Journal of Advanced Research xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Advanced Research

journal homepage: www.elsevier.com/locate/jare

Intracellular mitochondrial transfer in ischemic stroke: Mechanisms and therapeutic application

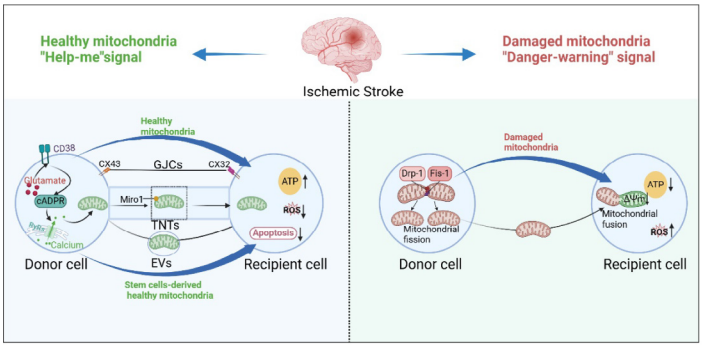
Rong Fu [a], Yang Zhao [b], Yayi Li [a], Yuliang Zhang [a], Meidan Wang [c], Zhuo Wang [a,d], Ming-sheng Zhou [a,*], Yueyang Liu [e,*]

[a] Science and Experiment Research Center & Shenyang Key Laboratory of Vascular Biology, Shenyang Medical College, Shenyang, China
[b] Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang, China
[c] Department of Vasculocardiology, The Second Hospital of Shenyang Medical College, Shenyang, China
[d] General Hospital of Northern Theater Command, Shenyang, China
[e] Department of Pharmacology, Shenyang Medical College, Shenyang, China

HIGHLIGHTS

- Summary of latest researches on IS-induced mitochondrial dysfunction.
- Overview of the healthy and damaged mitochondrial transfer among endogenous neural cells following IS.
- Overview of the healthy mitochondria donated by stem cell to treat IS.
- Discussion of the current issues and challenges pertaining to mitochondrial transfer.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 January 2025
Revised 11 March 2026
Accepted 15 March 2026
Available online xxxx

Keywords:
Cell to cell communication
Ischemic stroke

ABSTRACT

Background: Current therapeutic models for ischemic stroke (IS) are shifting from a narrow focus on neuroprotection to a broader concept of cytoprotection. This new paradigm emphasizes rescuing damaged brain cells and maintaining their structural and functional integrity through organelle transfer between healthy and damaged cells. Mounting evidence have supported that intracellular mitochondrial transfer is an intrinsic response to IS, playing a critical role in mitigating neural damage. Consequently, mitochondrial transplantation from stem cell is emerging as a therapeutic avenue for IS.

Aim of review: This article reviews the IS-induced mitochondrial dysfunction, the modes and mechanisms of endogenous intracellular mitochondrial transfer, and recent advances in using stem cell-derived mitochondrial transplantation to treat IS.

Abbreviations: ARF1, ADP-ribosylation factor 1; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; BECs, brain endothelial cells; BMSCs, bone marrow derived mesenchymal stem cells; cADPR, cyclic ADP ribose; CBF, cerebral blood flow; cGAS, cyclic GMP-AMP Synthase; CNS, central nervous system; Cx32, connexin 32; Cx43, connexin 43; Dmp1, dentin matrix protein 1; Drp1, dynamically-associated protein 1; cAMP, cyclic adenosine monophosphate; ECs, endothelial cells; EPCs, endothelial progenitor cells; ER, endoplasmic reticulum; EVs, extracellular vesicles; Fis1, mitochondrial fission 1 protein; GJCs, gap junction channels; GSH, glutathione; hNSCs, human neural stem cells; IL-1β, interleukin-1beta; IMM, inner Mitochondrial Membrane; IS, ischemic stroke; LRP1, low-density lipoprotein receptor-related protein 1; MB, mitochondrial biogenesis; Mfn1, mitofusins 1; Mfn2, mitofusins 2; Miro1, mitochondrial Rho GTPase 1; MMP, mitochondrial membrane potential; MMSCs, multipotent mesenchymal stem cells; mPTP, mitochondrial permeability transition pore; MSCs, mesenchymal stem cells; mtDNA, mitochondrial DNA; mtROS, mitochondrial reactive oxygen species; OGD, oxygen-glucose deprivation; OGD/R, OGD-reperfusion; OLs, oligodendrocyte; OMM, outer mitochondrial membrane; OPA1, optic atrophy 1; OPCs, oligodendrocyte precursor cells; ox-mtDNA, oxidative mtDNA; PGC-1α, peroxisome proliferator-activated receptor coactivator α; PINK1, PTEN induced kinase 1; pMCAO, permanent middle cerebral artery occlusion; STING, stimulator of interferon genes; TCA, tricarboxylic acid; tMCAO, transient middle cerebral artery occlusion; TNF-α, tumor necrosis factor-alpha; TNTs, tunneling nanotubes; Trkβ, tyrosine kinase beta; UC-MSCs, umbilical cord MSCs.

* Corresponding authors.
E-mail addresses: zhoums1963@163.com (M.-s. Zhou), yueyangliu1989@163.com (Y. Liu).

https://doi.org/10.1016/j.jare.2026.03.037

Please cite this article as: R. Fu, Y. Zhao, Y. Li et al., Intracellular mitochondrial transfer in ischemic stroke: Mechanisms and therapeutic application, Journal of Advanced Research, https://doi.org/10.1016/j.jare.2026.03.037

Mitochondrial transfer
Preserving neuronal function
Stem cell therapy

Key scientific concepts: This review emphasizes the dual roles of mitochondrial transfer in determining neural cells fate and neurological function recovery following IS. On one hand, health cells can donate intact mitochondria to damaged cells, to revitalize them by restoring cell metabolic function. On the other hand, damaged cell may expel dysfunction mitochondria, which can be cleared by healthy neighbors or, alternatively propagate injury. We discuss the current challenges in this field and propose that enhancing healthy mitochondrial transfer or preventing damaged mitochondrial release may hold great potential for alleviating IS injury.

Contents

Introduction

Stroke ranks as the second leading cause of mortality and permanent disability in adults worldwide. It is broadly classified into two main types: ischemic stroke (IS) and hemorrhagic stroke [1,2]. IS accounts for 87% of all stroke patients [3]. With the aging trend of the population, the incidence of IS has been progressively increasing, imposing a substantial burden on both society and families [4]. During IS, the sharp decline in cerebral blood flow (CBF) triggers a cascade of intricate physiological and pathological responses, referred to as the ischemic cascade, which ultimately culminate in neuronal damage. The ischemic cascade is complex, involving excitatory amino acid toxicity, oxidative stress, inflammation, calcium overload, apoptosis and autophagy [5,6]. Currently, there are two main clinical strategies for treatment of IS: vascular recanalization and neuroprotective therapy. Vascular recanalization includes surgical thrombectomy and medicant thrombolysis [7,8]. However, the time window for thrombolysis therapy is narrow, and there is also a risk of bleeding [9,10]. Neuroprotective therapy involves inhibiting the ischemic cascade following IS to prevent cell death or promote its survival in the ischemic penumbra, thereby alleviating cerebral ischemic injury

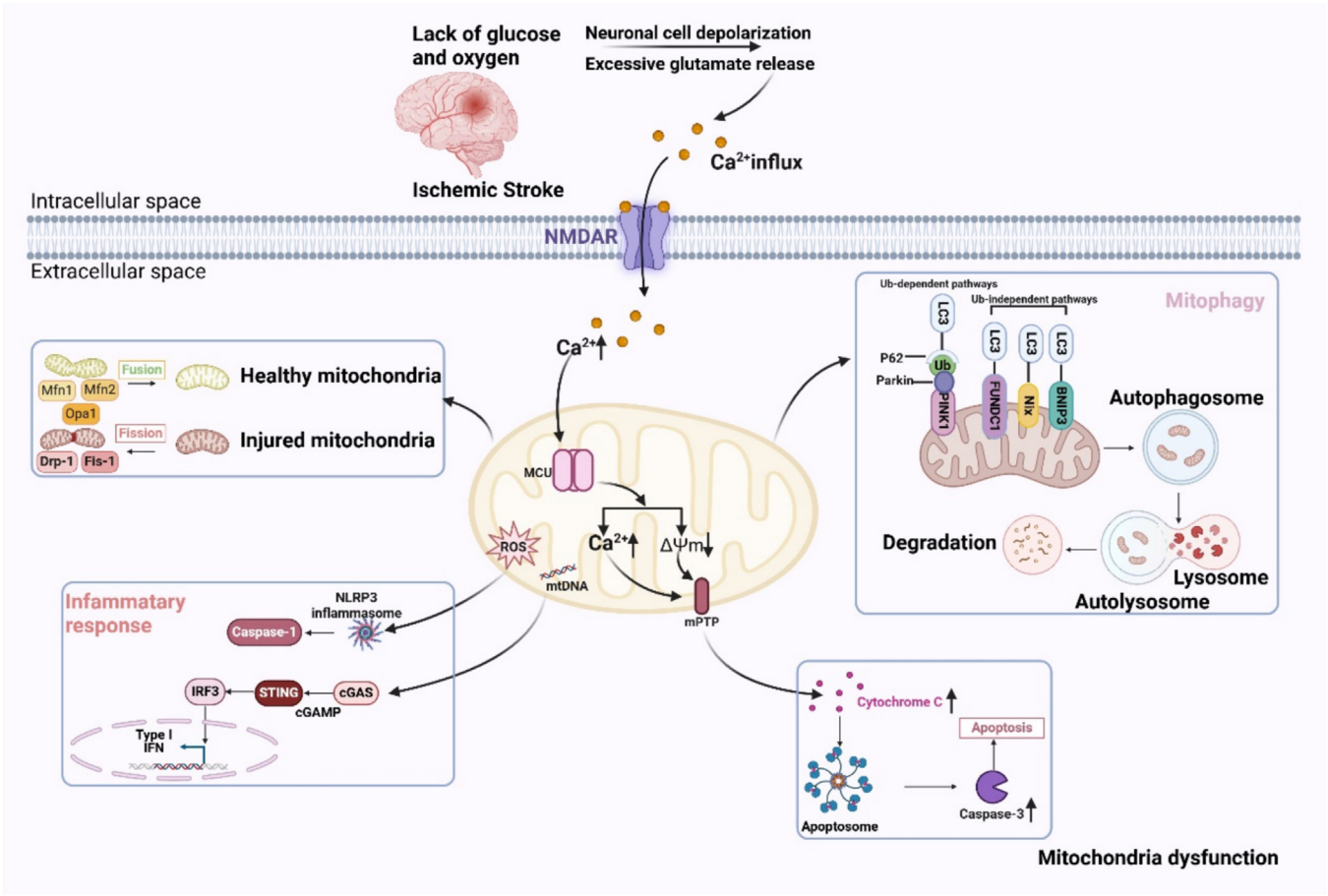


Fig. 1. The mechanisms of mitochondrial dysfunction in the ischemic cascade following ischemic stroke (IS). The diagram illustrates the pathophysiological events triggered by cerebral ischemia, with a focus on the pivotal mechanisms of mitochondrial failure. The initial energy deficit caused by oxygen and glucose deprivation leads to membrane depolarization and glutamate excitotoxicity, driving a pathological rise in intracellular calcium. This calcium is sequestered by mitochondria, disrupting the electron transport chain (ETC), collapsing the mitochondrial membrane potential ($\Delta\Psi m$) and triggering abnormal mitochondrial dynamics, mitophagy and mitochondrial DNA (mtDNA) release. Consequently, oxidative phosphorylation fails, ATP production ceases, reactive oxygen species (ROS) are overproduced and inflammatory response are activated. The simultaneous opening of the mitochondrial permeability transition pore (mPTP) releases pro-apoptotic factors (e.g., cytochrome *c*), initiating caspase-dependent apoptosis. Mitochondrial dysfunction thus acts as a critical hub, amplifying oxidative stress, propagating cell death signaling, and culminating in neuronal necrosis and infarction. This image was created in BioRender (*https://BioRender.com/9wbewj3*).

[11,12]. Despite promising results in preclinical studies, most clinical trials using neuroprotective agents have yielded disappointing outcome. Therefore, it is imperative to further clarify the pathogenesis of IS and explore new therapeutic strategies for treatment of IS.

Mitochondrial dysfunction and mitochondrial transfer in IS

Mitochondria are the primary site for energy metabolism and signaling hub of cells that integrate a wide range of interconnected process to maintain cellular homeostasis [13]. They are also important mediators of inflammation, endoplasmic reticulum (ER) stress, apoptosis and autophagy in ischemic cardiovascular and cerebrovascular diseases [14,15]. The reduction in CBF during IS can impair oxygen and glucose delivery, which promotes the dysfunction of mitochondria oxidative phosphorylation and increases cellular bioenergetic stress. Cells response to these stress by activating mitochondrial quality control mechanisms, such as mitochondrial biogenesis (MB), mitochondrial fission and fusion, mitophagy and intercellular mitochondrial transfer [16,17]. These adaptive responses help to maintain the integrity and function of the mitochondria network, avoiding the fate of neuron apoptosis and the disruption of neurovascular unit, thereby reducing IS-induced brain and neuron damage [18]. Furthermore, mitochondria are involved in various processes such as mitochondrial DNA (mtDNA) repair [19], mitochondrial reactive oxygen species (mtROS) production [20,21], MB kinetics, fusion dynamics, and proteomic regulation [22,23]. These alterations further damage blood–brain barrier (BBB) and worsen ischemic injury [24,25] (Fig. 1). Therefore, maintaining mitochondrial functional stability within cells is crucial for mitigating cerebral ischemic injury.

Is-induced alterations in mitochondrial function

Dysfunction of MB

MB refers to the process of increasing mitochondrial mass and generation of new mitochondria through the growth and division of pre-existing mitochondria. This process is predominantly governed by peroxisome proliferator-activated receptor coactivator α (PGC-1α), and the proteins encoded by both mitochondrial and nuclear genomes, as well as replication of mtDNA [26]. PGC-1α functions as a pivotal regulator of MB, primarily localized within the cytoplasm. It has been shown that cerebral ischemia is associated with alternations in MB. Yin et al. have reported that in a rat model of neonatal hypoxic-ischemic injury, mtDNA content, mitochondrial number and proteins such as heat shock protein 60 and cytochrome *c* oxidase subunit IV are significantly increased from 6 to 24 h after hypoxia[27,28]. Another study showed that hypoxic

preconditioning increases MB in a nitric oxide synthase-dependent manner [29]. Furthermore, Li et al. demonstrated that IS-induced increase in MB is neuroprotective, because reversing this change by silencing sestrin2 exacerbates ischemic injury in transient middle cerebral artery occlusion (tMCAO)-operated rats [30]. However, there are also several opposite results showing that IS may downregulate rather than upregulate MB. For example, IS has been shown to decrease mtDNA, impair mitochondrial function and worsen pathological damage [31]. Valerio et al. reported that oxygen-glucose deprivation (OGD)-induced neuronal damage is associated with decreased PGC1α and increased mtROS and apoptosis, these detrimental effects could be mitigated by a glycogen synthase kinase-3β inhibitor [32]. The ischemia-mediated decrease in MB was also observed in other studies, and reversal of this change through pharmacological intervention can ameliorate cerebral ischemic injury [33,34]. In spite of the existence of inconsistencies, most studies support the notion that upregulation of cerebral ischemia-induced MB plays a protective role in mitigating cerebral ischemic damage.

Imbalanced mitochondrial fission and fusion

Mitochondrial fission is the process by which a single mitochondrion undergoes division to generate two or more mitochondrial units [35]. In contrast, mitochondrial fusion involves merging two or more mitochondria into a single entity, a process that occurs in a coordinated sequence encompassing two distinct stages: outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) fusion [36].These two processes coordinate with each other to maintain the dynamic balance of mitochondrial morphology. The dedicated balance between division and fusion is crucial for mitochondrial health and cell survival; an imbalance between the two processes may cause mitochondrial dysfunction, which has been implicated in the pathogenesis of various diseases, including IS [37,38].

During IS, moderate mitochondrial fission maintains its health by facilitating the segregation of damaged mitochondria. However, excessive mitochondrial fission may exacerbate ischemic damage [39]. It has been shown that IS can increase mitochondrial fission and decrease mitochondrial membrane potential (MMP) in OGD-treated neurons and permanent middle cerebral artery occlusion (pMCAO)-operated mice, treatment with mdivi-1, an inhibitor of dynamically-associated protein 1 (Drp1), one of most important molecules to induce mitochondrial fission, or knockdown of Drp1 can block the translocation of Drp1 into mitochondria and alleviate cell death [40]. Drp1 activity is regulated by a variety of post-translational modification, for example, cyclic adenosine monophosphate (cAMP)-dependent protein kinase induces Drp1 phosphorylation at Ser-656 to inhibits its activity, while calcineurin dephosphorylates Drp1 to induce its activation, promoting neuronal death following OGD [41]. In addition to phosphorylation, recent studies have further identified acetylation and SUMOylation as new mechanisms in regulating Drp1 function during cerebral ischemia. For example, ischemia/hypoxia promotes Drp1 acetylation through the cyclin-dependent kinase 5-general control of amino acid synthesis 5 like-1 signaling axis, enhancing mitochondrial fission [42]. Inhibiting the excessive activation of Drp1 has been shown to improve cerebral ischemic injury by suppressing pathological fission [43,44]. On the contrary, some studies suggest that mitochondrial fission may also exert protective effects in certain context [45]. It has been shown that ligustilid, the main active ingredient of Angelica sinensis, mitigates ischemic injury by enhancing Drp1-mediated mitochondrial fission via adenosine monophosphate-activated protein kinase activation in both OGD-treated neurons and MCAO-operated rats [46].

Mitochondrial fusion requires close coordination of mitofusins 1 and 2 (Mfn1 and Mfn2) and optic atrophy 1 (OPA1). It is generally accepted that promoting mitochondrial fusion can repair mildly damaged mitochondria. Upregulation of OPA1 promotes mitochondrial fusion and alleviates ischemic injury[47,48]. Zhang et al. have reported that exercise increases OPA1 expression and mitochondrial fusion, alleviating ischemic injury in tMCAO-operated rats [49]. Similarly, sirtuin 3 has been found to repair mitochondrial structure and improve energy metabolism after ischemia by promoting OPA1 expression and regulating its deacetylation status [50]. In summary, the role of mitochondrial fission and fusion in cerebral ischemic injury are complex and context-dependent. Their overall impact likely hinges on the dedicated balance between these two processes in mitochondrial dynamics, and precisely modulating this balance may represent a critical therapeutic strategy for the management of IS.

