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Review

Mitochondrial dynamics and aging: Mitochondrial interaction preventing individuals from expression of respiratory deficiency caused by mutant mtDNA

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Abstract

In mammalian cells, there is an extensive and continuous exchange of mitochondrial DNA (mtDNA) and its products between mitochondria. This mitochondrial complementation prevents individuals from expression of respiration deficiency caused by mutant mtDNAs. Thus, the presence of mitochondrial complementation does not support the generally accepted mitochondrial theory of aging, which proposes that accumulation of somatic mutations in mtDNA is responsible for age-associated mitochondrial dysfunction. Moreover, the presence of mitochondrial complementation enables gene therapy for mitochondrial diseases using nuclear transplantation of zygotes.

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1. Introduction

Mitochondria are organelles producing 90% or more of all the energy made in the body by oxidative phosphorylation (OXPHOS). Mammalian mitochondria contain a covalently closed circular mitochondrial DNA (mtDNA) that is replicated and expressed within the organellar system [1,2]. Mammalian mtDNA contains 37 genes, encoding 13 proteins (all of which are OXPHOS subunits), 22 tRNAs, and 2 rRNAs [3]. The remaining mitochondrial OXPHOS proteins, the metabolic enzymes, the DNA and RNA polymerases and the ribosomal proteins are all encoded by nuclear genome. Individual cells contain several thousand copies of mtDNA, and , in normal individuals, nearly all of the mtDNA is thought to be identical (a state termed “homoplasmy”). However, in some cases, especially in mitochondrial diseases, wild-type and variant mtDNAs coexist at different levels (a state termed “heteroplasmy”).

The mutation rate of mtDNA is about 10 times higher in mtDNA than nuclear genome. There are three reasons for the high mutation rate in mtDNA. The first is that mtDNA is susceptible to reactive oxygen species (ROS)-induced damage due to highly oxidative circumstances in mitochondria. Mitochondria are the major cellular site of production of ROS. It is estimated that 1–5% of the oxygen consumed in the mitochondrial OXPHOS electron transport chain is converted to ROS. mtDNA associated with the mitochondrial inner membrane [4,5] is in close proximity to the ROS-generating electron transport chain [6,7]. The second reason is that mtDNA has no histone-like protection as nuclear genome, so that mtDNA is susceptible to ROS or other chemical reagent. The final reason is that mitochondria do not have significant repair systems. These circumstances cause mtDNA to accumulate various somatic mtDNA mutations in mitotic [8] and post-mitotic [9–11] tissues. This fact and the abundant evidence that a decline in the OXPHOS capacity of mitochondria occurs with aging in skeletal muscles, heart, brain, liver, and other organs [12–19] have given support to “the mitochondrial theory of aging” [7,20–22]. It proposes that age-associated mitochondrial

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dysfunction appears as the consequence of age-associated accumulation of somatic mutations in the mtDNA population. However, the recent findings indicate that the exchange of mtDNA and its products between mitochondria do not support the mitochondrial theory of aging. This "mitochondrial complementation" is a mitochondria-specific mechanism for avoiding expression of pathogenic mutant mtDNA.

In the present review, we aim to summarize the knowledge of mitochondrial complementation, and then describe gene therapy model for mitochondrial diseases using mitochondrial complementation.

2. Aging and mtDNA mutations

mtDNA-less (ρ°) cells are obtained by long-term treatment of cells with ethidium bromide (EtBr) [23]. Cybrids isolated by mtDNA transfer from mitochondrial disease patients to ρ° cells were used to investigate whether mutant mtDNAs were responsible for mitochondrial diseases [24]. This procedure can exclude involvement of the nuclear genome in expression of the mitochondrial dysfunction. mtDNA transfer from aged subjects to ρ° cells is useful to examine whether mtDNA mutations are responsible for age-related reduction in respiratory function.

