Review

Expert Opinion

Mitochondria targeting delivery of nucleic acids

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Background: Mitochondria are intracellular organelles involved in energy production, which play important roles in metabolism. Consequently, mutation in mitochondrial DNA may have adverse effects on the host organism. This hypothesis is supported by increasing number of reports that associate various diseases with the mutation of the mitochondrial genome. Therefore, new therapy methods for targeting mitochondria genome should be developed for the treatment of these diseases. *Objectives*: The current progress in mitochondrial targeting gene delivery is discussed and future direction is suggested. *Methods*: Recent research progress in this field is briefly introduced, and successes and obstacles in research are discussed. *Results/conclusion*: Delivery of antisense DNA using lipophilic cation showed possible therapeutic effect *in vitro*. Delivery of tRNA is also another possible approach to correct tRNA mutations. However, research into the delivery of protein expression system using liposome and polymer has been very limited. The results suggest that more research is required to address the problems in mitochondrial targeting gene delivery. Here, we suggest 'multifunctional multilamella vesicular or multifunctional multi-vesicular (MMV)' for efficient mitochondrial targeting DNA delivery.

Keywords: gene delivery, gene therapy, mitochondria, multifunctional multilamella vesicular, multifunctional multi-vesicular, targeting

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1. Introduction

Mitochondria are intracellular organelles involved in oxidative phosphorylation. In mitochondria, ATP is synthesized from ADP and phosphate in the process of oxidative phosphorylation. The driving force for ATP synthesis is the proton gradient across the mitochondrial inner membrane, which is established by the electron-transfer chain. The enzymes for the electron-transfer chain are located in the mitochondrial inner membrane. Most of the enzymes are encoded by the nuclear DNA, synthesized by cytosolic ribosomes and imported into the mitochondrial matrix. Citric acid cycle, urea cycle and β-oxidation also occur in mitochondria. The citric acid cycle is the process for the production of NADH, urea cycle the process for converting toxic ammonia to urea and β-oxidation the process for oxidation of fatty acid and eventually NADH production. Abnormality in some of the electron-transfer chain enzymes may lead to the production of reactive oxygen species (ROS). Increased ROS may be related to age-related neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [1-6].

Mitochondria have their own genome, which is composed of a circular DNA. The human mitochondrial DNA (mtDNA) is 16,569 bp long and located in the mitochondrial matrix [7]. Most mitochondrial proteins are imported from cytosol, but some of the proteins are produced in the mitochondrial matrix. The mtDNA genes encode 13 hydrophobic proteins and all of them are involved in the electron transfer. Twenty-two transfer RNAs and two ribosomal RNAs for the

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protein synthesis are also encoded by the mtDNA. As indicated by the gene composition, the mutations in the mitochondrial genome result in the production of abnormal protein or a deficiency of normal protein, which may impair the electron-transfer chain. For example, mutations in gene encoding cytochrome oxidase in complex IV may block electron transfer from complex III to oxygen and increase the level of ROS production [8,9].

A growing number of human diseases has been reported to be related to mitochondrial abnormality. Impaired mitochondrial protein production, which may be caused by point mutations in mtDNA, may induce ROS production and abnormal function of mitochondrial proteins. A high level of ROS production may be involved in the development of Alzheimer's disease [4,6], Parkinson's disease [5], myocardial infarction [10,11] and diabetes [12-15]. mtDNA mutations may also be involved in blindness, deafness and renal dysfunction [16,17].

Many genetic mutations that cause human diseases have been found in mitochondrial tRNA. The mitochondrial $tRNA^{Leu}$ mutations are related to mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibers (MERRF), progressive external ophthalmoplegia (PEO), Kearns–Sayre syndrome (KSS) and myopathy [16,18]. One point mutation may cause these various diseases. For example, A8344G mutation in $tRNA^{Leu}$ may cause MELAS, MERRF, or PEO. In addition, point mutations of other mitochondrial tRNA can cause MELAS, PEO and MERRF [16,18]. This suggests that these diseases share a common development pathway. Mutations in the protein encoding genes also induce various diseases. Mutations in NADH dehydrogenases, cytochrome b, or cytochrome oxidases are commonly related to various diseases such as MELAS and Leber's hereditary optic neuropathy [16].

Due to the extensive impact of mtDNA mutations, mitochondria have become a target for disease treatments. Various metabolic therapies and antioxidant therapy have been developed to treat these diseases. However, these therapies are temporal and gene therapy is needed for long-term treatment. Mitochondrial gene therapy was only theorized in the past due to technical restraints. However, recent progress in nucleic acids delivery to mitochondria makes it possible. In this review, the progress in mitochondrial gene delivery is presented and discussed.

