondria not only provide necessary energy for cells, but more importantly, they participate in the regulation of various biological functions and activities of cells. As one of the critical components of the body's genome, mitochondrial genome (mtDNA) is the key to cell bioenergetics and genetics. However, since no protection of histones and a complete self-repair system, mtDNA is extremely prone to mutate. Human diseases caused by mtDNA mutations are only transmitted through the maternal line. The same phenotype can come from multiple mtDNA mutations, and the same mtDNA mutation can lead to multiple phenotypes. This is the major reason that makes the diagnosis and identification of mtDNA genetic diseases difficult. Meanwhile, mtDNA mutations may be the culprit involved in mediating the aging and tumorigenesis. Currently, no effective therapeutics for diseases caused by mtDNA mutations, but with the deepening of research and technological advancement, it is promising that breakthroughs in the diagnosis and treatment of mitochondrial-related diseases in the near future.

Keywords: Mitochondrial DNA; Diseases; Genetics; Mutation; Therapeutics
Science Insights, 2021 September 17; Vol. 38, No. 4, pp.317-325.

A SINGLE cell in human body contains about 200-2,000 mitochondria with the largest number found in the most metabolically active cells like brain, heart, and skeletal muscles. The main function of mitochondria is to provide energy for cell activities through the respiratory chain, that is, the electron transport chain and the oxidative phosphorylation system, and also participate in some important metabolic pathways (1). In addition, mitochondria are also related to the production of reactive oxygen species (ROS), cell apoptosis, and autophagy (2, 3). There are more than 1,000 kinds of proteins that make up mitochondria. Except for the respiratory chain complex protein which is double-encoded by mitochondrion genome (mtDNA) and nuclear genes, all other proteins are encoded by nuclear genes. Therefore, mtDNA mutations or nuclear gene mutations may cause mitochondrial dysfunction (4, 5).

Mitochondrial disease is a general term for a large group of diseases caused by mitochondrial dysfunction. This concept was proposed by Luft et al. in 1962 in the skeletal muscle cells of a young woman with hypermetabolism but normal thyroid function, which discovered a large number of mitochondria with loose coupling of oxidative phosphorylation (6). In 1988, Holt et al. found large mtDNA deletion mutations in some patients with spontaneous neuromuscular diseases (7). Subsequently, Wallace et al. found a point mutation in mtDNA from a patient with Leber Hereditary Optic Neuropathy (LHON) (8, 9). So far, more than 250 mtDNA point mutations and a large number of mtDNA recombination mutations have been reported and are related to human diseases (11).

In this review, we summarize the genetic characteristics of mitochondria, mtDNA mutations and human genetic diseases,