Mitophagy dysfunction

Mitophagy is a form of selective autophagy which participate in maintenance of normal physiological activities and protection of cells from injury by facilitating lysosomes-mediating elimination of impaired mitochondria [51,52]. As an mitophagy process, damaged mitochondria are wrapped by autophagic vacuole to generate autophagosomes, which are subsequently fused with lysosomes and degraded by lysosome hydrolytic enzymes [53].

Mounting evidence have shown that IS alters mitophagy in the brain tissue. However, the role of mitophagy in cerebral ischemia may be a double-edged sword, it can be either beneficial or harmful, depending on the stage and pathological context of cerebral ischemia [54,55]. In the early stage, brain cells undergo a metabolic transformation from oxidative phosphorylation to anerobic glycolysis due to hypoxia, which reduces ATP generation and increases the accumulation of H^+, Na^+ and Ca^{2+} ion in mitochondria, resulting in mitochondrial dysfunction and injury [6,56]. To maintain cellular homeostasis, these damaged mitochondria must be cleared up. In addition, hypoxia diminishes antioxidant capacity, leading to increased cellular ROS production. As a result, mitophagy is activated to engulf the damaged mitochondria and reduce ROS levels [57,58]. Therefore, early induction of mitophagy followed cerebral ischemia may be beneficial for restoring cellular energy metabolism and homeostasis [59,60]. However, in the middle and late stages of ischemia, excessive mitophagy may impede recovery from brain injury due to mitochondria shortage by over-elimination of mitochondria [59,61]. Therefore, precisely regulating mitophagy is essential for the recovery of neurological function in IS.

The mechanisms of intercellular mitochondrial transfer in IS

Given that IS leads to mitochondrial dysfunction, as indicated above, delivery of functional mitochondria following ischemic injury holds significant therapeutic potential for the treatment of IS. Evidence has shown that the delivery of mitochondria into cells primarily occurs through functional connections formed either between endogenous cells or between exogenous stem cells and recipient endogenous cells. In directly contacted cells, the donor cells can transfer their mitochondria to the host cell via tunneling nanotubes (TNTs), gap junctions, or cell fusion [62,63]. In the non-contacted cells, mitochondrial transfer can achieve through extracellular vesicles (EVs) or direct release of unenveloped mitochondria. Mitochondrial transfer can improve mitochondrial function, mitigate oxidative stress, and preserve the fate of host cells [64] (Fig. 2). Although the molecular and signaling mechanisms underlying mitochondrial transfer in IS have not been fully elucidated,

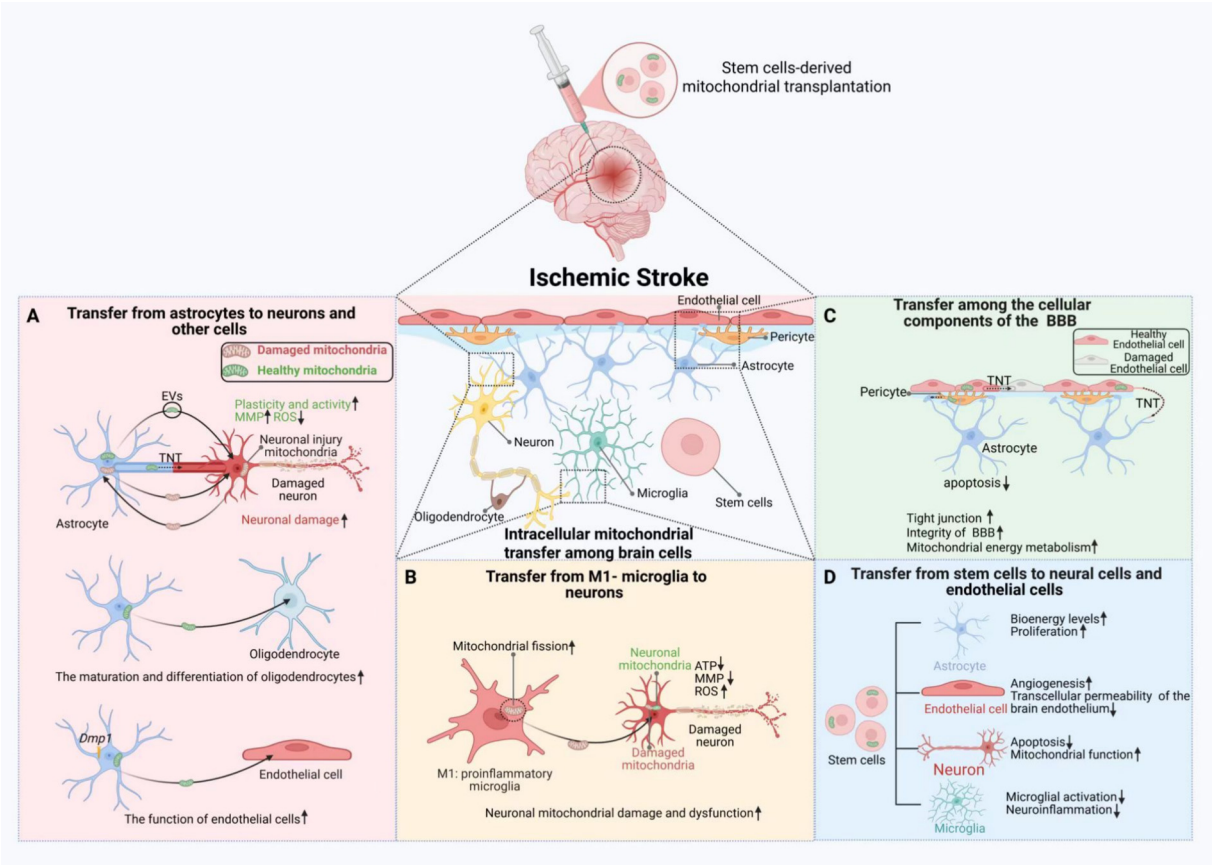


Fig. 2. An integrative model of endogenous mitochondrial transfer following IS and therapeutic strategies for exogenous stem cell-based mitochondrial transplantation. A. The dual role of astrocyte mitochondrial transfer: Astrocytes transfer healthy mitochondria to neurons and other neural cells, a process that enhances neuronal synaptic plasticity, oligodendrocyte maturation, and endothelial cell function. Conversely, the transfer of damaged mitochondria to neurons can exacerbating neuronal injury. Notably, a retrograde transfer of damaged mitochondria from neuron to astrocytes may occur as a compensatory signaling mechanism under ischemic stress. B. Detrimental transfer from microglia: Pro-inflammatory M1 microglia deliver dysfunction mitochondria to neurons, which increases oxidative stress in recipient neurons and further aggravation of neuronal damage. C. Blood-brain barrier (BBB) reinforcement: Intercellular mitochondrial transfer occurs among the cellular components of the BBB, including from pericytes to astrocytes, endothelial cells (ECs) to astrocytes, and from healthy to impaired ECs. These transfers enhance the bioenergetic capacity of recipient cells, upregulate tight junction proteins, and collectively support BBB structural and functional integrity. D. Therapeutic mitochondrial delivery from stem cells: The transportation of stem cell-derived mitochondria to both neural cells and cerebrovascular ECs improves astrocytic energy metabolism, stimulates angiogenesis, inhibits neuronal apoptosis, and attenuates microglial activation-mediated neuroinflammation, highlighting its therapeutic potential. This image was created in BioRender (*https://BioRender.com/wqup3wz*).

most studies suggest that TNTs and EVs may serve as the primary pathways for the intercellular transfer of functional mitochondria.

Tunneling nanotubes

TNTs are cytoskeleton-based intercellular pipe-like structures that primarily facilitating the intercellular exchange of lipids, nucleic acids, proteins, and even organelles [65]. These dynamic, actin-rich membrane protrusions play pivotal roles in regulating multiple biological processes, such as repairment of cellular damage, immune response, and cell metabolic reprogramming [66]. M–Sec, an earliest marker during TNTs formation, orchestrates actin polymerization [67,68]. The role of mammalian protein M–Sec in inducing membrane protrusions and influencing TNT formation depends on its interaction with RaI and the Exocyst complex [67]. These three components synergistically regulate the generation of membrane nanotubes.

Numerous studies have demonstrated that IS promotes mitochondrial transfer among neural cells by facilitating the formation of TNTs, thereby reducing IS-mediated cell injury [69]. The formation of TNTs has been observed in mesenchymal stem cells (MSCs)-mediated mitochondrial transfer to injured vascular endothelial cells (ECs). OGD-reperfusion (OGD/R) induced mitochondrial transfer through TNTs from MSCs to injured ECs, thereby rescuing aerobic respiration and suppressing apoptosis. This protective process is facilitated by phosphatidylserine exposure on the surface of apoptotic ECs, which is recognized by stem cells to activate a defensive rescue mechanism [70].

It has been shown that transplantation of MSCs can facilitate mitochondrial transfer into cerebral microvasculature and promote mitochondrial respiration and angiogenesis in tMCAO-operated rats. These effects can be reversed by TNTs inhibitor LatA or Annexin V [71]. TNTs can be formed between human induced pluripotent stem cells-derived MSCs and PC12 cells. Yang et al. reported that co-culture with MSCs significantly reduced apoptosis and restored mitochondrial function in the injured PC12 cells exposed to cobalt chloride. These benefits were partially abolished when TNT formation was inhibited [72].

Pericytes, astrocytes, ECs are essential components of the BBB. The TNTs-mediated signal transduction, material and energy exchange among these cells, participate in maintenance of the integrity and functionality of the BBB under both physiological and pathological circumstances. It has been reported that OGD/R can increase both the length and quantity of TNTs among these cells, enabling functional mitochondria to be transferred from healthy astrocytes and ECs to the damaged astrocytes, thereby inhibiting apoptosis, this protective effect is reversed by CytoD, an inhibitor of TNTs formation [73]. These results suggest that the formation of TNTs after IS may provide

a cytoprotective effect by facilitating mitochondrial transfer. Notably such transfer also occurred between damaged cells and partially intact cells with temporary functional integrity. In OGD/R-treated HT22 cells, melatonin was shown to enhance PGC-1α-dependent MB by stimulating sirtuin 3 pathway, inhibiting aberrant mitochondrial fission, promoting mitochondrial fusion, and improving mitochondrial morphology and function. Phalloidin staining further confirmed the presence of F-actin-rich TNTs transporting mitochondria between damaged and partially intact HT22 cells [74].

Mitochondrial Rho GTPase 1 (Miro1) is a calcium-sensitive adaptor protein located on the outer membrane of mitochondria. Miro1 is a crucial molecule in regulating the formation of TNTs and mediating the transport of mitochondria [75,76]. In the co-cultured MSCs and astrocyte subjected to OGD, Miro1 overexpression in MSCs [77] enhances TNT formation. In summary, TNTs serve as essential conduits for intercellular mitochondrial transfer, which helps recipient cells restore mitochondria function, combat against oxidative stress and cell death after IS, and promoting host cell survival.

Gap junction channel

Mitochondria contain connexins, a family of proteins known to form gap junction channels (GJCs) [78]. Connexins are synthesized in the ER and oligomerized in the Golgi apparatus to form hemichannels [78,79]. Hemichannels from adjacent cells dock with each other to form GJCs that aggregate into plaques and facilitate intercellular communication. The GJCs possess capability to transmit cellular signals and transport various ions, protein molecules, and cellular organelles such as mitochondria. Connexin 43 (Cx43) is a key protein involved in the formation of GJCs and mitochondrial transfer [80,81].

Li et al. demonstrated that bone marrow derived mesenchymal stem cells (BMSCs) are able to enhance energy supply and mitigate neuronal apoptosis by transferring their mitochondria to the OGD-treated motor neurons through GJCs. Furthermore, they observed that all-trans retinoic acid, a gap junction enhancer, facilitates mitochondrial transfer from BMSCs to neurons, while 18β-glycyrrhetinic acid, a gap junction inhibitor, impedes this process. Transplantation of mitochondria and BMSCs into the injured spinal cord improved locomotor functional recovery in spinal cord injury-operated rats. To further support this mechanism, BMSCs were found to express Cx43, while motor neurons expressed connexin 32 (Cx32), the heterotypic gap junctions containing both Cx43 and Cx32 suggest a structural basis for mitochondrial transfer from BMSCs to motor neurons [82].

Plasmogamy

Cell fusion is a biological process in which two autonomous cells merge their plasma membranes, enabling the exchange of cytoplasmic constituents and organelles while preserving nuclear integrity [83,84]. During partial fusion, transient intercellular communication allow for bidirectional organelle transfer, including mitochondria [62].

It has been shown that cell fusion occurs when MSCs and neurons are co-cultured, which allows mitochondria to transfer from MSCs to neurons. However, the cell fusion is disrupted when the two cells are co-cultured using a transwell system, which can inhibit cell physical contact. Compared to untreated MSCs, transplantation of MSCs co-cultured with neurons exhibits a more effective reduction in cerebral ischemic damage in tMCAO-operated rats. In addition, pre-co-culturing MSCs with neurons c can upregulate Miro1 expression and production of neurotrophic factors, thereby augmenting their neural protective effects [85].

Evs

EVs are nanoscale double-layered vesicles secreted by cells, that serve as stable transport carrier for lipids, proteins, RNA, microRNA, and organelles between cells [86]. EVs are primarily generated through the outward or inward protrusion of cell (organelle) membranes and are categorized into apoptotic bodies (800–5000 nm), microvesicles (150–1,000 nm), and exosomes (30–150 nm). Among them, microvesicles formed by cytosolic membrane budding have the ability to encapsulate mitochondria and release them outside the cell [87,88].

It has been reported that astrocytes release extracellular mitochondrial particles through a calcium-dependent mechanism involving CD38 and cyclic ADP ribose (cADPR) signaling pathways. These EVs-containing mitochondria can be transferred from astrocytes to neurons following IS, providing neuroprotective effects [89]. Similarly, it has been shown that microvesicles derived from human brain ECs (BECs) line hCMEC/D3, are capable of transferring polarized mitochondria to recipient BECs in cultured neurons. This transfer increases ATP generation in the recipient BECs exposed to OGD. Under hypoxia condition, only the transfer of microvesicles, but not exosomes, improve mitochondrial function. Compared with heterotypic macrophage-derived microvesicles, BEC-derived microvesicles have greater potential for transferring mitochondria and improving ECs survival under ischemic conditions [64]. Dave et al reported that ECs-derived medium-to-large EVs (m/l EVs) were enriched with functional mitochondria. Following OGD, the mitochondria from m/l EVs can integrate into the mitochondrial network of recipient BECs, boosting ATP generation. Intravenous administration of m/l EVs significantly decreases the brain infarct areas in MCAO-operated mice [90].

The process of donor cell-mediated mitochondrial transfer through microvesicles requires host cells to internalize mitochondria before they integrate into the existing mitochondrial network. Overall, microvesicles-mediated mitochondrial transfer may be a crucial mechanism for host cells to uptake extracellular mitochondria. Altering mitochondrial exocytosis and endocytosis can affect intercellular efficiency of mitochondrial transfer, thereby influencing the fate of recipient cells. Therefore, microvesicle-based mitochondrial transfer may offer a new avenue for exploring non-contact cellular interchanges and developing novel therapeutic strategies for mitochondrial transplantation.

Functional role of mitochondrial transfer in IS

IS is characterized by energy depletion resulting from the interruption of CBF. Mitochondria, as the central organelles for cellular energy production, play a critical role in maintaining neuronal homeostasis. Given that neurons are enriched in mitochondria, they are highly vulnerable to mitochondrial dysfunction, which leads to energy failure and triggers a cascade of pathological events, such as calcium overload, neuroinflammation, and oxidative stress. These ischemic cascades ultimately compromise BBB integrity and exacerbate brain injury. Restoring mitochondrial function has consequently emerged as a pivotal therapeutic strategy to preserve cellular metabolic stability in the context of cerebral ischemia. Notably, accumulating evidence indicates that the targeted delivery of functional mitochondria to ischemic cells can improve energy metabolism, suppress oxidative stress and apoptosis, and mitigate BBB disruption, offering a novel and promising avenue for stroke therapy.

Restoration of energy metabolism

Traditionally, brain energy metabolism has been closely linked to the delivery of oxygen and glucose via CBF. Under normal aerobic conditions, glucose is metabolized into pyruvate, which enters

the mitochondria and is further oxidized through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation to generate ATP [91]. Thus, mitochondria serve as essential organelles for cellular energy production and the maintenance of intracellular homeostasis. In the central nervous system (CNS), astrocytes account for only 10–15% of total brain energy consumption, neurons utilize approximately 85% of the glucose taken up by the brain [92]. Given their high metabolic demands, neurons primarily rely on oxidative phosphorylation for energy supply [93,94]. Following IS, ischemia and hypoxia trigger cellular energy failure, metabolic dysregulation, accumulation of pathological metabolites such as lactate, and subsequent induction of mitochondrial permeability transition pore (mPTP) opening [95]. The ensuing loss of MMP promotes excessive leakage of ROS from damaged mitochondria, which further exacerbates oxidative phosphorylation uncoupling and suppresses ATP production. This cascade establishes a self-amplifying cycle that intensifies neuronal injury.

A growing body of evidence indicates that functional mitochondrial transfer can rescue impaired cellular metabolism [96]. MSCs have been shown to transfer functional mitochondria to neurons under oxidative stress, significantly enhancing neuronal survival, ATP levels, mitochondrial respiratory function, and basal metabolic activity, thereby promoting neurofunctional recovery and cell viability after IS [77,96]. Moreover, during mitochondrial transfer, MSCs concurrently upregulate key TCA cycle enzymes, including citrate synthase, pyruvate dehydrogenase, and aconitase, providing additional support for the role mitochondrial transfer in the restoration of neuronal bioenergetics [97]. Recent studies have demonstrated that astrocytes transfer healthy mitochondria to damaged neurons via a regulatory mechanism mediated by low-density lipoprotein receptor-related protein 1 (LRP1), thereby reducing lactate production, improving neuronal energy metabolism, and alleviating cerebral ischemia–reperfusion injury [98]. In the MCAO mouse model, targeted delivery of MSCs-derived mitochondria to ECs in the lesion area improves mitochondrial function, reprograms the glutathione (GSH) metabolic pathway, promotes tip cell phenotype transition and angiogenesis, and ultimately enhances neurological functional recovery [99]. Collectively, these findings suggest that the therapeutic benefit of mitochondrial transfer may stem largely from the replacement of dysfunctional mitochondria in recipient neurons, potentially representing the principal mechanism underlying neuroprotection in ischemic brain injury.

