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Mitochondrial targeting as a novel therapy for stroke

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Abstract:

Stroke is a main cause of mortality and morbidity worldwide. Despite the increasing development of innovative treatments for stroke, most are unsuccessful in clinical trials. In recent years, an encouraging strategy for stroke therapy has been identified in stem cells transplantation. In particular, grafting cells and their secretion products are leading with functional recovery in stroke patients by promoting the growth and function of the neurovascular unit – a communication framework between neurons, their supply microvessels along with glial cells – underlying stroke pathology and recovery. Mitochondrial dysfunction has been recently recognized as a hallmark in ischemia/reperfusion neural damage. Emerging evidence of mitochondria transfer from stem cells to ischemic-injured cells points to transfer of healthy mitochondria as a viable novel therapeutic strategy for ischemic diseases. Hence, a more in-depth understanding of the cellular and molecular mechanisms involved in mitochondrial impairment may lead to new tools for stroke treatment. In this review, we focus on the current evidence of mitochondrial dysfunction in stroke, investigating favorable approaches of healthy mitochondria transfer in ischemic neurons, and exploring the potential of mitochondria-based cellular therapy for clinical applications. This paper is a review article. Referred literature in this paper has been listed in the references section. The data sets supporting the conclusions of this article are available online by searching various databases, including PubMed.

Keywords:

Bioenergetics, blood–brain barrier, cerebral ischemia, endothelial cells, impaired mitochondria, neurovascular unit, regenerative medicine, stem cell therapy, transfer of healthy mitochondria, vasculature

Introduction

Stroke is a leading cause of death and disability worldwide.[1] Although considerable advance in our understanding of the disease has been achieved over the last two decades, the available treatments are limited.[1] Mitochondrial dysfunction has been implicated in the secondary cell death associated with stroke. Accordingly, strategies designed to target mitochondria may prove beneficial for stroke. In this review, we provide an in-depth analysis of the role of mitochondria dysfunction in ischemic injury and examine current mitochondrial pharmacological and nonpharmacological therapeutic approaches. In particular, we explore the potential of mitochondria transfer-based stem cell therapies for the treatment of stroke.

Current Treatments for Stroke

To date, only one Food and Drug Administration (FDA)-approved drug, alteplase, is available for the treatment of ischemic stroke.[1] Alteplase, a recombinant tissue plasminogen activator, is a thrombolytic agent, and its mechanism of action consists in breaking down the clot from the occluded vessel, allowing the restoration of blood flow.[1] However, the efficacy of alteplase is limited within 4.5 h

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after stroke onset and therefore not all stroke patients can receive it in a timely manner.[1,2] In addition, anticoagulant agents increase the risk of hemorrhages.[1,2] Alternatively, an endovascular thrombectomy may be used as a supplement or substitute option for stroke patients ineligible for intravenous thrombolysis.[1,2] Other supportive medications for stroke patients include maintenance of normoglycemia and physiological body temperature as well as management of blood pressure.[1] Therefore, advancement in therapeutic options for stroke is an urgent necessity.[2] Increasing evidence suggest an encouraging potential of small molecules for the treatment of stroke.[2] Notably, the PA Stachybotrys microspora triprenyl phenol-7 reduced infarct area, hemorrhages, and improved neurological function in nonhuman primate stroke model.[2,3] In a mouse stroke model, the small molecule NSI-189 incremented neurogenesis, cell proliferation, and neurotrophic factors, as well as improvement in behavioral function along with the expansion of the time window to a 6-h for delivery after stroke.[2,4] Hence, these small molecules may represent an additional therapeutic approach to the current stroke treatments.

