Mitochondrial therapy: a vision of the outlooks for treatment of main twenty-first-century diseases

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Abstract
Mitochondria are dynamic organelles which constantly change their shape, size, and location within the cells. Mitochondrial dynamics is associated with mesenchymal metabolism or epithelial-mesenchymal transition to regulate the stem cell differentiation, proliferation, migration, and apoptosis. The transfer of mitochondria from one cell to another is necessary to improve and maintain homeostasis in an organism. Mitochondrial transplantation is a therapeutic approach that involves an introduction of healthy mitochondria into damaged organs. Recent evidence data have shown that the physiological properties of healthy mitochondria provide their ability to replace damaged mitochondria, with suggesting that replacing damaged mitochondria with healthy mitochondria may protect cells from further damage. Moreover, mitochondria can also be actively released into the extracellular space and potentially be transferred between the cells in the central nervous system. This increased interest in mitochondrial therapy calls for a deeper understanding of the mechanisms, which build the basis for mitochondrial transfer, uptake, and cellular defense. In this review, questions related to the involvement of mitochondria in the pathogenesis of cancer will be discussed. Particular attention will be paid to mitochondrial transplantation as a therapeutic approach to treat the mitochondrial dysfunction under some pathological conditions.

Keywords
Mitochondrial, Therapy, Cancer, Treatment, Cell

Introduction
The rediscovery and rethinking of the Warburg effect in 2000 eclipsed the issues related to the key functions of mitochondria in cancer cells for almost a decade. Until recently, the scientific community has indeed focused on constitutive glycolysis as a hallmark of cancer cells that is in fact not the case, with largely ignoring the contribution of mitochondria to malignancy of oxidative and glycolytic cancer cells being Warburgian or simply adapted to hypoxia [1]. Since most of the metabolic signals of cells occur in mitochondria or are regulated by the mitochondrial activity, knowledge of mitochondrial function is critical to discussing cancer cell metabolism [2].

Mechanisms of malignant transformation
There are at least five mechanisms, by which mitochondria may be involved in the development of the malignant phenotype as compared to the metabolic re-programming of cancer cells. First, it has been widely shown that a large number of diseases are associated with DNA mutations that affect mitochondria, mainly due to changes in subunits in the electron transport chain (ETC). It has been known for a long time that the subsets of hepatocellular carcinoma and prostate cancer have been associated with a mutation in the D-loop region of Complex I, and some neurological cancers contain mutations in succinate dehydrogenase - SDH; Complex II [3,4]. Second, oxidative stress caused by reactive oxygen species (ROS) is the most important stimulus for the onset of cancer and its progression towards malignancy [5]. ROS are mainly produced by mitochondria, which release superoxide as a by-product of oxidative respiration. Mitochondrial ROS (mtROS) can be generated either in the tricarboxylic acid (TCA) cycle or in the ETC [6].

Due to their high reactivity, ROS act as toxic particles for cellular macromolecules and, at low concentrations, as intracellular signaling agents, which regulate metabolic pathways. Elevated ROS levels are often found in cancer cells due to their increased metabolic activity and altered antioxidant capacity [7]. Third,
mitochondria are directly involved in the regulation of the cell death, including but not limited to apoptosis and necrosis [8]. In order to initiate apoptosis, proteins from the B-cell lymphoma-2 proteins (Bcl-2) family interact with mitochondria, since they bind to a voltage-gated anion channel (VDAC) to speed up its opening and release of cytochrome C. Thus, these proteins act as oncogenic or oncosuppressive triggers, participating in cancer progression and therapeutic resistance [9]. One of them, myeloid leukemia cell differentiation protein-1 (MCL-1), an anti-apoptotic member of the Bcl-2 family, is frequently overexpressed in human cancer and associated with the tumor aggressiveness. MCL-1 and Bcl-xL were found in different mitochondrial subcompartments. They exert their anti-apoptotic activity by counteracting the pro-apoptotic members of the Bcl-2 family, when they are located on the outer mitochondrial membrane (OMM), and when they are located in the mitochondrial matrix, by regulating mitochondrial homeostasis and bioenergetics maintaining the integrity of the inner mitochondrial membrane (IMM) and promoting assembly of ATP synthase oligomers in ETC. Mitochondria also control necroptosis, the regulated form of necrosis that requires the formation of mtROS and depends on changes in mitochondrial permeability. Fourth, metabolic reprogramming is also associated with several mutations in genes encoding TCA cycle enzymes, which promote the malignant transformation. Indeed, some TCA cycle intermediates, such as fumarate, succinate, aspartate, and D-2-hydroxyglutarate (2HG, a de novo metabolite from isocitrate dehydrogenases (IDHs) mutations, have important pro-carcinogenic effects when accumulated in cells following genetic mutations, and/or cancer-related modifications of protein expression [10]. Fifth, the hallmark of all tumors is persistent cell proliferation as a result of multiple molecular alterations. One of these alterations is the prevention of telomere erosion due to the constitutive expression of telomerase, which ensures the maintenance of the telomere length. Telomerase reverse transcriptase (TERT) has been shown to translocate from the nucleus to mitochondria under oxidative stress, preserving the mitochondrial functions and reducing oxidative stress, thereby protecting mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) from oxidative damage to avoid apoptosis [11].