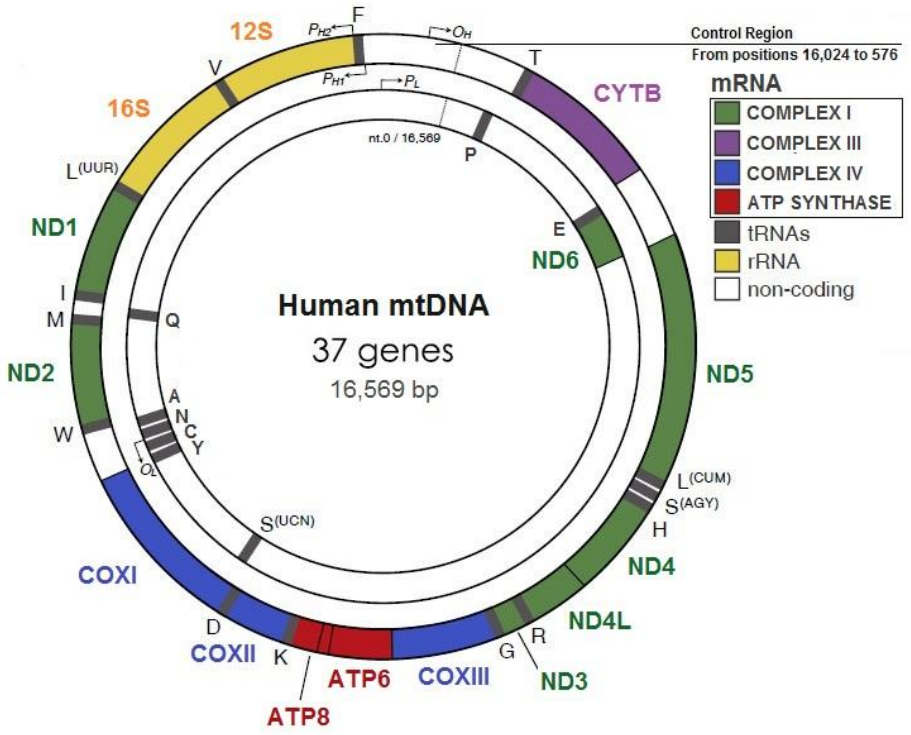

Figure 1. mtDNA Structure.
(Modified from *Mitochondrion 2016;30:105-116.*)

the role of somatic mtDNA mutations in aging and tumors, and the diagnosis and management of mtDNA diseases.

Mitochondrial Genetics

Mitochondria are the only organelles in human cells that have their own genetic material except for the nucleus. Compared with nuclear genes, the mitochondrial genome has its specific features (5). mtDNAs exist in mitochondria and cells in multiple copies. They encode 7 NADH-ubiquinone reductase ND subunits (ND1, ND2, ND3, ND4, ND4L, ND5 and ND6), 1 cytochrome b subunit (Cytb), and 3 cytochrome C oxidase subunits (COX I, COX II and COX III), 2 ATP synthase subunits (ATP6 and ATP8), 2 rRNAs (12S and 16S ribosomal RNA) and 22 tRNAs (**Figure 1**) (12). The characteristics of mitochondrial remains can be summarized in 4 aspects: (i) maternal inheritance; (ii) heterogeneity and mutation load; (iii) threshold effect; (iv) "bottleneck" and random allocation.

Maternal *versus* Paternal Inheritance

Giles et al. performed single nucleotide polymorphism analysis on mitochondrial genes of several European families and found that mtDNA molecules were transmitted strictly in accordance with the maternal inheritance method. Maternal inheritance means that only mothers can pass their mtDNA molecules to the next generation, and then to the offspring through their daughters (13). Schwartz et al. found a 2-bp deletion mutation in the MTND2 gene of mtDNA in the muscle tissue of a patient with mitochondrial myopathy (MM) (14). Even though the gene haplotype analysis showed that this mutated mtDNA molecule originated from the patient's father, although the mutation may spontaneously form in early embryogenesis (14), subsequent related studies have not found traces of mitochondrial paternal inheritance (15, 16). As mentioned by Sutovsky et al. that sperm mitochondria will be specifically recognized and degraded by ubiquitin hydrolase in the egg during the process of fertilization (17, 18). This explains the phenomenon that only maternal but not paternal mtDN be transmitted to the offspring. Interestingly, if the egg fails to prevent the mtDNA in the sperm from entering it during fertilization, the resulting fertilized egg will be lost due to abnormal development (19). Therefore, in the diagnosis and genetic consultation of mtDNA mutations and related diseases, maternal inheritance should be regarded as the basic rule of mitochondrial inheritance (see the sample pedigree diagram of mtDNA disease in **Figure 2**).