Roles of Mitochondria in Stroke

Mitochondria are widely known for their role as "energy powerhouse of the cell," being the primary generators of adenosine triphosphate (ATP), a high-energy molecule that stores and supplies energy for many biochemical processes. Mitochondria have crucial roles in energy metabolism regulation, cell cycle, survival and death, apoptosis, generation of reactive oxygen species (ROS), and calcium homeostasis.[5,6] About 90% of the whole cellular energy need is produced by the metabolic processes entailing glycolysis, fatty acid beta-oxidation, Krebs cycle, and oxidative phosphorylation (OXPHOS).[7,8] Under physiological conditions, approximately 2% of the total electrons transferred across the mitochondrial respiratory chain (MRC) leak during aerobic respiration, mainly from Complexes I and III, leading to the formation of superoxide anion.[9,10] Superoxide anion is the precursor of ROS such as hydrogen peroxide and hydroxyl radical, which can damage lipids, nucleic acids, and proteins, becoming critical players in the physiological process of aging as well as in the onset and progression of several diseases including myocardial infarction, inflammatory disorders, some cancers, and atherosclerosis.[2,7] The human brain is the most energy-consuming organ, with 20% of the total energy produced, being used by only 2% of the body mass. Most of this energy is used for crucial central nervous system functions such as generation action potentials and transmission of information through chemical synapses.[11] In this regard, mitochondrial dysfunction might have a critical role in neurodegeneration and in several diseases such as Alzheimer's, Parkinson's, Huntington's as well as psychiatric disorders such as depression and schizophrenia and neurodevelopmental illnesses like autism spectrum disorder.[12-17] In addition, Anderson–Fabry disease may be associated to a reduction in OXPHOS and energy production due to a mitochondrial dysfunction and it may generate ischemic stroke.[18-20]

Ischemic stroke occurs when a cerebral region is deprived of oxygen due to a decrease in local blood flow resulting from obstruction of a blood vessel such as embolism or thrombus formation. Ischemic stroke leads to deficits in neurological function, disability and, in many cases, death.[21,22] The risk of stroke increases with age, but there is a rising incidence of stroke cases in young adults that account for the 10%–15% of all the strokes.[23] Stroke in younger people has a higher economic and social impact than in older ones due to a disability in the most fruitful years of life.[24] Intravenous thrombolysis is efficacy within 4.5 h of stroke onset. A thrombectomy may be performed when thrombolytic agents cannot be used, but alternative options are very limited.[25-27] According to several evidence on the central role of mitochondria in neurons metabolism as well as in the ischemic/reperfusion cascade resulting in neuronal death,[28,29] this review focuses on the involvement of mitochondria in the pathophysiology of stroke and the promising mitochondria-based regenerative medicine for stroke treatment.

Mitochondria Dysfunction Inducer of Stress, Disease, and Death

Mitochondria have a central role in cellular biology. MRC is constituted by a series of mitochondrial complexes (Complex I–Complex CV) that serve as sites of electrons transport (Complex I–IV) coupled to ATP production (CV).[30] The OXPHOS machinery is organized into supercomplexes in the inner mitochondrial membrane.[31,32] Most of the ~90 subunits constituting the electron transport chain (ETC) complexes are of nuclear DNA origin, while 13 are encoded by mitochondrial DNA (mtDNA). In the ETC, electrons are transferred from $FADH_2$ and NADH to series of electron acceptors and donors, with molecular oxygen being the last acceptor and pumping protons across the inner membrane.[31,32] The electrochemical gradient thus generated is used to produce ATP through Complex V.[31,32] MRC is the central site of premature electron leak to oxygen.[33] The generation of superoxide anion, hydrogen peroxide, and hydroxyl radicals can result in oxidative stress.[34] Therefore, mitochondria are the main ROS producers and consequent oxidative stress is a key factor in degenerative processes.[35] OXPHOS disorders may affect 1–5/10,000 births and each mitochondrial complex may be crucial

in the genesis and progression of different diseases.[36] Some of these mitochondrial disorders are characterized by neuronal damage and encephalopathies (coenzyme Q10 deficiency, Complex I–IV deficiencies, Leigh disease, MIRA), epilepsy, seizures and ataxia (MERRF, MIRAS, Leigh disease, Friedreich's ataxia), and stroke-like episodes (MELAS).[36] Therefore, a brief overview of these disorders is imperative.