The hypothesis of an endosymbiotic relationship between mitochondria and their host cells was popularized by Lynn Margulis in 1986. An alternative hypothesis, namely the autogenous hypothesis, is currently less widely accepted. The symbiotic relationship probably originated between 1.7 and 2 billion years ago. From the evolutionary standpoint, an incorporation of mitochondria into the eukaryotic cell facilitated oxygen utilization (for OXPHOS and respiration) and its detoxification (to reduce oxidative stress caused by reactive oxygen species). The symbiosis between the bacterial organelles and the eukaryotic cells, with time, probably optimized an interaction between the two types of these bio-systems. The import of metabolites from the cytoplasm of the cell into the organelles occurs freely or through porin molecules. However, there is no export from the organelles to the cytoplasm available. This data on the origin (evolution), the structure (molecular and cell biology) and the function (biochemistry, physiology) of mitochondria is considered to be essential for a proper understanding of the pathophysiology of major human chronic diseases such as cardiovascular diseases, metabolic syndrome (MetS), neurodegenerative diseases, immune system disorders, and cancer [12].

The Systemic-Evolutionary Theory of the Origin of Cancer (SETOC) recently proposed is based on two important concepts: the evolution, interpreted as a process of cooperation and symbiosis, and the system, in terms of integration of various cellular components, where the whole is greater than the simple sum of the parts, as it is the case with any complex system. According to SETOC, cancer results from the de-emergence of the “eukaryotic cell system” and the re-emergence of some cellular subsystems such as archaeal-like (genetic information) and/or prokaryotic-like (mitochondrial) subsystems, which have uncoordinated behaviors [13]. There is evidence supporting the building blocks of the new theory, especially with regard to interactions between mitochondria and the nucleus. For example, a number of studies show that when the connection between mitochondria and the nucleus is disrupted, mitochondria become nonfunctional, and oncogenesis may be initiated [14,15]. This evidence is underscored by the importance of mitonuclear communication in human cancer, as supported by recent findings showing increased somatic mitochondrial DNA (mtDNA) transfer in colorectal tumors [16].

Recently, mitochondrial transplantation has attracted the attention of many researchers. Mitochondrial transplantation is considered as a potential therapeu-
tic method that can be used to treat some specific diseases associated with the mitochondrial dysfunction or mtDNA damage. Previous studies have shown that isolated mitochondria from various sources, including cultured stem cells or autologous tissues, give successful results in damaged tissues, organs, or cells [17].

