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Organelles in focus

Delivering healthy mitochondria for the therapy of mitochondrial diseases and beyond

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ABSTRACT

Mitochondrial transfer has been demonstrated to a play a physiological role in the rescuing of mitochondrial DNA deficient cells by co-culture with human mesenchymal stem cells. The successful replacement of mitochondria using microinjection into the embryo has been revealed to improve embryo maturation. Evidence of mitochondrial transfer has been shown to minimize injury of the ischemic-reperfusion rabbit heart model. In this mini review, the therapeutic strategies of mitochondrial diseases based on the concept of mitochondrial transfer are illustrated, as well as a novel approach to peptide-mediated mitochondrial delivery. The possible mechanism of peptide-mediated mitochondrial delivery in the treatment of the myoclonic epilepsy and ragged-red fiber disease is summarized. Understanding the feasibility of mitochondrial manipulation in cells facilitates novel therapeutic skills in the future clinical practice of mitochondrial disorder.

1. Introduction

Mitochondria are intracellular organelles containing DNA (mtDNA) inherited solely from the distaff side. Mitochondria play a crucial role in ATP production through oxidative phosphorylation, a process carried out by the respiratory chain complexes I–V. Mitochondria are also involved in amino acid, lipid, and steroid metabolism and serve as Ca^{2+} buffers, which are sources of free radicals and regulators of apoptosis. Mitochondrial dysfunction or disease represents a malfunction in the biochemical process of energy production, resulting from disruption of either the mtDNA or nuclear DNA genome (Orth and Schapira, 2001). Maintaining adequate mitochondria or mitochondrial mass is a critical issue for the longevity of postmitotic cells, such as neurons, retinal pigment epithelium, cardiac myocytes, and skeletal muscle fibers. These cells are all highly vulnerable to the aging process because of their intensive free radical exposure (Terman et al., 2010). Thus, mitochondrial replication, fission, fusion, degradation and mitochondrial shuttling may play a critical role in the homeostasis of mitochondria, especially for the longevity of postmitotic tissue.

2. Organelle function in mitochondrial transfer

Mitochondria are dynamic organelles. The equilibrium between fusion and fission events controls the elongated and fragmentary morphology of the mitochondria. Mitochondrial networks share information through inter-mitochondrial complementation, either in whole mitochondrial connections or genome exchanges, and enlighten potential therapeutic strategies in the treatment of mitochondrial disease with or without mtDNA mutations (Nakada et al., 2001; Ono et al., 2001). Nonetheless, no detailed information supports the evidence of mitochondrial transfer *in vivo* study. Spontaneous mitochondrial transfer can occur among cells *in vitro* and can play a physiological role by rescuing the respiration of deficient cells with increased ATP generation and oxygen consumption. Particularly in the co-culture with human mesenchymal stem cell (hMSCs), the progenitor cell can provide healthy mitochondria into the cell without mtDNA cell (ρ° cells) (Spees et al., 2006). However, mitochondrial function in ρ° cells is not restored after

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being co-cultured with isolated mitochondria or platelets, indicating the existence of an active cellular process in the pivot of mitochondrial transfer from hMSCs (Spees et al., 2006). Formations of tunneling nanotubes and microvesicles shuttling in progenitor cells were considered two possible routes of healthy mitochondria transferring from donator cells. Rustom et al. (2004) described highly sensitive nanotubular structures forming a de novo complex pathway between cells that facilitate the selective transfer of membrane vesicles and organelles, including mitochondria. The nanotubes traffic cell surface proteins and organelles between immune cells approximately 10 μm apart and connect a variety of cell types together, including human NK cells, macrophages, and EBV-transformed B cells (Onfelt et al., 2004). Microvesicles also play an important pleiotropic role in numerous biological processes in the exchange of bioactive molecules, including transfer membrane receptors, proteins, mRNA infectious pathogens, and organelles (e.g., mitochondria) (Ratajczak et al., 2006). The hMSCs transfer mitochondria into the ρ° cells and restore mitochondrial function in cybrid cells that have been treated with R6G, a non-genetic mitochondrial toxin. Thus, mitochondria-functional restoration by MSCs-mediated mitochondrial transfer in ρ° cells contributed to successfully transferring intact mitochondria, rather than mtDNA alone. However, such phenomena were not observed in the cybrid cells harboring pathogenic mtDNA mutations, such as the A3243G mutation, which failed to receive health mitochondria during the cell co-culture (Cho et al., 2012). Partial MSCs-fusion was thought to be the mechanism involved in the rescue of ρ° cells with severely depleted mtDNA (Bukoreshtliev et al., 2009).