However, it remains unclear whether transferred mitochondria can functionally integrate into the existing mitochondrial network of recipient cells, particularly under ischemic conditions where sustained calcium overload and inflammatory signaling promote excessive mitochondrial fission. Therefore, a comprehensive assessment of mitochondrial dynamics following mitochondrial transfer is critically needed. Notably, a recent groundbreaking study in a myocardial ischemia model revealed that even depolarized or mtDNA-deficient mitochondria, which lacks canonical bioenergetic capacity were capable of preserving ECs function without integrating into the host mitochondrial network [100]. This suggests that transferred mitochondria may not act merely as energy donors but could instead function as molecular "triggers" that initiate mitophagy in recipient cells, facilitating the selective clearance of damaged mitochondria. These findings challenge the classical "replacement hypothesis" and highlight an alternative, active role for mitochondrial transfer in stimulating endogenous repair mechanisms. Consequently, further investigation is warranted to determine, whether in the context of cerebral ischemia, transferred mitochondria confer protection through direct metabolic substitution or by activating quality control pathways such as mitophagy to eliminate compromised organelles.

Modulation of apoptosis

It is generally believed that IS induces neuron apoptosis, and inhibition of this process alleviates neuronal damage [101]. Evidence have shown that IS-induced mitochondrial dysfunction and injury promote the release of mtROS, Ca^{2+} and other apoptotic factors, which in turn trigger the opening of mPTP and the release of cytochrome *c* and apoptosis-inducing factor into cytoplasm, these events ultimately lead to the caspase-dependent apoptosis and neuronal damage [102]. Numerous studies have demonstrated that intervention in mitochondria-mediated apoptosis can ameliorate cerebral ischemic injury [103–106]. Accumulating evidence indicates that the transfer of functional mitochondria into damaged neurons significantly reduces the proportion of dysfunctional or apoptotic cells. Specifically, direct injection of astrocyte-derived extracellular mitochondria into the *peri*-infarct cortex of ischemic mice markedly upregulates the expression of the anti-apoptotic protein B-cell lymphoma-extra large and suppresses caspase-3 activation [89]. In another study, co-culturing healthy human neural stem cells (hNSCs) with SH-SY5Y cells subjected to OGD/R enables transfer of functional mitochondria from hNSCs to stressed neurons, accompanied by inhibiting caspase-3/7 activation [107]. Together, these findings suggest that mitochondrial delivery to neural cells may ameliorate ischemic brain injury through suppressing apoptotic pathways. However, it remains unclear whether transferred mitochondria integrate into endogenous mitochondrial networks, and whether their anti-apoptotic effects are mediated primarily by the replacement of damaged mitochondria or via alternative mechanisms.

Regulation of inflammation and oxidative stress

The OMM serves as a protective barrier, insulating the cell from innate immune responses. However, under pathological conditions such as IS, the inner and outer membranes may be damaged, leading to mitochondria release of mtROS and mtDNA to trigger an inflammatory response and oxidative stress, which in turn accelerates mitochondrial dysfunction [108].

It has been shown that IS induces an overproduction of mtROS, which activates nuclear factor-κB inflammatory pathways to increase the production of chemokines, interferon regulatory factor-1, and pro-inflammatory cytokines such as interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), which exacerbate brain tissue injury [109]. Global brain ischemia further aggravates mitochondrial oxidative stress by increasing mtROS and, and reducing GSH in the hippocampus. Treatment with mitochondrial-targeted antioxidant, Mitoquinol, reduces inflammatory cytokines TNF-α, and IL-1β, contributing to the improvement of cognitive function and hippocampal morphology [110].

Functional mitochondrial transfer exerts multifaceted antioxidant effects in the context of cerebral ischemia. Chen et al. demonstrated that umbilical cord MSCs (UC-MSCs) can transfer mitochondria to T cells, restoring their metabolic function, repairing mtDNA damage, reducing ROS accumulation, and ultimately mitigating oxidative stress and immune dysregulation [111]. The antioxidant mechanisms of this transfer are twofold: first, the delivered functional mitochondria activate the PTEN induced kinase 1 (PINK1)/Parkin pathway, triggering mitophagy and preventing sustained ROS production from damaged organelles [100]; second, exogenous mitochondria can fuse with impaired host mitochondria, effectively decreasing the population of mitochondria prone to excessive ROS generation [112]. Oxidative mtDNA (Ox-mtDNA) is known to activate the cyclic GMP-AMP Synthase (cGAS)-stimulator of interferon genes (STING) pathway, an important signaling pathway for detecting pathogenic DNA and initiating the inflammatory response, and limiting the release of ox-mtDNA into the cytoplasm has been shown to mitigate

inflammation and improve neurological functions in tMCAO-operated rats and OGD/R-treated neurons [113]. These studies suggest that delivering functional mitochondria to cells can reduce mtROS and ox-mtDNA levels, thereby alleviating inflammation and oxidative stress after IS.

It is noteworthy that potential crosstalk may exist between mitochondrial transplantation and inflammatory responses. Exogenous mitochondria such as mtDNA may act as a damage-associated molecular pattern, activating the host innate immune system through the cGAS-STING pathway and triggering an inflammatory response [114,115]. This immunogenicity represents a primary barrier to the clinical translation of exogenous mitochondrial transplantation. However, emerging evidence suggest counterbalancing mechanisms: transplanted mitochondria can activate PINK1-mediated mitophagy to suppress the cGAS-STING pathway and attenuate inflammation [116]. Therefore, the net effect of mitochondrial transfer on inflammatory signaling is complex and requires further elucidation. Combining mitochondrial transplantation with immunosuppressive agents or fine-tuning the cGAS-STING pathway may offer synergistic strategy for treating ischemic brain injury.

Maintenance of BBB integrity

The BBB is a specialized multicellular structure that regulates CNS homeostasis. Its core component is a continuous monolayer of ECs, which controls molecular exchange between blood and brain [117]. These cells are enveloped by pericytes, which are embedded within the basement membrane and are essential for capillary integrity, blood flow regulation, and diameter control [118]. The abluminal surface is covered by astrocytic endfeet, which secrete laminin to promote vascular adhesion and release signaling molecules to maintain BBB function and homeostasis

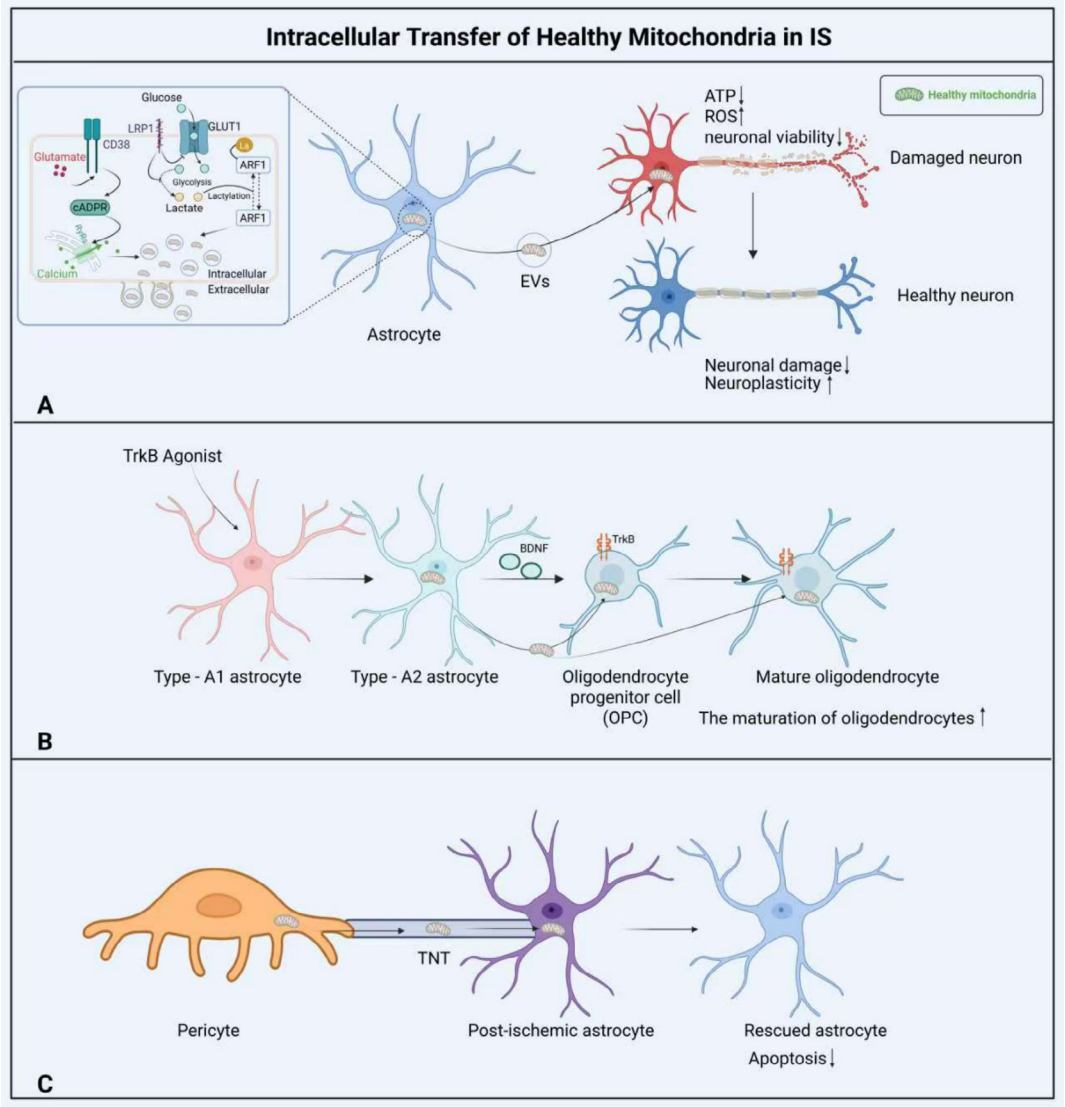


Fig. 3. Intracellular transfer of functional mitochondria among neural cells following IS. A. When cerebral ischemia occurs, adjacent astrocytes can release functional mitochondria which are taken up by damaged neurons that improve neuronal mitochondrial function, enhance neuronal activity and neuroplasticity. This process may be mediated by calcium-dependent activation of CD38 and cyclic ADP ribose (cADPR) pathways, while low-density lipoprotein receptor-related protein 1 (LRP1) facilitates mitochondrial transfer from astrocytes to neurons through extracellular vesicles (EVs) by reducing lactate production and lactylation of ADP-ribosylation factor 1 (ARF1). B. Astrocyte to oligodendrocyte precursor cells (OPCs) transfer: Type – A2 astrocytes can release and transfer functional mitochondria to OPCs, promoting maturation and proliferation of OPCs in chronic cerebral ischemia. C. Pericyte-to-Astrocyte Transfer: Transferring healthy mitochondria from pericytes to astrocytes can protect astrocytes from oxygen-glucose deprivation (OGD)-induced apoptosis. This image was created in BioRender (*https://BioRender.com/lzvavgo*).

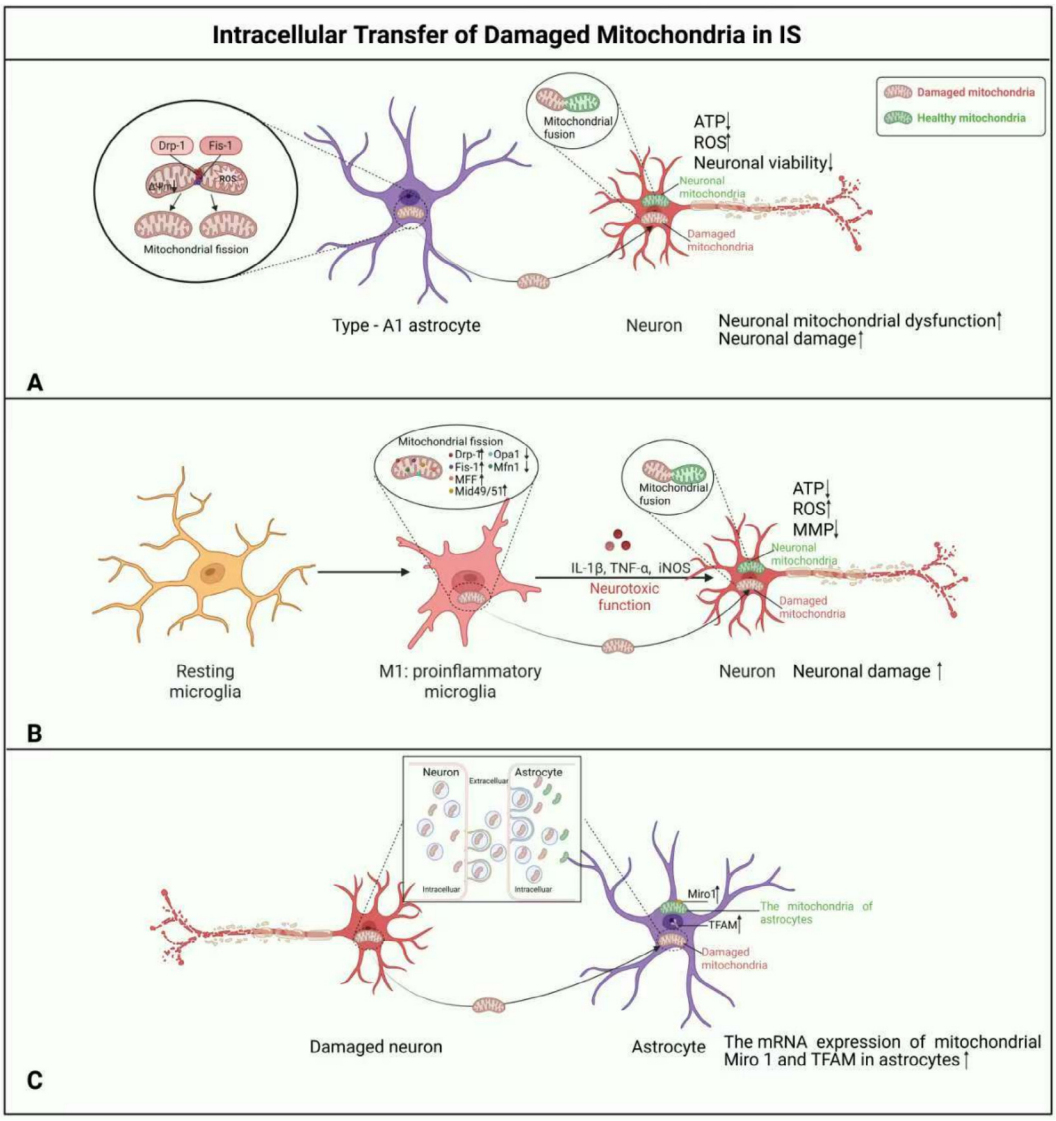


Fig. 4. Intracellular transfer of dysfunctional mitochondria among brain cells following IS. A. Type – A1 astrocytes can cause neuronal damage by releasing of dysfunctional mitochondria. This process enhances the interaction between mitochondrial dynamically-associated protein 1 (Drp1) and mitochondrial fission 1 protein (Fis1), which exaggerate mitochondrial fission processes, resulting in mitochondrial dysfunction. These damaged astrocytic mitochondria are released and enter neighboring neurons to fuse with the host neuronal mitochondria network, causing neuronal mitochondrial dysfunction and exacerbating neurological damage. B. Pro-inflammatory microglia propagate neuronal mitochondrial dysfunction: IS induces proinflammatory microglia which enhances the activities of mitochondrial fission and promotes the release of damaged mitochondria, subsequently transfer of these organelles to neurons, leading to neuronal mitochondrial damage and dysfunction. C. Astrocytic response to engulfed neuronal mitochondria: IS-injured neurons release damaged mitochondria, which are engulfed by adjacent astrocytes, leading to increased mRNA expression of mitochondrial Rho GTPase 1 (Miro1) and mitochondrial transcription factor A (TFAM) in astrocytes. This image was created in BioRender (*https://BioRender.com/qcuqoyq*).

[119,120]. Together with microglia, these elements form an integrated surveillance network that preserves BBB integrity and CNS function [121,122].

Following IS, excessive ROS production and sustained inflammation in ECs trigger the upregulation of matrix metalloproteinases. This enzymatic protease degrades tight junction proteins such as occludin and claudin-5, disrupting the BBB. The compromised barrier permits infiltration of peripheral immune cells, amplifies oxidative stress, activates glial cells, and ultimately promotes neuronal death [123]. In response, astrocytes undergo phenotypic transformation into reactive states, differentiating into either neurotoxic A1 or neuroprotective A2 subtypes, reflecting their dual roles in ischemic pathophysiology [124]. Notably, pericytes emerge as key contributors to BBB repair during the post-ischemic phase [125]. Possessing stem cell-like properties and transdifferentiation potential, pericytes migrate to injury sites to help restoring BBB integrity [126]. Pericytes also stimulate astrocyte activation and contribute to tissue remodeling and repair processes [127,128]. Thus, preserving the viability and functional competence of ECs and astrocytes is essential for maintaining BBB integrity and neurological recovery.

Emerging evidence indicates mitochondrial transfer as a critical mechanism for BBB repair. It has been shown that OGD/R can boost extensive TNT formation between healthy pericytes and astrocytes, facilitating functional mitochondrial transfer from pericytes to astrocytes, and help maintaining the structural and functional integrity of the BBB [73]. Xu et al. reported that healthy brain microvascular ECs can deliver functional mitochondria to OGD-damaged cells; however, hyperglycemia following IS impairs this transfer, exacerbating mitochondrial dysfunction, compromising the structural integrity of tight junctions, and increasing BBB permeability [129]. Further, Dave et al. revealed that endothelial-

Table 1
Mitochondrial transfer among neural cells following IS.