Heterogeneity and Mutation Load

Nuclear gene mutations occur on alleles, and the resulting mutants are divided into homozygote (homozygote, mutation load is 100%) versus heterozygote (heterozygote, mutation load is 50%). Unlike nuclear genes, mitochondrial gene mutations can occur in thousands of mtDNA molecules, resulting in mtDNA mutants with mutations ranging from 0% to 100%. Mutation load refers to the percentage of mtDNA that undergoes mutations in the total mtDNA, and it is an important indicator to measure the degree of heterogeneity of mtDNA mutants. The occurrence of mtDNA disease and its clinical phenotype often

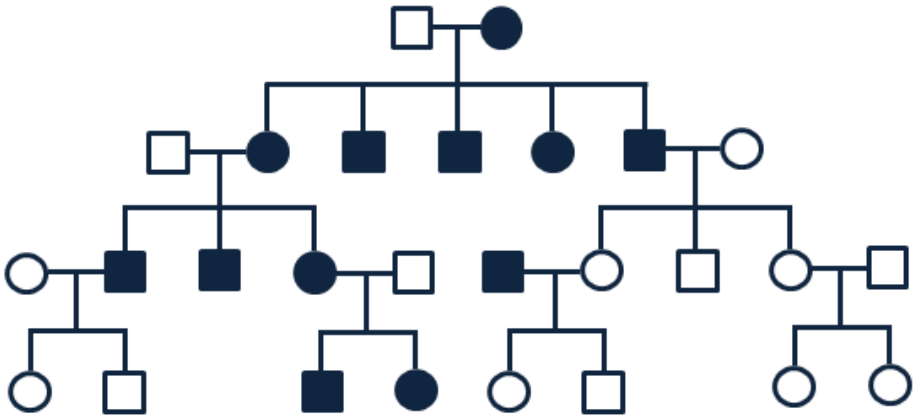

Figure 2. Pedigrees and Patterns of Mitochondrial Inheritance.

depend on the mutation load. Jeppesen and colleagues revealed that when the mutation load of the mt-A3243G point mutation in human muscle reaches 50%, it is sufficient to cause oxidative damage to skeletal muscle cells and abnormal muscle tissue morphology (20). In addition, the A3243G mutation load is positively correlated with the severity of the disease (21).

Threshold Effect

When the mutation load of the heterogeneous mtDNA mutant is low, the wild-type mtDNA coexisting with the mutant mtDNA will exert sufficient compensation to maintain the function of the mitochondrial respiratory chain. However, when the mutation load exceeds a certain range, making the amount of wild-type mtDNA insufficient to maintain the function of the respiratory chain that results in the tissues or organs be abnormal. This phenomenon is called the threshold effect (23). Different human tissues and organs have different susceptibility to mtDNA mutations. Those parts with high energy requirements such as skeletal muscle, brain, heart, renal tubules and endocrine glands, are susceptible to mutations, and thereby a lower mutation load will cause clinical symptoms in these tissues. But those parts with low energy requirements such as the lungs, skin, and ligaments are not sensitive to mutations, and so a higher mutation load is needed for causing abnormalities.

"Bottleneck" and Random Allocation

The mutation load of heterogeneous mtDNA changes significantly between different generations. This effect is the "bottleneck" of mitochondrial inheritance. A widely accepted hypothesis is that in the early stage of oogenesis, the amount of mtDNA in the primordial oocyte will decrease sharply, resulting in a "bottleneck" (23). However, the count of mtDNA in a single mouse germ cell showed that the primordial oocyte contains a stable and moderate number of mtDNA copies in the primary and intermediate stages of oogenesis (24, 25). At the maturation stage of primary oocytes, the number of mtDNA will increase substantially. This shows that the "bottleneck" is not caused by the sharp decrease in the number of mtDNA in the early stage of oogenesis, but because the oocyte has undergone multiple divisions so that the effective amount of mtDNA that is finally allocated to each egg is small.