The role of mtDNA and nuclear genome in human aging was examined by their intercellular transfer using skin fibroblasts and ρ° HeLa cells [25]. Human skin fibroblasts obtained from 16 donors of various ages (0–97 years) showed age-related reductions in the activity of cytochrome *c* oxidase (COX). The abnormality in mitochondria of the aged donors was attributable to significant decrease in mitochondrial translation activity. However, irrespective of whether the mtDNA population was transferred from fibroblasts of aged or fetal donors, all the cybrids showed similar activities of COX and mitochondrial translation. This observation suggests that nuclear-recessive mutations of factors involved in mitochondrial translation but not mtDNA mutations are responsible for the age-related respiration deficiency of human fibroblasts. This idea was supported by another observation that showed the restoration of respiratory function by nuclear transfer from ρ° HeLa cells to fibroblasts of aged subjects [26].

The mtDNA transfer from post-mitotic and highly oxidative tissues to ρ° cells was also carried out. The cybrids obtained by mtDNA transfer from postmortem mouse brain preserved for 1 month at 4 °C to ρ° mouse cells showed complete recovery of COX activity [27]. This observation suggests that mtDNA in brain tissue can survive for 1 month after death. Thus, this procedure was applied to autopsied human brain tissues from aged patient with Alzheimer's disease [28]. The cybrids were obtained by mtDNA transfer from autopsied brain tissues to ρ° HeLa cells. In brain tissues and their cybrids, the presence of pathogenic mutant mtDNA was observed. However, the cybrids restored mitochondrial respiration activity, suggesting the functional integrity of mtDNA in brain tissues of aged patient with Alzheimer's disease.

All of these observations obtained with mtDNA transfer from mitotic and post-mitotic cells to ρ° cells suggest that nuclear genome is responsible for the age-related decline of OXPHOS capacity. Accumulation of various somatic mtDNA mutations in tissues [8–11] would not have a causative role but, rather, it would be one of the manifestations in aging.

3. Mitochondrial complementation

In fact, mutations in mtDNA accumulate with aging. Some of them were the same mutations observed in patients with mitochondrial diseases such as mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), chronic progressive external ophthalmoplegia (CPEO) and Kearns–Sayre syndrome (KSS) [9–11]. However, why these pathogenic mtDNA mutations do not lead to respiration deficiency? One explanation for this question is the presence of "mitochondrial complementation". This is the idea that mitochondria and mtDNA function as a single dynamic cellular unit in living cells by the presence of exchanging mtDNA and its products between mitochondria. In addition to the classic kidney bean-shaped organelles, mitochondria are frequently found as extended reticular networks [29]. Moreover, timelapse microscopy of living cells reveals that mitochondria undergo constant migration, fission and fusion [30]. It was also observed that fused mitochondria exchanged their matrix contents [31,32] and mitochondrial fission and fusion activity declined with aging [33]. It is supposed that free mixing of mitochondrial genetic components throughout the mitochondrial network would protect mammalian mitochondria from direct expression of respiration defects caused by accumulated mtDNA mutations.

3.1. In vitro mitochondrial complementation

Exchange of mtDNA was observed by fusion of enucleated HeLa cells with ρ° HeLa cells [34]. By visualizing mtDNA with DNA-binding dyes EtBr and 4′,6-diamidino-2-phenylindole (DAPI), mtDNA was shown to spread rapidly to all mitochondria in the cybrids. This observation suggests the presence of mitochondrial fusion and subsequent diffusion of mtDNA. In another experiment, cells possessing deletion mutant mtDNA and HeLa cells were fused [35]. The deletion break point generated new fusion gene, but it was not translated because of the complete loss of mitochondrial translation activity by the absence of several tRNA genes. However, in fused cells, the fusion protein was observed. It showed that the fusion gene encoded by deletion mutant mtDNA was translated with the help of the tRNAs supplied from the HeLa mitochondria, suggesting unambiguously the occurrence of mitochondrial complementation.

