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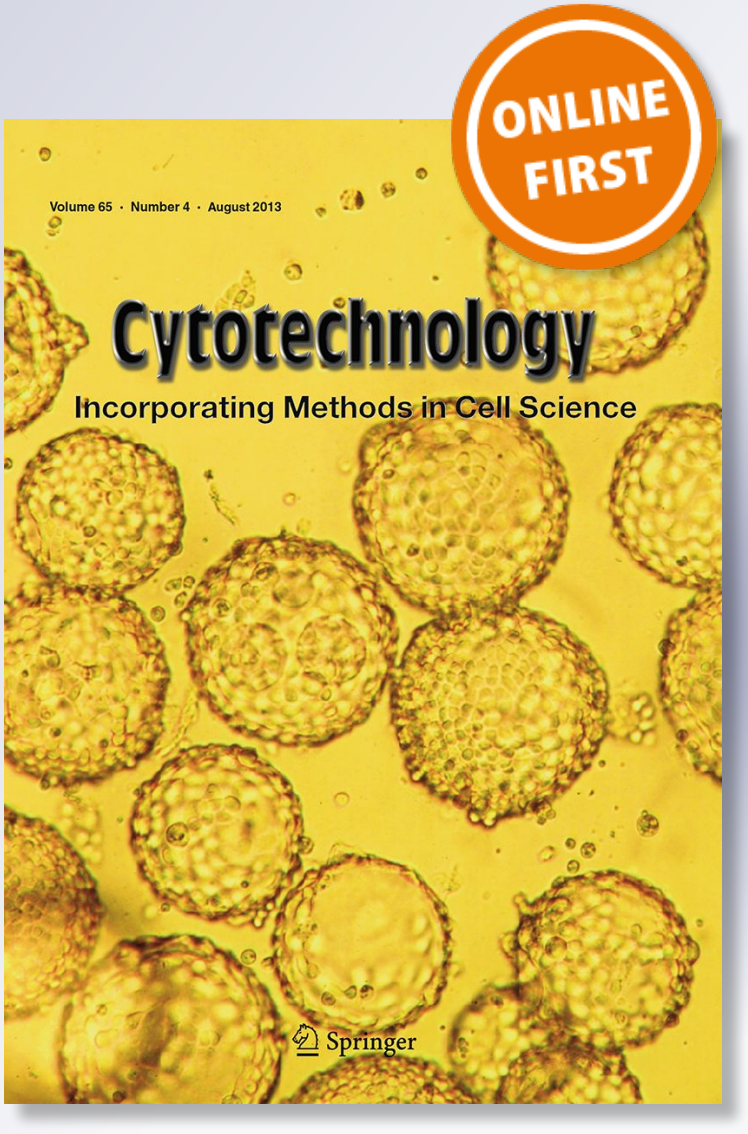

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REVIEW

Mitochondrial transplantation as a potential and novel master key for treatment of various incurable diseases

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Abstract Mitochondria are attractive cellular organelles which are so interesting in both basic and clinical research, especially after it was found that they were arisen as a bacterial intruder in ancient cells. Interestingly, even now, they are the focus of many investigations and their function and relevance to health and disease have remained open questions. More recently, research on mitochondria have turned out their potential application in medicine as a novel therapeutic intervention. The importance of this issue is highlighted when we know that mitochondrial dysfunction can be observed in a variety of diseases such as cardiovascular diseases, neurodegenerative diseases, ischemia, diabetes, renal failure, skeletal muscles disorders, liver diseases, burns, aging, and cancer progression. In other words, transplantation of viable mitochondria into the injured tissues would replace or augment damaged mitochondria, allowing the rescue of cells and restoration of the normal function. Therefore, mitochondrial transplantation would be revolutionary for the treatment of a variety of diseases in which conventional therapies have proved unsuccessful. Here, we describe pieces of evidence of mitochondrial transplantation, discuss and highlight the current and future directions to show why mitochondrial transplantation could be a master key for treatment of a variety of diseases or injuries.

Keywords Mitochondria dysfunction · Reactive oxygen species · Neurodegenerative diseases · Heart failure · Mitochondrial transplantation

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Introduction

Mitochondria are found in all mammalian cells except for mature red blood cells (Hoppel et al. 2009; Huang et al. 2013; McCully et al. 2016). Mitochondria have a variety of functions in the cells (Rub et al. 2017). It was classically believed that mitochondria were involved only in producing energy and were famous as energy chambers. However, they are involved in other important multiple cell functions such as heat regulation, calcium homeostasis, biogenesis and

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assembly of iron-sulfur proteins, control of apoptosis, reactive oxygen species ROS production, cell survival and proliferation, production of metabolites and coordination of metabolic pathways (Loureiro et al. 2017; McCully et al. 2016; Salimi et al. 2015, 2017). Of note is that they are key organelles in signaling platform and regulating many cells activity (Kabekkodu et al. 2015). It has been believed that mitochondria have been evolved from aerobic bacteria and/or early eukaryotic cells over a billion years ago. The mitochondria have their own DNA, mtDNA, with 16,569 bp length and encode 13 proteins for electron transport chain, 2 mitochondrial rRNAs and 22 mitochondrial tRNAs (Kuznetsova et al. 2017; Siira et al. 2017). Table 1 illustrates the proteins encoded by mtDNA. Although the mitochondria have their own genome, their biosynthetic pathways depend completely on nuclear DNA-encoded proteins (n-mitoproteins) (Berridge et al. 2018). For example, cytochrome c oxidase (COX), a very important enzyme in the function of mitochondria, consists of 14 subunits in which three of them encoded by mitochondrial DND and the rest eleven subunits encoded by nuclear DNA (Sinkler et al. 2017). Glycyl-tRNA synthetase gene (GARS) is another nuclear-encoded protein which is essential for protein translation in both cytoplasm and mitochondria. Orchestration of mitochondria-encoded and nuclear-encoded subunits or their crosstalk is necessary for the normal functions of mitochondria such as electron transport, ATP production and mitochondrial membrane potential (Boczonadi et al. 2018).

