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Treatment of human cells derived from MERRF syndrome by peptide-mediated mitochondrial delivery

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Abstract

Background aims. The feasibility of delivering mitochondria using the cell-penetrating peptide Pep-1 for the treatment of MERRF (myoclonic epilepsy with ragged red fibers) syndrome, which is caused by point mutations in the transfer RNA genes of mitochondrial DNA, is examined further using cellular models derived from patients with MERRF syndrome. *Methods.* Homogenesis of mitochondria (wild-type mitochondria) isolated from normal donor cells with about 83.5% preserved activity were delivered into MERRF fibroblasts by Pep-1 conjugation (Pep-1-Mito). *Results.* Delivered doses of 52.5 μg and 105 μg Pep-1-Mito had better delivered efficiency and mitochondrial biogenesis after 15 days of treatment. The recovery of mitochondrial function in deficient cells receiving 3 days of treatment with peptide-mediated mitochondrial delivery was comprehensively demonstrated by restoration of oxidative phosphorylation subunits (complex I, III and IV), mitochondrial membrane potential, adenosine triphosphate synthesis and reduction of reactive oxygen species production. The benefits of enhanced mitochondrial regulation depended on the function of foreign mitochondria and not the existence of mitochondrial DNA and can be maintained for at least 21 days with dramatically elongated mitochondrial morphology. In contrast to delivery of wild-type mitochondria, the specific regulation of Pep-1-Mito during MERRF syndrome progression in cells treated with mutant mitochondria was reflected by the opposite performance, with increase in reactive oxygen species production and matrix metalloproteinase activity. *Conclusions.* The present study further illustrates the feasibility of mitochondrial intervention therapy using the novel approach of peptide-mediated mitochondrial delivery and the benefit resulting from mitochondria-organelle manipulation.

Key Words: *MERRF syndrome, metalloproteinases, mitochondrial delivery, mitochondrial function, Pep-1*

Introduction

Myoclonic epilepsy with ragged red fibers (MERRF) syndrome is a maternally inherited mitochondrial encephalomyopathy characterized by myoclonus epilepsy, generalized seizures, ataxia and myopathy (1,2). Four different point mutations are known to be associated with MERRF. The most common one, an A-to-G transition at nucleotide 8344 (1), is connected with the *tRNALys* gene in mitochondrial DNA (mtDNA). The nucleotide 8344 mutation (mt^{8344}) has been associated with severe defects in protein synthesis, which lead to a general decrease in the respiration rate in cells and tissue mitochondria (3). mtDNA mutation may further compromise respiratory function and culminate in a vicious cycle derived from oxidative phosphorylation-generated reactive oxygen species (ROS), which promotes the progression of mitochondrial diseases (4,5). This fact is revealed in skin fibroblasts from patients with MERRF syndrome (5,6). These cells show broad expressions of imbalance in the gene expression of antioxidant enzymes, excessive ROS production and matrix metalloproteinases (MMPs), which are a progressive marker of neurodegenerative diseases (5,6). Similar to many mitochondrial disorders, no cure currently exists for MERRF syndrome. Treatment with high doses of coenzyme Q10, L-carnitine, and various vitamins has been attempted to improve mitochondrial function and reduce mitochondria-generated oxidative stress; however, success has been limited (7).

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Mitochondrial transfer (mito-transfer) is a variant of this approach. Embryonic development and quality improve through a direct micro-injection of mitochondrial concentrates (8,9). Spontaneous mito-transfer, which plays a physiologic role by rescuing the respiration of deficient cells, can occur among cells *in vitro* through two possible models of transfer: direct cytoplasmic transfer or discrete vesicles (e.g. synaptic communication among neurons) rather than transfer through cell fusion (10,11). Mitochondria are highly mobile and dynamic organelles in cells that continuously fuse and divide (12,13). These processes allow mitochondria to exchange their contents and maintain homeostasis, which ensures that cells maintain normal properties (12–14). Based on these findings, we hypothesize that active intervention in mitochondrial homeostasis via a peptide delivery system for regulating mitochondrial function would be a useful therapeutic approach for the treatment of mitochondrial diseases.