The idea contradictory to the presence of mitochondrial complementation has been proposed based on observations that the coexistence of human respiration-deficient mitochondria containing different pathogenic mutant mtDNAs derived from different patients within single cells did not restore reduced mitochondrial respiratory function [36,37]. The evidence supporting the absence of mitochondrial complementation between fused mitochondria is, however, indirect and based

on the observation that no cybrids generated by different types of respiration-deficient 'parental' cells grow in nutritional selection medium without pyruvate and uridine [37] (in which only cells with restored respiratory function can grow). It is possible that cybrids are excluded by the selection before respiratory function can be restored.

The conclusive evidence of mitochondrial complementation was obtained from fusion of two different types of respiration-deficient somatic cells caused by different pathogenic mutant mtDNAs from patients with mitochondrial diseases [38]. At first, two types of respiration-deficient cell lines were isolated. They possessed A4269G and A3243G pathogenic mutations in *tRNA*^{Ile} and *tRNA*$^{Leu(UUR)}$ genes, respectively. Because both cell lines showed homoplasmy of mutant mtDNAs, they had decreased translation and no COX activities. The coexistence of both mitochondria derived from different cell lines within hybrid cells restored translation and COX activity. This study showed that at least 10 days of pre-culture before nutritional selection was required to restore mitochondrial activities. Moreover, COX electron micrographs showed that the hybrid cells restored normal mitochondrial morphology and COX activity by 10–14 days after the fusion. Fusion of the different types of respiration-deficient mitochondria enables the exchange of genetic contents, including normal tRNA^{Ile} and tRNA$^{Leu(UUR)}$, resulting in the beginning of mtDNA-encoded polypeptide synthesis with the help of normal tRNA transcribed and supplied from other 'parental' mitochondria (Fig. 1).

On the other hand, when mutations were created within the same genes in different mtDNAs, exchange of genetic contents between mitochondria could not restore respiration deficiency [39].

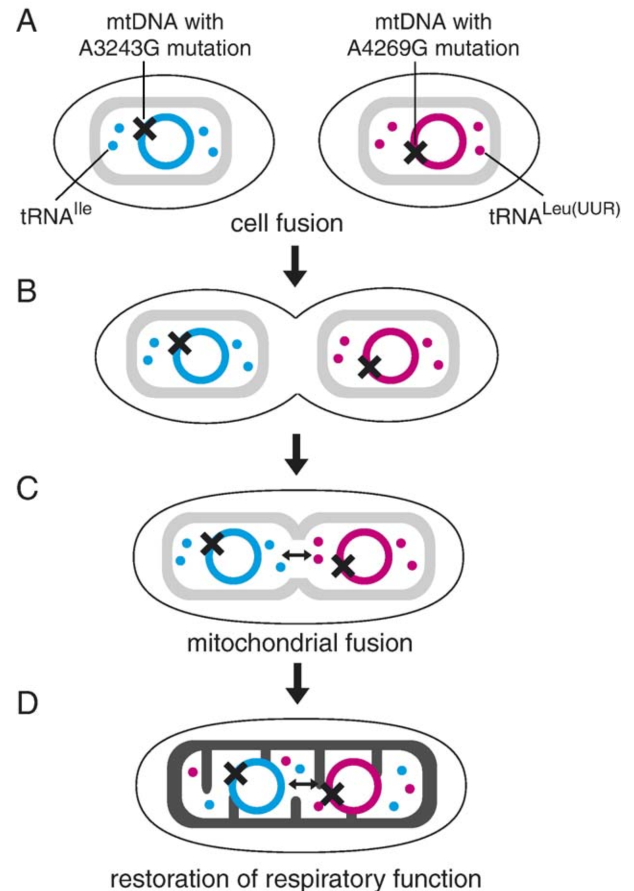

Fig. 1. Restoration of mitochondrial respiratory function. (A) Two cell lines possess A4269G and A3243G pathogenic mutations *tRNA*^{Ile} and *tRNA*$^{Leu(UUR)}$ genes, respectively. Both cell lines have no respiratory function. (B) Two cell lines are fused and hybrids are isolated. (C) Coexistence of mitochondria derived from these cells enables mitochondrial fusion and following exchange of genetic contents, including normal tRNA^{Ile} and tRNA$^{Leu(UUR)}$. (D) mtDNA-encoded polypeptide synthesis begins with the help of normal tRNA transcribed and supplied from other mitochondria, followed by restoration of respiratory function.