Table 1 Genes and proteins which are expressed by mitochondria DNA

Gene	Protein
MT-ATP6	ATP synthaseSubunit 6 (complex V)
MT-ATP8	ATP synthaseSubunit 8 (complex V)
MT-CYB	Cytochrome b (Complex III)
MT-CO1	Cytochrome c oxidaseSubunit 1 (complex IV)
MT-CO2	Cytochrome c oxidaseSubunit 2 (complex IV)
MT-CO3	Cytochrome c oxidaseSubunit 3 (complex IV)
MT-RNR2	Humanin
MT-ND1	NADH dehydrogenase Subunit 1 (complex I)
MT-ND2	NADH dehydrogenase Subunit 2 (complex I)
MT-ND3	NADH dehydrogenaseSubunit 3 (complex I)
MT-ND4	NADH dehydrogenaseSubunit 4 (complex I)
MT-ND5	NADH dehydrogenaseSubunit 5 (complex I)
MT-ND6	NADH dehydrogenase Subunit 6 (complex I)
MT-ND4L	NADH-ubiquinone oxidoreductase chain 4L

MT-ATP mitochondria adenosine triphosphate, *MT-CYB* cytochrome b, *MT-CO* cytochrome c oxidase, *RNR2* mitochondrially encoded 16S RNA, *NADH* nicotinamide adenine dinucleotide

Some tissues such as cardiac muscles, skeletal muscles, neurons, and oocyte have plenty of mitochondria but mammalian hepatocytes have only 800 mitochondria. Mitochondria not only are different in terms of number but also their size, shape, distribution, and feature vary from cell to cell. Moreover, the characters are changed and adapted depending on the cell physiological or pathological conditions (Bragoszewski et al. 2017).

Mitochondria have a variety of shapes. For example, they represent long, elongated, and tubular mitochondrial network shapes and small grain-shaped morphology. Interestingly, they can alter under the patho-physiological condition of cells (Simula and Campello 2018). This process is referred to mitochondrial dynamic or biogenesis (Chen and Chan 2009; Liesa et al. 2009). Fission and fusion are two important processes that control shape, size, number, motility, and inheritance of mitochondria. Fission and fusion occur during cell division, cell migration, cell proliferation, apoptosis and the localization of mitochondria throughout the cells (Simula and Campello 2018).

In fusion, two mitochondria are fused together by two steps. Firstly, the outer mitochondrial membranes (OMM) are fused together. Then, the inner mitochondrial membranes (IMM) are fused as well. In liver tissues, the mitochondria are fused and make a mega mitochondrion while in other tissues they have an arrangement in a network system. Mitochondrial fission is a multi-step process in which one mitochondrion is divided in two daughter mitochondria. These two processes define the structural and functional status of mitochondria. Fusion maintains the functionality and genetic and biochemical homogeneity of mitochondria. It also facilitates the communication between them and involved in cell homeostasis. On the other side, the fission is responsible to control number and distribution of the mitochondria. Of note, fission has a critical role in the removal of damaged mitochondria through mitophagy. In highly polarized cells such as neurons, mitochondrial dynamic is very important in synaptic transmission and vesicle

recycling. Mitochondrial dynamic also involves in neurogenesis during the development of the brain (Cantó 2018; Cid-Castro et al. 2018; Simula and Campello 2018; Tilokani et al. 2018).

The main proteins which are involved in mammalian mitochondria fusion are Mitofusion1 (Mfn1), Mitofusion2 (Mfn2), and optic atrophy type1 (Opa1); and the main ones for mitochondria fission are Dynamin-related protein 1 (Drp1), Fis1 (mitochondrial fission protein 1), and Mitochondrial Protein 18 (MTP18) (Arnoult et al. 2005; Bach et al. 2003; Ehses et al. 2009; Griparic et al. 2007; Liesa et al. 2009). It is noteworthy that the expression of these proteins and the balances between fusion and fission statuses of mitochondria alters in many mitochondrial dysfunctions and under pathological conditions (Chen et al. 2017; Mathis et al. 2017; Onyango et al. 2016; Schirone et al. 2017; Simula et al. 2017; You et al. 2017).

Mitochondrial dysfunction can be traced in a variety of diseases including cardiovascular diseases, diabetes, neurodegenerative diseases, ischemia, renal failure, skeletal muscles disorders, peripheral nerve injury, spinal cord injury, liver injury, aging, cancer, infertility, burn injury and even extensive exercise (Kuwahara et al. 2016; Ma et al. 2017; McCully et al. 2016; Wang et al. 1986; Zhang et al. 2008).

In tissues with high demand for energy such as cardiomyocytes, one-third of the cell's volume is occupied with mitochondria and approximately 30 kg of ATP/day is produced (Moreno-Lastres et al. 2012; Sabbah 2016; Tymoczko et al. 2011; von Hardenberg and Maack 2017). With mitochondrial dysfunction, the heart is like an engine without fuel. The higher the number of mitochondria, the more the level of the reactive oxygen species ROS produced. Figure 1 illustrates the role of mitochondria dysfunction in heart diseases (Aimo et al. 2016; Kanaan and Harper 2017; Lopez-Crisosto et al. 2017).

Tissues of the central nervous system (CNS) have a plenty of mitochondria, therefore it would be expectable to produce higher levels of metabolites and ROS (Onyango et al. 2017). Supporting this notion, mitochondrial dysfunction has been reported in several neurodegenerative diseases such as Alzheimer, Multiple Sclerosis (MS), Huntington (HD) and Amyotrophic Lateral Sclerosis (ALS), and Parkinson disease (PD), or other nervous system diseases such as spinal cord injury, peripheral nerve injury, and ischemic brain injury traces of mitochondrial dysfunctions can be found as well (Bonafede and Mariotti 2017; Di Domenico et al. 2017; Faizi et al. 2016; Ganie et al. 2016; Giannoccaro et al. 2017; Gollihue et al. 2017; Grimm et al. 2016; Kim et al. 2017; Kuo et al. 2017; Picone et al. 2016; Shaki et al. 2017). It is noteworthy that neurons are very sensitive to ROS and they consume 20% of the body's total basal oxygen (Grimm et al. 2016).

Another high prevalence and incurable disease, in which mitochondrial dysfunctions play an important role in the pathogenesis of the disease, is acute kidney injury (AKI) (Roushandeh et al. 2017). Kidneys are second to the heart in oxygen consumption and mitochondrial number. Therefore, it is obvious that mitochondria dysfunction is a key contributor to renal tubular cell death during acute kidney injury (Forbes 2016; Ralto and Parikh 2016). Therefore, restoring mitochondrial dysfunction in the aforementioned example diseases would be regarded as a new potential strategy for the treatment of a variety of diseases. The present review discusses highlights and describes pieces of evidence with regard to the restoration of mitochondrial dysfunction. We will try to introduce mitochondrial transplantation as a novel master key for treatment of various incurable diseases in the future.