Mitochondria are dynamic organelles which constantly change their shape, size, and location within the cells [18]. The transfer of mitochondria between the cells is required to improve and maintain homeostasis in an organism [19]. Transcellular transport of mitochondria involves various mechanisms, including tunneling nanotubes (TNTs), extracellular vesicles or microvesicles (EVs), gap junctions, and cell fusion/mitochondrial expulsion [20,21]. Tunneling nanotubes (TNTs) are considered to be the main cellular structures, which ensure the transfer of mitochondria from one cell to another [22]. TNTs provide for unidirectional exchange [23] or bidirectional transport [24,25] of various cargoes, including molecules (for example, Ca^{2+} ions), macromolecules (lipid droplets, ions, proteins, microRNAs, and pathogens), and organelles (mitochondria, endosomal vesicles, lysosomes etc.). TNTs cover the skeleton, mainly composed of F-actin and transport proteins, which provide the active transfer of mitochondria. The membrane of each cell builds a stable cell–cell contact formation, constructing a tightly connected bridge. Mitochondrial disordering could induce TNT generation and mitochondrial transport, but the p53 activation (the mechanism initiating these membrane protrusions) was the factor initiating TNT in response to cellular stress [24].

Mitochondrial expulsion, or extrusion, is another possible mechanism for the transfer of mitochondria from cell to cell. The extrusion releases the mitochondria or the mitochondrial content from the cells under certain conditions. Cytoplasmic vacuoles surround mitochondria, fusing with the plasma membrane and releasing free mitochondria into the extracellular environment during the TNFα-induced cell death [25]. Intact mitochondria or some mitochondrial components can also be transported through the processes of exocytosis and endocytosis [25]. Neutrophils can displace the mitochondrial content, including oxidized mitochondrial nucleoids [25].

Mitochondrial dynamics

Mitochondrial dynamics is associated with mesenchymal metabolism or the epithelial-mesenchymal transition to regulate the stem cell differentiation, proliferation, migration, and apoptosis [26]. Mitochondrial dynamics orchestrates the immune cell differentiation through metabolic programming involving aerobic glycolysis or fatty acid oxidation [27]. Mitochondrial dynamics covers the processes of fusion and fission. The mitochondrial fusion is a process consisting of three GTPases such as Mitofusin 1 (MFN1), Mitofusin 2 (MFN2) and Optical Atrophy 1 (OPA1) [27].

Mitochondrial transplantation has shown the fusion of exogenous mitochondria with endogenous mitochondria. A number of authors have reported that exogenous mitochondria fuse with endogenous mitochondria upon escaping endolysosomal sites after 0.5, 1, 2, and 4 h in the induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) and the human cardiac fibroblasts (HCFs) [28]. It has become known that the peptide-mediated mitochondrial transplantation increases the expression of proteins associated with the mitochondrial dynamics. An inhibition of the mitochondrial division and stimulation of the mitochondrial fusion have been found in the MERRF mitochondrial cells and in the ρ-cells [24].

Mitochondrial isolation and quality control

Many different methods have been used to isolate mitochondria. These methods include some commercially available automated cultured cell isolation systems or tissue isolation kits and manual isolation protocols. Typically, the procedures take about 60–90 minutes [29]. The duration of the mitochondrial isolation process should be shorter for therapeutic applications. The procedure for isolation of mitochondria should be carried out at a temperature of +4°C to keep the mitochondria healthy [29]. Quality control of the mitochondrial quantity, viability and functional integrity should be analyzed to verify the integrity of isolated mitochondria.

Mitochondria are surrounded by double lipoprotein membranes such as IMM (the inner mitochondrial membrane) and OMM (the outer mitochondrial membrane) [30]. Maintaining the integrity of mitochondrial membranes is vital for the functioning of the organelles and their actions and effects to be produced on a target cell [31]. OMM is flat, similar to the membrane of the eukaryotic cell, and rich in cholesterol. The OMM is both a barrier and a platform that controls the passage of substances between the cytoplasm and mitochondria. The OMM permeability can in-
crease due to some oxidative stress factors like ultraviolet rays and hypoxia [24]. This may cause irreversible mtDNA damage and the activation of pro-apoptotic proteins (Bcl-2, Bax and Bak). Mitochondria with the damaged OMM cannot enter the target cell or may lead to the cell death instead of correcting the target cellular functions [24].

In addition to these tests, some other tests are important for the mitochondrial transplantation. For example, hemocytometer and particle counter testing can be used to determine the number of mitochondria. Mitochondrial purity is assessed by Western blotting (cytochrome C, anti-COX IV, TOM20) and electron emission microscopy. Electron emission microscopy can also estimate the mitochondrial morphology. Oxygen consumption rate (OCR) or Clark electrode measurements are suitable methods for assessing the mitochondrial function. In addition, the ATP test is a practical method for assessing the functional activity of isolated mitochondria. We have already described the entire mitochondrial analysis in detail in our recent review article [32].

