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Research Article

Tunneling Nanotube-Mediated Mitochondrial Transfer: A New Approach to Cell Protection

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Abstract

Mitochondria are eukaryotic organelles involved in crucial biochemical processes in cells and owing to their importance in cellular functions; many studies have been conducted to demonstrate the effects of mitochondrial transfer between physically separated cells. Tunneling nanotubes have been denoted as the major route for mitochondrial transfer. Given that mitochondria regulate many cellular processes; perturbation in their internal organization ultimately leads to cellular failure. Interventions by using stem cells have shown that cell injury as a result of mitochondrial dysfunction caused by different insults can be reversed as miscellaneous studies have indicated

Keywords: Mitochondrial transfer; Tunneling nanotubes; Mesenchymal stem cells; Cell injury

Abbreviation: BMSCs: Bone marrow stromal cells; CEC: Corneal epithelial cells; CS: Cigarette smoke; ECs: Epithelial cells; EGF: Epidermal growth factor; EGFP: Enhanced green fluorescent protein; EGFR: Epidermal growth factor receptor; HUVECs: Human umbilical vein endothelial cells; iPSC-MSCs: Induced pluripotent stem cell-derived mesenchymal stem cells; MCAO: Middle cerebral artery occlusion; MMSCs: Multipotent mesenchymal stem cells; MPs: Membrane protrusions; mTOR: Mammalian target of rapamycin; NSCs: Neural stem cells; OGD: Oxygen-glucose deprivation; OXPHOS: Oxidative phosphorylation; PS: Phosphatidylserine; RCN: Rat cortical neurons; Rot: Rotenone; TNTs: Tunneling nanotubes; WJMSCs: Wharton's jelly-derived mesenchymal stem cells

Introduction

Many cellular metabolic pathways are dependent on sufficient supply of ATP; NAD; and FADH2 which are produced in the mitochondria; double-membrane eukaryotic organelles containing 16.569 base-pair mtDNA [1]. Although mitochondria are considered as the "*powerhouses*" of the cell; they have a variety of critical disparate functions by which many cellular processes are regulated such as fatty acid and cholesterol synthesis; amino acid synthesis; management of redox balance; waste management; apoptosis; autophagy and senescence [2-4].

Lynn et al. has proposed that not only mitochondria and chloroplast but also eukaryotic flagellum and mitotic apparatus originated from endosymbiotic organisms [5]. It is considered that early "proto-mitochondria" evolved from alpha-proteobacterium and many genomic and proteomic changes occurred throughout the evolutionary processes [6,7]. Given that mitochondria have crucial functions for cell metabolism; perturbation of their internal integrity may culminate in abnormal cell dynamics which can lead to disturbed cell functioning and even cell death.

Mitochondrial Transfer

In vitro mitochondrial transfer was first exemplified with an experiment in which mtDNA mutated and depleted A549 cells were rescued by human bone marrow stem cells [8]. It has been demonstrated that mitochondria can be donated intercellularly by means of different mechanisms such as tunneling nanotubes; cell fusion; extracellular vesicles; and mitochondrial extrusion [9]. Recently, many studies have been demonstrated regarding beneficial effects of mitochondrial transfer with both *in vitro* and

in vivo studies. Intercellular mitochondrial transfer may result in enhanced cell survival or cellular reprogramming and cell stress may enhance intercellular organelle transport to stressed cells [10]. The latter one has been illustrated by many experimental studies in which stressors stimulated mitochondrial transport towards stimulated cells. Chondrocytes that are stressed by Interleukin-1 beta or mitochondria specific stressors received mitochondria from MSCs [11]. ROT-induced corneal epithelial cells (CECs) enhanced their mitochondrial uptake from MSCs that have functional mitochondria [12]. This result highlights the fact that donor cells must have functional mitochondria to be able to have therapeutic effects on injured cells. Treatment of human umbilical vein endothelial cells (HUVECs) with ethidium bromide resulted in MSC-derived tunneling nanotubes through which mitochondria are transported to injured HUVECs. An intriguing finding has also been detected that phosphatidylserine (PS) domains on HUVECs' cell surfaces were necessary for complete TNT formation. Annexin V; which binds to phosphaditylserine (PS); significantly reduced the number of TNTs but caused insignificant change in the number of membrane protrusions (MPs) [13]. Apparently, PSs on MPs are recognized by MSCs to fully form TNTs (All of the related points summarized in the (Table 1).

Table 1: A brief summary of mitochondrial transfer from donor cells to recipient cells and therapeutic effects.

Donor Cell	Recipient Cell	Disease Model	Consequence of Transportation	References
MSCs[1]	Chondrocytes	IL-1ß, oligomycin, and rotenone-mediated chondrocyte injury.	Not mentioned.	[11]
MSCs	HUVECs[2]	Oxygen-glucose deprivation (OGD)[3]-mediated HUVECs injury.	Reduced apoptosis, improved cell viability.	[13]
MSCs	CECs[4]	Rotenone (Rot)[5]-induced mitochondrial impairment of CECs.	Increased respiratory function, protection against cell death and proliferation inhibition.	[12]
iPSC-MSC[6]	Bronchial cells	Cigarette Smoke-induced lung damage.	Reverting CS-induced injury.	[28]
BMSCs[7]	Alveolar epithelial cells	Sepsis-induced acute lung injury.	Increased ATP production.	[27]
MSCs	NSCs[8]	Cisplastin-induced neuronal damage.	Normalized mitochondrial membrane potential.	[14]
MSCs	Cardiac myocytes	Not mentioned.	Not mentioned.	[35]
MMSCs[9]	PC12, astrocytes	Oxygen-glucose deprivation (OGD) astrocytes, ethidium bromide-treated PC12 cells, simulated ischemic lesion by middle cerebral artery occlusion (MCAO)[10].	Decreased lactate production and normalized proliferation in PC12 cell, reduced ischemic damage.	[26]
T24	RT4	Demonstration of invasive capability of non-malignant RT4 cells after mitochondrial transfer.	Enhanced invasive ability.	[32]
MMSCs	RCNs[11], astroglial cells	Simulated ischemic lesion by middle cerebral artery occlusion (MCAO).	Neuroprotection	[31]
WJMSCs[12]	Osteosarcoma cells	Not mentioned.	Rescued oxidative phosphorylation (OXPHOS)	[34]