Mitochondrial transplantation

Mitochondria are the organelles playing fundamental roles in the cellular function and metabolism and are also viewed as a key player in the cell pathology and death (McCully et al. 2016). Recently, the term "mitochondrial transplantation" opens a novel horizon for many diseases (Elliott et al. 2015; Emani et al. 2017; McCully et al. 2016). As described above, the mitochondrial dysfunction threatens cell homeostasis, disturbs energy production and finally leads to cell death and diseases (Lim et al. 2015; Morris and Berk 2015; Onyango et al. 2017; Ralto and Parikh 2016; Sasaki and Iwata 2007; Simula et al. 2017). "Mitochondrial transplantation" seeks to find the strategies to replace or restore damaged mitochondria.

The concept of mitochondrial transplantation originated from fact that the mitochondria can be transferred from one cell to another cell. This especially occurs when the mitochondria are damaged to support

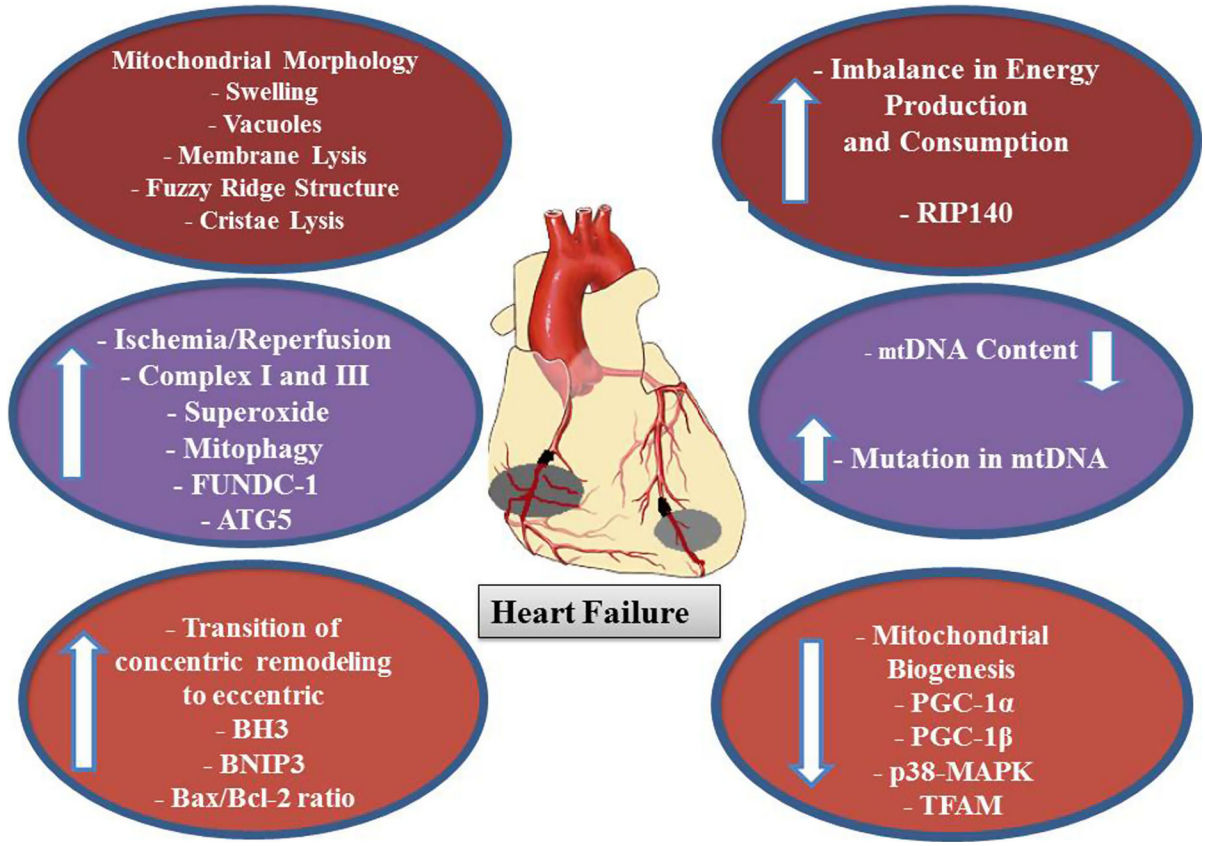

Fig. 1 Schematic representation of mitochondrial dysfunctions in heart failure. Mitochondria present abnormal configurations such as swelling and vacuoles, membrane lysis, fuzzy ridge structure (indistinct) and cristae lysis. Abbreviation: *RIP140* receptor-interacting protein 140, *PGC-1a* Peroxisome proliferator-activated receptor gamma coactivator-1 a, *PGC-1b* Peroxisome proliferator-activated receptor gamma coactivator-1 b, *p38-MAPK* p38 mitogen-activated protein kinases, *TFAM* mitochondrial transcription factor A, *FUNDC-1* Fun14 domain containing 1, *ATG5* Autophagy related gene 5

the injured cells (Hayakawa et al. 2016, 2018; McCully et al. 2016; Rocca et al. 2017).

Mitochondria are transferred between cells by various contact modes including; junction, cell fusion, and tunneling nanotube formation (Lu et al. 2017; Paliwal et al. 2018).

It has been shown that when the neurons encounter the ischemia, astrocytes deliver generously their own healthy mitochondria to the neurons in order to prevent them from death. More recently, it has been revealed that the extracellular mitochondrial particles which were released by astrocytes were mediated by a calcium-dependent mechanism involving CD38 and cyclic ADP ribose signaling. Moreover, microglia, other supporting cells of the nervous system, followed this pattern (Hayakawa et al. 2016; Rocca et al. 2017). It has also been shown that endothelial progenitor cells-derived conditioned medium containing functionally viable mitochondria was successfully incorporated into normal brain endothelial cells. It promoted angiogenesis and decreased the trans-cellular permeability of brain endothelial cells as well. Furthermore, it enhanced the copy number of mtDNA, mitochondrial proteins expression and, ATP production (Hayakawa et al. 2018).

Macropinocytosis is another mechanism underlying mitochondria internalize into the cells. Several studies have also been suggested that mitochondria enter into cells by an actin-dependent macropynocytosis; however, the precise mechanism and the endocytosis pathway are still unclear. Of note, it has been reported that $< 10\%$ of injected mitochondria was taken up by cardiomyocytes in an animal model of cardiac ischemia. Nonetheless, they exerted their

therapeutic properties and improved ATP production (Kumar 2017; Pacak et al. 2015).