Complex I, well known as NADH dehydrogenase, is involved in different neurodegenerative disorders characterized by deficits in mitochondrial energy metabolism.[37-39] Complex I is the first protein of the MRC involving the electron transfer from NADH to ubiquinone. Along with this reaction, a premature transfer of electrons to oxygen may happen, leading to superoxide anion production. Therefore, Complex I represents a source of ROS production.[40] ROS directly can damage mitochondrial proteins, mtDNA, and lead to disruption of membrane integrity with consequent depolarization and triggering of the apoptotic pathway.[41]

Although Complex II represents only the 2% of all MRC defects, its deficits extend from cancer to Leigh syndrome, infantile leukodystrophies up to cardiomyopathies.[42] Complex II is involved in apoptosis induction. As such, Complex II detects pH changes generated by apoptosis-inducer transmembrane protein Fas ligand (FasL) leading to ROS production and cellular death.[43]

Similarly, Complex III dysfunction may result in cell death. Pesticide exposure has been associated with inhibition or destruction of Complex III with consequent impairment of electron transport and ROS production leading to apoptotic pathway activation.[44] Interestingly, different studies suggest an association between pesticide exposure and Parkinson's disease.[45] In addition, exercise intolerance and ischemic cardiomyopathy have been correlated with cytochrome b or other Complex III subunits gene mutations.[46,47]

Complex IV or cytochrome c oxidase (COX) has the function to reduce oxygen to water by electron transfer from the reduced cytochrome c.[48] Mutations in the mitochondrial COX gene cause a number of rare autosomal recessive diseases.[48] COX deficiencies are associated with Leigh Syndrome, hypertrophic cardiomyopathy and myopathy, and fatal infantile lactic acidosis.[48,49] Moreover, COX deficit associated to iron-deficiency anemia may exacerbate the oxidative stress.[50]

ATP synthase, also known as Complex V, is the final OXPHOS enzyme that uses the energy of the proton electrochemical gradient to synthesize ATP from ADP and phosphate.[51,52] ATP synthase plays a role in mitochondrial cristae morphology and in the formation of the permeability transition pore complex (mPTP).[51,52] Despite being somewhat uncommon, ATP synthase disorders are highly severe.[53] ATP synthase deficiencies involve structural modifications characterized by energy deprivation.[54,55] Genetic mutations in mtDNA coding for ATP synthase subunits determine incorrect assembly and/or function of the enzyme. Disorders related to ATP-synthase deficits are neuropathy, ataxia, retinitis pigmentosa (NARP), Leigh syndrome (MILS), and encephalo (cardio) myopathy.[55,56] On the other hand, quantitative deficits present serious symptoms and usually fatal in newborns with hyperlactacidemia, hypertrophic cardiomyopathy, and high levels of 3-methylglutaconic acid.[56,57]

An abnormal ROS production can overload the cerebral antioxidant defense system leading to further cell death and degeneration.[58] Numerous evidence have indicated oxidative stress as a key factor in stroke, pointing to mitochondria as potential target to treat this disease.[58] In addition to mitochondrial dysfunction, alternative mechanisms can contribute to oxidative stress. NADPH oxidases (NOX) in microglia, neurons, and endothelial cells represent significant ROS sources during stroke.[59] NOX2, a member of NOX family located in brain phagocytes, is a key factor in the stroke-dependent ROS production leading to cell death.[60] As a consequence, the inhibition of NOX activity might be suitable therapeutic strategy for the treatment of stroke.[60]

As discussed above, mitochondria may trigger apoptotic cell death. The apoptotic pathway is characterized by several mitochondria-centered events, such as cytochrome c release, modifications in the MRC, loss of mitochondrial membrane potential, impaired cellular redox state, and implicating the action of pro- and anti-apoptotic B-cell lymphoma 2 (Bcl-2) proteins.[61] Bcl-2 family members have a key role in the cytosolic release of mitochondrial molecules that activate the effectors caspases, cysteine proteases involved in degradation, and removal of cellular components.[62] Two different pathways can initiate apoptosis: intrinsic and extrinsic.[63] The intrinsic pathway is triggered by the binding of proapoptotic factors to the outer mitochondrial membrane (OMM) causing the damage of mPTP and, consequently, the release into the cytosol of proapoptotic molecules normally located in the intermembrane space of mitochondria, such as second mitochondria-derived activator of caspases (Smac), apoptosis-inducing factor (AIF), and cytochrome c.[64] In the cytosol, Smac inhibits the inhibitor-of-apoptosis proteins, which role is to inhibit procaspase activation and caspases activity.[64] Instead, AIF can migrate into the nucleus where induces caspase-independent chromatin