The mitochondrial transplantation is a therapeutic approach that involves an introduction of healthy mitochondria into some damaged organs. Recent evidence data have shown that the physiological properties of healthy mitochondria provide their ability to replace damaged mitochondria [33], suggesting that replacing damaged mitochondria with healthy mitochondria may protect the cells from their further damage. Moreover, mitochondria can also be actively released into the extracellular space and potentially be transferred from one cell to another in the central nervous system [34]. This increased interest in mitochondrial therapy requires a deeper understanding of the mechanisms constituting the basis of the mitochondrial transport, absorption, and cellular defense [35].

In vitro studies

The first artificial mitochondrial transplantation was carried out in 1982 by Clark and Shay by the method of co-incubation. In the above study, the antibiotics chloramphenicol (CAP) and efrapeptine (EP) were used to induce cell death by inhibiting mitochondrial protein synthesis and ATPase activity. The isolated mitochondria from the antibiotic-resistant CAP and EP cells were transplanted into some antibiotic-sensitive cells by a simple coincubation method. They reported that transplanted mitochondria internalized by endocytosis improved the antibiotic resistance and increased the viability of the recipient cells. It has been found that mitochondrial transplantation into a large number of cells by coincubation is possible [24]. In other experiments that followed that study, mitochondrial transplantation was described as a promising approach [36]. However, there have been reports available to present some missing points whether isolated mitochondria are internalized by the recipient cells, discussing the share of internalized mitochondria and their long-term effect [37]. Considering all the above mentioned aspects, the co-incubation method can be found useful for studying various features of mitochondrial transplantation.

The injection of exogenous mitochondria directly into the target cell has been defined [24]. They reduced mtDNA with ethidium bromide and transplanted isolated mitochondria from the antibiotic-resistant CAP cells into some damaged cells. As a result, the resistance to antibiotics developed in the cultured cells, and the donor mtDNA appeared in the recipient cells. In the protocol of the microinjection method, mitochondria were delivered to the cells with a plunger-type syringe in an average volume of 10–20 μl. The offered method was less efficient than the co-incubation method, since the amount of the transplantable cells and the number of transplantable mitochondria per cell were limited. In addition, there was a high probability of damage to the recipient cells or transplanted mitochondria. However, it is useful for some studies where larger cells such as oocytes and needles of larger diameters can be utilized. Moreover, according to some experiments, the transferred mitochondria could not be detected or replicated in the recipient cell [38].

In vivo methods

The mitochondrial transplantation in vivo is carried out by a direct injection into a tissue, delivery to a damaged tissue through the systemic circulation, or by some alternative methods such as an intranasal introduction. There are many in vivo studies on the impact made by the mitochondrial transplantation on various models of diseases. Ischemia is one of the most studied patterns. The role of mitochondria in ischemic damage has been identified. Some structural alterations in the electron transport chain (ETC) proteins, especially in complexes I and III, are among the main damages, which may cause a decrease in the activity of
mitochondrial complex proteins after ischemia. ATP is generated by mitochondria via OXPHOS generated by ETC, which is located on the inner mitochondrial membrane [39]. Oxygen and the respiratory substrates are necessary for the formation of ATP, and the lack of these molecules impairs this vital mitochondrial function [40]. ATP hydrolysis and anaerobic glycolysis during ischemia initiate an increase in the production of free inorganic phosphates and induce an increase in the membrane permeability. Moreover, the leakage of electrons after the damaged ETC complexes becomes possible. Following these alterations, the ATP depletion may appear, and this has been identified as one of the most important damage mechanisms [40].