3.2. *In vivo mitochondrial complementation*

In vivo mitochondrial complementation was investigated using mitochondrial disease model mice, mito-mice [40,41]. They were generated by introduction of respiration-deficient mitochondria carrying a predominant amount of 4,696 bp deletion mutant mtDNA (ΔmtDNA) and a residual amount of wild-type mtDNA from cultured mouse cells into mouse zygotes [40]. Mito-mice with predominant proportion of ΔmtDNA showed low body weight, lactic acidosis, systemic ischaemia, COX deficiency in skeletal and cardiac muscles, auriculoventricular block with Wenckebach periodicity and hearing loss. Most of mito-mice died about 6 months after birth because of renal failure with high blood urea nitrogen and creatinine levels [40,41].

In mito-mice, COX activity of individual mitochondria in single cells was examined by COX electron micrographs. COX electron micrographs clearly showed that all mitochondria in tissues with ΔmtDNA were COX-positive until ΔmtDNA became predominant. The appearance of COX-negative mitochondria was limited to tissues with more than 85% ΔmtDNA. Moreover, it was not observed that the coexistence of COX-positive and COX-negative mitochondria within single cells, irrespective of whether the tissues contained low or high concentrations of ΔmtDNA. If exchange of mtDNA did not occur between exogenous COX-negative mitochondria carrying ΔmtDNA and COX-positive mitochondria from recipient zygotes, or even if it occurred, but did not occur frequently and continuously, mitochondria in the mito-mice would continue to possess either ΔmtDNA or wild-type mtDNA. Under these circumstances, cells in tissues with certain ratio of ΔmtDNA should have the same ratio of COX-negative mitochondria, but this was not the case [40]. These observations could be explained by assuming in vivo mitochondrial complementation between COX-negative mitochondria carrying ΔmtDNA and COX-positive mitochondria carrying wild-type mtDNA.

The presence of in vivo mitochondrial complementation was further confirmed by mito-mice possessing ΔmtDNA of *Mus musculus domesticus* and wild-type mtDNA of *M. spretus* [42]. The previous study [41] contained the possibility that exogenously introduced wild-type mtDNA contributed to the restoration of COX activity in mito-mice. If it was the case, the restoration of COX activity observed was not due to mitochondrial complementation [43]. However, newly

generated mito-mice showed transmission of exogenous ΔmtDNA, but not exogenous wild-type mtDNA of *M. m. domesticus* to following generations. Complete elimination of exogenous wild-type mtDNA would be due to stochastic segregation, whereas transmission of exogenous ΔmtDNA would be due to its smaller size leading to a propagational advantage. Tissues in the mito-mice showed complete protection from respiration deficiency until ΔmtDNA accumulated predominantly. The coexistence of COX-positive and COX-negative mitochondria within single cells was not observed. This protection from expression of mitochondrial dysfunction was attained by the help of endogenous wild-type mtDNA of *M. spretus*, since the mito-mice did not possess exogenous wild-type mtDNA of *M. m. domesticus*. These observations indicate the presence of complementation between exogenous mitochondria carrying ΔmtDNA and endogenous mitochondria carrying *M. spretus* wild-type mtDNA.

3.3. mtDNA recombination

Extensive recombination between mtDNAs from both parental cells has been proved in yeast [44] and plant cells [45]. Moreover, the presence of recombinant mtDNA was reported in mussel species [46]. In mammalian species, however, the issue of mtDNA recombination has been controversial [47,48]. The activity of enzymes involved in homologous recombination was identified in mammalian mitochondria [49]. The presence of recombinant or recombinant intermediates was suggested by using electrophoresis [50,51]. Some studies suggested the presence of mtDNA recombination using indirect evidence derived from statistical analyses of mtDNA sequence data [52–54], although they were criticized on several grounds [55–58].