2. The modification of nucleic acids for delivery to mitochondria

2.1 Leader sequence conjugated nucleic acids

Most mitochondrial proteins are encoded by nuclear DNA. After the translation in cytosol, the proteins are delivered to the mitochondrial matrix. Proteins cannot pass through inner and outer mitochondrial membranes. The translocation of the proteins is mediated by translocator outer membrane (TOM)/translocator inner membrane (TIM) complex. The nuclear-encoded mitochondrial proteins commonly have amino-terminal leader sequence, which is recognized by the mitochondrial membrane translocators. Thus, this protein translocation machinery has been investigated for nucleic acids delivery to mitochondria.

An early study investigated mitochondrial translocation of protein–DNA conjugate [19]. In this study, mitochondrial leader sequence of yeast cytochrome oxidase was linked to a modified dihydrofolate reductase. Then, the leader sequence-dihydrofolate reductase fusion peptide was covalently conjugated to a single-stranded or double-stranded DNA. This protein–DNA conjugate was tested for mitochondrial entry. The study showed that 5 – 10% of total protein–DNA conjugates were completely imported to the mitochondrial matrix. This amount of the imported protein–DNA conjugates was approximately five times lower than the fusion protein without DNA. The results suggest that leader sequence-conjugated DNA can enter mitochondria efficiently. This result was confirmed by another study using the ornithine carbamoyl transferase leader sequence [20]. The ornithine carbamoyl transferase leader sequence was covalently conjugated to double-stranded DNA and the mitochondrial import was evaluated. In this report, Seibel *et al.* conjugated the leader sequence to DNA of 7 bp or 322 bp in size. Leader sequence conjugated DNAs were imported to the mitochondria regardless of the size, suggesting that comparatively large DNA with a size of more than 300 bp could be transported to mitochondrial matrix by attaching leader sequence. The mitochondrial leader sequence conjugated DNA was also used for liposome-mediated DNA delivery to mitochondria [21]. Liposome released the mitochondrial leader sequence conjugated DNA near the mitochondrial sites, then the DNA was imported to the mitochondrial matrix.

The mitochondrial leader sequence was also conjugated to peptide nucleic acids (PNA) [22,23]. PNA is a synthetic polynucleotide, in which each nucleotide is linked via peptide bond. PNAs form strong double strands with complementary DNA and RNA molecules in a sequence-specific manner. Therefore, PNA can be applied to antisense gene therapy. Mitochondrial DNA (mtDNA) with mutation may be suppressed with a sequence-specific PNA, while wild-type mtDNA produces its normal gene products. For antisense gene therapy, the mitochondrial PNA import technology was developed using mitochondrial leader sequence. The leader sequence of cytochrome c oxidase (COX) subunit VIII was covalently attached to PNA [22,23]. The leader sequence-PNA entered the isolated mitochondria. In addition, confocal microscopy studies showed that the leader sequence-PNA was localized at the mitochondrial sites, strongly suggesting that the leader sequence-PNA conjugates were imported into the intact mitochondria.

2.2 Lipophilic cation conjugated nucleic acids

Another approach for nucleic acids delivery to mitochondria is to use amphiphilic molecules with delocalized positive

charges. It was previously reported that hydrophobic molecules with cationic charges could diffuse through mitochondrial membranes and accumulate in the mitochondrial matrix [24]. Mitochondria have highly negative mitochondrial membrane potential, and lipophilic cations such as triphenylphosphomium (TPP) and rhodamine are attracted to mitochondria due to this negative potential. Also, delocalization of cationic charges on the molecules can decrease activation energy required for diffusion through hydrophobic mitochondrial membranes. For example, TPP conjugated ubiquinol (MitoQ) was developed as an antioxidant drug targeting mitochondria [25-27].

TPP conjugated PNA was developed as an antisense mitochondrial gene therapy system [28]. To suppress the point mutation in mitochondrial DNA in MERRF, a complementary PNA to the sequence containing A8344G mutation was linked to TPP. In intact cells, TPP conjugated PNA was transported across the mitochondrial membrane and accumulated in mitochondria. In many patients, wild-type and mutant mtDNAs coexist and selective repression of mutant mtDNA replication may be required for the treatment of the disease. Therefore, sequence-specific inhibition of the antisense PNA is an important factor for mitochondrial antisense gene therapy. *In vitro* assay showed the efficacy of the strategy [29]. In this study, TPP conjugated PNA bound to its target mutant DNA and inhibited its replication. Meanwhile, wild-type DNA was normally replicated even in the presence of the TPP conjugated PNA, suggesting its sequence specificity. However, in intact cells, TPP conjugated PNA did not inhibit mutated mitochondrial DNA, although it was accumulated in mitochondria. One possible explanation for the lack of effect is that the TPP conjugated PNA cannot access its target sequence or is removed after binding to its target sequence. If this is the case, covalent binding to its target DNA may increase the efficiency. As a model system, a photoreactive crosslinker, benzophenone, was attached to the TPP conjugated PNA [29]. *In vitro* binding assay showed that benzophenone–TPP conjugated PNA bound to its target irreversibly in a sequence-specific manner. The inhibition of the replication was not observed. Therapeutic evaluation of TPP-mediated PNA delivery for antisense therapy is in progress and no positive result has yet been confirmed. Irreversible binding of PNA to target sequence may inhibit the replication of mutated DNA. However, possible side effects such as non-specific binding of benzophenone and cytotoxicity should be evaluated. Although there are numerous obstacles to be overcome to realize TPP-mediated antisense therapy, it is clear that TPP-mediated antisense technology is one of the more promising technologies for the treatment of mitochondrial diseases.