During mitosis (including oogenesis), mtDNA is randomly allocated to progeny cells. In oocytes, about 150,000 mtDNA molecules exist. After oogenesis, only part of mtDNA enters the primary oocytes, forming a population of oocytes with very different levels of heterogeneity; the experience of fertilized eggs after fertilization During cleavage and embryo development, only a few copies of mtDNA molecules finally enter the neonatal tissue cells (24, 26). Therefore, the disease phenotype between members of the same maternal family and the mutation burden between the same patient's tissues are often very different. Every time a somatic cell undergoes mitosis, mtDNA will be randomly assigned to the progeny cells along with the mitochondria. Therefore, the mutation load of mtDNA in the tissue will change with the division of tissue cells. Furthermore, the disease phenotype of the same patient can also show variability over time.

mtDNA Mutations and Diseases

Mutations in mtDNA and Human Genetic Diseases

The mutation rate of mtDNA is extremely high due to the lack of histone protection and intact mutation repair function, (27). The high mutation rate of the mitochondrial genome not only produces a large number of pathogenic mutants, but also produces more sequence polymorphisms. Pathogenic mtDNA mutations generally have the following characteristics: (i) The mutation site is relatively conservative, and the mutation results in nucleotide or amino acid substitution, or loss of biological function of the gene-coded product. (ii) The biochemical damage caused by the mutation can be separated from the clinical phenotype of the disease. (iii) When the mutation is a heterogeneous mutation, the degree of tissue damage is positively correlated with the mutation load. (iv) The same mutation can be found in

genetically independent patients.

Pathogenic mtDNA mutations are usually located in genes encoding proteins, tRNAs or rRNAs, and can cause obvious clinical symptoms (28). The relationship between mtDNA mutation and phenotype is complex. The same mtDNA mutation can cause different disease phenotypes. For example, the A3243G mutation in the tRNALeu (UUR) gene can appear in patients with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), as well as chronic progressive external ophthalmoplegia (CPEO), mitochondrial myopathy, and diabetic patients with deafness. In addition, the same disease phenotype can also be caused by different mutations. For example, MELAS can be caused by more than 20 point mutations, or it can be caused by recombinant mutations. Some independent factors can affect the clinical manifestations of mtDNA diseases, including: the level of heterogeneity of mutants, tissue distribution, and the degree of dependence of organs on the respiratory chain, nuclear background, and environmental factors.

mtDNA mutations can be divided into two categories: point mutations and recombination mutations.

Point Mutation

Human genetic diseases related to mtDNA point mutations mainly include: LHON, MELAS, myoclonus epilepsy associated with ragged-red fibers (MERRF), neuropathy ataxia and retinitis pigmentosa (NARP), maternally inherited Leigh-like syndrome (MILS), maternally inherited diabetes and deafness (MIDD), nonsyndromic hearing impairment (NSHI), cardiomyopathy and myoglobinuria (27). In addition, mtDNA point mutations are also related to the susceptibility of some metabolic diseases (such as hypertension, diabetes, hypercholesterolemia, etc.) and neurodegenerative diseases (such as Parkinson's disease, Alzheimer's disease, etc.) (29).

● LHON

LHON was the first maternal genetic disease identified with mtDNA point mutations (8). Clinically, it is characterized by continuous acute or subacute central vision loss on both sides, which mainly affects adolescent males. Most of the point mutations associated with LHON are located in the MTND gene. Three primary mtDNA mutations G11778A, G3460A and T14484C are located on the MTND4, MTND1 and MTND6 genes, respectively. The cases caused by these mutations account for about 95% of all LHON patients. The penetrance of LHON-related mutants varies greatly, and different mutants have different penetrance, even if the penetrance of the same mutant is different among different individuals (30).

● MELAS

MELAS is a group of mtDNA diseases with high clinical variability and genetic heterogeneity. Its main characteristics are: (i) Focal damage to the parieto-occipital lobes and the resulting stroke-like episodes; (ii) Lactic acidosis and ragged red fiber (RRF); (iii) Other centers Neurological manifestations, such as dementia, repeated headaches and vomiting, seizures, retinitis pigmentosa, and deafness; (iv) Some patients have manifestations of ataxia; (v) A small number of patients have diabetes, intestinal pseudo-obstruction and cardiomyopathy; (vi) Biochemically, mitochondrial complex I is often defective, while complex IV is not easily affected, and the broken red fiber appears to be positive for COX [29]. There are more than 20 mtDNA point mutations related to MELAS. Most cases of MELAS are caused by a heterogeneous mutation A3243G in the mt-tRNALeu (UUR) gene. A few are caused by other mtDNA point mutations and large fragment recombination mutations. There are also some MELAS patients whose mtDNA is normal, suggesting that mutations may occur in nuclear genes (31).