Extensive exchanges of mtDNAs between mitochondria in human hybrid cells [34,35,38] and in mouse tissues [41,42] suggest exclusion of physical barriers against recombination between heteroplasmic mtDNAs derived from different individuals or even from different species. Therefore, this issue could be resolved by precise sequence analysis of the heteroplasmic mtDNAs.

Recently, several sequence analyses of PCR products of heteroplasmic human mtDNAs suggested the presence of recombination [59–61]. However, studies using PCR for identification of recombinant mtDNA could not completely exclude the possibility that artificial recombinants were generated by PCR jumping [62]. When DNA polymerase encounters the end of a template molecule, the premature terminated product jumps to another template and polymerase reaction continues, creating an in vitro recombination product.

For examination of in vitro mtDNA recombination, previously generated human hybrid cells possessing A4269G and A3243G pathogenic mutant mtDNA [38] were used. Cloning and sequence analysis of the 4878 bp PCR products of mtDNAs in the hybrid cells was carried out. Of the 100 clones, 64 clones possessed mutation sites specific to parental A3243G mtDNA and to A4269G mtDNA, suggesting that they corresponded to recombinant mtDNA. However, cloning and sequence analysis of mtDNA purified by EtBr-CsCl centrifugation showed no recombinant, suggesting that all recombinants observed in PCR products corresponded to PCR jumping products [63].

For examination of in vivo mtDNA recombination, mito-mouse possessing ΔmtDNA of *M. m. domesticus* and wild-type mtDNA of *M. spretus* [42] were used. To avoid PCR jumping artifacts, PCR products were not used, but mtDNAs purified from mito-mouse tissues by EtBr-CsCl centrifugation were used for cloning and sequence analysis. Of the 318 clones, only three clones possessed *M. m. domesticus* and *M. spretus* mtDNA specific mutation sites [63].

Considering the high concentration of ROS around the mtDNA and its frequent strand breakage, recombinant clones would correspond to gene conversion products created by repair of nucleotide mismatches.

The presence of recombinant mtDNA indicates that the exo- and endogenous mtDNAs come into close enough proximity to convert their sequences.

4. Gene therapy for mitochondrial diseases

The presence of extensive and continuous complementation between fused mitochondria could correspond to a unique defense system to compensate for defects induced by various kinds of mtDNA mutations, regardless of whether they are transmitted maternally [41,42] or are newly acquired [25–28]. The system is also applied to gene therapy for mitochondrial diseases. When normal mitochondria with wild-type mtDNA are introduced into cells with mutant mtDNA, cellular respiration activity would be restored by mitochondrial complementation. The issue of gene therapy for mitochondrial diseases was addressed using zygotes of mito-mice [40]. Since inheritance of mtDNA is strictly maternal [64–66], gene therapy of zygotes would be effective procedure to obtain normal progeny.

4.1. Preimplantation genetic diagnosis

Before treatment, the preimplantation genetic diagnosis of mitochondrial disease was important [67]. Because mtDNA is distributed randomly to the offspring, it is possible that the offspring would possess various proportions of mutant mtDNA. Moreover, bottleneck effect, reduction of mtDNA copy number in primordial germ cells, is thought to contribute to rapid mtDNA segregation [68,69].

It is important whether the proportion of mutant mtDNA in biopsy samples used for preimplantation genetic diagnosis correlates with that of embryos or individuals. In human fetuses or neonates with pathogenic mutant mtDNAs, it was suggested that mtDNA did not segregate much during embryogenesis [70,71]. In preimplantation embryos, polar bodies and blastomeres were shown to be effective for deducing the status of heteroplasmy in embryos of mice carrying NZB and BALB wild-type mtDNAs [72]. Also, in mito-mice possessing pathogenic ΔmtDNA, the proportions of ΔmtDNA in zygotes

and their polar bodies were correlated [73]. Then, polar bodies were used as biopsy samples of zygotes.