2.3 Naked DNA

Recently, it was reported that the isolated rat mitochondria had the ability to uptake naked DNA [30]. Furthermore, the imported DNA could be replicated and expressed in a mitochondrial promoter dependent manner. The mechanism of the uptake is not clear. However, it was proved that the uptake might not require respiration or membrane potential [30]. Research with plant mitochondria suggests that VDAC and ANT are involved in the uptake of DNA into the plant mitochondria [30]. These studies suggest that naked DNA itself may be delivered to mitochondria without further modification. Further research should be conducted to fully identify the mechanism and apply the naked DNA delivery to mitochondrial gene therapy.

3. Delivery of cytosolic tRNA into mitochondria

Protozoan, plants and yeasts have import machinery of cytosolic tRNA to mitochondria. However, cytosolic tRNAs are not imported to mitochondria in mammalian cells. In MERRF A8344G, mt-tRNA^{Lys} is poorly aminoacylated and induces premature termination of translation [31]. Recently, it was suggested that a hypomodification at the anticodon wobble position was one of the primary causes of MERRF [32,33]. Thus, the import of cytosolic tRNA^{Lys} into mitochondria is an attractive option for the treatment of MERRF. Two enzymes are involved in the import of yeast tRNA^{Lys} to mitochondria [34-36]. The cytosolic lysyl–tRNA synthetase (KRS1p) aminoacylates the tRNA^{Lys} before the import. The other enzyme is a cytosolic precursor of the mitochondrial aminoacyl synthetase, preMSK1p. This enzyme acts as a carrier and targeting factor to mitochondria. When yeast tRNA^{Lys} was applied to isolated human mitochondria with the yeast enzymes, the yeast tRNA^{Lys} was imported into the mitochondria [37,38]. Interestingly, the yeast enzyme could be replaced with the human cell extracts, suggesting that human cell may have a protein with the yeast preMSK1p function [38]. Recently, the yeast tRNA^{Lys} gene was transfected into human cells and the effect of the tRNA^{Lys} expression was evaluated [39]. The expressed yeast tRNA^{Lys} was partially imported into the human mitochondria and the import of the yeast cytosolic tRNA^{Lys} partially restored the function of mutated mitochondrial DNA [39].

Another approach for cytosolic tRNA delivery to mitochondria for MERRF gene therapy is to use leishmania RNA import complex (RIC). Leishmania RIC is a multi-subunit protein located in mitochondrial inner membrane [40-42]. RIC interacts with cytosolic tRNA and induces the translocation of the tRNA into mitochondria in an ATP-dependent way [42]. Interestingly, the purified RIC induced tRNA import into isolated human mitochondria. After the import, tRNA was lysylated and participated in mitochondrial translation providing lysine. When RIC was added to human cells, it internalized into the cells in a caveolin-1-dependent pathway and subsequently facilitated the import of endogenous cytosolic tRNA^{Lys} [43]. Therefore, the treatment partially restored the mitochondrial malfunction caused by mutant mt-tRNA^{Lys} gene.

4. Liposome-mediated DNA delivery to mitochondria

Liposome-mediated systems, which are self-assembled structures composed of amphiphilic unit materials, provide control over the composition, the size of vesicle and the trapped volume, making it possible to contain more spaces for drugs or genetic materials either on the surface or inside if the same number of particulates are taken up by cells. Considering the number of target mitochondria, which ranges from dozens to several thousands per cell depending upon the tissue [44], one of the benefits of a liposomal vesicle-mediated delivery system is the larger amount of cargo compared to the conventional polymeric carrier systems. Given the difference in lipid composition and proteins of mitochondrial membranes compared to the plasma membrane [29], the liposome system is considered to possess flexibility over the current polymer-mediated system for potential mitochondria targeting activity.

Until now, the two representative mitochondria-targeted cationic liposome systems containing delocalized charge centers are TPP derivatives and bolasomes with dequalinium moiety [45,46]. However, a recent study shows the limitations of the mitochondria targeting system even when it is conjugated with cell penetration peptide [47]. Therefore, the liposome system with mitochondria-targeting activity should also be equipped with endosome destabilizing reagents (DOPE, chemicals, pH-sensitive linkage, oxidation-reduction sensitive linkage, other peptides, etc.) like the conventional cationic liposomes used for gene transfection. After following receptor-mediated endocytosis pathway on the plasma membrane, leading to translocation inside the primary target cells, the liposomes, equipped with additional mitochondria-specific secondary targeting activity, should be released into cytoplasm as much as possible.