condensation and large-scale DNA fragmentation following ischemia.[65,66] In addition, the association of cytochrome c with APAF-1 creates the apoptosome that recruits and active the procaspase-9 in caspase-9. Once activated, caspase-9 can activate effector caspase-3. Consequently, caspase-3 activates endonucleases and proteases which causing breakdown of DNA. This controlled cell death pathway is associated with the expression of ligands for phagocytic receptors, resulting in phagocytosis.[67,68] On the other hand, extrinsic pathway is triggered after binding of FasL or tumor necrosis factor to their respective receptors and promoting the assembly of the death-induced signaling complex (DISC). DISC activates procaspase-8 to caspase-8, which converge in the activation of caspase-3 and in the final part of the intrinsic pathway.[69] In the same way, cytotoxic T-cells trigger perforin/granzyme-induced apoptosis also activating caspase-3.[70] Apoptosis, along with necrosis and aponecrosis, can be associated to inflammation response.[71] Moreover, an inflammatory response can be also secondary to the cell death damaging the adjacent cells with consequent extension of the primary injury.[72] Conversely, an anti-apoptotic signaling balances the death pathway involving Akt which inhibits proapoptotic factors such as Bcl-2-associated X protein (BAX) and Bcl-2-associated-death promoter (BAD).[73]

Excitotoxicity, characterized by large calcium cellular influx, is associated with stroke as well as traumatic brain injury and neurodegenerative diseases such as multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis, fibromyalgia, Parkinson's disease, and Huntington's disease. The activated calcium/calmodulin phosphatase calcineurin (CaN) determines the activation of proapoptotic factors.[74] In this regard, the translocation to the OMM of activated BAD results in the inhibition of survival proteins Bcl-2 and B-cell lymphoma-extra large inducing BAX to open the mPTP causing the release of cytochrome c and the consequently formation of apoptosome.[75] Moreover, CaN can stimulate mitochondrial fission by dephosphorylating the dynamin-related protein 1 triggering cell death.[76] Even though mitochondrial fission is a normal process in physiological conditions, the presence of spherical mitochondrial lacking of cytochrome c is a hallmark of a pathological apoptotic condition.[77]

Mitochondria in Regenerative Medicine

Mitochondrial impairment has been shown in stroke, neurodegenerative disorders as well as in aging and other metabolic diseases. Therefore, mitochondrial pharmacological and nonpharmacological therapy could be novel strategies to treat several diseases. Strengths and weaknesses of mitochondria based-regenerative medicine are discussed in this section.

As mentioned above, impaired mitochondrial function is implicated in ROS production.[78,79] The different pathways of ROS production could be critical therapeutic targets. In this respect, the activation of sirtuin 1 (SIRT1) has been investigated. SIRT1 is a NAD-dependent deacetylase with a main role of sensor of redox state and energy reducing oxidative stress and improving mitochondrial function.[80,81] It has been shown its crucial function in metabolism of lipids and glucose through insulin signaling in the liver, skeletal muscle, and adipose tissue.[82,83] SIRT1 induces mitochondrial biogenesis and glucose uptake, through activation of PPARs and PGC1, as well as reduce inflammation and oxidative stress.[84,85] Resveratrol, an activator of SIRT1, can act like a free radical scavenger.[86] It has been shown that the pretreatment with resveratrol is neuroprotective following cerebral ischemia through SIRT1–UCP2 pathway.[87]