Donor cells	Recipient cells	IS Model	Transfer mode	Mitochondrial status	Mechanisms or outcome	Ref
Astrocytes	Neurons	OGD/R tMCAO	EVs	Health	Improve their mitochondrial function, enhances neuronal activity and neuroplasticity in a CD38-dependent manner	[89]
Astrocytes	Neurons	OGD/R	/	Health	Rb1 increases MMP and oxygen consumption rate within the OGD/R-treated neurons co-cultured with conditioned medium of astrocytes in a CD38-dependent manner	[139]
Astrocytes	Neurons	tMCAO	EVs	Health	LRP1 facilitates mitochondrial transfer from astrocytes to neurons through EVs by reducing lactate production and lactylation of ARF1	[98]
Astrocytes	Neurons	OGD/R	/	Health	Trigger post-translational modification via O-GlcNAcylation of mitochondria protein in astrocytes	[138]
A2-type Astrocyte	Oligodendrocyte	BCAS	/	Health	The transferred functional mitochondria enhance the maturation and differentiation of OPCs	[155]
Pericyte	Astrocytes	OGD/R	TNTs	Health	Protect astrocytes from OGD-induced apoptosis, thereby maintaining the structural and functional integrity of the BBB	[73]
A1-type astrocytes	Neurons	tMCAO	/	damage	Treatment with inhibitor that prevents Drp/Fis1 interaction reduces mitochondrial fission, thereby reducing neuronal damage through improved neuronal mitochondrial function	[147]
M1-type Microglia	Neurons	tMCAO	/	damage	The damaged mitochondria released from microglia fused with neuronal mitochondria, resulting in neuronal mitochondrial damage and dysfunction,	[112]
Neurons	Astrocytes	Acidosis tMCAO	/	damage	Dysfunctional mitochondria are prone to be expelled into the extracellular space by injured neurons and subsequently engulfed by adjacent astrocytes	[174]
Human BECS Mouse BECS	Brain endothelial cells (BECs)	tMCAO	EVs	Health	Enhancing mitochondrial function and increased ATP production, reduced infarct volume and improved neurological outcomes.	[179]

derived EVs transport functional mitochondria to recipient ECs, reinforcing tight junction assembly, improving BBB integrity, and reducing cerebral infarct volume [90]. Collectively, these findings indicate that targeted mitochondria delivery to astrocytes and ECs following IS restores cellular bioenergetics and rescues critical cellular functions, thereby enhancing BBB stability and mitigating neural injury.

Intracellular mitochondrial transfer among neural cells as a therapeutic target for IS

Mitochondrial transfer, as a part of cell therapy, is gaining great attention for treatment of IS due to its ability to provide healthy mitochondria for compromised cells, thereby restoring cellular metabolism and preserving BBB integrity. It should be noted that the mitochondrial transfer after IS is bidirectional. On one hand, intact mitochondria from healthy cells can be transported into the damaged cells to promote metabolic recovery and cellular revitalization [130](Fig. 3). On the other side, mitochondria released from injured cells may serve as a danger-warning signal to trigger an inflammatory response which in turn aggravate ischemic injury [131] (Fig. 4). In addition, mitochondrial transfer after IS can occur among all brain cells, with mitochondrial transfer between astrocytes and neuronal cells being the most common pathway.

Current research on mitochondrial transfer is highly complex and fragmented. There is an urgent need to systematically review existing evidence and categorize findings according to specific sources of donor cells. Such an effort would help clarify research priorities and guild future strategies aimed at ameliorating ischemic brain injury through cell-specific enhancement of mitochondrial function. Table 1 summarizes key studies investigating intracellular mitochondrial transfer between various donor and recipient cell types in cerebral ischemia.

Intracellular mitochondrial transfer from astrocytes

Astrocytes are widely distributed through the entire brain, exhibiting significant heterogeneity in morphology, functionality, and molecular properties [132,133]. They play diverse roles and display distinct gene expression traits that are related to their regional localization and interactions with other brain cells [134,135]. Due to their widespread distribution in the CNS, astrocytes become the earliest donor cells to establish cellular communication and transport mitochondria to damaged neurons and other brain cells after IS, which plays an important role in reducing IS-induced neuronal injury.

Mitochondrial transfer from astrocytes to neurons

Neurons are the earliest and most studied recipients to accept mitochondrial donors. When cerebral ischemia occurs, adjacent astrocytes release functional mitochondria, which are taken up by damaged neurons to improves their mitochondrial function, enhances neuronal activity and neuroplasticity. This process is potentially mediated by calcium-dependent activation of CD38 and cycle ADP pathways, as the inhibition of CD38 diminishes mitochondrial transfer and worsens neurological outcomes following cerebral ischemia [89]. CD38 is mainly produced by glial cells in the brain and activated by IS-induced glutamate release. CD38 increases the synthesis of calcium messenger cADPR, activating ryanodine receptors in the ER, thereby amplifying intracellular signals within organelle network that includes mitochondria [136,137].

Interestingly, activation of CD38-cADPR signaling triggers post-translational modification via O-GlcNAcylation of mitochondria protein in astrocytes, while OGD/R treatment result in transient and mild protein modification. Inhibition of ER-Golgi trafficking using Brefeldin A or siRNA targeting slc35B4 reduces O-GlcNAcylation, MMP and mtDNA content. These results suggest that CD38-cADPR signaling pathway-mediated post-translational modification by O-GlcNAc may be essential for maintaining the functionality and neuroprotective properties of astrocyte-released mitochondria [138]. It is worth noting that this pathway can be activated under IS, but its activity is relatively low and transient, enhancing this pathway may promote mitochondrial transfer to restore neuronal function.

Ginsenoside Rb1 has been demonstrated to enhance healthy mitochondrial transfer from astrocytes to OGD/R-induced damaged neurons via regulating CD38 signaling pathway. Specifically,

Rb1 increases MMP and oxygen consumption rate within the OGD/R-treated neurons co-cultured with conditioned medium of astrocytes. Blockade of mitochondrial transfer by CD38 knockdown diminished the neuroprotective effects of Rb1 in mice subjected to photochemical cerebral thrombosis [139]. Recent studies indicate that electroacupuncture therapy can inhibit neuronal apoptosis and improve the neurological function of MCAO rats by promoting the transfer of mitochondria from astrocytes to neurons, potentially through the regulation of the CD38-cADPR-Ca^{2+} signaling pathway [140]. These findings indicate the critical role of the CD38 signaling pathway in mediating healthy mitochondrial transfer from astrocytes to ischemic neurons.

Zhou et al. demonstrated a novel mechanism underling IS-mediated mitochondrial transfer from astrocyte to damaged neuron. LRP1 is a cell-surface receptor involved in endocytic and signaling processes, LRP1 facilitates mitochondrial transfer from astrocytes to neurons through EVs by reducing lactate production and lactylation of ADP-ribosylation factor 1 (ARF1) which is a cytosolic protein facilitating vesicular trafficking. Clinically, stroke patients with higher cerebrospinal fluid lactate levels have low level of astrocytic mitochondria [98]. These findings underscore the pivotal role of LRP1 in mitigating ischemic injury through facilitating mitochondria-mediated astrocyte-neuron communication, providing clinical evidence for the release of mitochondria by astrocytes after cerebral ischemia.

Astrocytes exhibit significant heterogeneity and morphological diversity across distinct brain regions [135]. Under pathophysiological conditions, activated astrocytes can be classified into A1 or A2 phenotypes [141]. It has been reported that A1-type astrocytes can cause neuronal damage by the release of dysfunctional mitochondria [131,142]. In response to stress, astrocytes rapidly activate into the A1-type astrocytes that enhances the interaction between mitochondrial Drp1 and mitochondrial fission 1 protein (Fis1), exaggerates mitochondrial fission processes, and increases mitochondria fragmentation, mtROS and loss of MMP, ultimately resulting in mitochondrial dysfunction [143,144]. The damaged astrocytic mitochondria are released and enter neighboring neurons to fuse with neuronal mitochondria, causing neuronal mitochondrial dysfunction and exacerbating neurological damage [89,145]. Heptapeptide (Hep), a peptide inhibitor P110 targeting Drp1-Fis1 interaction, can protect against IS-induced mitochondrial dysfunction [146]. Liu et al. reported that Hep-loaded macrophage-derived exosomes can mitigate mitochondrial dysfunction in lipopolysaccharide-induced A1-type astrocytes by inhibiting Drp/Fis1-mediated mitochondrial fission. As a result, more functional mitochondria are released from astrocytes and transferred into neurons, improving neuronal mitochondrial function and reducing neuronal damage as well as cerebral ischemia infarct size in tMCAO rats [147].

Mitochondrial transfer from astrocytes to oligodendrocyte (OLs)

The CNS consists of gray matter and white matter, the latter accounts for a major brain volume and is composed of axons and myelin sheaths [148,149]. The major cell type that forms myelin sheaths is OLs, which are vulnerable to ischemia [150,151]. It is generally accepted that ischemia-induced white matter lesions can damage oligodendrocyte precursor cells (OPCs), preventing them from differentiation into OLs, leading to demyelination [152,153]. Astrocytes are main cell type of white matter, which facilitates nutrient transport, providing structure and metabolic support for the adjacent neurons and OLs that produce myelin sheath [154]. Magami et al. have shown that A2-type phenotype of astrocytes release functional mitochondria which are taken up by OPCs, promoting maturation and differentiation of OPCs under chronic cerebral hypoperfusion. Cobalt chloride-induced chronic ischemia typically disrupts OPC maturation, which is reversed by incubation with astrocyte-conditioned medium. Further studies shows that treatment with tyrosine kinase beta (Trkβ) agonist induces astrocyte transformation into A2 astrocytes and facilitates the release and transfer of functional mitochondria from A2 astrocytes, this process enhances OLs maturation and differentiation, and ultimately alleviating white matter damage and memory disturbance in bilateral common carotid artery stenosis-operated mice [155]. It is well-known that Trkβ is the main receptor of brain-derived neurotrophic factor (BDNF), which is a neurotrophin that plays a crucial role in neuron survival and differentiation and OPC maturation [156].

Mitochondrial transfer from astrocytes to ECs

Astrocytes and ECs are critical cellular constituents of the BBB [157]. Recent studies indicate that dentin matrix protein 1 (Dmp1)-expressing astrocytes deliver functional mitochondria to ECs via their endfeet processes, a process essential for maintaining BBB integrity. Genetic deletion of Mfn2 in Dmp1-positive astrocytes impairs mitochondrial transfer, resulting in BBB leakage. Furthermore, age-related decline in astrocytic MFN2 expression diminishes the efficiency of mitochondrial trafficking, contributing to progressive BBB dysfunction [158]. Given that cerebral ischemia induces significant BBB disruption, these findings suggest that enhancing Dmp1 expression in astrocytes could ameliorate ischemic injury by promoting intercellular mitochondrial transfer and preserving BBB integrity.

Mitochondrial transfer derived from microglia

Microglia, as resident immune cells within the CNS, play a pivotal role in the pathophysiology of cerebral ischemia [159,160]. When cerebral ischemia occurs, local brain tissue undergoes oxygen deprivation and metabolic disorders. Microglial cells promptly sense this pathological alteration through various cell surface receptors, such as toll-like receptors, and activated in response for the stimuli [161,162]. Microglia can be classified into two phenotypes: pro-inflammatory M1 phenotype and anti-inflammatory M2 phenotype. The dynamic balance between M1 and M2 microglia significantly influences IS progress. In the early stage, M1 microglial activation facilitates pathogen clearance and tissue damage resolution [163,164]. However, their persistent activation promotes the release of inflammatory factors, ROS and proteases, which further exacerbates ischemic injury [164,165]. In the contrary, M2 microglia play a critical role in later-stage for tissue repairing and neural regeneration [166,167]. Liu et al. have shown that IS induced microglia activation may worsen neuronal injury through promoting mitochondrial transfer from microglia into neurons, because activated microglia increase mitochondrial fission and the release of damaged mitochondria, which may be taken up by neurons and fused with neuronal mitochondria, resulting in neuronal mitochondrial damage and dysfunction. Furthermore, they demonstrate that the injection of mitochondria derived from M1 microglia into the ischemic cortex exacerbates neuronal impairment in tMCAO rats [112]. These results suggest that inhibiting microglia activation and preventing dysfunctional mitochondrial transfer from microglia to neuros could be a promising strategy for reducing IS-induced neuronal damage.

Mitochondrial transfer derived from pericytes

It is well-known that pericytes, ECs and astrocytes play a crucial role in maintaining the integrity and function of the BBB under physiological conditions [122,168]. IS induces pericyte contraction, reducing capillary diameter and aggravating hypoxia and ischemia [169,170], and may eventually promotes pericyte death due to energy failure, oxidative stress and inflammation [171,172]. Peri-

cyte demise further compromises the integrity of the BBB and intensifies cerebral ischemic injury [171]. Pisani et al. have shown that OGD/R can boost extensive TNT formation between healthy pericytes and astrocytes, facilitating functional mitochondrial transfer between these cells. Transferring healthy mitochondria from pericytes to astrocytes protects astrocytes from OGD-induced apoptosis, and help maintaining the structural and functional integrity of the BBB. Interestingly, when astrocytes are treated with staurosporine, a cellular stressor that impairs mitochondrial function, the number of pericyte-astrocyte TNTs and pericytes-derived mitochondria within staurosporine-treated astrocytes increases [73]. These findings suggest that increased TNT formation may represent a compensatory "help-me" mechanism, by which damaged astrocytes recruit functional mitochondria from pericytes to promote survival under ischemic conditions.

Mitochondrial transfer derived from neurons

Neurons are fundamental entities in the structure and function of the nervous system, and remain the most widely studied cell type in IS models [5,173]. Mounting evidence have demonstrated that IS-mediated mitochondrial dysfunction, predominantly occur in neurons, implicating neurons as the primary source of injured mitochondria following IS [174,175]. Interestingly, a recent study suggests that injured mitochondria derived from neurons may also act as a "help-me" signal to facilitate neuron-astrocyte communication following IS. It has been reported that the release of dysfunction mitochondria from neurons increases in response for stress challenges such as acidosis, hydrogen peroxide, N-methyl-D-aspartate, or glutamate exposure, concurrent with reduction in mitochondrial basal respiration and MMP. These dysfunctional mitochondria are expelled into the extracellular space and subsequently engulfed by adjacent astrocytes, leading to increased mRNA expression of mitochondrial Miro1 and mitochondrial transcription factor A in astrocytes, suggesting the activation of MB pathway [174]. Although it is still unclear whether neurons can directly obtain rescue from astrocytes through this "help-me" signal, clearance of neuronal mitochondrial debris by astrocyte engulfment is undoubtedly helpful for restoring ischemic neuronal recovery.

Mitochondrial transfer derived from ECs

Mitochondrial respiratory function in brain ECs is frequently compromised following IS, resulting in impaired vascular repair and diminishes angiogenic capacity [176]. These findings underscore the central role of mitochondria in maintaining ECs function and supporting vascular integrity [177,178]. Supporting this concept, studies have demonstrated that administration of mitochondrial-enriched EVs derived from BECs significantly attenuates OGD-induced endothelial damage, highlighting therapeutic potential for preserving mitochondrial content in BECs for resilience against ischemic stress [47]. A recent comparative study evaluated the therapeutic efficacy of intravenous delivery of mitochondria-containing EVs derived from human BECs and mouse BECs in a mouse tMCAO model. The findings revealed that mitochondria from both sources were effectively internalized by neighboring BECs, leading to enhanced mitochondrial function and increased ATP production. Importantly, mouse BEC-derived EVs contained higher mitochondrial content than human BEC-derived EVs and homologous pairing (mouse to mouse) yielded superior improvements in ATP levels and mitochondrial respiration compared to heterologous pairing. *In vivo*, intravenous administration of mouse BEC-derived EVs significantly reduced infarct volume and improved neurological outcomes in tMCAO mice. These results indicate that mitochondria-containing EVs secreted by BECs represent a promising strategy for enhancing post-stroke recovery [179,180]. Nevertheless, whether endogenous intercellular mitochondrial transfer occurs from ECs to other cell types remains unclear, representing a critical direction for future investigation.

Stem cells-derived mitochondrial transplantation as a novel therapeutic strategy for IS

Endogenous mitochondrial transfer among brain cells is typically transient and may be insufficient to confer robust or sustained neuroprotection. A growing body of evidence have demonstrated that transplantation of healthy mitochondria from autologous and exogenous sources has emerged as a promising therapeutic approach for restoring mitochondrial function following IS [69,181]. Stem cell transplantation therapy is widely investigated for IS treatment due to their low immunogenicity, high differentiation capacity and paracrine activity which produce various growth factors, immunomodulatory factors, and EVs to promote neurogenesis and functional recovery. The transfer of mitochondria from donor stem cells to recipient brain cells can modulate various cellular processes, including proliferation, differentiation, metabolism, inflammation, cell senescence, cell stress, and cell migration, position the stem cell-derived mitochondria transplantation as a novel therapeutic avenue for treating IS. Indeed, the healthy mitochondria derived from stem cells have been demonstrated to exert multiple beneficial effects on neurons, ECs and glia to improve IS outcome (Table 2). The administration of exogenous stem cells after IS has been shown to facilitate mitochondrial transfer between the stem cells and all brain cells in the CNS, the following section will separately discuss the protective role and potential mechanisms of mitochondrial transfer between stem cells and individual brain cells, such as neurons, ECs, astrocytes and microglia for clarity (Fig. 5).

Mitochondrial transfer from stem cells to neurons

Stem cell transplantation, especially using MSCs, offers an attractive strategy for IS treatment, since MSCs can secret trophic factors which promote axonal regeneration and neuron survival. It has been demonstrated that mitochondria could be transferred from BMSCs to OGD-injured neurons in a GJCs-dependent manner, which is attenuated by GJCs inhibitor 18β glycyrrhetinic acid. Further study shows that gap junctions Cx43 and Cx32 are expressed in BMSCs and motor neurons, respectively. This mitochondrial transfer enhances the bioenergetic profile in OGD-treated motor neurons, mitigating apoptosis, and facilitating cell survival. In addition, both mitochondrial and BMSCs transplantation have been shown potential to promote motor function recovery in rats with SCI [82]. Another study revealed bidirectional transfer of intercellular contents between multipotent MSCs (MMSCs) and neurons in co-culture. This involves the transfer of cellular contents from neurons to MMSCs and reversal mitochondrial transfer from MMSCs back to neural cells. In rats subjected to tMCAO, intravenous administration of cell suspension from neuron co-cultured with MMSC upregulates the expression of BDNF and Miro1 in MMSCs, reducing infarct volume and improving neurological function [77]. Wei et al. reported that low temperature can increase Miro1 expression in MSC, combining administration of MSC and cooling saline (therapeutic hypothermia) enhances MSC mitochondrial transfer and neurological recovery in OGD/R-treated SH-SY5Y cells and tMCAO-operated rats, the mechanisms underlying may involve upregulating Miro1 and inhibiting inflammation and apoptosis [182]. Upregulation of Miro1 in MSCs enhances mitochondrial transfer, protects against oxidant injury and improves metabolism in neurons exposed to hydrogen perox-

Table 2
Mitochondrial transfer from stem cells to neural cells following IS.