It is obscure whether the delivering of mitochondria into the cells is active or passive? Some studies have been indicated that mitochondrial internalization is passive because when the internalization of molecular pathways were inhibited with specific blockers, mitochondria delivered to the cells. However, the endocytosis was dependent on the cell types. It is noteworthy that when the mitochondria transplanted, they might be delivered into the different cells including macrophages, vessels endothelial cells, pericytes, neurons and neuroglia such as oligodendrocytes, microglia, and astrocytes (Paliwal et al. 2018). It is interesting to mention that mitochondria were detected in CSF of patients with subarachnoid hemorrhage or in experimental subarachnoid hemorrhage rat model. It has been revealed that mitochondria in the CSF were originated from astrocytes. This findings suggest that it would be feasible to transplant the isolated mitochondria through CSF when the brain or spinal cord are injured in order to restore the damaged tissues (Zussman et al. 2017).

It has been reported that some strategies such as peptide-mediated mitochondrial delivery (PMD) can be employed to enhance mitochondrial intake and increase the transplantation efficiency especially in the patients who suffer from a mitochondrial disease. The PMD strategy positively had impacts on the rescue of the myoclonic epilepsy and ragged-red fiber (MERRF) model cell line. It is expecting that transplantation of mitochondria by PMD would have a better outcome for clinical use in the future. Although this approach is promising, however, further studies are required (Liu et al. 2014).

Dextran, a biocompatible polymer, can increase the delivery rates of isolated mitochondria. However, this is also in the beginning steps and requires further investigations (Wu et al. 2018).

Sources and methods of mitochondrial isolation

Isolation of mitochondria from skeletal muscles has been suggested as an appropriate candidate for mitochondrial transplantation. Pectoralis major, rectus abdominis, gastrocnemius and even neck strap muscles such as sternohyoid or deltoid and lattissimus dorsi are the suitable sources to obtain mitochondria (Emani et al. 2017; Masuzawa et al. 2013; McCully et al. 2016, 2017). Due to cosmetic reason, pectoralis major muscles are not a suitable source to isolate mitochondria for women (Table 2).

Mesenchymal stem cells (MSCs) are multipotent and attractive cells in regenerative medicine that can be obtained from a variety of sources (Roudkenar et al. 2018). Recently it has been suggested that stem cells, especially MSCs, are suitable and substitute source to isolate mitochondria (Wang et al. 2018). Interestingly, it has been shown that the therapeutic effects of MSCs can be exerted by secretome which are containing various molecules including mitochondria (Abbasi-Malati et al. 2018). Moreover, MSCs donate their mitochondria to injured cells via tunneling nanotube (Wang et al. 2018). Stem cell-derived mitochondrial transplantation could be a novel strategy for tissue injury especially in the patients with mitochondrial diseases (Paliwal et al. 2018; Wang et al. 2018). Another, the source of mitochondria could be spermatozoa. They have a plenty of mitochondria. However, further studies are required to clarify whether it would be possible to isolate healthy mitochondria from these cells and utilize them in the clinic or not?

A number of studies have been demonstrated that the efficacy of mitochondrial transplantation highly depends on viable and functional mitochondria. Non-viable mitochondria, previously frozen mitochondria, mitochondrial fractions (proteins, complex I–V), mitochondrial DNA and RNA, and exogenous ATP or ADP do not provide any cytoprotection (Masuzawa et al. 2013; McCully et al. 2009; Preble et al. 2013).

Recently, Mc Cully and his team established a rapid isolation protocol using different filters that took less than half an hour. Therefore, this is valuable for clinical application. For clinical application of mitochondria transplantation, it is essential to meet the criteria of good manufacturing practice (GMP). Quality control of size, number, purity, shape, viability, and function of the organelles must be analyzed. Tissue dissociator and multisizer counter are essential and helpful devices for isolation, counting, control the shape, and size of the organelles. All parts of the isolation procedure must be performed on the ice. In some laboratories, the hemocytometer could be helpful but it is prone to personal mistakes. The viability of the organelles can be assayed by mitochondrial fluorescent probes such as MitoTracker Orange CMTMRos (Preble et al. 2014b).

Table 2 Summary of the mitochondrial dysfunction in nervous system diseases

Nervous system diseases	Mitochondrial dysfunction
Alzheimer	Mitochondria fragmentation
	Abnormal cristae
	Quantity of the mitochondria
	NDUFA2, NDUFB3, UQCR11, COX7C, ATPD, ATP5L, ATP50
	NRF1, NRF2, TFAM and PGC-1α hippocampal tissue↓
	Mutation of mtDNA (mitochondrial 5-methylcytosine in entorhinal cortex)
	Complex IV and Δψm index↓
Multiple Sclerosis	NAA↓
	Complexes I, III and IV↓
	mtHSP70↑
	Mitochondrial membrane potential↓
	Cytochrome C expression↓
Parkinson	mtDNA mutations↑
	PINK-1↓
	PARKIN-1↓
	MDVs↓
	PARK genes↓
Huntington	Abnormal Huntingtin gene↑
	Ca^{++} loading capacity↓
	Activation of the excitotoxicity↑
	PGC-1 a↓
	Cytochrome b and c oxidase 1↑
	8-hydroxy-2-deoxyguanosine↑
Amyotrophic Lateral Sclerosis	Swelling, Fragmented and Vacuolated mitochondria
	Intermembrane space dilation, disorganized crista↑
	HK1↑
	SOD1↓
Spinal Cord Injury	Ca^{++} loading capacity↓
	Activation of the excitotoxicity↑
	CtBP gene↓
	PDHC, complex I, complex IV disturbance↑
	Permeability of mitochondria membrane
	Drp1, Fis1↑
Peripheral Nerve Injury	Cycling of futile proton↑
	ATP synthase↓
	Electron transport chain
	Mutation in OPA1, MFN2 and mitofusin 2↑
	Interaction of mitochondria, microtubules and endoplasmic reticulum↓
	Swollen and vacuolated mitochondria
	Mutation of mtDNA↑
Ischemic Brain	Depolarization and swollen disturbance in calcium homeostasis↑
	ROS and MDA↑
	Mfn2 gene expression↓