5. Polymer-mediated nucleic acids delivery to mitochondria

DNA delivery to mitochondria has been researched with the mitochondrial leader peptide conjugated polyethylenimne (mLP–PEI) [48,49]. Polyethylenimine (PEI) has been widely investigated for gene delivery to the nucleus [50-52]. PEI has several advantages as a gene delivery carrier to the nucleus. First, PEI has high positive charge density, which facilitates the tight complex formation between DNA and the polymer. Second, PEI has been known to have a proton buffering effect, which can induce endosomal escape of internalized polymer/DNA complex. Third, PEI can be easily modified to redirect the DNA/PEI complex to the targeting tissues or intracellular organelles. To apply these advantages to gene delivery to mitochondria, the ornithine carbamoyltransferase mitochondrial leader sequence peptide (mLP) was conjugated to high molecular weight PEI (PEI25k, 25 kDa) (**Figure 1**) [48]. After the conjugation, mLP–PEI formed complexes with DNA equally well as PEI. *In vitro* gene delivery assay with mLP–PEI showed that the DNA was delivered to mitochondrial sites in a live cell. However, PEI without mLP did not localize the DNA at mitochondrial sites, suggesting that mLP directed the complexed DNA to mitochondria. This result showed that the DNA delivery to mitochondrial site using mLP-conjugated PEI may be a possible strategy. However, several problems should be addressed before the application of this technique. First, it is not clear if mLP–PEI/DNA complex is dissociated at mitochondrial sites. In the nuclear targeting gene delivery, it is believed that DNA delivered by PEI should be released for transcription from the polymer after delivery. Although there have been many efforts to address this question, it is not clear which step enables the release of DNA from the complex. Likewise, in the mitochondrial DNA delivery, DNA should be released from its polymeric carrier in mitochondrial matrix. Further studies should be conducted to answer this question. Second, it is not yet known whether DNA/mLP–PEI complex is internalized into the mitochondrial matrix or bonded to the surface of the mitochondrial membrane. The mitochondrial protein encoded by the nuclear DNA and produced in the cytoplasm should be transported into the mitochondrial matrix through the TIM/TOM complex. The upper size limit of the TIM/TOM complex has not been reported, but the mLP–PEI/DNA complex may be produced to have a smaller size than the size limit of the TIM/TOM complex by careful preparation. Third, the cytotoxicity of mLP–PEI should be minimized. The polymers for gene delivery commonly have positive charges for complexation with negative charged DNA. Also, complexation of DNA with cationic polymers has been performed in the excess of cationic polymer, resulting in positively surface charged complex. The positive surface charge may be beneficial for internalization of the complex into the mitochondrial matrix, since negative potential exists over the barrier of the mitochondrial inner membrane. However, cationic charge also may induce cytotoxicity. As previously reported, cationic polymer PEI may induce channel formation on the mitochondrial outer membrane and release of cytochrome c [53].

To test the correlation between molecule size and cytotoxicity, mLP-conjugated PEI was synthesized with low molecular weight PEI (mLP–PEI2K, M.W. 2,000) [49]. It was previously reported that low molecular weight PEI had low cytotoxicity [54]. In cytotoxicity assay, mLP–PEI2K showed much less cytotoxicity than mLP–PEI25K. This suggests that low positive density of PEI may reduce the cytotoxicity of the carrier. However, low charge density of the carrier may hamper some important characteristics of the gene carrier. For instance, a greater amount of PEI is required for the formation of stabilized PEI/DNA complexes and also the complex may have moderate transfection efficiency [54]. Therefore, careful modification is required for high delivery efficiency and low cytotoxicity. Recent results from nuclear