● MERRF

MERRF's main characteristics are: (i) Myoclonus, epilepsy, muscle weakness and weight loss with broken red fibers; (ii) Cerebellar ataxia, deafness and dementia; (iii) Neurons in the dentate nucleus and secondary lobules of the cerebellum Loss and hypergliosis; (iv) Some patients have symptoms of symmetrical fat increase in the trunk; (v) Biochemically, mitochondrial complex I often has defects, while complex IV is not easily affected, with broken red fibers appearing and most of them are COX negative (32). Most of the mtDNA mutations associated with MERRF are located in the tRNALys gene, of which the A8344G point mutation is the most common. Sometimes, MERRF and MELAS disease phenotypes coexist in the same patient, forming MERRF/MELAS overlap syndrome. Mutations such as mt-A3243G, T8356C and G12147A can respectively cause MERRF/MELAS overlap syndrome (33).

● NARP and MILS

The main characteristics of NARP are: (i) Ataxia, retinitis pigmentosa, and peripheral neuropathy; (ii) MRI examination revealed that the cerebellum and brain of NARP patients have mild diffuse atrophy, and in severe cases, basic ganglion damage may occur; (iii) Muscle biopsy often fails to detect broken red fibers. MILS is a neurodegenerative disease related to mtDNA mutation in Leigh disease (34). NARP and MILS are usually caused by two heterogeneous mutations 8993T>G/C located at one locus of the MTA6 gene. The severity of the disease is related to the level of mutant heterogeneity. NAPR can be caused when the mutation load is between 70%-95%, and MILS can be caused when the mutation load is higher than 90%.

● Mitochondrial Deafness

The mt-12S rRNA gene is a mutation hotspot related to deafness. Some point mutations on it such as A1555G, T961C and C1494T, have been confirmed to cause NSHI (35). Another hot spot for mutations is the mt-tRNASer (UCN) gene, such as A7445G, 7472insC and T7511C, which are also related to DEAF. In addition, the point mutation A3243G in the mt-tRNALeu (UUR) gene can cause MIDD (36).

Recombinant Mutation

Although the length of mtDNA fragments undergoing recombination mutations can vary from one base to several thousand bases, recombination mutations are mainly divided into: deletion and duplication of large fragments.

Deletion mutations can exist alone in the patient, or they can occur at the same time as the doubling. The most common type of recombination mutation is the deletion/doubling of a

single large fragment of mtDNA that is about 5 kb in length and spans the region between the Cytb gene and the Cox II gene. This large-segment recombination mutation is usually associated with some typical disease phenotypes, such as Pearson's syndrome, Kearns-Sayre syndrome (KSS), Chronic Progressive External Ophthalmoplegia (CPEO) and Pearson's bone marrow-pancreatic syndrome. However, recombination mutations are not limited to the above-mentioned disease phenotypes, and can also involve diabetes, hearing loss and almost all mitochondrial encephalomyopathy (37).

A single large fragment of mtDNA recombination mutation may be formed during oogenesis or early embryonic development (38). Most of the diseases associated with large deletion/doubling mutations are sporadic and have a low risk of recurrence. Chinnery and colleagues reported that the next generation risk of diseased women is only 4.11% (39).