It has long been established that mitochondria are extremely dynamic organelles, undergoing constant morphological remodeling through fission and fusion events crucial for their functions, transport along axons, and survival of the cell.[88] Imbalance in these events underlies several diseases and as such becomes strategic target for new therapies.[88] In this regard, drug inhibitors of fission (e.g., mdivi-1, dynasore, and P110) and activators of fusion, such as leflunomide, have been investigated[89,90] and have proved to reduce oxidative stress and offer neuroprotection and improvement of energy metabolism following hypoxia.[89-94]

Purines act as neurotransmitters and their signaling represents the link between neuronal activity energy charge and homeostasis.[95] It has been shown that the modulation of purinergic pathways may be a novel therapeutic strategy for the treatment of stroke. In particular, purinergic receptor agonists regulate the Ca^{2+} levels reducing the glutamate release and, consequently, the excitotoxicity following stroke.[96] Moreover, the activation of P2Y1 receptor has been associated with increased astrocyte mitochondrial metabolism and reduced infarct size and edema formation.[97] Other studies showed that purinergic modulation resulted in normalization of mPTP with a consequent reduction of apoptosis.[98,99]

Large interest is arising for methylene blue, currently approved by the FDA for the treatment of Alzheimer's and Parkinson's diseases, for stroke patients.[100] It has been shown that methylene blue can modulate the electrons flow through the ETC. In particular, being a carrier of electrons between NADH and cytochrome c, it can allow electrons to bypass Complex I and III resulting in decrease of electron leakage and improvement in ATP production along with a consequent reduction of

ROS and oxidative stress.[101] Moreover, in an *in vitro* stroke model, methylene blue increased the activity of Complex IV improving the mitochondrial function.[102] In addition, in a rat stroke model, methylene blue restored cerebral blood flow and glucose uptake to normal conditions as well as reduced the infarct size leading to improved behavioral functions.[103] Taken together, these studies support the efficacy of methylene blue for stroke treatment.

Detrimental effects caused by ROS accumulation following stroke are mitigated by conversion of superoxide to hydrogen peroxide through mitochondrial superoxide dismutase 2 (SOD2 or MnSOD).[104-106] Overexpression of SOD2 and its cytoplasmic counterpart SOD1 (or Cu/Zn SOD) decrease deficits associated with stroke.[106,107] On the other hand, increase of infarct volumes leads to SOD deficiencies.[106,107] Despite the beneficial effects related to SOD activity, its therapeutic utilization is hampered by its short half-life, high molecular weight, and the low oral bioavailability. To overcome the issues connected with the pharmacological application of such enzymes, SOD mimetics with lower molecular weight, high diffusion rate and permeability, lack of immunogenicity, and resistance to peroxynitrite inactivation along with elevated efficiency have been developed.[108] Manganese (Mn) exerts a key role in the regulation of the redox activity of SOD.[109,110] In this regard, Mn (III) tetrakis(1-methyl-4-pyridyl)porphyrin (MnTm4PyP) reduced cytochrome c and superoxide radical in a dose-dependent manner in a stroke model.[111] In addition, it decreased active caspase-3 and preserved intracellular calcium level.[111] Similarly, Mn (II) pentaazomacrocyclic mimetic M40403 targets superoxide but, interestingly, it has reported higher redox abilities then endogenous SOD when linked with triphenylphosphonium (TPP) forming the MitoSOD compound.[110] Moreover, reduction of oxidative and nitrosative stress has been obtained with Mn (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP).[112] However, serious side effects such as edema formation and even increased cell death following stroke can occur.[113] In addition, the utility of SOD mimetics in hemorrhagic stroke should be investigated.