Four animal studies have demonstrated the advantages of the mitochondrial transplantation in case of stroke. Using a 60-minute model of the focal carotid occlusion in the male C57BL6 mice, Nakamura et al. (2020) [41] assessed the effect of transfusion of mitochondria-rich fractions, derived from frozen placenta, produced on the infarct size. Treatment with placental mitochondria significantly reduced the infarct size as assessed by 2,3,5-triphenyltetrazolium chloride (TTC) staining 72 hours post-reperfusion; in addition, Mitotracker Deep Red fluorescence imaging demonstrated diffuse mitochondrial absorption in the brain, the lungs, the liver, and the kidney tissues 2 hours after treatment [41] (Nakamura et al. 2020). Excess reactive oxygen species and oxidative stress are major causes of brain damage after acute ischemic stroke. Pourmohammadi-Bejarpasi Z. et al. (2020) [42] reported on the intracerebroventricular transplantation of isolated mitochondria from human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) in a rat model of the middle cerebral artery occlusion. [The occlusion of the middle cerebral artery in rats for 70 min followed by reperfusion and mitochondrial transplantation demonstrated the normal brain cytoarchitecture and the neurons with a reduced number of the pynotic cells [42]. The brain injury following an acute stroke can lead to astrocyte hypertrophy, and the subsequent release of glial fibrillary acidic protein (GFAP) may result in scarring that restricts the recovery of the neurons. Zhang Z. and a team of researchers (2019) [43] demonstrated the neuroprotective effects of the transplantation of autologous mitochondria, derived from the major pectoralis muscle, into the lateral ventricles after 90 min of the occlusion of the middle cerebral artery. 24 hours after the middle cerebral artery occlusion, the number of viable mitochondria observed in the cerebrospinal fluid increased, leading to the improved neurological outcomes, suggesting the potential use of the mitochondrial transplantation after stroke. An infusion of isolated mitochondria into the lateral post-stroke ventricles resulted in an increased accumulation of mitochondria in the penumbra area with a rise in the total ATP content and an elevated expression of complex IV. The mitochondrial transplantation after the occlusion of the middle cerebral artery also reduced apoptosis, attenuated astrogliosis with enhanced neurogenesis, and lowered the volume of cerebral infarct [43]. Huang PJ. et al. (2016) [44] used a model of cerebral stroke in male rats with the middle cerebral artery occlusion to compare the effects of direct intracerebral versus intrafemoral introduction produced on the functional status and the infarct size. The intracerebral and intrafemoral transplantation improved the functional characteristics assessed by the rotating pole testing and grip strength measured within 1 month after transplantation, significantly reduced the size of the lesion as assessed by the terminal deoxynucleotidyl transferase dUTP (TUNEL) end labeling test, and showed a diffuse distribution of grafted mitochondria into neurons, astrocytes, and microglia. Mitochondria treated with antimycin A and oligomycin failed to provide the equivalent protection against oxygen and glucose deprivation (OGD) stress, suggesting that the benefits of the transplantation required the intact mitochondrial function [44].

Nine experimental models of a cardiac ischemic reperfusion injury were performed with employing a porcine model [45-48], a rabbit model [49] and a murine model [50], including seven models of focal ischemia [45] and one model of global ischemia [46]. The types of transplantation were autologous [45-48,51] and allografts [50]. An introduction was intra-arterial and by a direct injection. Kaza AK. et al. (2017) [52] introduced autologous mitochondria into the area at risk (AAR) of the heart 24 minutes after temporary regional ischemia, which was produced by the circumflex artery ligation in a porcine model. Mitochondria isolated from the dissected major pectoralis muscle were injected into the risk area (1.3 × 10^7 per injection site × 8 injection sites) that significantly increased the viability of the myocardial cells and reduced the infarction size. Magnetic resonance imaging showed that the injected mitochondria were present at least for four weeks after injection [52]. Shin B. et al (2019) [54]
demonstrated that autologous mitochondria (1×10^9 mitochondria) injected into the left coronary artery after a 30-minute regional ischemia due to the temporary ligation of the middle of the anterior left descending artery significantly improved the myocardial function, the coronary blood flow and regressed the infarct size. Thus, the authors concluded that the intracoronary delivery of mitochondria is a safe and effective therapy method for ischemia-reperfusion injury of the myocardium [53]. A group of scientists guided by Guariento A. (2020) [54] performed an autologous mitochondrial transplantation as a therapeutic strategy for preventive myocardial protection in a model of regional ischemic reperfusion injury in swine.