Donor cells	Recipient cells	IS Model	Transfer mode	Mitochondria status	Mechanisms and/or outcome	Ref
bone marrow derived mesenchymal stem cells (BMSCs)	Neurons	OGD	GJC	Health	Mitigate apoptosis, and facilitate cell survival	[82]
multipotent mesenchymal stem cells (MMSCs)	Neurons Astrocytes	OGD MCAO	TNTs	Health	Upregulate BDNF and Miro1, restore the bioenergy levels in astrocytes and stimulated their proliferation	[77]
MMSCs	Neurons	tMCAO	/	Health	Upregulate Miro1 and BDNF, and promote neurological recovery	[85]
MSCs	Neurons	hydrogen peroxide exposure	/	Health	Upregulate Miro1 and protect neurons from oxidant injury and improve metabolism.	[96]
MSCs	SH-SY5Y cells	OGD/R tMCAO	/	Health	Upregulate Miro1, reduce inflammation and apoptosis, and promote neurological recovery	[182]
umbilical cord derived (UC-MSCs)	Neurons	MCAO	/	Health	Decrease blood creatine phosphokinase level and astroglyosis as well as microglia activation, inhibition of apoptosis	[183]
Metabolic switching manipulated MSCs	Neurons	OGD	/	Health	Enhance cell viability, improve cel metabolism, decrease mitochondrial reactive oxidative species, and increase mitochondria ATP levels	[184]
UC-MSCs	Neurons Endothelial cells Astrocytes	OGD/R tMCAO	/	Health	Decrease ROS and enhance cell survival	[185]
Human induced Pluripotent stem cells-derived mesenchymal stem cells (iPSC-MSCs)	PC12 cells	$CoCl_2$	TNTs	Health	The transferred mitochondria reduce apoptosis and restored mitochondrial function in the injured PC12 cells	[72]
Endothelial progenitor cells (EPCs)	Endothelial cells	OGD	/	Health	Promote angiogenesis and reduce transcellular permeability of the brain endothelium	[186]
MSCs	Human umbilical vein endothelial cells	OGD/R	TNTs	Health	Protect mitochondrial function and reduce apoptosis	[70]
MSCs	Cerebral microvasculature	tMCAO	TNTs	Health	Improve mitochondrial activity of injured microvasculature and enhance angiogenesis and neurological function	[71]
MSCs	Endothelial cells	tMCAO	EVs	Health	MSCs-derived mitochondrial transfer rescued mitochondrial function in endothelial cells and reprogrammed glutathione metabolism to facilitate tip cell transition, enhance angiogenesis and facilitate functional recovery	[99]
BMSCs	Microglia Astrocytes	OGD/R tMCAO	/	Health	Reduce ROS and HIF-1α levels, inhibit microglia activation and neuroinflammation	[187]

ide. On the contrary, downregulating Miro1 have the opposite effect [96]. In addition, co-culture of MMSCs with neurons enhances neuroprotective effects, accompanied by increased Miro1 expression. This suggests that MMSC therapy promotes intracellular exchange between neurons and stem cells to protect neuronal damage, contributing to the recovery of neurological function in tMCAO-operated rats [85]. These results identify Miro1 as an important molecule to mediate mitochondrial transfer.

It has been reported that intracerebroventricular transplantation of healthy mitochondria from UC-MSCs into MCAO-operated rats reduced the ischemic injury. This was evidenced by decreased blood creatine phosphokinase, attenuated astroglyosis and microglia activation, reduced apoptosis, and infarct size, and improved neurological function [183]. Gorsky et al. reported that the MSC culture using alternating galactose and glucose medium c can generate "super mitochondria" with high ATP production and metabolic rate. Importantly, co-culturing neurons with these MSCs enriched with super mitochondria can increase cell viability and ATP production, improving cell metabolism, and decreasing mtROS, compared to standard OGD-treated MSCs [184].

Li et al. compared the ability of different brain cells to internalize UC-MSCs-derived mitochondria after cerebral ischemia. They found that neurons and ECs exposure to OGD/R or from tMCAO-operated mice have a higher ability to internalize mitochondria than astrocytes. The internalized mitochondria effectively reduced ROS levels and promoted cell survival. Furthermore, stress-induced ROS further enhanced host cell's capacity to internalize mitochondria after cerebral ischemia [185]. These results demonstrate that application of MSC-derived mitochondrial transfer is beneficial for neurons survival after IS.

Mitochondrial transfer from stem cells to ECs

IS induces angiogenesis as a compensatory mechanism for repairing damaged blood vessels. However, post-ischemic microenvironment, such as inflammation and oxidative stress, can impair BECs and impede reparative angiogenesis. Therefore, repairing BECs is crucial for the recovery of IS.

A growing body of evidence supports the therapeutic potential of mitochondrial transfer from stem cells to ECs [178]. For instance, it has been shown that endothelial progenitor cells (EPCs) –derived extracellular mitochondria can incorporate into normal brain ECs, promoting angiogenesis and reducing endothelial permeability. This incorporation promotes angiogenesis, reduces endothelial permeability, and restores bioenergetics by increasing mtDNA and intracellular ATP levels following OGD [186]. Liu et al. demonstrated that MSCs improve the mitochondrial function and protect human umbilical vein ECs against OGD/R-induced apoptosis by transferring functional mitochondria to improve their mitochondrial function via TNTs [70]. This transfer improved mitochondrial activity in the damaged microvasculature, leading to reduced infarct volume, enhanced angiogenesis and neurological functional recovery in tMCAO-operated rats [71].

The link between mitochondrial transfer and the stimulation of angiogenesis was further elucidated by Zhang et al., who developed a targeted delivery system for MSC-derived mitochondria.

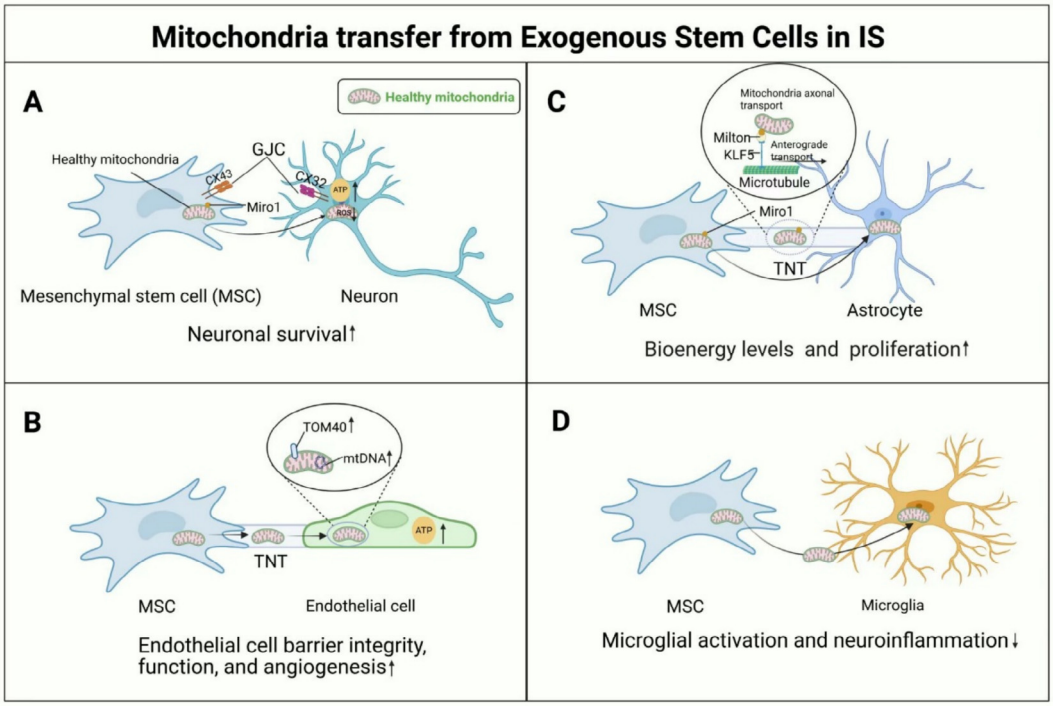


Fig. 5. Exogenous mitochondria-derived from stem cells protect neural cells against IS injury. A. Mesenchymal stem cells (MSCs) transfer mitochondria to OGD-injured neurons through a gap junction channels (GJCs)-dependent manner, primarily involving increased gap junctions connexin 43 (Cx43) and connexin 32 (Cx32), and this transfer enhances the bioenergetic profile, inhibits apoptosis and facilitates cell survival. B. Mitochondrial transfer from MSCs to brain endothelial cell improves cerebral endothelial function and angiogenesis. C. IS promotes tunneling nanotubes (TNTs) formation and mitochondrial transfer from MSCs to astrocytes, thereby improving the bioenergy metabolism and cell proliferation of astrocytes. D. MSCs-derived mitochondria taken up by microglia inhibit its activation and neuroinflammation following IS. This image was created in BioRender (*https://BioRender.com/drte122*).

Using an EV-mimetic platform functionalized with Arg-Gly-Asp (RGD) peptide, they selectively delivered mitochondria to *peri*-lesional ECs. This intervention restored mitochondrial function, reprogrammed GSH metabolism, and promoted a tip cell phenotype, a critical initiator of vessel sprouting, thereby enhancing angiogenesis and functional recovery in mice [99]. Collectively, these studies build a compelling case for the "replacement hypothesis," where transferred mitochondria act as direct energy suppliers to restore endothelial function.

However, a pivotal breakthrough from Melero-Martin's team challenges this very premise. Their groundbreaking study revealed that even depolarized or mtDNA-deficient mitochondria, which lack canonical bioenergetic capacity, can preserve endothelial cell function without integrating into the host mitochondrial network [100]. This discovery suggests that transplanted mitochondria may act not merely as energy suppliers but as molecular "triggers" that activate mitophagy in recipient cells, thereby selectively clearing damaged organelles and stimulating endogenous repair mechanisms.

Mitochondrial transfer from stem cells to astrocytes

Babenko et al. investigated mitochondrial transfer between MMSCs and astrocytes under normal and damaged mitochondrial condition. They found that MMSCs effectively transfer healthy mitochondria to injured astrocytes exposed to OGD. The transfer improves the energy metabolism and proliferation of astrocytes. They also found that mitochondrial damage in astrocytes promoted the efficiency of mitochondrial transfer and TNTs formation from MMSCs to astrocytes. Furthermore, administrating MMSCs with overexpression of Miro 1 into experimental IS animals was shown to increase the transfer of MMSCs mitochondria to damaged neuronal and astrocytes, thereby alleviating neurological dysfunction [77]. This support the proposal when mitochondria of recipient cells are damaged, these damaged mitochondria may send signals to promote mitochondrial transfer.

Mitochondrial transfer from stem cells to microglia

Dai et al. compared the ability of microglia and astrocytes to take up functional mitochondria derived from BMSCs following cerebral ischemia. They observed that microglia have higher ability to take up functional mitochondria compared to astrocytes. This uptake of functional mitochondria reduces ROS and HIF-1α expression and improves neurological outcome in tMCAO-operated rats. Notably, the study found that ROS produced by microglia cells following ischemia is a critical regulator of mitochondrial internalization, and that enhancing autophagy in microglia cells may subsequently reduce both ROS and HIF-1α [187].

Challenges and future directions of stem cell-derived mitochondrial therapy for IS

Limitations and key scientific problems

The translation of stem cell-derived mitochondrial therapy for IS faces several fundamental limitations. A primary concern is the low efficiency of mitochondrial delivery and cellular uptake. Isolated mitochondria lack active homing capabilities; after systemic administration, they are extensively sequestered in the pulmonary microvasculature and struggle to cross the BBB, resulting in minimal delivery to the ischemic penumbra. Even if delivered, the uptake of these large organelles by stressed neurons is inefficient, and their fate remains unclear, whether they integrate functionally into the host's mitochondrial network or are degraded in

lysosomes is a critical unanswered question [188]. Another significant challenge is ensuring mitochondrial quality and viability. The processes of isolation, purification, and storage can severely compromise MMP, respiratory function, and structural integrity. Administering dysfunctional mitochondria is not only ineffective but also risky, as they may release mtDNA and formyl peptides, which act as damage-associated molecular patterns. These can trigger innate immune responses via pathways like cGAS-STING, potentially exacerbating neuroinflammation [114,115]. Furthermore, the field currently lacks standardized protocols for mitochondrial isolation, quality assessment, and dosage, leading to considerable batch-to-batch variability.

Risks and challenges

The clinical application of mitochondrial transplantation is fraught with risks. Immunogenicity is a foremost risk; given their bacterial origins, allogeneic mitochondria may provoke immune recognition and rejection [189,190]. Although an ongoing early-phase clinical trial is assessing the safety of autologous mitochondrial transplantation in brain ischemia (NCT04998357), the long-term safety profile of exogenous mitochondria, including their potential to disrupt host metabolic or signaling networks, remains largely unknown. From a technical perspective, achieving targeted delivery to the brain remains a major hurdle. Standard intravenous injections lead to widespread systemic distribution and poor CNS engagement. Direct intracranial injection, while more local, is highly invasive and carries risks of additional damage in already compromised brain tissue.

Potential solutions and future directions

To overcome these barriers, innovative strategies are being explored. Advanced delivery systems are paramount. For instance, engineering mitochondria with targeting ligands, such as RGD peptide-functionalized mitochondrial vesicles (mitoEVMs), has been shown to enhance specific binding to integrins on ischemic ECs, improving both delivery precision and therapeutic efficacy in mouse stroke models [99]. Similarly, packaging mitochondria into engineered EVs or biocompatible nanoparticles can protect them during circulation and facilitate BBB crossing [191]. Optimizing administration routes, such as the intranasal pathway, offers a non-invasive alternative to bypass the BBB and directly access the CNS [192]. To address quality control, developing robust biomarkers for mitochondrial potency, such as membrane potential, oxygen consumption rate, and establishing good manufacturing practice (GMP)-compatible production pipelines are essential for clinical-grade products. Preconditioning stem cells through hypoxia or genetic engineering to upregulate proteins like Miro1 can enhance the intrinsic quality and transfer competence of their mitochondria. Finally, combining mitochondrial therapy with biomaterials, for example, hydrogels for sustained release, or pharmacological agents that promote cellular uptake and endosomal escape could significantly improve functional integration and long-term benefits in patients[193–196].

In summary, while stem cell-derived mitochondrial transplantation holds revolutionary potential for IS, its clinical success hinges on resolving the intertwined challenges of targeted delivery, functional integration, consistent quality, and comprehensive safety. A multidisciplinary approach, integrating bioengineering, molecular biology, and clinical neurology, will be crucial to advancing this promising "organelle therapy" from the bench to the bedside.

Concluding remarks and future perspectives

The therapeutic landscape for IS is undergoing a paradigm shift, moving beyond traditional pharmacologic and cellular therapies to embrace the innovative concept of mitochondrial transfer as a form of "organelle therapy." This approach capitalizes on the fundamental biological process of intercellular mitochondrial sharing, yet faces the dual challenge of deciphering its complex endogenous regulatory mechanisms while developing effective exogenous application strategies. The transition from cellular to subcellular medicine represents a frontier in stroke therapeutics that demands a concerted multidisciplinary effort.

Advancing the understanding of endogenous mitochondrial transfer

Endogenous mitochondrial transfer represents a double-edged sword in cerebral ischemia. While the donation of healthy organelles from supportive cells like astrocytes to stressed neurons provides well-documented neuroprotection, the reverse transfer of damaged mitochondria can propagate injury signals across neural networks. This duality necessitates future strategies that selectively promote beneficial transfer while inhibiting detrimental dissemination through pharmacological or genetic approaches.

Critical knowledge gaps remain regarding the molecular mechanisms governing mitochondrial transfer. Key unanswered questions include the precise triggers initiating transfer, with evidence suggesting roles for ischemia-induced ATP depletion as a "help-me" signal [107,197] and calcium overload regulating Miro1 protein function [174,198]. The kinetic characteristics of mitochondrial movement also require investigation, particularly whether transferred mitochondria can reach energy-demanding synaptic regions to support neural function effectively.

The balance between functional and damaged mitochondrial transfer under pathological conditions constitutes another critical research direction. Future therapeutic strategies should not aim to generally enhance mitochondrial transfer but rather to selectively promote the transfer of functional mitochondria. This may be achieved by: (1) improving mitochondrial homeostasis in donor cells (e.g., astrocytes) through pharmacological or genetic approaches to ensure source quality; (2) developing targeting systems that specifically guide healthy mitochondrial subpopulations for transfer; and (3) identifying specific signaling pathways that block the transfer of damaged mitochondria. Intriguingly, recent evidence from myocardial ischemia models suggests that even depolarized or mtDNA-deficient mitochondria can preserve endothelial function without integrating into the host mitochondrial network [100], indicating that transferred mitochondria may function not merely as energy donors but as molecular "primers" that activate mitophagy in recipient cells, facilitating clearance of dysfunctional organelles.

The possibility of peripheral-to-central mitochondrial transfer presents another fascinating research avenue. Current studies predominantly focus on mitochondrial transfer among CNS cells, yet emerging evidence suggests ECs which are key components of the BBB may receive mitochondria from peripheral sources through circulating EVs. Elucidating peripheral-central mitochondrial communication could not only provide novel therapeutic approaches for delivering mitochondria via peripheral circulation but also identify early diagnostic biomarkers for stroke. However, given the structural and functional complexity of the BBB, However, given the structural and functional complexity of the BBB, future research should employ rigorous in vivo tracking techniques to confirm the contribution of peripheral mitochondria to the CNS following IS. Key approaches include: (i) genetic labeling of peripheral mitochondria using cell type–specific transgenic reporters; (ii) real-time intravital imaging to visualize transfer across the BBB; (iii) single-cell multi-omics and mtDNA haplotype analysis to identify cells acquiring exogenous mitochondria; (iv) characterization of circulating extracellular vesicles as potential long-distance carriers; and (v) functional perturbation studies to establish physiolog-

ical relevance. Integrating these strategies will provide a comprehensive framework for deciphering peripheral–central mitochondrial communication.

Overcoming challenges in exogenous mitochondrial transplantation

Despite promising preclinical results and ongoing clinical trials, mitochondrial transplantation faces significant translational hurdles. Primary limitations include mitochondrial instability outside native environments, storage difficulties, and sourcing challenges – particularly for patients with pre-existing mitochondrial dysfunction where autologous transplantation may be impractical.

Critical uncertainties surround the fate of transplanted mitochondria, which often undergo lysosomal degradation rather than functional integration. Enhancing endosomal escape represents a key challenge, potentially addressed through bio-inspired engineering approaches. To address the critical bottleneck of lysosomal degradation of transplanted mitochondria, recent advances in bioengineering offer several promising strategies to enhance cytosolic delivery and functional integration. First, pH-dependent endosomal escape peptides can be conjugated to the mitochondrial surface. These peptides, often engineered by substituting cationic residues with histidine, remain inert at neutral pH but undergo protonation within the acidic endosomal environment, disrupting the endosomal membrane and facilitating mitochondrial release into the cytosol[199]. Second, co-delivery of small molecules with endosomolytic activity, such as chloroquine or siramesine, within extracellular vesicle carriers can compromise endosomal integrity, promoting mitochondrial escape prior to lysosomal fusion[200]. However, this approach requires careful targeting modifications to confine activity to ischemic tissue and avoid off-target cytotoxicity. Third, fusion-based delivery systems that bypass the endosomal pathway entirely represent a transformative approach. By encapsulating mitochondria within fusogenic liposomes composed of lipids like DOPE and cationic components, the carrier can fuse directly with the target cell membrane, depositing mitochondria directly into the cytosol and circumventing endosomal entrapment altogether[201,202]. These strategies, while at varying stages of development, provide a mechanistic roadmap for improving mitochondrial delivery efficiency to the brain and other target tissues.