NDUFA2 NADH dehydrogenase (ubiquinone)1 alpha subcomplex subunit 2, *UQCR11* ubiquinol-cytochrome c reductase complex, *COX7C* cytochrome c oxidase subunit 7c, *ATP5L* synthase subunit g, *NRF1* nuclear respiratory factor1, *NRF2* nuclear respiratory factor 2, *TFAM* transcription factor A, *PGC-1α* Peroxisome proliferator-activated receptor gamma coactivator-1 a, *NAA* *N*-acetyl aspartate, *mtHSP70* mitochondrial heat shock protein 70, *PINK1* PTEN-induced kinase 1, *MDV* mitochondria delivery vesicle, *HK1* Hexokinase 1, *SOD1* super oxide dismutase1, *CtBP* C-terminal-binding protein, *PDHC* pyruvate dehydrogenase complex, *Drp1* dynamin-related protein1, *Fis1* fusion protein 1, *OPA1* optic atrophy type1, *MFN2* Mitofusion 2, *MDA* malondialdehyde, *ROS* reactive oxygen species

The function of the mitochondria can be evaluated by polarographic or spectrophotometric techniques. Moreover, Clark electrode measurements, oxygen consumption rate (OCR), are the reliable and suitable method for the evaluation of the function of mitochondria. Finally, ATP measurement is the another and versatile method to evaluate the functional activity of isolated mitochondria (Preble et al. 2013).

Labeling of mitochondria

Fluorescent dyes are used to visualize intercellular transplanted mitochondrial. Rosamine-based dyes (MitoTracker Red CMXRos and MitoTracker Red CMH2XRos) and carbocyanine-based dyes (MitoTracker Green FM, MitoTracker Red FM, and MitoTracker Deep Red FM) are usually employed for mitochondrial labeling. Mitochondrial fluorescent proteins (MFP) are other options for mitochondrial labeling. MitoGFP, mitoRFP, mitoYFP, and mitoDsRed are examples of MFP. To visualize the stained mitochondria confocal microscope or time-lapse confocal microscope is necessary (Berridge et al. 2018).

Delivery methods of mitochondrial transplantation

Mitochondrial transplantation can be performed by several routes. In situ and systemic transplantations are two main routes could be employed in the clinic. By in situ approaches, it is assured that all the isolated mitochondria can be delivered to the tissues. However, over-accumulation of the organelle would be challenging. Several studies have been tried to inject the mitochondria directly into the cardiac tissue using a suitable syringe. Supporting this notion, mitochondria have been directly injected into the myocardium of ischemic heart patients by a 27–32 gauge syringe (Emani et al. 2017; McCully et al. 2017). Direct transplantation of mitochondrial usually requires surgery in order to access the injured tissues. However, transplantation of mitochondria during surgery, for example in the ischemic heart, may not be repeated for several times. In situ transplantation of mitochondria into CNS by syringe and steriotax are feasible in preclinical studies, but their application in the clinic would be difficult (Gollihue et al. 2017).

The sonography-guided catheter carrying mitochondria through a carotid system for brain and renal artery for kidney might be applicable. Systemic injection such as intravenous transplantation is another way for mitochondrial transplantation. By systemic injection, it would be possible to transplant mitochorndria several times. However, it not only needs a high number of mitochondria but also the transplanted mitochondria might distribute vastly throughout the body including in the injured tissue. As mentioned above, recently it has been found that after subarachnoid hemorrhage, the extracellular mitochondria were found in CSF that in turn, could highlight and recommend a new route for mitochondrial transplantation for brain deficits and spinal cord injuries i.e. through subarachnoid space or brain ventricles (Chou et al. 2017).

Mitochondria can be transferred in vitro by different protocols as well. Co-culture of healthy cells with cells containing damaged mitochondria resulted in delivering of the healthy mitochondria into injured cells (Ahmad et al. 2014; Jiang et al. 2016; Wang and Gerdes 2015). Microinjection is another method for delivering isolated mitochondria into the recipient cells (Oktay et al. 2015).

MitoCeption is a new and efficient protocol for delivering of mitochondria in vitro. In this method, the isolated mitochondria enforced to deliver into cells by centrifuging of culture plates followed by 24 h incubation. For the first time, Caicedo et al. in 2015 successfully delivered the mitochondria which were isolated from mesenchymal stem/stromal cells to cancer cells by mitoCeption method (Caicedo et al. 2015; Nzigou et al. 2017).

More recently, a simple and quick method was introduced for delivering of exogenous mitochondria into culture cells. The new protocol only requires centrifugation of the cells and mitochondria at $1500\times g$ for 5 min without additional incubation (Kim et al. 2018).

Doses of mitochondria for transplantation

It is noteworthy that the suitable dose of mitochondria for transplantation has not been optimized so far. Moreover, almost all of available information in this regard is based on preclinical studies. However, according to McCulley and et al. study, administration of approximately 2×10^5 mitochondria per gram of tissue wet weight is a suitable dose for treatment of ischemic heart. Of noted, they revealed that only 10% of injected mitochondria can be found in the cardiomyocytes however, they had therapeutic effects (Emani et al. 2017). Administration doses of mitochondria both in vitro and in vivo studies are summarized in Table 3. It is worth to mention that the healthy, function and viability of the isolated mitochondria are much more important than the dose of administration.

Clinical trial of mitochondria transplantation

Clinical application of mitochondria transplantation is very limited and needs more preclinical studies to know about its optimization and develop its efficiency and safety with improvements in its quality control protocols. The first attempt in this regard was initiated in 2016 and conducted by Mc Cully research team in Boston children hospital at Harvard University in the United States. The primary results were released in 2017 and opened a new hopeful window for many patients. A novel strategy termed "mitochondrial auto transplantation" helps to treat the patients suffering from heart ischemia/reperfusion. Five children patients, age from 2 days to 2 years old, with cardiac ischemia participated in this study. Epicardial

Table 3 Summary of some preclinical studies dealing with mitochondrial transplantation

Mitochondrial isolation from:	Mitochondria transplantation to:	Animal model Or Human disease	Route of transplantation	Dose of injected mitochondria	Mitochondrial transplantation outcomes	Reference
Rectus Abdominis	Heart	Human heart ischemic/ reperfusion	In situ to ischemic area	1×10^7	Improved cardiac functions Independent to ECMO support	Emani et al. (2017)
Pectoralis major	Heart	Ischemic/ reperfusion of heart in Pig	Subendocardial injection	9.9×10^7	Decreased infarct size Improve the cardiac tissue histologically Long survival of transplanted mitochondria	Kaza (2017)
Non-ischemic skeletal muscle	Heart	Occlusion of left descending artery in rabbit	In situ injection into ischemic area	$9.7 \times 10^6 \pm 1.7 \times 10^6$/ ml	Decrease Infarct size Improve cardiac function Decrease cardiomyocyte apoptosis and necrosis	Akihiro Masuzawa et al. (2013)
PC12 cell line	Spinal cord	Spinal cord injury rat	Intraspinal injection	12.5 µg mitochondria	Presence of transplanted mitochondria in spinal cord	Gollihue et al. (2017)
BHK-21 cells	Sciatic nerve	Sciatic nerve crush model in rat	Injection into epineurium	195 µg mitochondria	Improve the cytoskeleton of injured sciatic nerve Decrease the ROS production Improved the muscles function and nerve conduction Develop neurobehavioral activity	Kuo et al. (2017)
Hamster cells	Brain	Ischemic/ reperfusion of brain in rat	Intra cerebral and intra-arterial injection	75 µg of mitochondria	Restore the motor function of the ischemic rat brain Decrease of apoptotic cells and infarct size	Huang et al. (2016)