Mutations of Somatic mtDNA and Senescence and Tumors

Mutation and Aging

Somatic mtDNA mutations will gradually accumulate as individual's age. Abnormal mtDNA is basically undetectable in the skeletal muscle of people under the age of 40, while a large number of mtDNA recombination mutations can be found in the skeletal muscle of people over 50, which was considered related to the aging of muscle tissue (40). High-frequency mtDNA deletion mutations were found in the substantia nigra neurons of elderly Parkinson's patients and normal elderly people with higher levels in Parkinson's patients that was speculated to the aging of brain tissue (41). Meanwhile, ROS-related oxidative damage is another crucial contributor to the somatic mtDNA mutations, and the accumulation of mutations will further damage the function of the mitochondrial respiratory chain, and generate more ROS and mutations, thereby accelerating the aging process (42).

Knock-in was used to establish an mtDNA-mutated mouse model to study the effect of mtDNA mutation on the phenotype of mice and found that these mice have obvious manifestations of premature aging, such as: shortened lifespan, weight loss, osteoporosis, hunchback, cardiac hypertrophy, and fertility decline (43). The in-depth study of mtDNA mutant mice by Kujoth and colleagues revealed that the accumulation of mtDNA mutations does not cause cell proliferation defects, nor is it related to oxidative stress markers, but is closely related to apoptosis markers (44). They also observed similar levels of apoptosis markers in normally aging mice. Therefore, it is speculated that apoptosis caused by the accumulation of somatic mtDNA mutations may be the core mechanism that promotes the aging of mammals. Theoretically, the mtDNA mutant mouse model is a good tool for studying the mechanism of aging, whereas its experimental results cannot confirm absolutely that mtDNA mutations will definitely accelerate the natural aging process of feeding animals. After all, it is impossible to have such a high mtDNA mutation rate in normal aging mice, moreover, mouse models that do not contain mtDNA mutants can also exhibit a premature aging phenotype (45). However, it can still provide indirect evidence that somatic mtDNA mutations may play a key role in human aging.

Mutations and Tumors

It has been suspected that mitochondrial defects may play an important role in the development of tumors and cancers (46). Although somatic mtDNA mutations can be observed in a variety of tumor cells (47, 48), the exact mechanism of these mutations on tumor development is rarely known. Mutations in the MTCOX I gene occurred in 11%-12% of prostate cancer patients in comparison to 7.8% of healthy controls; the mutation sites on the MTCOX I gene in cancer patients were more conservative in evolution; on the MTATP6 gene, the T8993G point mutation accelerated the formation of tumors of PC3 prostate cancer cells in nude mice, and promoted the production of ROS in tumor tissues (49). When the mitochondrial hybridoma cells containing mutant or wild-type mtDNA were implanted into nude mice, the morphology of tumors formed in mice, and found that mutant mtDNA could obviously promote tumor growth (50). However, when a section of normal mitochondrial gene complementary to the mutant gene is introduced into the nucleus of transmitochondrial hybridoma cells containing mutant mtDNA, the newly constructed tumor cells are then used to infect nude mice. Then the effect of mutant mtDNA to promote tumor growth was substantially inhibited suggesting that mtDNA mutations may promote tumor or cancer development (51).

Diagnosis and Treatment of mtDNA Diseases

Diagnosis

The diagnosis of patients with clinical syndromes is relatively easy. But for patients with atypical symptoms, the diagnosis of mtDNA disease is difficult (52). The precise diagnosis of mtDNA diseases also requires investigations in several aspects: (i) Using histochemical and biochemical methods to determine the exact nature of respiratory chain damage; (ii) Using genetic analysis techniques to find common mtDNA mutations; (iii) The entire mtDNA is sequenced to find rare or new mutations (53). Given the mutation load of heterogeneous mutants in different tissues might be quite different, only by collecting samples from the site of the disease or other sites with higher mutation load can get an ideal diagnosis result (54).