In mito-mice, expression of disease phenotype is strictly dependent on proportion of ΔmtDNA [40,41]. In human, it is well known that deletion mutant mtDNAs possess replication advantages over wild-type mtDNA due to its smaller size [24,74,75]. In mito-mice, ΔmtDNA also possesses replication advantages, and it leads to ΔmtDNA accumulation during aging. Thus, for success of preimplantation genetic diagnosis, the change of ΔmtDNA proportions during in utero development, postnatal stage and aging should be addressed.

When the proportions of ΔmtDNA in the polar bodies of zygotes and whole bodies of neonates developed from the same zygotes were compared, the zygotes used possessed 2–78% ΔmtDNA in their polar bodies, and the resultant neonates possessed 0–75% ΔmtDNA. No neonates developed from zygotes carrying more than 60% ΔmtDNA. Considering that the average increase in the proportion of ΔmtDNA during gestation (for 19 days) was 17±10% (mean±S.D.), neonates developed from such zygotes possessing more than 60% ΔmtDNA would carry more than 77% ΔmtDNA during gestation, and would be lethal in the uterus.

The following increase rate of ΔmtDNA proportion in mito-mice tails was 17% during gestation (19 days after fertilization), 8% before weaning after birth (for 30 days after birth), 8% in the first 100 days after weaning, and 6% in every subsequent 100 day period. The significant decrease in the rate of ΔmtDNA accumulation after birth would correspond to decrease of the mtDNA replication frequency due to decrease in frequency of cell division [72].