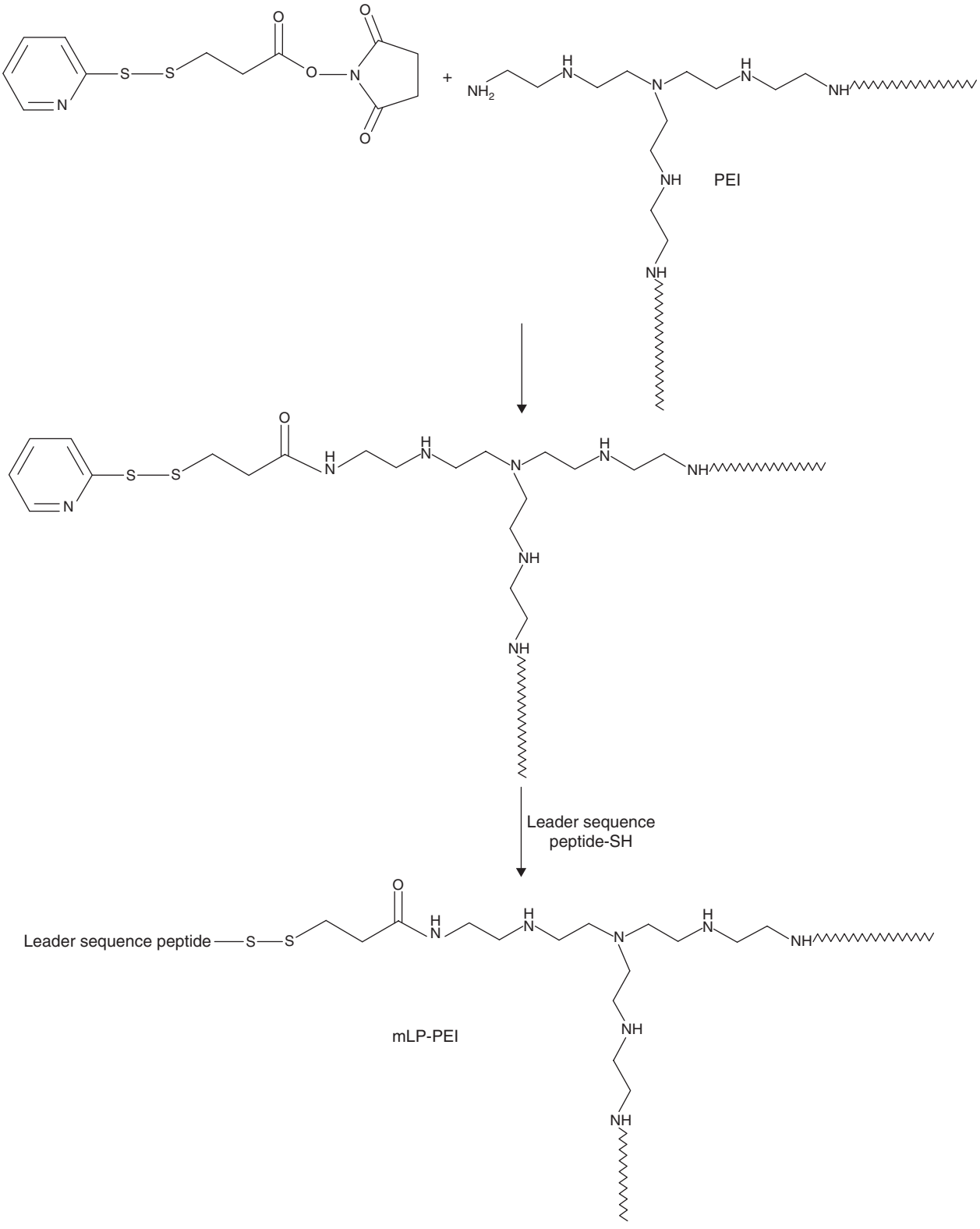

Figure 1. Synthesis of mitochondrial leader peptide conjugated polyethyleneimine.

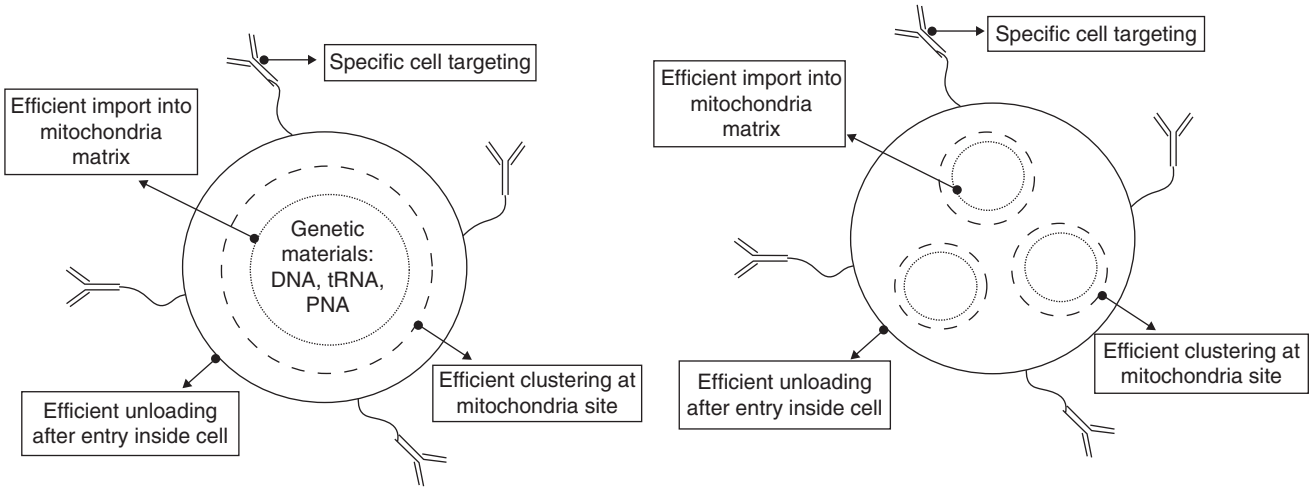

Figure 2. Schematic presentation of MMV (multifunctional multilamella vesicular or multifunctional multi-vesicular) system.

targeting gene delivery suggest that degradation of polymer using disulfide linkages after delivery is one of the effective strategies [55]. This technique may be an option for low cytotoxicity mitochondrial gene delivery.