3. Cell physiology

Can exogenous mitochondria isolated from an allogeneic individual adapt and survive in the recipient cell, co-existing in cooperation with the host cell? Pinkert et al. (1997) presented an experimental transfer of foreign mitochondria between two distantly related species of mice. They microinjected mitochondria that had been isolated from the liver of *Mus spretus* mice into fertilized zygotes of *Mus musculus domesticus* origin Foreign mitochondria were still detected by nested PCR at the blastocyst-stage of the embryo after 4.5 days in culture (Pinkert et al., 1997). Exogenous young mitochondrial implantation may have improved embryonic development in the fertilized murine zygotes from younger or older mice. Yi et al. (2007) collected mitochondria from murine hepatocytes and fertilized murine zygotes from young and old mice in the 2PN stage. After the in vitro culture, 38% of the embryos from the young mice developed to the blastocyst stage in the injected group, but only 21% of those in the control groups. Zygotes from the older mice that received mitochondrial transfer also exhibited a more favorable outcome at the active stage of blastocyst development (Yi et al., 2007). However, microinjection of stimulated mitochondria by serum-starvation derived from somatic cells enhanced the parthenogenetic development of bovine or murine oocytes. Thus, different situations, including diversity of mtDNA heteroplasmy, nuclear-mitochondrial interaction, preparation in mitochondrial purification, and epigenetic modification, all impact the outcome of mitochondrial transfer (Takeda et al., 2010). In 2013, a feasible and novel method of mtDNA, not mitochondrial organelle, replacement in human oocytes was developed as spindle–chromosomal complex transfer. All zygotes of embryonic stem cell lines derived from spindle–chromosomal complex transfer contained normal euploid karyotypes and, exclusively, donor mtDNA. This achievement can be applied in the fertility clinic, especially for the prevention of mitochondrial disease with maternal transmission in offspring (Tachibana et al., 2012).

4. Organelle pathology

4.1. Restoration of mitochondrial dysfunction in the ischemic perfusion model of the heart by mitochondria transfer

Ischemia induces mitochondrial damage and dysfunction that persist throughout reperfusion and may determine the severity of post-ischemic functional deterioration and cellular apoptosis in the heart (McCully et al., 2007). McCully et al. (2009) demonstrated that the injection of isolated myocardial mitochondrial into the ischemic zone of myocardial tissue before reperfusion partially restores postischemic functional recovery, including left ventricular peak-developed pressure and systolic shortening in ischemic heart. Creatine kinase-MB, cardiac troponin-I, and infarct size relative to area at risk were substantially decreased in the group of mitochondrial injections. However, they noted that the injected mitochondria were only observed in the interfibrillar space of epicardial surfaces and not inside the myocytes (McCully et al., 2009). This works by intervention of xenogeneic mitochondrial transfer, and the research team demonstrated that applying autologously derived mitochondria by injection at the ischemic zone prior to reperfusion of ischemic rabbits heart model may dwindle the infarction size and suppress the plasma level of creatine kinase MB, cardiac troponin-I, and cell apoptosis activity. The transferred mitochondria restored partial function, including oxygen consumption, ATP production, cytokine mediators generation, and remodeling of proteomic pathways. Finally, the transplanted mitochondria can be successfully translocated from the interstitial spaces of myocytes into the host cells after injection for 2–8 h, either in vivo or in vitro (Masuzawa et al., 2013).

4.2. Restoration of mitochondrial dysfunction in the cancer model by mitochondria transfer

Elliott et al. (2012) disclosed that the mitochondria purified from immortalized, untransformed mammary epithelial MCF-12A cells could successfully enter human breast cancer cell lines and suppress cancer cell proliferation in a dose-dependent pattern. Mitochondria from MCF-12A cells could also be transferred into human breast cancer MCF-7 cell lines, accompanied by increased sensitivity of doxorubicin, abraxane, or carboplatin chemotherapy. This is the first publication regarding mitochondria transfer, creating a niche of cancer treatment that promotes cancer cell apoptosis and drug sensitivity (Elliott et al., 2012). Tumorigenesis was generated when the genomic background of the HeLa-cybrid cancer cell line was switched from wide type mtDNA to 8993 or 9176-np mtDNA mutations (gene of ATP synthase subunit-6). These findings indicated that tumor growth depended upon the pathogenic or somatic mtDNA mutations that provide defective oxidative phosphorylation, glycolysis-dependent shifting, and finally promote tumor growth (Shidara et al., 2005).

4.3. Restoration of mitochondrial dysfunction in the mitochondrial disease by mitochondria transfer

Occurrence of mitochondrial disease with mtDNA mutation is attributed to the pregnable mitochondrial genome and a high frequency of free radical generation in mitochondria. The severity of mtDNA defects, either from congenital or acquired etiology, may determine the time table of exhaustion in the neuro-cardio-gastro-endocrine-reproductive system during the ageing process (Wallace, 2005). Schaefer et al. (2008) reported the prevalence of mtDNA disease in the working-age population of North-East England from 1990 to 2004, that 9.2 in 100,000 people clinically manifest mtDNA disease, and 16.5 in 100,000 children and adults younger than retirement age are at risk of suffering from mtDNA