Table 3 continued

Mitochondrial isolation from:	Mitochondria transplantation to:	Animal model Or Human disease	Route of transplantation	Dose of injected mitochondria	Mitochondrial transplantation outcomes	Reference
Allogenic peptid-labled mitochondria from PC12 cells and human osteosarcoma cybrids (xenogeneic source)	Brain	6-OHDA induced Parkinson model	Local injection to MFB	1.05 µg of Pep-1–conjugated mitochondria	Protects neurons in substantia nigra and nigristriate circuit Improve motor function Decrease cytotoxic effects of 6-OHDA Increase of mitochondrial function	Chang et al. (2016)
Oogonial precursor cells	Human oocyte	Infertile woman	Intracytoplasmic injection	Not mentioned	Increase of IVF rate	Oktay et al. (2015)
Young donor oocyte cytoplasm	Human oocyte	Infertile woman DOR	Intracytoplasmic injection	Not mentioned	Increase pregnancy Increase oocyte quality Decrease maternally inherited diseases	Woods and Tilly (2015)
Granular cell	Human oocyte	Infertile woman	Intracytoplasmic injection	Not mentioned	Two normal babies were born	Kong et al. (2003a, b)
Granular cell	Human oocyte	Infertile and old woman	Intracytoplasmic injection	Not mentioned	Formation of embryo	Kong et al. (2003a, b)
Gastrocnemius and quadriceps femoris muscle	Syngeneic injection (isolated from BALB/cJ mice donors) or allogeneic injection mitochondria (isolated from C57BL/6J mice donors)	Skin graft	IP	1×10^5, 1×10^6 or 1×10^7 mitochondria	No immuonological rsponese and DAMPs were seen	Ramirez-Barbieri et al. (2018)
Epstein–Barr virus transformed lymphocytes or rat brain	Co-culture in in vitro	Rodent model of SZ	In situ	In vitro: 10–50 µg protein/10^6 cells	Long-lasting improvement in mitochondrial functions	Robicsek et al. (2017)
	Intracerebral in in vivo			In vivo: 100 µg/4.5 µl in in vivo	Attenuated SZ-related deficits	

ECMO extracorporeal membrane oxygenation, *ROS* reactive oxygen species, *BHK-21* baby hamster kidney, *6-OHDA* 6-Hydroxydopamine hydrobromide, *IVF* in vitro fertilization, *DOR* diminished ovary reservoir, *IP* intraperitoneal, *SZ* schizophrenia, *DAMPs* damage-associated molecular pattern molecules

echocardiography was performed to detect akinesias or hypo kinesis. The mitochondria were extracted during 20–30 min from 2 pieces of samples obtained from rectus abdominis muscle. $1 \times 10^7 \pm 1 \times 10^4$ mitochondria were injected using a tuberculin syringe directly into 10 ischemic areas according to echocardiography findings. After the transplantation, none of the patients had bleeding or arrhythmia because of the injection. In four of the patients, cardiac functions improved according to echocardiographic findings and were separated from extracorporeal membrane oxygenation (ECMO) support. Two of them passed away for cardiac problems but also died for respiratory and other disorders. The dose applied in this study was based on previous animal studies and needs to be developed. The authors proposed more clinical trials

in the future in other diseases to optimize the protocol and efficiency (Emani et al. 2017). It is worth mentioning, according to www.clinicaltrail.gov, only one clinical trial dealing with mitochondria transplantation can be found.

Preclinical trial of mitochondria transplantation

Our knowledge about preclinical studies of mitochondrial transplantation is also owing to Mc Cully research group. In 2016, they injected viable, functional autologous mitochondria to ischemic pig heart. They isolated the healthy mitochondria from the non-ischemic area, pectoralis major muscle. 9.9 $3 \times 10^7 \pm 1.4$ 3×10^7/mL; 1.3 3×10^7 mitochondria per injection site was transplanted by sub-endocardial injection into eight areas at risk. The results showed that there was no significant difference in terms of heart and left ventricle weight. There was no immune response in animals receiving the mitochondria. Myocardial damage significantly was higher in vehicle animals according to the results of a creatine kinase-MB isoenzyme analysis. Infarct size in the group receiving mitochondria was significantly less that the vehicle. Histopathological and ultrastructural findings showed an increase in the longitudinal and transverse interfibrillar separation and mitochondrial damage in the vehicle heart compared to the mitochondria transplanted group. However, the transplanted mitochondria did not change the heart fibrosis (Kaza et al. 2017).

Electrocardiography (EKG), recorded 2 h and 4 weeks after injection, did not show fibrillation, bradycardia or conducting system defects in the ventricle of two groups. Interestingly, transplanted mitochondria were detected 4 weeks after injection in the pig's heart (Kaza et al. 2017).

Masuzawa et al. (2013) extracted the functional mitochondria from the non-ischemic skeletal muscle and injected then into regional ischemic area induced with occlusion of rabbit left anterior descending artery. They injected $9.7 \pm 1.7 \times 10^6$/mL mitochondria immediately before reperfusion in eight ischemic regions and recovered the animals for 4 weeks. The mitochondria resided in interstitial spaces surrounding cardiomyocytes at 0, 2, 4 and 24 h after the injection. The results revealed that no arrhythmogenic was detected after mitochondrial transplantation. Infarct size and cardiomyocytes necrosis decreased in the experimental group and the cardiac function developed. Infarct size did not increase after 28 days indicating the effectiveness of mitochondrial transplantation. Dimensional echocardiography demonstrated that after mitochondrial transplantation the myocardial function increased significantly and diastolic and systolic blood pressure did not change. Of note is that cardiomyocyte apoptosis decreased in the group that received mitochondria after 28 days. Proteomic analysis showed that after mitochondrial transplantation 26 proteins were upregulated and 23 were down-regulated. These proteins were precursors for energy production and cell respiration. Overall, their results revealed that administration of mitochondria enhanced cardiac function and decreased necrosis and apoptosis in cardiomyocytes (Masuzawa et al. 2013).