Cowan DB. et al. (2016) [49] used the New Zealand white rabbits to investigate whether exogenous mitochondria could be effectively delivered through the coronary vasculature to protect the ischemic myocardium and studied the fate of those transplanted organelles in the heart. Xenotransplanted mitochondria have been observed in interstitial spaces, and they have been associated with blood vessels and cardiomyocytes. They also found that autologous liver mitochondria markedly reduced the infarct size and improved myocardial function [49]. Masuzawa A. with a group of scientists (2013) [55] transplanted mitochondria (9.7 ± 1.7×10^6/ml), which were autologously obtained from the major pectoralis muscle, in rabbits subjected to regional ischemia, 1 min before reperfusion. Regional ischemia was produced by temporary narrowing of the anterior left descending artery for 30 minutes. The animals were then allowed to recover for 4 weeks to measure their cardiac function. Mitochondrial transplantation significantly reduced the infarct size, the creatine kinase levels, the cardiac troponin-I levels, and apoptosis in the area of the reperfusion injury compared to the vehicle group. The authors also showed that mitochondria transplanted in vivo and in vitro were observed in the interstitial space and internalized by cardiomyocytes 2–8 h after the transplantation. Transplanted mitochondria increase the oxygen consumption and enhance the high-energy phosphate synthesis [55] (Masuzawa A, et al. 2013). In a model of the mouse heart transplantation, Moskowitzova K. et al. (2019) [50] reported that 1×10^8 allogeneic mitochondria isolated from the gastrocnemius muscle were delivered antegradely to the coronary arteries by an injection at the mouth of the coronary arteries before donor heart harvesting and after the transplantation. Mitochondrial therapy (1×10^9 in respiratory buffer) prolonged cold ischemia time, significantly improved the graft function, and reduced graft tissue damage [50].

Studies on mitochondrial transplantation have also been performed in other experimental models, including the models of acute limb ischemia [56], ischemic reperfusion lung injury [57], spinal cord injury [58], and acute liver injury [59]. Thus, a C57BL/6J male mouse acute limb ischemia model was applied to compare the effects of dose-dependent mitochondrial injection (1×10^6, 1×10^7, 1×10^8 and 1×10^9) mitochondria/g of muscle) after IRI with f-rhodamine 6G-labeled mitochondria. Positron emission tomography demonstrated diffuse mitochondrial absorption by the injected muscle group, and muscle histology showed a significant reduction in the infarct size; in addition, some dose-dependent significant differences were achieved between the lowest and highest dosing regimens. The mitochondrial injections were associated with an improved functional status as measured by the visual gait assessment and increased the IL-10 expression as measured by multiplex analysis [57]. In addition, mitochondrial transplantation has been shown to be effective in a mouse IRI lung model. The male C57BL/6 J mice received either vehicle or vehicle-containing mitochondria, either by the pulmonary vascular delivery or tracheal aerosol delivery (nebulization). Upon expiration of 24 post-reperfusion hours, the lung function was found to be increased, whereas the tissue damage was significantly reduced in the mitochondrial treated groups as compared to the corresponding vehicle treated groups [58].

Studies in humans

At Boston Children’s Hospital, from May 2002 to December 2018, 10 children with severe heart diseases who required central ECMO support for the IRI-associated myocardial dysfunction after heart surgery were eligible for autologous mitochondrial transplantation. These 10 subjects were compared with the 14 reference cases. Mitochondria were collected from non-ischemic rectus abdominis and isolated within 20–30 min under sterile conditions in the cardiology intensive care unit or in the operating room. A 6 mm x 6 mm sheath of heather rectus abdominis was harvested, and mitochondria isolated, yielding approximately 2 x 10^10 viable and respirable mitochondria from a 0.18 ± 0.04 g (wet weight) tissue sample. The isolated
mitochondria were suspended in a 1 ml volume of the respiratory buffer at a concentration approximately of $1 \times 10^7$ to $1 \times 10^8$ particles/ml. During the same intervention, mitochondria were delivered by a direct injection using a tuberculin syringe (28-gauge needle) into the myocardium affected by IRI that was identified by epicardial echocardiography. After the procedure, the cardiac function estimated by echocardiography was checked by two blind examiners for median circumferential tension and qualitative assessment [24].