Common mutations can be detected from the patient's tissues through genetic analysis with the combination of the patient's clinical features, the disease can be diagnosed. If a new or rare mtDNA mutation is detected, functional analysis is needed to distinguish the nature of the mutant (55). As indicated that clinical bioinformatics would be a critical tool for analyzing variant and sequencing data available at MSeqDR, MitoMap, and HmtDB in interpreting mitochondrial DNA variant and helping diagnose the disease (56).

The functional analysis of mutants generally includes: (i) Conservative analysis of the gene site where the mutation is located; (ii) The potential impact of the mutation on the changes in polypeptide hydrophobicity, changes in protein spatial conception, and changes in rRNA and tRNA spatial structure, etc.; (iii) The pathogenic function of mutants. The most commonly used tools for functional studies of mtDNA mutants are transmitochondrial cytoplasmic hybrid and animal model (57). In view of the high variability of mtDNA mutation-related diseases, accurate genetic diagnosis can provide useful clues for

patient treatment and genetic counseling.

Treatment

Clinically, medications or supportive therapies are generally used to relieve the symptoms of patients, such as vitamin supplementation, nutrition improvement, treatment of epileptic seizures, adjustment of blood lactic acid levels, surgical correction of blepharoptosis, etc. (58). These methods alleviate the suffering of patients to a certain extent and play a palliative role.

Endurance training can increase the enzyme activity of mitochondria, and it is possible to activate resting cells located between the basal thin layer of muscle fibers and the protoplasmic layer, and promote their differentiation into muscle cells, thereby regulating the mutational load of mtDNA in muscles (59). Endurance training is safe and effective for the treatment of mitochondrial myopathy (60, 61). For example, aerobic exercise can improve the respiratory chain function of patients with mitochondrial myopathy, and can reduce the mutation load of mtDNA mutants (62). Continuous endurance training can significantly improve the life and work abilities of patients with mitochondrial myopathy. However, when endurance training is stopped for a period of time, the patient's exercise ability will return to the pre-training level (63).

Antioxidants can relieve the oxidative stress of cells, and can alleviate mitochondrial dysfunction caused by mtDNA mutations (64). Antioxidants N-acetylcysteine, dihydrolipoic acid or CoQ10 to act on cytoplasmic hybrid cells carrying the mt-T8993G mutation and fibroblasts of NARP patients and found that: intracellular reactive oxygen species were decreased; the function of cellular respiratory chain returned to normal; and ATP synthesis ability was also improved (65, 66).

In addition, gene therapy uses genetic engineering technology to transplant normal mtDNA genes into the patient's cells to replace or correct the patient's mutant genes, so as to achieve the goal of eradicating mtDNA diseases. In recent years, there has been a lot of research on gene therapy of mtDNA diseases. The wild-type mitochondrial genes were adapted from generalized codons through the nucleus, and the translated protein can use the mitochondrial targeting signal peptide located at its own amino terminus to enter the mitochondria (67). By using nuclear genes to express exogenous mt-tRNA, and got these rRNAs enter the mitochondria of patient cells cultured in vitro, and can be correctly aminoacylated to participate in the mitochondrial protein translation system, thereby protecting cells from mt-tRNA genes. Apoptosis caused by mutation (68). An promising method is that selectively recognize and degrade mutant mtDNA molecules but not the wild-type mtDNA by importing specific restriction endonucleases into human mitochondria, which can achieve the purpose of treatment by reducing the mutation load of some heterogeneous mutants (69-71).

Concluding Remarks

mtDNA mutations have attracted widespread attention due to its strong causal relationship with human genetic diseases, potential to provide reliable forensic identification, and the underlying key role in the aging and tumorigenesis. However, so far, the pathogenic mechanism of mtDNA mutations has not been clarified at the molecular level, and no effective therapeutics available for mtDNA diseases. However, with the deepening of research and technological progress, it is hopefully obtaining breakthroughs in the diagnosis and treatment of diseases from mtDNA mutations.

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Received: June 22, 2021 | Revised: August 26, 2021 | Accepted: August 30, 2021