More recently, Gollihue et al. designed a strategy in which the isolated mitochondria were labeled with turbo-green fluorescent protein (tGFP) in vivo. The labeled mitochondria were injected into the spinal cord injury model of a rat and were detected in the rat tissue. The transplanted mitochondria were found within microglia and neurons of the spinal cord 24 h and 48 h after the injection (Gollihue et al. 2017, 2018).

Kuo et al. (2017) tried to know mitochondrial transplantation potential in neuroprotection and regeneration after sciatic nerve crush in a rat model. They also co-cultured the sciatic nerve explants with mitochondria in in vitro where the results were exciting. Mitochondria improved the cytoskeleton of injured sciatic nerve explants and decreased the ROS production. The electrophysiology findings showed that the ttransplanted mitochondria into the perineurium improved the muscles function and nerve conduction. The ROS production decreased significantly and animal neurobehavioral activity developed. This will be good news for those suffering from peripheral neuropathies (Kuo et al. 2017).

Some studies have demonstrated that the exogenous mitochondria transplantation has protective effects on brain and liver after ischemia. In a study, the mitochondria isolated from hamster cells were injected into the brain ischemic model in a rat (Huang et al. 2016). The findings revealed that transplanted mitochondria restored the motor function of the ischemic rat brain. Furthermore, apoptotic cells and infarct size significantly decreased in the brain tissue

of rat which received exogenous mitochondria. Based on these observations, it was strongly suggested that mitochondria transplantation has protective effects on neurons of CNS after ischemia (Huang et al. 2016).

In another study conducted by Chang et al. (2016), the exogenous and allogenic peptide-labeled mitochondria that were transplanted to an animal model of Parkinson's disease induced with 6-hydroxydopamine (6-OHDA) showed protective effects on neurons in the substantia nigra and nigristriate circuit. The isolated mitochondria were transplanted into medial forebrain bundle of PD model. After a three-month follow up, the motor function of PD model improved, the mitochondrial function increased and the cytotoxic effects of 6-OHDA decreased (Chang et al. 2016).

Immune cell therapy is one of the new and well-known approaches to cancer therapy. However, the metabolic functions of adult stem cells, immune cells, and somatic cells are attenuated with aging. More recently, Kim et al. demonstrated that the combination of immunotherapy and mitochondrial transplantation increased two folds cytotoxicity effects of immunotherapy against cancer cells (Kim et al. 2018).

More recently mitochondria transplantation were performed in the experimental rat model of schizophrenia (SZ). Mitochondria were isolated from lymphocytes (lymphoblasts) of three healthy persons or from whole brains (except the cerebellum) of 10 rats using a percoll gradient method. The isolated mitochondria were transferred to the lymphoblasts of schizophrenia patients and also were transplanted to the experimental rat model. Moreover, the isolated mitochondria were transferred to differentiated dopaminergic neurons of iPCs of healthy and SZ patients. The results revealed that approximately 50% of the lymphoblasts up took the isolated active normal mitochondria and recovered the impaired respiration of lymphoblasts of SZ. This study demonstrated that heterologous isolated active normal mitochondria entered into different cell types and induced long-lasting improvement in mitochondrial functions and differentiation of SZ-iPSCs into neurons. Furthermore, in the animal model of SZ, Intra-prefrontal cortex transplantation of isolated active normal mitochondria in young rats exposed prenatally to a viral mimic prevented mitochondrial $\Delta\psi$ m and attenuated SZ-related deficits (Robicsek et al. 2017).

More recently, Moskowitzova et al. reported that mitochondrial transplantation prolonged cold preservation time and increased the heart transplantation outcomes. In their study, donor's hearts received 1×10^8 mitochondria or respiration buffer, as a vehicle group, before excision and at the beginning of reperfusion during graft to the recipient mice. The donor's hearts maintained for 27–30 h in saline before graft. The results were promising. Ejection fraction and beating scores improved in the hearts that received mitochondria transplantation compared to the vehicle group. In addition, apoptosis and histopathological changes were decreased in the heart of the mitochondrial transplanted group. Overall, these findings strongly suggested that mitochondria transplantation might be employed to improve organ transplantation outcomes (Moskowitzova et al. 2018).

The oocyte is one of the richest cells in terms of mitochondria in women (Van Blerkom 2011). It has been believed that mitochondria have a crucial role in the oogenesis and in the early stages of development as well (Oktay et al. 2015). The mitochondria can be considered as a landmark to estimate the quality of the oocyte (May-Panloup et al. 2016). Aging affects the mitochondria quality and increases the mtDNA mutations and results in defects in the granulosa and oocyte development (May-Panloup et al. 2016).

Based on a study on 72 infertile men, ten types of nucleotide variants were found in their mitochondrial DNA. These nucleotide variants lead to disturbance in mitochondria energy production and in turn interfere with sperm motility and function (Heidari et al. 2016).

In another study, it was found that mitochondrial maternal mito-nuclear incompatibility leads to severe effects on oogenesis and embryo survival (Zhang et al. 2017). Overall, mitochondria replacement might be considered as a new strategy for infertility treatment and ovary juvenilization (May-Panloup et al. 2016).

It has been proved that aging decreases the oocyte quality and decreases the rate of pregnancy. In a clinical trial that was conducted by Oktay K in 2015, the healthy mitochondria isolated from oogonial precursor cells were injected with a needle during intracytoplasmic sperm injection. Again, the result was amazing. In autologous mitochondrial injection group, the rate of the in vitro fertilization (IVF) outcome was higher than the group received no mitochondrial injection (Oktay et al. 2015).

Diminished ovarian reserve (DOR) leads to a low response to stimulation in IVF cycle (Alborzi et al.

2015). It has been suggested that mitochondrial transplantation could be a new hope for the treatment of infertility in assisted reproductive technology (ART) outcome (Alborzi et al. 2015). Transplantation of young donor oocyte cytoplasm including healthy mitochondria into aged fertilized eggs is a promising and interesting strategy in ART field and would increase the rate of pregnancy improve the quality of the oocyte and decrease maternally inherited diseases especially mitochondrial disorders (Woods and Tilly 2015).