6. Expert opinion on mitochondrial gene delivery

1. Direct modification of DNA or RNA with mitochondrial targeting ligands is a promising technology for mitochondrial gene delivery. However, there are problems that should be addressed. Modified DNA or RNA may pass through the mitochondrial membrane but have difficulty entering the plasma membrane. Therefore, another strategy is required for the delivery of DNA or RNA to the mitochondrial site from extracellular space. Direct modification of DNA or RNA and the application of polymeric or liposomal carriers may be combined to get the best results.
2. Liposomal or polymeric carriers are promising. However, cytotoxicity, release of DNA/RNA from the complex and delivery efficiency are not fully assessed and optimized. Only a few examples of the carriers have been reported for this technique. We propose such a 'multifunctional multilamella vesicular or multifunctional multi-vesicular (MMV)' system strategy (**Figure 2**). First, MMV should be decorated with primary active targeting ligands for the plasma membrane (either endocytosis-mediated or receptor-mediated endocytosis) on the target cell. Second, after evading endosomes, MMV should be able to exert secondary active targeting function to mitochondria, using reagents that have been reported to be efficient, such as TPP, cationic bolaamphiphiles, or Rhodamine 123 [46]. As mitochondria are known to be aligned along with cytoskeleton structures inside cells [56], further active targeting to myotubles may increase the possibility of MMV to physically accumulate around mitochondria, leading to enhanced targeting. In addition, the ester derivatives of dietary fatty acids may be incorporated to utilize the carnitine shuttle-mediated mitochondria entry machinery [57].
3. Minicircle DNA may be useful for mitochondrial gene therapy. At present, DNA vector for mitochondrial gene therapy is not available. Previously, it was suggested that minicircle DNA without bacterial DNA elements would be useful for appropriate mitochondrial transcription and transcriptional cleavage [58]. The minicircle DNA is a circular DNA in which unnecessary elements such as antibiotics resistant genes or replication origin are eliminated by active recombination after the replication in living bacteria. The first reported production of a minicircle DNA was a minicircle DNA by phage lambda recombinase-mediated recombination [59,60]. The target DNA was integrated between attB and attP, constructing the parent plasmid. The parent plasmid also had the lambda recombinase expression unit. After the culture of the plasmid transformed bacteria, the expression of lambda recombinase was induced. The expression of lambda recombinase facilitated the recombination and eventually produced the two minicircle DNAs, one of which contained the target DNA and the other the unnecessary DNA.

Minicircle DNA has attracted much interest in the gene therapy field due to its advantages, and various techniques have been developed for the efficient and simple production of minicircle DNA. However, direct application of minicircle DNA to mitochondrial gene therapy has not been reported. In addition, there are problems to be addressed for mitochondrial therapeutic applications of minicircle DNA. Gene expression systems such as transcription and translation in mitochondria

should be firmly established, which requires a thorough understanding of the molecular biology of mitochondrial gene expression.

4. The molecular biology of mitochondrial gene expression system should be thoroughly studied. Increasing knowledge of the system will be useful in establishing an artificial gene expression system in mitochondria. Without the knowledge, antisense therapy may currently be the only option for mitochondrial gene therapy.

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Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Perez-Severiano F, Santamaria A, Pedraza-Chaverri J, et al. Increased formation of reactive oxygen species, but no changes in glutathione peroxidase activity, in striata of mice transgenic for the Huntington's disease mutation. Neurochem Res 2004;29(4):729-33
2. Perry JJ, Fan L, Tainer JA. Developing master keys to brain pathology, cancer and aging from the structural biology of proteins controlling reactive oxygen species and DNA repair. Neuroscience 2007;145(4):1280-99
3. Jiang H, Jiang Q, Liu W, Feng J. Parkin suppresses the expression of monoamine oxidases. J Biol Chem 2006;281(13):8591-9
4. Pratico D, Uryu K, Leight S, et al. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. J Neurosci 2001;21(12):4183-7
5. Xu J, Kao SY, Lee FJ, et al. Dopamine-dependent neurotoxicity of alpha-synuclein: a mechanism for selective neurodegeneration in Parkinson disease. Nat Med 2002;8(6):600-6
6. Nunomura A, Perry G, Aliev G, et al. Oxidative damage is the earliest event in Alzheimer disease. J Neuropathol Exp Neurol 2001;60(8):759-67
7. Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome. Nature 1981;290(5806):457-65
8. Villani G, Attardi G. In vivo control of respiration by cytochrome c oxidase in wild-type and mitochondrial DNA mutation-carrying human cells. Proc Natl Acad Sci USA 1997;94(4):1166-71
9. Palmer G. Current issues in the chemistry of cytochrome c oxidase. J Bioenerg Biomembr 1993;25(2):145-51
10. Crow MT, Mani K, Nam YJ, Kitsis RN. The mitochondrial death pathway and cardiac myocyte apoptosis. Circ Res 2004;95(10):957-70
11. Rodriguez M, Lucchesi BR, Schaper J. Apoptosis in myocardial infarction. Ann Med 2002;34(6):470-9
12. Malecki MT. Genetics of type 2 diabetes mellitus. Diabetes Res Clin Pract 2005;68(Suppl1):S10-21
13. Silva JP, Kohler M, Graff C, et al. Impaired insulin secretion and beta-cell loss in tissue-specific knockout mice with mitochondrial diabetes. Nat Genet 2000;26(3):336-40
14. Ballinger SW, Shoffner JM, Hedaya EV, et al. Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. Nat Genet 1992;1(1):11-5
15. Ballinger SW, Shoffner JM, Gebhart S, et al. Mitochondrial diabetes revisited. Nat Genet 1994;7(4):458-9
16. Pulkes T, Hanna MG. Human mitochondrial DNA diseases. Adv Drug Deliv Rev 2001;49(1-2):27-43
17. Wallace DC, Lott MT, Shoffner JM, Brown MD. Diseases resulting from mitochondrial DNA point mutations. J Inherit Metab Dis 1992;15(4):472-9
18. Mukhopadhyay A, Weiner H. Delivery of drugs and macromolecules to mitochondria. Adv Drug Deliv Rev 2007;59(8):729-38
19. Vestweber D, Schatz G. DNA-protein conjugates can enter mitochondria via the protein import pathway. Nature 1989;338(6211):170-2