Safety assessment must address mtDNA-mediated immune activation via cGAS-STING signaling and potential long-term effects from persistent mitochondrial presence. Finally, standardized protocols for isolation, quality control and delivery are urgently needed to ensure reproducible clinical translation.

A roadmap for clinical Translation: From mechanisms to medicine

To systematically address these challenges, we propose a structured research roadmap comprising three integrated phases. The initial Foundational & Mechanistic Understanding phase should resolve core biological questions by confirming transfer dynamics, assessing mitochondrial functionality, determining integration within recipients, and elucidating mechanisms governing release, uptake, and endosomal escape. The subsequent Technology & Therapeutic Development phase must translate these insights into practical strategies by enhancing donor mitochondrial quality, optimizing targeted delivery platforms, and establishing standardized GMP-compatible protocols. Finally, the Translational & Clinical Strategy phase should pave the way for clinical application through rigorous preclinical testing, biomarker validation, and early-phase clinical trials progressing toward allogeneic products.

In conclusion, mitochondrial transfer represents a paradigm shift in IS treatment, moving therapeutic strategies from the cellular to the subcellular level. The successful clinical translation of this approach requires addressing interconnected challenges in understanding endogenous transfer mechanisms, developing targeted delivery systems, ensuring functional integration, maintaining consistent quality, and comprehensively evaluating safety profiles. A multidisciplinary approach integrating bioengineering, molecular biology, and clinical neurology will be essential to safely harness this powerful biological phenomenon, ultimately restoring metabolic function and promoting neural repair after ischemic brain injury.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the grants from the National Natural Science Foundation of China (Nos. 82270434, 81970357) to M-SZ, and the National Natural Science Foundation of China (82574406), Key R&D Project of Liaoning Provincial Department of Science and Technology (2025JH2/101300019), the U35 Outstanding Young Talent Program of Shenyang (RC240612) and the Research Project of Educational Commission of Liaoning Province (LJ212410164003) to Y-YL.

References

[1] . Lancet Neurol 2021;20:795–820. doi: https://doi.org/10.1016/s1474-4422(21)00252-0.
[2] Hilkens NA, Casolla B, Leung TW, de Leeuw FE. Stroke. Lancet 2024;403:2820–36. doi: https://doi.org/10.1016/s0140-6736(24)00642-1.
[3] Ajoolabady A, Wang S, Kroemer G, Penninger JM, Uversky VN, Pratico D, et al. Targeting autophagy in ischemic stroke: from molecular mechanisms to clinical therapeutics. Pharmacol Ther 2021;225:107848. doi: https://doi.org/10.1016/j.pharmthera.2021.107848.
[4] . EClinicalMedicine 2023;59:102028. doi: https://doi.org/10.1016/j.eclinm.2023.102028.
[5] Mao R, Zong N, Hu Y, Chen Y, Xu Y. Neuronal Death Mechanisms and Therapeutic Strategy in Ischemic Stroke. Neurosci Bull 2022;38:1229–47. doi: https://doi.org/10.1007/s12264-022-00859-0.
[6] Qin C, Yang S, Chu YH, Zhang H, Pang XW, Chen L, et al. Signaling pathways involved in ischemic stroke: molecular mechanisms and therapeutic interventions. Signal Transduct Target Ther 2022;7:215. doi: https://doi.org/10.1038/s41392-022-01064-1.
[7] Wardlaw JM, Murray V, Berge E, del Zoppo G, Sandercock P, Lindley RL, et al. Recombinant tissue plasminogen activator for acute ischaemic stroke: an updated systematic review and meta-analysis. Lancet 2012;379:2364–72. doi: https://doi.org/10.1016/s0140-6736(12)60738-7.
[8] Widimsky P, Snyder K, Sulzenko J, Hopkins LN, Stetkarova I. Acute ischaemic stroke: recent advances in reperfusion treatment. Eur Heart J 2023;44:1205–15. doi: https://doi.org/10.1093/eurheartj/ehac684.
[9] Goncalves A, Su EJ, Muthusamy A, Zeitelhofer M, Torrente D, Nilsson I, et al. Thrombolytic tPA-induced hemorrhagic transformation of ischemic stroke is mediated by PKCβ phosphorylation of occludin. Blood 2022;140:388–400. doi: https://doi.org/10.1182/blood.2021014958.
[10] Ma H, Campbell BCV, Parsons MW, Churilov L, Levi CR, Hsu C, et al. Thrombolysis Guided by Perfusion Imaging up to 9 Hours after Onset of Stroke. N Engl J Med 2019;380:1795–803. doi: https://doi.org/10.1056/NEJMoa1813046.
[11] Haupt M, Gerner ST, Bähr M, Doeppner TR. Neuroprotective strategies for Ischemic Stroke-Future Perspectives. Int J Mol Sci 2023;24. doi: https://doi.org/10.3390/ijms24054334.
[12] Tao T, Liu M, Chen M, Luo Y, Wang C, Xu T, et al. Natural medicine in neuroprotection for ischemic stroke: challenges and prospective. Pharmacol Ther 2020;216:107695. doi: https://doi.org/10.1016/j.pharmthera.2020.107695.
[13] Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. Cell 2012;148:1145–59. doi: https://doi.org/10.1016/j.cell.2012.02.035.
[14] Wang J, Pu X, Zhuang H, Guo Z, Wang M, Yang H, et al. Astragaloside IV alleviates septic myocardial injury through DUSP1-Prohibitin 2 mediated mitochondrial quality control and ER-autophagy. J Adv Res 2025;75:561–80. doi: https://doi.org/10.1016/j.jare.2024.10.030.
[15] Marchi S, Guilbaud E, Tait SW, Yamazaki T, Galluzzi LJNRI. Mitochondrial control of inflammation 2023;23:159–73. doi: https://doi.org/10.1038/s41577-022-00760-x.

[16] Liu BH, Xu CZ, Liu Y, Lu ZL, Fu TL, Li GR, et al. Mitochondrial quality control in human health and disease. Mil Med Res 2024;11:32. doi: https://doi.org/10.1186/s40779-024-00536-5.
[17] Liu Y, Fu T, Li G, Li B, Luo G, Li N, et al. Mitochondrial transfer between cell crosstalk - an emerging role in mitochondrial quality control. Ageing Res Rev 2023;91:102038. doi: https://doi.org/10.1016/j.arr.2023.102038.
[18] Zhou D, Yang S, Wu J, Li Y, Jin H, Luo Y, et al. Mitochondrial transfer in the Neurovascular Unit, not only for Energy rescue: a Systematic Review. Aging Dis 2024. doi: https://doi.org/10.14336/ad.2024.0461.
[19] Bazzani V, Equisoain Redin M, McHale J, Perrone L, Vascotto C. Mitochondrial DNA Repair in Neurodegenerative Diseases and Ageing. Int J Mol Sci 2022;23. doi: https://doi.org/10.3390/ijms231911391.
[20] Kowaltowski AJ, de Souza-Pinto NC, Castilho RF, Vercesi AE. Mitochondria and reactive oxygen species. Free Radic Biol Med 2009;47:333–43. doi: https://doi.org/10.1016/j.freeradbiomed.2009.05.004.
[21] Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev 2014;94:909–50. doi: https://doi.org/10.1152/physrev.00026.2013.
[22] Pfanner N, Warscheid B, Wiedemann N. Mitochondrial proteins: from biogenesis to functional networks. Nat Rev Mol Cell Biol 2019;20:267–84. doi: https://doi.org/10.1038/s41580-018-0092-0.
[23] Tábara LC, Segawa M, Prudent J. Molecular mechanisms of mitochondrial dynamics. Nat Rev Mol Cell Biol 2024. doi: https://doi.org/10.1038/s41580-024-00785-1.
[24] Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders - a step towards mitochondria based therapeutic strategies. Biochim Biophys Acta Mol basis Dis 1863;2017:1066–77. doi: https://doi.org/10.1016/j.bbadis.2016.11.010.
[25] Chen W, Zhao H, Li Y. Mitochondrial dynamics in health and disease: mechanisms and potential targets. Signal Transduct Target Ther 2023;8:333. doi: https://doi.org/10.1038/s41392-023-01547-9.
[26] Johnson J, Mercado-Ayon E, Mercado-Ayon Y, Dong YN, Halawani S, Ngaba L, et al. Mitochondrial dysfunction in the development and progression of neurodegenerative diseases. Arch Biochem Biophys 2021;702:108698. doi: https://doi.org/10.1016/j.abb.2020.108698.
[27] Xie S, Jiang X, Doycheva DM, Shi H, Jin P, Gao L, et al. Activation of GPR39 with TC-G 1008 attenuates neuroinflammation via SIRT1/PGC-1α/Nrf2 pathway post-neonatal hypoxic-ischemic injury in rats. J Neuroinflammation 2021;18:226. doi: https://doi.org/10.1186/s12974-021-02289-7.
[28] Yin W, Signore AP, Iwai M, Cao G, Gao Y, Chen J. Rapidly increased neuronal mitochondrial biogenesis after hypoxic-ischemic brain injury. Stroke 2008;39:3057–63. doi: https://doi.org/10.1161/strokeaha.108.520114.
[29] Gutsaeva DR, Carraway MS, Suliman HB, Demchenko IT, Shitara H, Yonekawa H, et al. Transient hypoxia stimulates mitochondrial biogenesis in brain subcortex by a neuronal nitric oxide synthase-dependent mechanism. J Neurosci 2008;28:2015–24. doi: https://doi.org/10.1523/jneurosci.5654-07.2008.
[30] Li L, Xiao L, Hou Y, He Q, Zhu J, Li Y, et al. Sestrin2 Silencing Exacerbates Cerebral Ischemia/Reperfusion Injury by Decreasing Mitochondrial Biogenesis through the AMPK/PGC-1α Pathway in Rats. Sci Rep 2016;6:30272. doi: https://doi.org/10.1038/srep30272.
[31] Zhang Z, Yang D, Zhou B, Luan Y, Yao Q, Liu Y, et al. Decrease of MtDNA copy number affects mitochondrial function and involves in the pathological consequences of ischaemic stroke. J Cell Mol Med 2022;26:4157–68. doi: https://doi.org/10.1111/jcmm.17262.
[32] Valerio A, Bertolotti P, Delbarba A, Perego C, Dossena M, Ragni M, et al. Glycogen synthase kinase-3 inhibition reduces ischemic cerebral damage, restores impaired mitochondrial biogenesis and prevents ROS production. J Neurochem 2011;116:1148–59. doi: https://doi.org/10.1111/j.1471-4159.2011.07171.x.
[33] Li X, Li Y, Wang K, Qi S, Zhang Z, Cai S. Isoquercitrin alleviates OGD/R-induced oxidative stress and impaired mitochondrial biogenesis in SH-SY5Y cells via the NRF1/TFAM pathway. Cell Biochem Biophys 2024;82:2455–64. doi: https://doi.org/10.1007/s12013-024-01355-0.
[34] Lin CH, Nicol CJB, Cheng YC, Yen C, Wang YS, Chiang MC. Neuroprotective effects of resveratrol against oxygen glucose deprivation induced mitochondrial dysfunction by activation of AMPK in SH-SY5Y cells with 3D gelatin scaffold. Brain Res 2020;1726:146492. doi: https://doi.org/10.1016/j.brainres.2019.146492.
[35] Al Ojaimi M, Salah A, El-Hattab AW. Mitochondrial Fission and Fusion: Molecular Mechanisms, Biological Functions, and Related Disorders. Membranes (Basel) 2022;12. doi: https://doi.org/10.3390/membranes12090893.
[36] Mishra P, Chan DC. Mitochondrial dynamics and inheritance during cell division, development and disease. Nat Rev Mol Cell Biol 2014;15:634–46. doi: https://doi.org/10.1038/nrm3877.
[37] Tilokani L, Nagashima S, Paupe V, Prudent J. Mitochondrial dynamics: overview of molecular mechanisms. Essays Biochem 2018;62:341–60. doi: https://doi.org/10.1042/ebc20170104.
[38] Zacharioudakis E, Gavathiotis E. Mitochondrial dynamics proteins as emerging drug targets. Trends Pharmacol Sci 2023;44:112–27. doi: https://doi.org/10.1016/j.tips.2022.11.004.
[39] An H, Zhou B, Ji X. Mitochondrial quality control in acute ischemic stroke. J Cereb Blood Flow Metab 2021;41:3157–70. doi: https://doi.org/10.1177/0271678x211046992.
[40] Grohm J, Kim SW, Mamrak U, Tobaben S, Cassidy-Stone A, Nunnari J, et al. Inhibition of Drp1 provides neuroprotection in vitro and in vivo. Cell Death Differ 2012;19:1446–58. doi: https://doi.org/10.1038/cdd.2012.18.
[41] Slupe AM, Merrill RA, Flippo KH, Lobas MA, Houtman JC, Strack S. A calcineurin docking motif (LXVP) in dynamin-related protein 1 contributes to mitochondrial fragmentation and ischemic neuronal injury. J Biol Chem 2013;288:12353–65. doi: https://doi.org/10.1074/jbc.M113.459677.
[42] Zhang J, Wang S, Zhang H, Yang X, Ren X, Wang L, et al. Drp1 acetylation mediated by CDK5-AMPK-GCN5L1 axis promotes cerebral ischemic injury via facilitating mitochondrial fission. Mol Med 2024;30:173. doi: https://doi.org/10.1186/s10020-024-00948-y.
[43] Shen N, Kong L, Wang X, Zhang Y, Li R, Tao C, et al. Elabela ameliorates neuronal pyroptosis and mitochondrial fission via APJ/ZBP1 signaling in ischemic stroke. Exp Neurol 2024;378:114802. doi: https://doi.org/10.1016/j.expneurol.2024.114802.
[44] Wang W, Pang M, Zhang Y, Hou S, Xia Y, Shi Y, et al. Semaglutide Mitigates Ischemic Brain Injury by Inhibiting Ferroptosis via Modulation of FoXO1 and DRP1 Pathways. Mol Neurobiol 2025;62:16168–88. doi: https://doi.org/10.1007/s12035-025-05253-1.
[45] Wang J, Zhuang H, Jia L, He X, Zheng S, Ji K, et al. Nuclear receptor subfamily 4 group a member 1 promotes myocardial ischemia/reperfusion injury through inducing mitochondrial fission factor-mediated mitochondrial fragmentation and inhibiting FUN14 domain containing 1-depedent mitophagy. Int J Biol Sci 2024;20:4458–75. doi: https://doi.org/10.7150/ijbs.95853.
[46] Wu Q, Liu J, Mao Z, Tian L, Wang N, Wang G, et al. Ligustilide attenuates ischemic stroke injury by promoting Drp1-mediated mitochondrial fission via activation of AMPK. Phytomedicine 2022;95:153884. doi: https://doi.org/10.1016/j.phymed.2021.153884.
[47] Ge S, Wang L, Jin C, Xie H, Zheng G, Cui Z, et al. Unveiling the neuroprotection effects of Volvalerenic acid a: Mitochondrial fusion induction via IDO1-mediated Stat3-Opa1 signaling pathway. Phytomedicine 2024;129:155555. doi: https://doi.org/10.1016/j.phymed.2024.155555.
[48] Wang L, Qin N, Gao S, Zhu T. Ginsenoside Rb3 Promotes Opa1-Mediated Regenerative Neurogenesis via Activating the Ido1 Pathway in Ischemic Stroke. Phytother Res 2025;39:564–80. doi: https://doi.org/10.1002/ptr.8392.
[49] Zhang L, He Z, Zhang Q, Wu Y, Yang X, Niu W, et al. Exercise pretreatment promotes mitochondrial dynamic protein OPA1 expression after cerebral ischemia in rats. Int J Mol Sci 2014;15:4453–63. doi: https://doi.org/10.3390/ijms15034453.
[50] Chen H, Liu J, Chen M, Wei Z, Yuan J, Wu W, et al. SIRT3 facilitates mitochondrial structural repair and functional recovery in rats after ischemic stroke by promoting OPA1 expression and activity. Clin Nutr 2024;43:1816–31. doi: https://doi.org/10.1016/j.clnu.2024.06.001.
[51] M. Onishi, K. Yamano, M. Sato, N. Matsuda, K. Okamoto, Molecular mechanisms and physiological functions of mitophagy, Embo j, 40 (2021) e104705. 10.15252/embj.2020104705.
[52] Chang X, Zhou H, Hu J, Ge T, He K, Chen Y, et al. Targeting mitochondria by lipid-selenium conjugate drug results in malate/fumarate exhaustion and induces mitophagy-mediated necroptosis suppression. Int J Biol Sci 2024;20:5793–811. doi: https://doi.org/10.7150/ijbs.102424.
[53] Wang S, Long H, Hou L, Feng B, Ma Z, Wu Y, et al. The mitophagy pathway and its implications in human diseases. Signal Transduct Target Ther 2023;8:304. doi: https://doi.org/10.1038/s41392-023-01503-7.
[54] Li J, Wu J, Zhou X, Lu Y, Ge Y, Zhang X. Targeting neuronal mitophagy in ischemic stroke: an update, Burns. Trauma 2023;11:tkad018. doi: https://doi.org/10.1093/burnst/tkad018.
[55] Tang YC, Tian HX, Yi T, Chen HB. The critical roles of mitophagy in cerebral ischemia, Protein. Cell 2016;7:699–713. doi: https://doi.org/10.1007/s13238-016-0307-0.
[56] Daniele SG, Trummer G, Hossmann KA, Vrselja Z, Benk C, Gobeske KT, et al. Brain vulnerability and viability after ischaemia. Nat Rev Neurosci 2021;22:553–72. doi: https://doi.org/10.1038/s41583-021-00488-y.
[57] Ashrafi G, Schwarz TL. The pathways of mitophagy for quality control and clearance of mitochondria. Cell Death Differ 2013;20:31–42. doi: https://doi.org/10.1038/cdd.2012.81.
[58] Uoselis L, Nguyen TN, Lazarou M. Mitochondrial degradation: Mitophagy and beyond. Mol Cell 2023;83:3404–20. doi: https://doi.org/10.1016/j.molcel.2023.08.021.
[59] Jia J, Jin H, Nan D, Yu W, Huang Y. New insights into targeting mitochondria in ischemic injury. Apoptosis 2021;26:163–83. doi: https://doi.org/10.1007/s10495-021-01661-5.
[60] Huang J, Chen L, Yao ZM, Sun XR, Tong XH, Dong SY. The role of mitochondrial dynamics in cerebral ischemia-reperfusion injury. Biomed Pharmacother 2023;162:114671. doi: https://doi.org/10.1016/j.biopha.2023.114671.
[61] Palikaras K, Lionaki E, Tavernarakis N. Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. Nat Cell Biol 2018;20:1013–22. doi: https://doi.org/10.1038/s41556-018-0176-2.
[62] Torralba D, Baixauli F, Sánchez-Madrid F. Mitochondria know No Boundaries: Mechanisms and Functions of Intercellular Mitochondrial transfer. Front Cell Dev Biol 2016;4:107. doi: https://doi.org/10.3389/fcell.2016.00107.
[63] Paliwal S, Chaudhuri R, Agrawal A, Mohanty S. Regenerative abilities of mesenchymal stem cells through mitochondrial transfer. J Biomed Sci 2018;25:31. doi: https://doi.org/10.1186/s12929-018-0429-1.
[64] D'Souza A, Burch A, Dave KM, Sreeram A, Reynolds MJ, Dobbins DX, et al. Microvesicles transfer mitochondria and increase mitochondrial function in