Pep-1, an amphipathic peptide that consists of three domains—a hydrophobic tryptophan-rich motif, a hydrophilic lysine-rich domain and a spacer domain—is a member of the cell-penetrating peptide family. It can efficiently deliver various, fully biologically active peptides and proteins, even nanoparticles, into cells by electrostatic and hydrophobic interaction with the cell membrane, without the need for prior cross-linking or chemical modification (15–17). The mechanism of Pep-1-mediated cell translocation is independent of the endosome pathway (18). Cytotoxicity of Pep-1 is not observed in primary mammalian cells. It does not affect the competitive binding of receptors, reporter genes, receptor internalization, or intracellular calcium release in different cell lines (19–21).

We demonstrated more recently the functional recovery of human cells harboring the mtDNA mutation MERRF *A8344G* (MERRF cybrid cells) through peptide-mediated mitochondrial delivery (PMD) (22). The internalization of foreign mitochondria labeled with Pep-1 (Pep-1-Mito) and their mtDNA replication can be observed in host cells; internalized Pep-1-Mito prevent mitochondria-dependent cell death under conditions of starvation and regulate mitochondrial dynamics (22). It would be interesting to know whether the performance of the PMD system is dependent on the cell type, whether the performance varies with the amount of mitochondria to be delivered, and whether these effects are determined from the specific manipulation of delivering foreign mitochondria. In the present study, we examined the feasibility of PMD using various Pep-1-Mito doses in fibroblasts derived from patients with MERRF syndrome (MERRF fibroblasts) and B lymphocyte cell lines. In addition, matrix metalloproteinase type 1 (MMP-1) expression was analyzed in the MERRF fibroblasts after delivery of mutant mitochondria ($mtDNA^{8344}$) isolated from MERRF cybrid cells or wild-type mitochondria isolated from control cybrid cells.

Through tracking the reporter gene of green fluorescent protein (GFP) from *Aequorea coerulescens* (Ac-GFP) targeted to donor mitochondrial matrix (mito-GFP), we demonstrated that Pep-1-Mito could be internalized into the cells. Recovery of mitochondrial function and return of significantly elevated matrix metalloproteinase (MMP) activity to almost normal levels were observed in treated MERRF fibroblasts but not in MERRF fibroblasts with $mtDNA^{8344}$. In addition, the importance of mitochondrial functionality after mitochondrial isolation in the effectiveness of PMD therapy was revealed by delivery of dysfunctional mitochondria induced by rhodamine 6G (R6G) without mtDNA depletion; restoration of mitochondrial function disappeared in cells delivered of R6G-treated mitochondria at the same manipulation. The present study further illustrates the potential of mitochondrial intervention therapy using a novel approach of PMD. It is hoped that this study will help clarify the related molecular mechanisms of mitochondria-related diseases.

Methods

Cell culture and culture conditions

The primary skin fibroblasts from healthy females and female patients with MERRF syndrome were kindly provided by Professor Y. H. Wei (23). The proportion of mtDNA with A3844G mutation in the skin fibroblasts of female patients was determined to be >85% by polymerase chain reaction (PCR)-restriction fragment length polymorphism, as described previously (23). The skin fibroblasts were cultivated with Dulbecco's modified Eagle's medium (DMEM; low glucose DMEM; Gibco/Invitrogen, Carlsbad, California, USA) supplemented with 10% fetal bovine serum (Gibco), 100 μg/mL sodium pyruvate (Gibco) and 1% PS (100 U/L penicillin G sodium, 100 mg/L streptomycin sulfate) (Gibco). By following the detailed procedures described previously (24), B lymphoblastoid cells (lymphoblastoid cell line [LCL]) from one patient with MERRF (MERRF LCL) were derived by Epstein-Barr virus transformation of peripheral blood mononuclear cells. LCLs from healthy individuals, which were used as the normal control, were obtained from Dr Show-Yow Li (Chung Shan Medical University, Taiwan). All LCLs were grown in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine (Gibco) and 1% PS. Cell cultures were maintained at 37°C in a humidified