An alternative strategy for the reduction of ROS is the use of antioxidants such as coenzyme Q, N-acetylcysteine and Vitamins C and E.[114] Despite the well-established mechanisms of action, few clinical trials have provided evident efficacy of Vitamin E for the treatment of cardiovascular diseases.[114] Interestingly, some antioxidants can directly target mitochondria. In experimental models of cardiac hypertrophy and aging, MitoQ, a derivative of ubiquinone is able to freely access mitochondria and decreased lipid peroxidation.[115-117] In addition, the mitochondrial access of MitoQ has been reported extremely increased when associated with the lipophilic TPP cation.[118] Currently, clinical trials for the treatment of PD or liver damage with MitoQ are in progress.[119,120]

Tiron, antioxidant and iron chelator, is another molecule that can penetrate in the mitochondria protecting human dermal fibroblast from photoaging damage.[121,122] In several *in vitro* and *in vivo* models, MitoVit E (or [2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-yl) ethyl] triphenylphosphonium bromide) was 350-fold more potent in reducing oxidative stress than nontargeted antioxidants such as Vitamin E or its water-soluble analog trolox.[114,123] Similarly, MitoPeroxidase (2-[4-(4-triphenylphosphoniobutoxy) phenyl]-1,2-benzisoselenazol)-3 (2H)-one iodide), a mitochondrially targeted analog of ebselen (glutathione peroxidase analog), protected by oxidant-induced apoptosis by catalyzing the breakdown of H_2O_2.[124]

Among the mitochondria-targeted antioxidant, glutathione analogs protect the mitochondrial redox system and its signaling ability in cardiovascular diseases.[125] Taken together, these evidence show that mitochondria-targeted antioxidants are more advantageous than the nontargeted ones.[126,127] The free-radical trapping agent NXY-059 has been reported neuroprotective in stroke animal models.[128] However, no difference between NXY-059-treated and placebo stroke patients in a large-scale clinical trial.[129,130] Moreover, further investigations are needed for stilbazulenyl nitrone (STAZN) due to its elevated ability in inhibition of lipid peroxidation and its lipophilic characteristics making it highly applicable for brain delivery.[131,132]

Exercise may contribute to the recovery from different neurological disorders by inducing mitochondrial biogenesis and boosting OXPHOS capacity.[133,134] Exercise has been linked to increased mitochondrial biogenesis and density, through AMPK signaling pathway and PGC1 expression modulation, leading to increased mitochondrial respiration and decreased oxidative stress.[135-138] Moreover, exercise may moderate the age-dependent decline in mitochondrial functions.[139] Similarly, caloric restriction (CR) may increase lifespans and reduce negative outcomes of metabolic disorders.[140] However, few studies in human have reported that CR reduced ROS level and improved mitochondrial functions.[140,141] It has been shown that CR action may involve Sir2/SIRT1 and AMPK/PGC1 pathways improving mitochondria biogenesis and function.[142,143]

Mitochondria Transfer as New Therapeutic Strategy

The discovery of mitochondrial transfer into ischemic cells paved the way for the treatment of mitochondria

dysfunction-related diseases. The demonstration of mitochondria transfer from astrocytes to ischemic neurons supports the concept to use stem cells as source of healthy mitochondria for stroke therapy.[144]

The current stem cells repair mechanisms involve the secretion of growth factors, the direct replacement of injured neuronal cells, as well as the stimulation of migration of the endogenous neural stem cells from the neurogenic niches to the lesion site.[145] The latest findings emphasize the replacement of dysfunctional mitochondria as a tool to restore mitochondrial function and to recover cell damage after stroke.[146-149] The finding that not only mitochondria but also microvesicles, lysosomes, exosomes, and endosomes can be transferred from stem cells to ischemic host cells, suggest that the stem cells may be considered as organelles donors.[150]

It has been proposed that the transfer of mitochondrial genes may be implicated in restoring mitochondrial function,[151] increasing respiration in cells with impaired mitochondria.[152] However, to date, the mechanism of mitochondria transfer is still uncertain. Evidence show that the mitochondrial transfer may occur through the formation of tunneling nanotubes (TNTs) or extracellular vesicles (EVs), but the passive uptake of mitochondrial is not still confirmed.[153] Several studies have demonstrated that stem cells can donor healthy mitochondria to different recipient cells.[154] In an *in vitro* ischemic-reperfusion model, mesenchymal stem cells (MSCs) transferred their mitochondria to human umbilical vein endothelial cells (HUVECs) restoring aerobic respiration.[155] In addition, damaged cells can produce phosphatidylserine, inducing the TNTs formation in MSCs promoting mitochondrial transfer.[155] Similarly, TNTs mitochondrial transfer from MSCs to cardiomyocytes improved survival and reduced cellular damage in an *in vitro* ischemia/reperfusion model[156] and mitochondrial transfer from MSCs to lung epithelium alleviated cigarette smoke damage.[157]