brain endothelial cells. J Control Release 2021;338:505–26. doi: https://doi.org/10.1016/j.jconrel.2021.08.038.
[65] O. Korenkova, A. Pepe, C. Zurzolo, Fine intercellular connections in development: TNTs, cytonemes, or intercellular bridges?, Cell Stress, 4 (2020) 30-43. 10.15698/cst2020.02.212.
[66] Zurzolo C. Tunneling nanotubes: Reshaping connectivity. Curr Opin Cell Biol 2021;71:139–47. doi: https://doi.org/10.1016/j.ceb.2021.03.003.
[67] Hase K, Kimura S, Takatsu H, Ohmae M, Kawano S, Kitamura H, et al. M-Sec promotes membrane nanotube formation by interacting with Ral and the exocyst complex. Nat Cell Biol 2009;11:1427–32. doi: https://doi.org/10.1038/ncb1990.
[68] Kimura S, Hase K, Ohno H. Tunneling nanotubes: emerging view of their molecular components and formation mechanisms. Exp Cell Res 2012;318:1699–706. doi: https://doi.org/10.1016/j.yexcr.2012.05.013.
[69] Lu M, Guo J, Wu B, Zhou Y, Wu M, Farzaneh M, et al. Mesenchymal Stem Cell-Mediated Mitochondrial transfer: a Therapeutic Approach for Ischemic Stroke. Transl Stroke Res 2021;12:212–29. doi: https://doi.org/10.1007/s12975-020-00853-6.
[70] Liu K, Ji K, Guo L, Wu W, Lu H, Shan P, et al. Mesenchymal stem cells rescue injured endothelial cells in an in vitro ischemia-reperfusion model via tunneling nanotube like structure-mediated mitochondrial transfer. Microvasc Res 2014;92:10–8. doi: https://doi.org/10.1016/j.mvr.2014.01.008.
[71] Liu K, Guo L, Zhou Z, Pan M, Yan C. Mesenchymal stem cells transfer mitochondria into cerebral microvasculature and promote recovery from ischemic stroke. Microvasc Res 2019;123:74–80. doi: https://doi.org/10.1016/j.mvr.2019.01.001.
[72] Yang Y, Ye G, Zhang YL, He HW, Yu BQ, Hong YM, et al. Transfer of mitochondria from mesenchymal stem cells derived from induced pluripotent stem cells attenuates hypoxia-ischemia-induced mitochondrial dysfunction in PC12 cells. Neural Regen Res 2020;15:464–72. doi: https://doi.org/10.4103/1673-5374.266058.
[73] Pisani F, Castagnola V, Simone L, Loiacono F, Svelto M, Benfenati F. Role of pericytes in blood-brain barrier preservation during ischemia through tunneling nanotubes. Cell Death Dis 2022;13:582. doi: https://doi.org/10.1038/s41419-022-05025-y.
[74] Nasoni MG, Carloni S, Canonico B, Burattini S, Cesarini E, Papa S, et al. Melatonin reshapes the mitochondrial network and promotes intercellular mitochondrial transfer via tunneling nanotubes after ischemic-like injury in hippocampal HT22 cells. J Pineal Res 2021;71:e12747. doi: https://doi.org/10.1111/jpi.12747.
[75] Zhang S, Kazanietz MG, Cooke M. Rho GTPases and the emerging role of tunneling nanotubes in physiology and disease. Am J Physiol Cell Physiol 2020;319:C877–84. doi: https://doi.org/10.1152/ajpcell.00351.2020.
[76] Panchal K, Tiwari AK. Miro (Mitochondrial Rho GTPase), a key player of mitochondrial axonal transport and mitochondrial dynamics in neurodegenerative diseases. Mitochondrion 2021;56:118–35. doi: https://doi.org/10.1016/j.mito.2020.10.005.
[77] Babenko VA, Silachev DN, Popkov VA, Zorova LD, Pevzner IB, Plotnikov EY, et al. Miro1 Enhances Mitochondria transfer from Multipotent Mesenchymal Stem Cells (MMSC) to Neural Cells and Improves the Efficacy of Cell Recovery. Molecules 2018;23. doi: https://doi.org/10.3390/molecules23030687.
[78] Cetin-Ferra S, Francis SC, Cooper AT, Neikirk K, Marshall AG, Hinton Jr A, et al. Mitochondrial Connexins and Mitochondrial Contact Sites with Gap Junction Structure. Int J Mol Sci 2023;24. doi: https://doi.org/10.3390/ijms24109036.
[79] Yamasaki R. Connexins Control Glial Inflammation in Various Neurological Diseases. Int J Mol Sci 2023;24. doi: https://doi.org/10.3390/ijms242316879.
[80] Bell CL, Shakespeare TI, Smith AR, Murray SA. Visualization of Annular Gap Junction Vesicle Processing: the Interplay between Annular Gap Junctions and Mitochondria. Int J Mol Sci 2018;20. doi: https://doi.org/10.3390/ijms20010044.
[81] Zhu Y. Gap Junction-Dependent and -Independent Functions of Connexin43 in Biology. Biology (Basel) 2022;11. doi: https://doi.org/10.3390/biology11020283.
[82] Li H, Wang C, He T, Zhao T, Chen YY, Shen YL, et al. Mitochondrial transfer from Bone Marrow Mesenchymal Stem Cells to Motor Neurons in Spinal Cord Injury Rats via Gap Junction. Theranostics 2019;9:2017–35. doi: https://doi.org/10.7150/thno.29400.
[83] Ogle BM, Cascalho M, Platt JL. Biological implications of cell fusion. Nat Rev Mol Cell Biol 2005;6:567–75. doi: https://doi.org/10.1038/nrm1678.
[84] Martens S, McMahon HT. Mechanisms of membrane fusion: disparate players and common principles. Nat Rev Mol Cell Biol 2008;9:543–56. doi: https://doi.org/10.1038/nrm2417.
[85] Babenko VA, Silachev DN, Zorova LD, Pevzner IB, Khutornenko AA, Plotnikov EY, et al. Improving the Post-Stroke Therapeutic Potency of Mesenchymal Multipotent Stromal Cells by Cocultivation with Cortical Neurons: the Role of Crosstalk between Cells. Stem Cells Transl Med 2015;4:1011–20. doi: https://doi.org/10.5966/sctm.2015-0010.
[86] Iorio R, Petricca S, Di Emidio G, Falone S, Tatone C. Mitochondrial Extracellular Vesicles (mitoEVs): Emerging mediators of cell-to-cell communication in health, aging and age-related diseases. Ageing Res Rev 2024;101:102522. doi: https://doi.org/10.1016/j.arr.2024.102522.
[87] Zappulli V, Friis KP, Fitzpatrick Z, Maguire CA, Breakefield XO. Extracellular vesicles and intercellular communication within the nervous system. J Clin Invest 2016;126:1198–207. doi: https://doi.org/10.1172/jci81134.
[88] Meng W, He C, Hao Y, Wang L, Li L, Zhu G. Prospects and challenges of extracellular vesicle-based drug delivery system: considering cell source. Drug Deliv 2020;27:585–98. doi: https://doi.org/10.1080/10717544.2020.1748758.
[89] Hayakawa K, Esposito E, Wang X, Terasaki Y, Liu Y, Xing C, et al. Transfer of mitochondria from astrocytes to neurons after stroke. Nature 2016;535:551–5. doi: https://doi.org/10.1038/nature18928.
[90] Dave KM, Stolz DB, Venna VR, Quaicoe VA, Maniskas ME, Reynolds MJ, et al. Mitochondria-containing extracellular vesicles (EV) reduce mouse brain infarct sizes and EV/HSP27 protect ischemic brain endothelial cultures. J Control Release 2023;354:368–93. doi: https://doi.org/10.1016/j.jconrel.2023.01.025.
[91] Sifat AE, Nozohouri S, Archie SR, Chowdhury EA, Abbruscato TJ. Brain Energy Metabolism in Ischemic Stroke: Effects of smoking and Diabetes. Int J Mol Sci 2022;23. doi: https://doi.org/10.3390/ijms23158512.
[92] Gjedde A, Marrett S, Vafaee M. Oxidative and nonoxidative metabolism of excited neurons and astrocytes. J Cereb Blood Flow Metab 2002;22:1–14. doi: https://doi.org/10.1097/00004647-200201000-00001.
[93] Liu H, Wang S, Wang J, Guo X, Song Y, Fu K, et al. Energy metabolism in health and diseases. Signal Transduct Target Ther 2025;10:69. doi: https://doi.org/10.1038/s41392-025-02141-x.
[94] S. Rumpf, N. Sanal, M. Marzano, Energy metabolic pathways in neuronal development and function, Oxf Open Neurosci, 2 (2023) kvad004. 10.1093/oons/kvad004.
[95] Guan R, Zou W, Dai X, Yu X, Liu H, Chen Q, et al. Mitophagy, a potential therapeutic target for stroke. J Biomed Sci 2018;25:87. doi: https://doi.org/10.1186/s12929-018-0487-4.
[96] Tseng N, Lambie SC, Huynh CQ, Sanford B, Patel M, Herson PS, et al. Mitochondrial transfer from mesenchymal stem cells improves neuronal metabolism after oxidant injury in vitro: the role of Miro1. J Cereb Blood Flow Metab 2021;41:761–70. doi: https://doi.org/10.1177/0271678x20928147.
[97] Sarmah D, Datta A, Rana N, Suthar P, Gupta V, Kaur H, et al. SIRT-1/RHOT-1/PGC-1α loop modulates mitochondrial biogenesis and transfer to offer resilience following endovascular stem cell therapy in ischemic stroke. Free Radic Biol Med 2024;225:255–74. doi: https://doi.org/10.1016/j.freeradbiomed.2024.09.022.
[98] Zhou J, Zhang L, Peng J, Zhang X, Zhang F, Wu Y, et al. Astrocytic LRP1 enables mitochondria transfer to neurons and mitigates brain ischemic stroke by suppressing ARF1 lactylation. Cell Metab 2024;36:2054–2068.e2014. doi: https://doi.org/10.1016/j.cmet.2024.05.016.
[99] Zhang Q, Huang J, Chen X, Li L, Chen L, Zhou X, et al. Mesenchymal Stem Cell-Derived Mitochondrial transfer Promotes Tip Cell Phenotype via Glutathione Metabolic Reprogramming in Stroke mice. ACS Nano 2025;19:20452–71. doi: https://doi.org/10.1021/acsnano.4c15759.
[100] Lin RZ, Im GB, Luo AC, Zhu Y, Hong X, Neumeyer J, et al. Mitochondrial transfer mediates endothelial cell engraftment through mitophagy. Nature 2024;629:660–8. doi: https://doi.org/10.1038/s41586-024-07340-0.
[101] Jittiwat J, Suksamrarn A, Tocharus C, Tocharus J. Dihydrocapsaicin effectively mitigates cerebral ischemia-induced pathological changes in vivo, partly via antioxidant and anti-apoptotic pathways. Life Sci 2021;283:119842. doi: https://doi.org/10.1016/j.lfs.2021.119842.
[102] Li Y, Sun J, Wu R, Bai J, Hou Y, Zeng Y, et al. Mitochondrial MPTP: a Novel Target of Ethnomedicine for Stroke Treatment by Apoptosis Inhibition. Front Pharmacol 2020;11:352. doi: https://doi.org/10.3389/fphar.2020.00352.
[103] Andrabi SS, Tabassum H, Parveen S, Parvez S. Ropinirole induces neuroprotection following reperfusion-promoted mitochondrial dysfunction after focal cerebral ischemia in Wistar rats. Neurotoxicology 2020;77:94–104. doi: https://doi.org/10.1016/j.neuro.2019.12.004.
[104] Lv J, Guan W, You Q, Deng L, Zhu Y, Guo K, et al. RIPC provides neuroprotection against ischemic stroke by suppressing apoptosis via the mitochondrial pathway. Sci Rep 2020;10:5361. doi: https://doi.org/10.1038/s41598-020-62336-w.
[105] Yang L, Tao Y, Luo L, Zhang Y, Wang X, Meng X. Dengzhan Xixin injection derived from a traditional chinese herb Erigeron breviscapus ameliorates cerebral ischemia/reperfusion injury in rats via modulation of mitophagy and mitochondrial apoptosis. J Ethnopharmacol 2022;288:114988. doi: https://doi.org/10.1016/j.jep.2022.114988.
[106] Huang S, He Q, Sun X, Qu Y, Abuduxukuer R, Ren J, et al. DL-3-n-butylphthalide Attenuates Cerebral Ischemia-Reperfusion Injury by Inhibiting Mitochondrial Omi/HtrA2-Mediated Apoptosis. Curr Neurovasc Res 2023;20:101–11. doi: https://doi.org/10.2174/1567202620666230228100653.
[107] Capobianco DL, De Zio R, Profico DC, Gelati M, Simone L, D'Erchia AM, et al. Human neural stem cells derived from fetal human brain communicate with each other and rescue ischemic neuronal cells through tunneling nanotubes. Cell Death Dis 2024;15:639. doi: https://doi.org/10.1038/s41419-024-07005-w.
[108] Mishra SR, Mahapatra KK, Behera BP, Patra S, Bhol CS, Panigrahi DP, et al. Mitochondrial dysfunction as a driver of NLRP3 inflammasome activation and its modulation through mitophagy for potential therapeutics. Int J Biochem Cell Biol 2021;136:106013. doi: https://doi.org/10.1016/j.biocel.2021.106013.