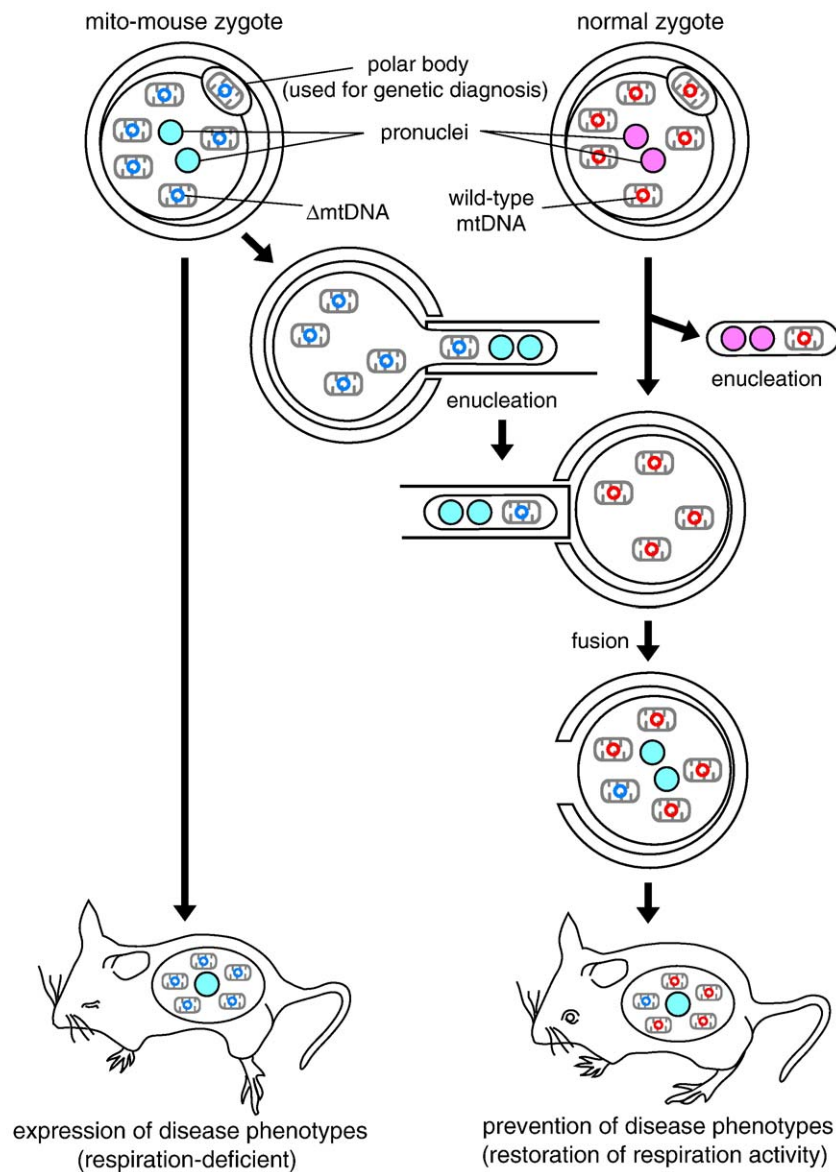

Fig. 2. Schematic representation of gene therapy. Polar bodies of mito-mouse zygotes are used to estimate the proportion of ΔmtDNA. Mito-mice developed from zygotes with more than 5% ΔmtDNA die because of respiration deficiency. Male and female pronuclei are enucleated from mito-mouse zygotes and introduced into enucleated normal zygotes by electrofusion. Small amount of mitochondria with ΔmtDNA are cointroduced into normal zygotes. Nuclear-transplanted mito-mice possess low levels of ΔmtDNA and expression of disease phenotypes is prevented.

Considering that respiration deficiency was observed in cells possessing more than 80% ΔmtDNA [40,41] and the rate of ΔmtDNA accumulation, mito-mice carrying less than 5% ΔmtDNA at their zygote stage can avoid expression of disease phenotypes caused by ΔmtDNA-induced respiration deficiency within their life times.

4.2. Gene therapy of mito-mice by nuclear transplantation

Effective procedures to obtain normal progeny from affected mothers would be either nuclear transplantation from mito-mouse zygotes to enucleated normal zygotes, or cytoplasmic transplantation of mitochondria from normal to mito-mouse zygotes [67,76]. Ooplasmic transfer has been used as an effective technique to restore normal developmental potential of oocytes from patients with embryonic development failure [77,78]. However, the amount of mtDNA copies that could be introduced into mouse zygotes by cytoplasmic transplantation is relatively small. Nuclear transplantation inevitably cointroduces a small volume of cytoplasm and mitochondria, but the amount of mtDNA cointroduced with nucleus is 6% of all mtDNA in zygotes [73]. Nuclear transplantation would result in production of zygotes with less than 5% ΔmtDNA from mito-mice, even when the mito-mouse zygotes possess the maximum proportion of ΔmtDNA (78%). It indicates that we can prevent all progeny of mito-mice from expression of disease phenotypes. Then, nuclear transplantation was considered to be more effective than cytoplasmic transplantation for sufficient dilution of ΔmtDNA in zygotes of mito-mice (Fig. 2).

First, proportions ΔmtDNA in mito-mouse zygotes were estimated using polar bodies as biopsy samples. Next, from 39 zygotes possessing 35±7% ΔmtDNA, karyoplasts were prepared and fused with enucleated normal zygotes. They were transferred to pseudopregnant females, and the resultant 11 mice at the weaned stage possessed 11±5% ΔmtDNA in their tails. On the other hand, when 34 mito-mouse zygotes with 32±12% ΔmtDNA were transferred without nuclear transplantation, obtained 9 mice possessed 66±9% ΔmtDNA. They showed respiration deficiency and died because of renal failure at 218–277 days after birth, while nuclear-transplanted mito-mice showed no abnormalities throughout their lives [73].

Recently, it was reported that the UK Human Fertilization and Embryology Authority (HFEA) would allow scientists at the University of Newcastle upon Tyne to transfer the nucleus of a human fertilized egg into an egg donated by a second woman. The study using mito-mice unambiguously showed that the technique could also be applied to human patients with mitochondrial diseases.

In human cases, however, there are several problems to be resolved before applying gene therapy to zygotes. One is that human unfertilized oocytes, but not zygotes, are preferable for nuclear transplantation. When unfertilized oocytes are used for nuclear transplantation, following in vitro fertilization with partner's sperm should be carried out as suggested by Thorburn and Dahl [76].