Interestingly, along with the transfer of healthy mitochondria, the protective effect of MSCs seem to involve the endocytosis and degradation of dysfunctional mitochondria reducing the oxidative stress in damaged cells.[158,159] The mitochondrial transfer may be in response to a "help me" signal from oxidative stress since it rarely occurs in healthy conditions.[153,160,161] In this regard, it has been reported that the formation nanotubes and vesicles can be associated with the connexins of gap junctions allowing mitochondria transfer.[162] Moreover, overexpression of Miro1, involved in connection and movement of mitochondria through cytoskeleton, increased mitochondrial transfer by TNTs from MSCs to stressed epithelial cells reducing inflammatory cell infiltration, cellular apoptosis, collagen deposition, and hypersecretion of mucus in the lungs.[163]

In cancer cells, mitochondria transfer form MSCs is linked with resistance to doxorubicin and promotion of survival by increment of ATP production (50%) and content (4.5 fold).[164] However, further investigation is granted to better understand the molecular mechanisms of the transfer process, the level of cellular damage that promotes the mitochondrial transfer as well as the signals that allow the interaction between damaged and stem cells.[153]

Although the signaling mechanisms between mitochondria donor and recipient cells are still unclear, the TNTs formation is well-documented *in vitro* and *in vivo* studies.[153] The formation of filopodium triggers the TNT formation, but it is retracted releasing an ultrafine structure upon arriving to recipient cell that allows unidirectional or bidirectional mitochondrial transfer.[165-167] Mitochondrial transfer impairment has been reported when stressed cells are exposed to TNT inhibitors supporting the fundamental role of TNTs in this fine mechanism.[168] In addition, stress can modulate TNT formation.[169]

Mitochondrial transfer can occur also by EVs release that may be used as biomarkers for different disease.[170,171] Despite the limited knowledge about this mechanism, the delivery of mitochondria by EVs promotes the recovery of mitochondrial function.[152,153,162] Furthermore, cell fusion has been reported as another mechanism of mitochondrial transfer between stem cells and epithelium cells of the respiratory tract and cardiomyocytes.[172-174] Cell fusion has been shown also after bone marrow transplantation in rodent in which leaded with liver regeneration.[175,176] Extrusion of whole mitochondria or their components has been reported as an alternative mechanism of mitochondria transfer.[177,178]

Despite the presence of clinical trials for the use of stem cells for stroke, this therapeutic strategy is still experimental.[179] By restoring cell bioenergetics proliferation, mitochondria transfer could be a promising tool for the treatment of ischemic stroke.[180] Studies in stroke model have shown that targeting TNTs may promote mitochondria transfer.[163,180] MSCs overexpressing Miro1, a key protein involved in TNT formation, increased mitochondrial transfer reducing neurovascular unit deficits after stroke.[180] To this end, targeting key elements of mitochondria transfer could be a safe and effective therapeutic strategy for stroke.

Conclusions

ATP and nutrients depletion in the ischemic penumbral implicate the key role of mitochondria in stroke pathology. The rapid degeneration of the penumbral neurovascular unit requires the urgent identification

of novel and effective therapeutic strategy for stroke. Recent findings of mitochondria transfer, by TNT formation, EVs or cellular fusion, and its potential to restore mitochondrial function in promoting cell survival in several preclinical trials support stem cell-mediated mitochondria transfer therapies for stroke. Further investigations are needed to advance our knowledge on mitochondria transfer, and consequently, its application for the treatment of stroke and other mitochondrial dysfunction-related diseases.

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Conflicts of interest
There are no conflicts of interest.

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