•• **Excellent example of leader peptide-conjugated DNA for mitochondrial gene delivery.**

20. Seibel P, Trappe J, Villani G, et al. Transfection of mitochondria: strategy towards a gene therapy of mitochondrial DNA diseases. Nucleic Acids Res 1995;23(1):10-7
21. D'Souza GG, Boddapati SV, Weissig V. Mitochondrial leader sequence-plasmid DNA conjugates delivered into mammalian cells by DQAsomes co-localize with mitochondria. Mitochondrion 2005;5(5):352-8
22. Chinnery PF, Taylor RW, Diekert K, et al. Peptide nucleic acid and delivery to human mitochondria. Gene Ther 2000;7(9):813
23. Chinnery PF, Taylor RW, Diekert K, et al. Peptide nucleic acid delivery to human mitochondria. Gene Ther 1999;6(12):1919-28
24. Rottenberg H. Membrane potential and surface potential in mitochondria: uptake and binding of lipophilic cations. J Membr Biol 1984;81(2):127-38
25. Kelso GF, Porteous CM, Coulter CV, et al. Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. J Biol Chem 2001;276(7):4588-96
26. Adlam VJ, Harrison JC, Porteous CM, et al. Targeting an antioxidant to mitochondria decreases cardiac ischemia-reperfusion injury. FASEB J 2005;19(9):1088-95
27. James AM, Sharpley MS, Manas AR, et al. Interaction of the mitochondria-targeted antioxidant MitoQ with phospholipid bilayers and ubiquinone oxidoreductases. J Biol Chem 2007;282(20):14708-18
28. Muratovska A, Lightowlers RN, Taylor RW, et al. Targeting peptide nucleic acid (PNA) oligomers to mitochondria within cells by conjugation to lipophilic cations: implications for mitochondrial DNA replication, expression and disease. Nucleic Acids Res 2001;29(9):1852-63

29. Ross GF, Smith PM, McGregor A, et al. Synthesis of trifunctional PNA-benzophenone derivatives for mitochondrial targeting, selective DNA binding, and photo-crosslinking. Bioconjug Chem 2003;14(5):962-6

30. Koulintchenko M, Temperley RJ, Mason PA, et al. Natural competence of mammalian mitochondria allows the molecular investigation of mitochondrial gene expression. Hum Mol Genet 2006;15(1):143-54

•• **Demonstration of naked DNA uptake by mitochondria. Mitochondrial expression unit using a mitochondrial promoter is developed.**

31. Enriquez JA, Chomyn A, Attardi G. MtDNA mutation in MERRF syndrome causes defective aminoacylation of tRNA(Lys) and premature translation termination. Nat Genet 1995;10(1):47-55

32. Kirino Y, Yasukawa T, Ohta S, et al. Codon-specific translational defect caused by a wobble modification deficiency in mutant tRNA from a human mitochondrial disease. Proc Natl Acad Sci USA 2004;101(42):15070-5

33. Kirino Y, Goto Y, Campos Y, et al. Specific correlation between the wobble modification deficiency in mutant tRNAs and the clinical features of a human mitochondrial disease. Proc Natl Acad Sci USA 2005;102(20):7127-32

34. Tarassov I, Entelis N, Martin RP. Mitochondrial import of a cytoplasmic lysine-tRNA in yeast is mediated by cooperation of cytoplasmic and mitochondrial lysyl-tRNA synthetases. EMBO J 1995;14(14):3461-71

35. Entelis NS, Kieffer S, Kolesnikova OA, et al. Structural requirements of tRNALys for its import into yeast mitochondria. Proc Natl Acad Sci USA 1998;95(6):2838-43