Another problem is that maternal transmission of deletion mutant mtDNA is rare in human [79,80]. When deletion mutant mtDNA is transmitted to oocytes [81], the 13 times longer gestation period in humans than in mice would result in accumulation of a sufficient proportion of deletion mutant mtDNA in nuclear-transplanted embryos to be lethal before birth. Therefore, application of nuclear transplantation would be restricted to patients with mitochondrial diseases, only when pathogenic mtDNAs in patients were inherited maternally, and did not possess significant replication advantages over wild-type mtDNA. For example, zygotes from patients with MELAS and MERRF caused by 3243 and 8344 point mutations in mtDNAs, respectively, would be appropriate for applying nuclear transplantation to rescue their F_0 progeny. In these cases, a small proportion of wild-type mtDNAs was sufficient to suppress disease phenotypes [82,83].

An additional biological problem is that nuclear transplantation results in zygotes possess nuclear genomes from both parents but possess mitochondrial genomes from normal donors. Moreover, there was no evidence and no rational explanation that the heteroplasmic state itself or resultant creation of mtDNA recombinants induced respiration deficiency.

Recently, Spees et al. demonstrated adult nonhematopoietic stem/progenitor cells from human bone marrow or fibroblast rendered mitochondria into ρ° cells when they were cocultured [84]. If such a transfer of mitochondria is also possible in vivo, adult mito-mice can be rescued.

5. Accelerated aging and mtDNA mutations

Recently, Trifunovic et al. [85] and Kujoth et al. [86] addressed the mitochondrial theory of aging by creating knock-in mice "mutator mice" expressing a proofreading-deficient version of the mitochondrial DNA polymerase γ (POLG). The mutator mice showed increased levels (3–8 times) of mtDNA mutations, premature aging phenotypes, such as reduced lifespan, weight loss, hair loss, curvature of the spine, loss of bone mass, decrease in red blood cells and testicular atrophy [85,86], and no increase of ROS production [86,87]. Although ROS has been thought to be the major contributor of the aging, their findings indicated the premature aging phenotypes were not caused by increase of oxidative stress accompanied by accumulation of mtDNA mutations [86–88].

There are several different phenotypes between the mutator mice created by the two groups, although the same amino acid residue was substituted. First, Kujoth et al. [86] showed increased levels of caspase-3, key mediator of apoptosis, and TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling)-positive cells in several tissues of mutator mice. They suggested that the increased apoptosis caused premature aging phenotypes. On the other hand, Trifunovic et al. showed no increased sensitivity to oxidative stress induced apoptosis in mouse embryonic fibroblasts (MEFs) [87]. Second, Trifunovic et al. showed progressive reduction of respiratory function and ATP production rates [85,87] but Kujoth et al. did not [86]. Trifunovic et al. associated the respiration deficiency with the premature aging phenotypes [85]. However, random mutation occurred in

mutator mice should not lead to respiration deficiency due to the presence of mitochondrial interaction. Thus, even though clonal accumulation of mutant mtDNA in each cell is indispensable for respiratory dysfunction, Trifunovic et al. showed no evidence for clonal accumulation of certain mutant mtDNA both in adult mutator mice [85] and MEFs [87]. It is ambiguous whether mitochondria are contributing to the premature aging phenotypes.

6. Conclusions

The mitochondrial theory of aging proposes that age-associated accumulation of somatic mutations in mtDNAs is responsible for age-associated mitochondrial dysfunction. In fact, mtDNA is continuously exposed to ROS generated in mitochondria and accumulation of various kinds of mtDNA mutations was reported. However, the mitochondrial theory of aging is based on the circumstantial evidence. Random somatic mtDNA mutations are complemented by exchange of mtDNAs and its products. This mitochondrial complementation is a very unique and effective defense system of the highly oxidative organelles for preventing individuals from expression of respiratory deficiency caused by mutant mtDNAs.

It is generally accepted that mitochondria are the symbionts into eukaryotic cells. After the symbiosis, mitochondria might have lost individuality due to the development of mitochondrial complementation. Thus, mitochondria function as a single cellular unit in mammalian cells.

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