ELSEVIER

Direct Human Mitochondrial Transfer: A Novel Concept Based on the Endosymbiotic Theory

T. Kitani[a], D. Kami[b], T. Kawasaki[b], M. Nakata[a], S. Matoba[a], and S. Gojo[b,*]

[a]Department of Cardiovascular Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan; [b]Department of Regenerative Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

ABSTRACT

Mitochondria play an essential role in eukaryotes, and mitochondrial dysfunction is implicated in several diseases. Therefore, intercellular mitochondrial transfer has been proposed as a mechanism for cell-based therapy. In addition, internalization of isolated mitochondria cells by simple coincubation was reported to improve mitochondrial function in the recipient cells. However, substantial evidence for internalization of isolated mitochondria is still lacking, and its precise mechanism remains elusive. We tested whether enriched mitochondria can be internalized into cultured human cells by simple coincubation using fluorescence microscopy and flow cytometry. Mitochondria were isolated from endometrial gland–derived mesenchymal cells (EMCs) or EMCs stably expressing mitochondrial-targeted red fluorescent protein (EMCs-DsRed-mito), and enriched by anti-mitochondrial antibody-conjugated microbeads. They were coincubated with isogeneic EMCs stably expressing green fluorescent protein (GFP). Live fluorescence imaging clearly showed that DsRed-labeled mitochondria accumulated in the cytoplasm of EMCs stably expressing GFP around the nucleus. Flow cytometry confirmed the presence of a distinct population of GFP and DsRed double-positive cells within the recipient cells. In addition, transfer efficiency depended on mitochondrial concentration, indicating that human cells may possess the inherent ability to internalize mitochondria. Therefore, this study supports the application of direct transfer of isogeneic mitochondria as a novel approach for the treatment of diseases associated with mitochondrial dysfunction.

MITOCHONDRIAL dysfunction is associated with a wide range of health problems, such as cancer, aging, and metabolic, cardiovascular, and neurodegenerative diseases [1,2]. Accordingly, the transfer of exogeneous mitochondria into human cells is currently envisioned as a mechanism in cell-based therapy [3,4].

Cellular uptake of exogeneous mitochondria, and the subsequent functional recovery of the recipient cells have been reported [5,6]. However, previous studies used mitochondrial dyes, which may leak from the donor mitochondria and result in artifactual transfer of exogeneous mitochondria. In the present study, we used genetically labeled mitochondria instead of dyes to determine whether exogeneous mitochondria may be internalized into isogeneic mesenchymal cells. Internalization was monitored by fluorescence microscopy and flow cytometry using mitochondria isolated from human uterine endometrial gland–derived mesenchymal cells (EMCs) stably expressing mitochondrial-targeted red fluorescent protein (EMCs-DsRed-mito) and EMCs stably expressing green fluorescent protein (GFP).

MATERIALS AND METHODS

Cell Culture

Human EMCs were kindly provided by Dr Umezawa [7]. EMCs stably expressing GFP or DsRed-mito were generated with a recombinant retrovirus carrying GFP or DsRed-mito driven by the pMX retroviral vector, as described previously [8]. Both cell lines were maintained in Dulbecco's modified Eagle's medium (Life

*Address correspondence to Satoshi Gojo, MD, PhD, Department of Regenerative Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 465 Kajii cho, Kamigyo ku, Kyoto 602-8566, Japan. E-mail: gojos@koto.kpu-m.ac.jp

0041-1345/14/$–see front matter
http://dx.doi.org/10.1016/j.transproceed.2013.11.133

Technologies, Tokyo, Japan) supplemented with 10% fetal bovine serum (Life Technologies) and 1% penicillin/streptomycin (Life Technologies).

Mitochondrial Isolation and Transfer

Mitochondria were isolated from EMCs or EMCs-DsRed-mito using the magnetic beads isolation kit (Miltenyi Biotec, Tokyo, Japan) with a modified protocol for further enrichment. In brief, harvested cells were ruptured by 20 strokes of a 27-gauge needle. The homogenate was centrifuged (400 × *g*; 5 minutes) twice to remove unbroken cells. After magnetic labeling with antimitochondria outer membrane receptor 22 microbeads (Miltenyi Biotec), the suspension was loaded onto a MACS Column (Miltenyi Biotec) placed in the magnetic field of a MACS Separator (Miltenyi Biotec). After removing the column from the magnetic field, the retained mitochondria were flushed out and collected. The concentration of isolated mitochondria was expressed relative to protein concentration using a Bio-Rad protein assay kit (Bio-Rad, Richmond, Calif, United States). The surface charge (zeta potential: electrostatic potential generated by the accumulation of ions at the surface of colloidal particles) of isolated mitochondria was determined using a Zetasizer Nano ZS (Malvern Instruments, Malvern, United Kingdom) [9]. Mitochondria (100 μg) were fixed with 2% paraformaldehyde (TAAB Laboratory Equipment Ltd, Aldermaston, United Kingdom) and 2% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA, United States) in 0.1 mol/L cacodylate buffer (pH 7.4) (Electron Microscopy Sciences) at 4°C overnight. After fixation, the samples were analyzed by transmission electron microscopy. For mitochondrial transfer, EMCs were incubated in 6-well plates (1×10^5 cells/well) with various concentrations (2.5, 5, or 10 μg/mL) of enriched mitochondria for 24 hours at 37°C under 5% CO_2.