Potential mechanisms responsible for the beneficial effects of mitochondrial transplantation

The mechanisms responsible for the mitochondrial transplantation remain to be fully elucidated. Astrocytes play a wide range of roles in the regulation of the nervous system development, neurotransmission, formation of the blood-brain barrier as well as protection against oxidative stress and excitotoxicity. Recent studies have shown that astrocytes can release and transport extracellular mitochondrial particles, which enter damaged neurons to support neuroprotection and neurorepair through calcium-dependent CD38 signaling [60]. In some experimental models of cerebral ischemia, it has been found that transplanted mitochondria are included in neurons, astrocytes, and microglial cells of the peri-infarct region that leads to an increase in the total ATP content and the enhanced expression of complex IV [61].

The mitochondrial transplantation significantly attenuates cellular oxidative stress, apoptosis, reduces astrogliosis and microglial activation, and promotes neurogenesis after ischemia [61]. Furthermore, fluorescence imaging showed that, in addition to the ischemic brain, labeled mitochondria were found in various organs, including the lungs, the liver, the kidneys, and the heart [62]. Thus, further research is needed to determine how the intravenous introduction of mitochondria makes its effect on other organs outside the central nervous system after stroke. In models of cardiac IRI, transplanted mitochondria have been shown to act both extracellularly and intracellularly [63]. Injected mitochondria were found to localize near their delivery site, while vascular perfusion of mitochondria resulted in a rapid and extensive distribution throughout the heart [64]. Transplanted mitochondria increased the total ATP content in tissues and ATP synthesis, as well as enhanced the viability of myocardial cells [52]. This increase in high-energy molecules rapidly improves the cardiac function. In addition, transplanted mitochondria enhance biological pathways important for maintaining myocardial energy production and cell viability [65]. The proteomic analysis showed the activation of proteomic pathways for the energy production, the mitochondrial function, and cellular respiration [65]. The mitochondrial transplantation reduced pro-inflammatory markers, increased levels of anti-inflammatory cytokines, and inhibited endoplasmic reticulum stress and the caspase-3 expression [66]. The mitochondrial transplantation did not change the levels of pro-inflammatory cytokines [57]. Moreover, the metabolic analysis has shown that the mitochondrial electron transport chain is among the top 10 pathways involved in the altered metabolic profile of the heart received mitochondria [54].

The precise mechanisms by which exogenous mitochondria can be integrated by the cells remain unclear; it has been detected that transplanted exogenous mitochondria sequentially move along the endolysosomal system from early endosomes to late endosomes and lysosomes [67]. Most exogenous mitochondria leave endosomal and lysosomal compartments and effectively fuse with endogenous mitochondria in the heart cells that is accompanied by an increase in the ATP content, the oxygen consumption rate, and the replacement of depleted mitochondrial DNA [68]. However, further research to explain the molecular mechanism of exogenous mitochondria is needed in order to develop this innovative treatment method.

In terms of the clinical interpretation, a potential alternative source of viable respiratory competent mitochondria might be from another individual of the same species (allogeneic) or another species (xenogeneic). Ramirez-Barbieri G (2019) [69] investigated the immune response and that associated with damage-associated molecular patterns (DAMP) after an injection of allogeneic mitochondria into the graft rejection system of completely MHC-incompatible skin allografts. Their results demonstrated the absence of any direct or indirect, acute or chronic alloreactivity, alloreognition, or DAMP response to single or serial injections of allogeneic mitochondria [69].

Thus, the potential application of the mitochondrial transplantation in experimental and clinical studies has been generalized and summarized hereby. The potential of mitochondrial therapy remains to be explored and understood, however, the data available so
far indicate a vector of the direction for studying the metabolism and pathways of actions and effects made on mitochondria.

Under the conditions of the tumor growth, the study of the mitochondrial dysfunction will provide a deeper understanding of the critical problems in cancer. Therefore, the obtained knowledge of the state of mitochondria in the pathologically altered cells and the degree of their dysfunction supply us with essential information for a better understanding of the pathophysiology of cancer that gives impetus to the development of mitochondrial pharmacology.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References