Kong et al. (2003a, b) had two successful experiences for autologous granular cell mitochondria transfer. In the first study, a 37 years old woman with several times failure in ART, received autologous granular cell mitochondria transfer in five oocytes in which four of them fertilized but three of the embryos implanted. One of the embryos was aborted at 4th week of pregnancy and finally, two normal babies were born for the first time by this method in China (Kong et al. 2003a). In the other case, a 46-year-old woman underwent an autologous granular cell mitochondria transfer for her MII oocytes. The oocytes fertilized and one of them was transferred to the uterus. The fetus was formed but spontaneous abortion occurred at 9th week of pregnancy. This was the first pregnancy with this method in an aged woman (Kong et al. 2003b).

It is obvious that autologous transplantation of mitochondria could have more efficient outcomes. However, in some cases including mitochondrial related diseases or in some sickest patients, isolation of their own mitochondria is not feasible. On the other hand, some patients need several series of injections. Therefore, in this regard heterologous mitochondria transplantation is inevitable. The main problems of heterogenous or allergenic mitochondria transplantation are immune system responses and damage-associated molecular pattern (DAMPs). Of note, in all previous studies, only a single injection of mitochondria has been reported. What would happen following serial injections of mitochondria into the damaged tissues? Mc Cully in 2018 conducted a study to know the immune system behavior after direct or indirect autogenetic and allogeneic injections, single and serial injections and also a different number of isolated mitochondria (1×10^5, 1×10^6 or 1×10^7 mitochondria). The findings have been demonstrated that the level of immune system profiles including IL-1, IL-4, IL-6, IL-12, IL-18, IP-10, macrophage inflammatory protein MIP-1 α, and MIP-1 β did not change. Single or serial injections of mitochondria did not show any DAMP in recipient tissues. Based on previous studies, circulating free mtDNA activated DAMPs and induced an immune response and cell injury which resulted in remarkable damages to lung or heart tissues (Kuck et al. 2015; Simmons and Gillespie 2015; Cloonan and Choi 2016). However, more recently it has been demonstrated that there was no increase in circulating free mtDNA following 10 days of recovery from serial i.p. injections of either syngeneic or allogeneic mitochondria compared with the control mice, received only respiration buffer. In addition, as histopathological analysis revealed, no evidence of inflammation, cellular damages such as necrosis and fibrosis in heart and lung tissues were observed (Ramirez-Barbieri et al. Ramirez-Barbieri 2018).

Challenges of mitochondrial transplantation

It is exciting for a scientific committee to use mitochondrial transplantation in the clinic for the treatment of several diseases. However, there are many unsolved problems including safety and efficiency. The isolation of healthy and functional mitochondria is very important and crucial. It is noteworthy that only functional mitochondria can exert their therapeutic efficiency. Therefore, the quality control of healthy mitochondria is crucial (McCully et al. 2016; Preble et al. 2014a). We also do not exactly know for how long mitochondria can be functional after transplantation. The transplanted mitochondria can exert their therapeutic effects both extracellular and intracellular. The transplanted mitochondria in the ischemic heart immediately increased myocardial ATP levels, rapidly altered the myocardial proteome to upregulate pathways for energy generation, cellular respiration and upregulated cardioprotective cytokines (Masuzawa et al. 2013; Pacak et al. 2015). These cytokines are associated with reduced cardiomyocyte apoptosis and enhanced functional cardiac recovery and cardiac remodeling independent of cardiac myocyte regeneration (Masuzawa et al. 2013). The mitochondria were rapidly internalized (2.5–60 min) into myocardial cells by actin-dependent endocytosis where they improved cellular function

(Cowan et al. 2017). The transplanted, mitochondria maintained viable and had function for at least 28 days and had no pro-arrhythmic, inflammatory, immune or auto-immune response (Emani and McCully 2018).

It has been recommended to obtain tissue biopsy from non-ischemic skeletal muscle mainly from pectoralis major or rectus abdominis. Usually, 6 mm tissue biopsy is suitable to take enough mitochondria for injection. Approximately, the number of mitochondria which can be obtained from a 6 mm biopsy is $2.4 \times 10^{10} \pm 0.1 \times 10^{10}$ (Emani et al. 2017; McCully et al. 2016; Preble et al. 2014a). The availability of other tissues to replace skeletal muscle for the sake of obtaining functional and higher number of mitochondria requires further studies. Some considerations in terms of quality control must be applied before clinical application of mitochondria including; the number of mitochondria, mitochondrial viability, mitochondrial purity and mitochondrial function (Preble et al. 2014a, c). Although mitochondrial transplantation has been optimized for cardiac transplantation, the method should get optimized and revised for other tissues as well.

The route of injection could be another challenge. The clinical trial and preclinical studies used the in situ injection. In 2017, Gollihue optimized intraspinal delivery of mitochondria in SCI model of rat (Gollihue et al. 2017). However, clinical application of in situ injection into the spinal cord or other parts of CNS such as hippocampus or substantia nigra seems not feasible. Therefore, it might be feasible to inject the mitochondria through other routes such as intravenous or intramuscular or even orally.

Currently, autologous transplantation is recommended for the clinical trial (Emani et al. 2017). However, we do not know what would happen if heterogonous mitochondrial transplantation is employed. Isolation of the healthy mitochondria from patients suffering from ischemic diseases is limited. Therefore, finding another source more healthy organs and easier isolation process would be helpful. Currently, the storage of mitochondria is impossible and it must be injected immediately after isolation. Therefore, addressing this challenge would be revolutionary in mitochondrial medicine.

Conclusion

All body cells except red blood cells contain mitochondria which are involved in a large number of important cellular and metabolic processes. The importance of mitochondrion in the maintenance and regulation of cellular homeostasis and function is well-established and there are sufficient supporting studies showing that mitochondrial injury or loss of function is harmful. In other words, mitochondrial dysfunction plays a crucial role in the pathogenesis of a variety of diseases. Therefore, transplantation of viable mitochondria isolated from a healthy tissue and then its delivery into the injured organ/tissue/cells would replace or augment the damaged mitochondria, allowing the rescue of cells and restoration of normal function. Supporting our notion, more recently, the efficacy of mitochondrial transplantation for cardioprotection has been shown in a clinical trial which strongly suggests that mitochondria might be considered beyond their initial role to supply energy i.e. as a novel therapeutic intervention. Overall, mitochondrial transplantation might not only act as a novel unique therapeutic strategy but also could be applicable for the treatment of many diseases/injuries in which conventional therapies have proved unsuccessful. However, mitochondrial transplantation is in the beginning steps of development and further and comprehensive studies including the precise mechanisms of protection are required.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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