Flow Cytometry

Cells were dispersed with 0.25% trypsin–ethylenediaminetetraacetic acid and subjected to flow cytometry. The cell population was evaluated using 488- and 561-nm laser excitation lines to detect GFP and Ds-Red, respectively. Fluorescence data were collected using the Cell Sorter SH800 (Sony, Tokyo, Japan), and the flow cytometry data were analyzed using FlowJo software (TreeStar, San Carlos, Calif, United States).

RESULTS

Isolated Mitochondria from EMCs-DsRed-mito

Mitochondria were isolated from EMCs-DsRed-mito (Figs 1A, B). Flow cytometry revealed that isolated mitochondria may be efficiently enriched (Fig 1C). Transmission electron microscopy confirmed a morphologically maintained ultrastructure of cristae and intact outer membranes with microbeads after enrichment (Fig 1D). Zetasizer measurement showed that isolated mitochondria had a negatively charged surface (mean = −18.7 ± 10.1 [standard deviation] mV), indicating that mitochondria could have viable functionality (Fig 1E).

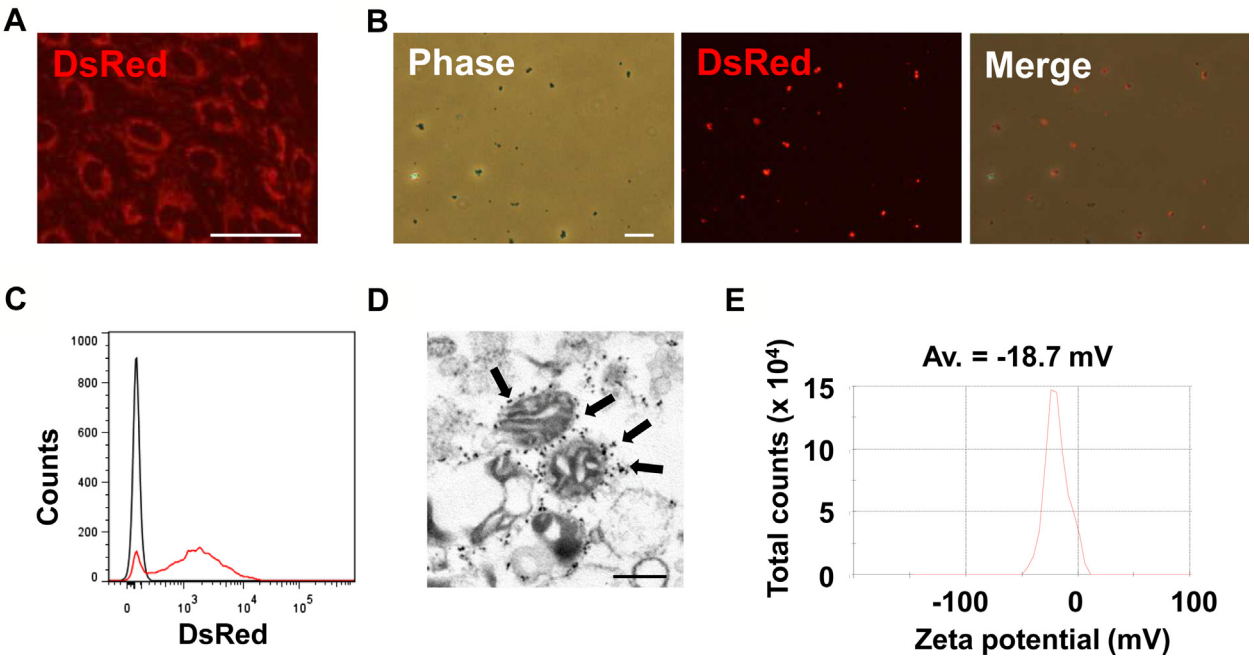

Fig 1. Mitochondria were isolated and enriched from human uterine endometrial gland-derived mesenchymal cells (EMCs) or EMCs stably expressing mitochondrial-targeted red fluorescent protein (EMCs-DsRed-mito). **(A)** Representative image of EMCs-DsRed-mito by fluorescence microscopy. Scale bar, 100 μm. **(B)** Representative images of mitochondria isolated from EMCs-DsRed-mito: phase contrast (*left*), fluorescence (*middle*), and merged images (*right*). Scale bar, 20 μm. **(C)** Flow cytometry of isolated mitochondria. *Black and red curves* represent mitochondria from EMCs and EMCs-DsRed-mito, respectively. **(D)** Transmission electron microscopy of isolated mitochondria. *Black arrows* indicate microbeads attached to the mitochondrial outer membrane. Scale bar, 500 nm. **(E)** Zeta potential distribution of isolated mitochondria. (AV, average zeta potential.)