Antineoplastic drugs have detrimental effects on healthy cells and investigators have been researching therapeutic approaches against chemotherapy-induced cell damage. Cisplastin; as a neoplastic drug; induce neuronal cell damage and nasal administrations of MSCs *in vivo* normalized mitochondrial function and membrane potential [14]. These results imply that mitochondrial transfer can be accomplished in in vivo conditions following cellular dysfunction as a result of perturbation of mitochondrial bioenergetics and specific manipulations that enhance TNT development.

Tunneling Nanotubes (TNTs)-Mediated Intercellular Transport System

Although many intercellular transport mechanisms have been described so far; one of them has a pronounced involvement in intercellular transport *in vitro* and *in vivo*; named as tunneling nanotubes (TNTs). TNTs were first discovered between PC12 cell line and have a diameter range from 20 to 500 nm mediating cellular communication between cells [15]. TNTs are membrane spanning protrusions formed between physically separated cells and are composed of F-actin and microtubules; the latter was exemplified in macrophages as thin and thick TNTs containing only actin or both actin and microtubules; respectively [16]. However, a recent study demonstrated the co-distribution of actin filaments; intermediate filaments and microtubules in tunneling nanotubes [17]. Following *Rustom et al*.; TNT configuration was observed in different cell types such as neurons; rat cardiac myocytes; rat kidney; myeloid cells and endothelial progenitor cells [18]. *Kumar et al*. has illustrated that TNTs are formed between epithelial cell lines and following TEM analyses have revealed that many mitochondria and ribosomes are shuttled in TNTs [19]. Several mechanisms have been proposed in terms of induction of TNT development. Treatment with stressful

insults culminates in enhanced TNTs. Subsequent analyses have proposed that proto-oncogene p53 expression is an important factor for TNT development. p53-deficient cells were unable to form TNTs.

In addition; EGFR and downstream pathways involving Akt; PI3K and mTOR were shown to be important regulators of TNT formation [20]. These results are consistent with the studies unveiled that mTORC2-mediated signaling pathways have been implicated in actin cytoskeleton organization [21,22]. Furthermore, inhibition of mTOR has led to the reduced TNT development [23]. Activation of EGFR; akin to mTOR; culminated in increased actin filament formation at the apical membrane of the cells which were treated with EGF [24]. Moreover, Wnt/Ca^{2+} signaling pathway has been shown to induce TNT formation [25]. Notably, mitochondrial transportation through TNTs is enhanced by overexpression of Miro1 in multipotent mesenchymal stem cells (MMSCs) and intravenous administrations of MMSCs reversed the effects of ischemia on neuronal functions more efficiently than those MMSCs without overexpression of Miro1 [26]. Taken together; these results suggest that miscellaneous mechanisms impact TNT development.

Impacts of Mitochondrial Transfer on *in vivo* Disease Models

In vitro studies emerge a number of questions regarding whether mitochondrial transfer is seen in only *in vitro* conditions or it can be seen in *in vivo* conditions as well. *Islam et al*. has noted that bone marrow stromal cells (BMSCs) are able to transfer mitochondria to injured alveolar epithelial cells in an in vivo acute lung injury model [27]. In another study; induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) were shown to have beneficial effects on Cigarette-Smoke induced lung damage; whereas bone marrow-derived mesenchymal stem cells (BM-MSCs) are less effective in attenuating lung damage compared to iPSC-MSCs in a CS-induced rat model [28]. Additionally, mitochondrial transfer from iPSC-MSCs to epithelial cells (ECs) via TNT alleviates asthmatic inflammation [29]. Similarly, iPSC-MSCs and BM-MSCs can donate mitochondria to cardiomyocytes with doxycycline-induced damage [30]. Stroke is an important disease owing to its high risk of sequelae. That's why it might has drawn attention to investigate therapeutic approaches.

Babenko et al. illustrated therapeutic effects of MMSCs in the setting of stroke by the study in which neuroprotection has been accomplished following mitochondrial transfer to rat cortical neurons (RCN) [31]. On the other hand, mitochondrial transfer might have adverse effects. Such condition has been demonstrated in a study in which malignant T24 cells enhanced non-malignant RT4 cells' invasive ability by transferring mitochondria through TNTs [32]. Proliferation and invasiveness of cancer cells increased due to recovered oxidative phosphorylation (OXPHOS) followed by mitochondrial transfer [33]. A similar research determined that osteosarcoma cells rescued from impaired oxidative phosphorylation by WJMSCs [34, 35]. It is also possible that chemoresistance can be gained through transfer of mitochondria. Such finding has been observed in a study in which endothelial cells were found to be contributed to chemoresistance development by transferring mitochondria to cancer cells [36].

Conclusion

As we reviewed here; TNT formation mechanism depends on many signaling pathways, but it remains to be fully elucidated. Understanding this peculiar cellular communication system would increase our chance to develop more efficient therapeutic approaches. Whilst stem cell therapy per se remains to be an important entity; mitochondrial transfer through stem cell donation may serve as a therapeutic treatment in the future.

Conflicts of Interest

The authors declare no conflict of interest.

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