36. Entelis NS, Kolesnikova OA, Martin RP, Tarassov IA. RNA delivery into mitochondria. Adv Drug Deliv Rev 2001;49(1-2):199-215

37. Kolesnikova OA, Entelis NS, Mireau H, et al. Suppression of mutations in mitochondrial DNA by tRNAs imported from the cytoplasm. Science 2000;289(5486):1931-3

38. Entelis NS, Kolesnikova OA, Dogan S, et al. 5 S rRNA and tRNA import into human mitochondria. Comparison of in vitro requirements. J Biol Chem 2001;276(49):45642-5

39. Kolesnikova OA, Entelis NS, Jacquin-Becker C, et al. Nuclear DNA-encoded tRNAs targeted into mitochondria can rescue a mitochondrial DNA mutation associated with the MERRF syndrome in cultured human cells. Hum Mol Genet 2004;3(20)2519-34

40. Goswami S, Dhar G, Mukherjee S, et al. A bifunctional tRNA import receptor from Leishmania mitochondria. Proc Natl Acad Sci USA 2006;103(22):8354-9

41. Mukherjee S, Basu S, Home P, et al. Necessary and sufficient factors for the import of transfer RNA into the kinetoplast mitochondrion. EMBO Rep 2007;8(6):589-95

42. Mahata B, Bhattacharyya SN, Mukherjee S, Adhya S. Correction of translational defects in patient-derived mutant mitochondria by complex-mediated import of a cytoplasmic tRNA. J Biol Chem 2005;280(7):5141-4

43. Mahata B, Mukherjee S, Mishra S, et al. Functional delivery of a cytosolic tRNA into mutant mitochondria of human cells. Science 2006;314(5798):471-4

44. Szewczyk A, Wojtczak L. Mitochondria as a pharmacological target. Pharmacol Rev 2002;54(1):101-27

45. Smith RA, Porteous CM, Gane AM, Murphy MP. Delivery of bioactive molecules to mitochondria in vivo. Proc Natl Acad Sci USA 2003;100(9):5407-12

46. Weissig V, Torchilin VP. Cationic bolasomes with delocalized charge centers as mitochondria-specific DNA delivery systems. Adv Drug Deliv Rev 2001;49(1-2):127-49

•• **Excellent review of liposome-based DNA delivery to mitochondria.**

47. Ross MF, Filipovska A, Smith RA, et al. Cell-penetrating peptides do not cross mitochondrial membranes even when conjugated to a lipophilic cation: evidence against direct passage through phospholipid bilayers. Biochem J 2004;383(Pt. 3):457-68

48. Lee M, Choi JS, Choi MJ, et al. DNA delivery to the mitochondria sites using mitochondrial leader peptide conjugated polyehtylenimine. J Drug Target 2007;15:115-22

• **Good example of polymer-mediated DNA delivery to mitochondria.**

49. Choi JS, Choi MJ, Ko KS, et al. Low molecular weight polyethylenimine-mitochondrial leader peptide conjugate for DNA delivery to mitochondria. Bull Kor Chem Soc 2006;27(9):1335-40

50. Kang HC, Lee M, Bae YH. Polymeric gene carriers. Crit Rev Eukaryot Gene Expr 2005;15(4):317-42

51. Lee M, Kim SW. Polymeric gene carriers. Pharm News 2002;9:407-15

52. Han S, Mahato RI, Sung YK, Kim SW. Development of biomaterials for gene therapy. Mol Ther 2000;2(4):302-17

53. Moghimi SM, Symonds P, Murray JC, et al. A two-stage poly(ethylenimine)-mediated cytotoxicity: implications for gene transfer/therapy. Mol Ther 2005;11(6):990-5

54. Lee M, Rentz J, Han SO, et al. Water-soluble lipopolymer as an efficient carrier for gene delivery to myocardium. Gene Ther 2003;10(7):585-93

55. Read ML, Bremner KH, Oupicky D, et al. Vectors based on reducible polycations facilitate intracellular release of nucleic acids. J Gene Med 2003;5(3):232-45

56. Heggeness MH, Simon M, Singer SJ. Association of mitochondria with microtubules in cultured cells. Proc Natl Acad Sci USA 1978;75(8):3863-6

57. Steiber A, Kerner J, Hoppel CL. Carnitine: a nutritional, biosynthetic, and functional perspective. Mol Aspects Med 2004;25(5-6):455-73

58. Bigger BW, Tolmachov O, Collombet JM, et al. An araC-controlled bacterial cre expression system to produce DNA minicircle vectors for nuclear and mitochondrial gene therapy. J Biol Chem 2001;276(25):23018-27

• **First example of minicircle DNA for nuclear and mitochondrial gene therapy.**

59. Darquet AM, Rangara R, Kreiss P, et al. Minicircle: an improved DNA molecule for in vitro and in vivo gene transfer. Gene Ther 1999;6(2):209-18

60. Darquet AM, Cameron B, Wils P, et al. A new DNA vehicle for nonviral gene delivery: supercoiled minicircle. Gene Ther 1997;4(12):1341-9

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