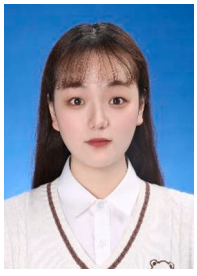
[109] Esenwa CC, Elkind MS. Inflammatory risk factors, biomarkers and associated therapy in ischaemic stroke. Nat Rev Neurol 2016;12:594–604. doi: https://doi.org/10.1038/nrneurol.2016.125.
[110] Ibrahim AA, Abdel Mageed SS, Safar MM, El-Yamany MF, Oraby MA. MitoQ alleviates hippocampal damage after cerebral ischemia: the potential role of SIRT6 in regulating mitochondrial dysfunction and neuroinflammation. Life Sci 2023;328:121895. doi: https://doi.org/10.1016/j.lfs.2023.121895.
[111] Chen S, Han C, Shi Z, Guan X, Cheng L, Wang L, et al. Umbilical mesenchymal stem cells mitigate T-cell compartments shift and Th17/Treg imbalance in acute ischemic stroke via mitochondrial transfer. Stem Cell Res Ther 2025;16:134. doi: https://doi.org/10.1186/s13287-025-04224-6.
[112] Liu W, Qi Z, Li W, Liang J, Zhao L, Shi Y. M1 Microglia Induced Neuronal Injury on Ischemic Stroke via Mitochondrial Crosstalk between Microglia and Neurons. Oxid Med Cell Longev 2022;2022:4335272. doi: https://doi.org/10.1155/2022/4335272.
[113] Li Q, Yang L, Wang K, Chen Z, Liu H, Yang X, et al. Oxidized mitochondrial DNA activates the cGAS-STING pathway in the neuronal intrinsic immune system after brain ischemia-reperfusion injury. Neurotherapeutics 2024;21:e00368. doi: https://doi.org/10.1016/j.neurot.2024.e00368.
[114] Chen Z, Liu Y, Lin Z, Huang W. cGAS-STING pathway in ischemia-reperfusion injury: a potential target to improve transplantation outcomes. Front Immunol 2023;14:1231057. doi: https://doi.org/10.3389/fimmu.2023.1231057.
[115] Ouyang W, Wang S, Yan D, Wu J, Zhang Y, Li W, et al. The cGAS-STING pathway-dependent sensing of mitochondrial DNA mediates ocular surface inflammation. Signal Transduct Target Ther 2023;8:371. doi: https://doi.org/10.1038/s41392-023-01624-z.
[116] Xu J, Wu D, Zhou S, Hu H, Li F, Guan Z, et al. MLKL deficiency attenuated hepatocyte oxidative DNA damage by activating mitophagy to suppress macrophage cGAS-STING signaling during liver ischemia and reperfusion injury. Cell Death Discov 2023;9:58. doi: https://doi.org/10.1038/s41420-023-01357-6.
[117] Yuan Y, Sun J, Dong Q, Cui M. Blood-brain barrier endothelial cells in neurodegenerative diseases: Signals from the "barrier". Front Neurosci 2023;17:1047778. doi: https://doi.org/10.3389/fnins.2023.1047778.
[118] Armulik A, Genové G, Mäe M, Nisancioglu MH, Wallgard E, Niaudet C, et al. Pericytes regulate the blood-brain barrier. Nature 2010;468:557–61. doi: https://doi.org/10.1038/nature09522.
[119] Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. Nat Med 2013;19:1584–96. doi: https://doi.org/10.1038/nm.3407.
[120] Segarra M, Aburto MR, Cop F, Llaó-Cid C, Härtl R, Damm M, et al. Endothelial Dab1 signaling orchestrates neuro-glia-vessel communication in the central nervous system. Science 2018;361. doi: https://doi.org/10.1126/science.aao2861.
[121] Liu LR, Liu JC, Bao JS, Bai QQ, Wang GQ. Interaction of Microglia and Astrocytes in the Neurovascular Unit. Front Immunol 2020;11:1024. doi: https://doi.org/10.3389/fimmu.2020.01024.
[122] Sweeney MD, Ayyadurai S, Zlokovic BV. Pericytes of the neurovascular unit: key functions and signaling pathways. Nat Neurosci 2016;19:771–83. doi: https://doi.org/10.1038/nn.4288.
[123] Mathias K, Machado RS, Stork S, Dos Santos D, Joaquim L, Generoso J, et al. Blood-brain barrier permeability in the ischemic stroke: an update. Microvasc Res 2024;151:104621. doi: https://doi.org/10.1016/j.mvr.2023.104621.
[124] Cheng J, Zheng Y, Cheng F, Wang C, Han J, Zhang H, et al. Different roles of astrocytes in the blood-brain barrier during the acute and recovery phases of stroke. Neural Regen Res 2026;21:1359–72. doi: https://doi.org/10.4103/nrr.Nrr-d-24-01417.
[125] Sun J, Huang Y, Gong J, Wang J, Fan Y, Cai J, et al. Transplantation of hPSC-derived pericyte-like cells promotes functional recovery in ischemic stroke mice. Nat Commun 2020;11:5196. doi: https://doi.org/10.1038/s41467-020-19042-y.
[126] Geranmayeh MH, Rahbarghazi R, Farhoudi M. Targeting pericytes for neurovascular regeneration. Cell Commun Signal 2019;17:26. doi: https://doi.org/10.1186/s12964-019-0340-8.
[127] Birbrair A, Zhang T, Files DC, Mannava S, Smith T, Wang ZM, et al. Type-1 pericytes accumulate after tissue injury and produce collagen in an organ-dependent manner. Stem Cell Res Ther 2014;5:122. doi: https://doi.org/10.1186/scrt512.
[128] Pekny M, Wilhelmsson U, Tatlisumak T, Pekna M. Astrocyte activation and reactive gliosis-a new target in stroke? Neurosci Lett 2019;689:45–55. doi: https://doi.org/10.1016/j.neulet.2018.07.021.
[129] Xu T, Yang J, Xu Y, Wang X, Gao X, Sun J, et al. Post-acute ischemic stroke hyperglycemia aggravates destruction of the blood-brain barrier. Neural Regen Res 2024;19:1344–50. doi: https://doi.org/10.4103/1673-5374.385851.
[130] Borcherding N, Brestoff JR. The power and potential of mitochondria transfer. Nature 2023;623:283–91. doi: https://doi.org/10.1038/s41586-023-06537-z.
[131] Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn 2nd GW, et al. Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. Nat Neurosci 2019;22:1635–48. doi: https://doi.org/10.1038/s41593-019-0486-0.
[132] Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 2005;6:626–40. doi: https://doi.org/10.1038/nrn1722.
[133] Zhou B, Zuo YX, Jiang RT. Astrocyte morphology: Diversity, plasticity, and role in neurological diseases. CNS Neurosci Ther 2019;25:665–73. doi: https://doi.org/10.1111/cns.13123.
[134] Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 2006;7:41–53. doi: https://doi.org/10.1038/nrn1824.
[135] Batiuk MY, Martirosyan A, Wahis J, de Vin F, Marneffe C, Kusserow C, et al. Identification of region-specific astrocyte subtypes at single cell resolution. Nat Commun 2020;11:1220. doi: https://doi.org/10.1038/s41467-019-14198-8.
[136] Aksoy P, White TA, Thompson M, Chini EN. Regulation of intracellular levels of NAD: a novel role for CD38. Biochem Biophys Res Commun 2006;345:1386–92. doi: https://doi.org/10.1016/j.bbrc.2006.05.042.
[137] Hattori T, Kaji M, Ishii H, Jureepon R, Takarada-Iemata M, Minh Ta H, et al. CD38 positively regulates postnatal development of astrocytes cell-autonomously and oligodendrocytes non-cell-autonomously. Glia 2017;65:974–89. doi: https://doi.org/10.1002/glia.23139.
[138] Park JH, Nakamura Y, Li W, Hamanaka G, Arai K, Lo EH, et al. Effects of O-GlcNAcylation on functional mitochondrial transfer from astrocytes. J Cereb Blood Flow Metab 2021;41:1523–35. doi: https://doi.org/10.1177/0271678x20969588.
[139] Ni XC, Wang HF, Cai YY, Yang D, Alolga RN, Liu B, et al. Ginsenoside Rb1 inhibits astrocyte activation and promotes transfer of astrocytic mitochondria to neurons against ischemic stroke. Redox Biol 2022;54:102363. doi: https://doi.org/10.1016/j.redox.2022.102363.
[140] Guo Y, Fu T, Cheng Y, Li Y, Zhang R, Ma Q, et al. Mechanisms of electroacupuncture-induced neuroprotection in acute stroke rats: the role of astrocyte-mediated mitochondrial transfer. Cell Commun Signal 2025;23:316. doi: https://doi.org/10.1186/s12964-025-02287-9.
[141] Escartin C, Galea E, Lakatos A, O'Callaghan JP, Petzold GC, Serrano-Pozo A, et al. Reactive astrocyte nomenclature, definitions, and future directions. Nat Neurosci 2021;24:312–25. doi: https://doi.org/10.1038/s41593-020-00783-4.
[142] Lawrence JM, Schardien K, Wigdahl B, Nonnemacher MR. Roles of neuropathology-associated reactive astrocytes: a systematic review. Acta Neuropathol Commun 2023;11:42. doi: https://doi.org/10.1186/s40478-023-01526-9.
[143] Tian L, Potus F, Wu D, Dasgupta A, Chen KH, Mewburn J, et al. Increased Drp1-Mediated Mitochondrial Fission Promotes Proliferation and Collagen Production by right Ventricular Fibroblasts in Experimental Pulmonary Arterial Hypertension. Front Physiol 2018;9:828. doi: https://doi.org/10.3389/fphys.2018.00828.
[144] Shi H, Yu Y, Liu X, Yu Y, Li M, Wang Y, et al. Inhibition of calpain reduces cell apoptosis by suppressing mitochondrial fission in acute viral myocarditis. Cell Biol Toxicol 2022;38:487–504. doi: https://doi.org/10.1007/s10565-021-09634-9.
[145] Shanmughapriya S, Langford D, Natarajaseenivasan K. Inter and Intracellular mitochondrial trafficking in health and disease. Ageing Res Rev 2020;62:101128. doi: https://doi.org/10.1016/j.arr.2020.101128.
[146] Qi X, Qvit N, Su YC, Mochly-Rosen D. A novel Drp1 inhibitor diminishes aberrant mitochondrial fission and neurotoxicity. J Cell Sci 2013;126:789–802. doi: https://doi.org/10.1242/jcs.114439.
[147] Liu W, Su C, Qi Y, Liang J, Zhao L, Shi Y. Brain-targeted heptapeptide-loaded exosomes attenuated ischemia-reperfusion injury by promoting the transfer of healthy mitochondria from astrocytes to neurons. J Nanobiotechnology 2022;20:242. doi: https://doi.org/10.1186/s12951-022-01425-6.
[148] Timmler S, Simons M. Grey matter myelination. Glia 2019;67:2063–70. doi: https://doi.org/10.1002/glia.23614.
[149] Nozais V, Forkel SJ, Petit L, Talozzi L, Corbetta M, Thiebaut de Schotten M, et al. Atlasing white matter and grey matter joint contributions to resting-state networks in the human brain. Commun Biol 2023;6:726. doi: https://doi.org/10.1038/s42003-023-05107-3.
[150] Mifsud G, Zammit C, Muscat R, Di Giovanni G, Valentino M. Oligodendrocyte pathophysiology and treatment strategies in cerebral ischemia. CNS Neurosci Ther 2014;20:603–12. doi: https://doi.org/10.1111/cns.12263.
[151] Kuhn S, Gritti L, Crooks D, Dombrowski Y. Oligodendrocytes in Development, Myelin Generation and beyond. Cells 2019;8. doi: https://doi.org/10.3390/cells8111424.
[152] Chen D, Huang Y, Shi Z, Li J, Zhang Y, Wang K, et al. Demyelinating processes in aging and stroke in the central nervous system and the prospect of treatment strategy. CNS Neurosci Ther 2020;26:1219–29. doi: https://doi.org/10.1111/cns.13497.
[153] Huang S, Ren C, Luo Y, Ding Y, Ji X, Li S. New insights into the roles of oligodendrocytes regulation in ischemic stroke recovery. Neurobiol Dis 2023;184:106200. doi: https://doi.org/10.1016/j.nbd.2023.106200.
[154] Naveed M, Smedlund K, Zhou QG, Cai W, Hill JW. Astrocyte involvement in metabolic regulation and disease. Trends Endocrinol Metab 2024. doi: https://doi.org/10.1016/j.tem.2024.08.001.
[155] Miyamoto N, Magami S, Inaba T, Ueno Y, Hira K, Kijima C, et al. The effects of A1/A2 astrocytes on oligodendrocyte linage cells against white matter injury under prolonged cerebral hypoperfusion. Glia 2020;68:1910–24. doi: https://doi.org/10.1002/glia.23814.

[156] Miyamoto N, Maki T, Shindo A, Liang AC, Maeda M, Egawa N, et al. Astrocytes Promote Oligodendrogenesis after White Matter damage via Brain-Derived Neurotrophic factor. J Neurosci 2015;35:14002–8. doi: https://doi.org/10.1523/jneurosci.1592-15.2015.
[157] Pang B, Dong G, Pang T, Sun X, Liu X, Nie Y, et al. Emerging insights into the pathogenesis and therapeutic strategies for vascular endothelial injury-associated diseases: focus on mitochondrial dysfunction. Angiogenesis 2024;27:623–39. doi: https://doi.org/10.1007/s10456-024-09938-4.
[158] Liu D, Liao P, Li H, Tong S, Wang B, Lu Y, et al. Regulation of blood-brain barrier integrity by Dmp1-expressing astrocytes through mitochondrial transfer. Sci Adv 2024;10:eadk2913. doi: https://doi.org/10.1126/sciadv.adk2913.
[159] Borst K, Dumas AA, Prinz M. Microglia: Immune and non-immune functions. Immunity 2021;54:2194–208. doi: https://doi.org/10.1016/j.immuni.2021.09.014.
[160] Fan PL, Wang SS, Chu SF, Chen NH. Time-dependent dual effect of microglia in ischemic stroke. Neurochem Int 2023;169:105584. doi: https://doi.org/10.1016/j.neuint.2023.105584.
[161] Anttila JE, Whitaker KW, Wires ES, Harvey BK, Airavaara M. Role of microglia in ischemic focal stroke and recovery: focus on Toll-like receptors. Prog Neuropsychopharmacol Biol Psychiatry 2017;79:3–14. doi: https://doi.org/10.1016/j.pnpbp.2016.07.003.
[162] Tajalli-Nezhad S, Karimian M, Beyer C, Atlasi MA, A.. Azami Tameh, the regulatory role of Toll-like receptors after ischemic stroke: neurosteroids as TLR modulators with the focus on TLR2/4. Cell Mol Life Sci 2019;76:523–37. doi: https://doi.org/10.1007/s00018-018-2953-2.
[163] Guo S, Wang H, Yin Y. Microglia Polarization from M1 to M2 in Neurodegenerative Diseases. Front Aging Neurosci 2022;14:815347. doi: https://doi.org/10.3389/fnagi.2022.815347.
[164] Long Y, Li XQ, Deng J, Ye QB, Li D, Ma Y, et al. Modulating the polarization phenotype of microglia - a valuable strategy for central nervous system diseases. Ageing Res Rev 2024;93:102160. doi: https://doi.org/10.1016/j.arr.2023.102160.
[165] Zhao SC, Ma LS, Chu ZH, Xu H, Wu WQ, Liu F. Regulation of microglial activation in stroke. Acta Pharmacol Sin 2017;38:445–58. doi: https://doi.org/10.1038/aps.2016.162.
[166] Cherry JD, Olschowka JA, O'Banion MK. Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. J Neuroinflammation 2014;11:98. doi: https://doi.org/10.1186/1742-2094-11-98.
[167] Qin C, Zhou LQ, Ma XT, Hu ZW, Yang S, Chen M, et al. Dual Functions of Microglia in Ischemic Stroke. Neurosci Bull 2019;35:921–33. doi: https://doi.org/10.1007/s12264-019-00388-3.
[168] Manu DR, Slevin M, Barcutean L, Forro T, Boghitoiu T, Balasa R. Astrocyte Involvement in Blood-Brain Barrier Function: a critical Update Highlighting Novel, complex, Neurovascular Interactions. Int J Mol Sci 2023;24. doi: https://doi.org/10.3390/ijms242417146.
[169] Korte N, Ilkan Z, Pearson CL, Pfeiffer T, Singhal P, Rock JR, et al. The Ca2+-gated channel TMEM16A amplifies capillary pericyte contraction and reduces cerebral blood flow after ischemia. J Clin Invest 2022;132. doi: https://doi.org/10.1172/jci154118.
[170] Buizza C, Enström A, Carlsson R, Paul G. The Transcriptional Landscape of Pericytes in Acute Ischemic Stroke. Transl Stroke Res 2024;15:714–28. doi: https://doi.org/10.1007/s12975-023-01169-x.
[171] Cai W, Liu H, Zhao J, Chen LY, Chen J, Lu Z, et al. Pericytes in Brain Injury and Repair after Ischemic Stroke. Transl Stroke Res 2017;8:107–21. doi: https://doi.org/10.1007/s12975-016-0504-4.
[172] Gautam J, Yao Y. Roles of Pericytes in Stroke Pathogenesis. Cell Transplant 2018;27:1798–808. doi: https://doi.org/10.1177/0963689718768455.
[173] Tuo QZ, Zhang ST, Lei P. Mechanisms of neuronal cell death in ischemic stroke and their therapeutic implications. Med Res Rev 2022;42:259–305. doi: https://doi.org/10.1002/med.21817.
[174] Gao L, Liu F, Hou PP, Manaenko A, Xiao ZP, Wang F, et al. Neurons Release Injured Mitochondria as "Help-me" Signaling after Ischemic Stroke. Front Aging Neurosci 2022;14:785761. doi: https://doi.org/10.3389/fnagi.2022.785761.
[175] Li X, Li H, Xu Z, Ma C, Wang T, You W, et al. Ischemia-induced cleavage of OPA1 at S1 site aggravates mitochondrial fragmentation and reperfusion injury in neurons. Cell Death Dis 2022;13:321. doi: https://doi.org/10.1038/s41419-022-04782-0.
[176] Dave KM, Stolz DB, Manickam DS. Delivery of mitochondria-containing extracellular vesicles to the BBB for ischemic stroke therapy. Expert Opin Drug Deliv 2023;20:1769–88. doi: https://doi.org/10.1080/17425247.2023.2279115.
[177] Pang B, Dong G, Pang T, Sun X, Liu X, Nie Y, et al. Advances in pathogenesis and treatment of vascular endothelial injury-related diseases mediated by mitochondrial abnormality. Front Pharmacol 2024;15:1422686. doi: https://doi.org/10.3389/fphar.2024.1422686.
[178] Im GB, Melero-Martin JM. Mitochondrial transfer in endothelial cells and vascular health. Trends Cell Biol 2025. doi: https://doi.org/10.1016/j.tcb.2025.04.004.
[179] Dave KM, Venna VR, Rao KS, Stolz DB, Brady B, Quaicoe VA, et al. Mitochondria-containing extracellular vesicles from mouse vs. human brain endothelial cells for ischemic stroke therapy. J Control Release 2024;373:803–22. doi: https://doi.org/10.1016/j.jconrel.2024.07.065.
[180] Dave KM, Zhao W, Hoover C, D'Souza A, S.m.. D, Extracellular Vesicles Derived from a Human Brain Endothelial Cell Line increase Cellular ATP Levels. AAPS PharmSciTech 2021;22:18. doi: https://doi.org/10.1208/s12249-020-01892-w.
[181] Zhang Z, Ma Z, Yan C, Pu K, Wu M, Bai J, et al. Muscle-derived autologous mitochondrial transplantation: a novel strategy for treating cerebral ischemic injury. Behav Brain Res 2019;356:322–31. doi: https://doi.org/10.1016/j.bbr.2018.09.005.
[182] Wei W, Wu D, Duan Y, Elkin KB, Chandra A, Guan L, et al. Neuroprotection by mesenchymal stem cell (MSC) administration is enhanced by local cooling infusion (LCI) in ischemia. Brain Res 2019;1724:146406. doi: https://doi.org/10.1016/j.brainres.2019.146406.
[183] Pourmohammadi-Bejarpasi Z, Roushandeh AM, Saberi A, Rostami MK, Toosi SMR, Jahanian-Najafabadi A, et al. Mesenchymal stem cells-derived mitochondria transplantation mitigates I/R-induced injury, abolishes I/R-induced apoptosis, and restores motor function in acute ischemia stroke rat model. Brain Res Bull 2020;165:70–80. doi: https://doi.org/10.1016/j.brainresbull.2020.09.018.
[184] Gorsky A, Monsour M, Nguyen H, Castelli V, Lee JY, Borlongan CV. Metabolic Switching of Cultured Mesenchymal Stem Cells creates Super Mitochondria in Rescuing Ischemic Neurons. NeuroMol Med 2023;25:120–4. doi: https://doi.org/10.1007/s12017-022-08720-3.
[185] Li Y, Wang Y, Yang W, Wu Z, Ma D, Sun J, et al. ROS-responsive exogenous functional mitochondria can rescue neural cells post-ischemic stroke. Front Cell Dev Biol 2023;11:1207748. doi: https://doi.org/10.3389/fcell.2023.1207748.
[186] Hayakawa K, Chan SJ, Mandeville ET, Park JH, Bruzzese M, Montaner J, et al. Protective Effects of Endothelial Progenitor Cell-Derived Extracellular Mitochondria in Brain Endothelium. Stem Cells 2018;36:1404–10. doi: https://doi.org/10.1002/stem.2856.
[187] Dai L, Wu Z, Yin L, Cheng L, Zhou Q, Ding F. Exogenous Functional Mitochondria Derived from Bone Mesenchymal Stem Cells that Respond to ROS can rescue Neural Cells following Ischemic Stroke. J Inflamm Res 2024;17:3383–95. doi: https://doi.org/10.2147/jir.S463692.
[188] Al Amir Dache Z, Chevé M, Dancourt J, Lavieu G. Quantitative cellular characterization of extracellular mitochondria uptake and delivery. Nat Commun 2025;16:9053. doi: https://doi.org/10.1038/s41467-025-64147-x.
[189] Maleki F, Rabbani S, Shirkoohi R, Rezaei M. Allogeneic mitochondrial transplantation ameliorates cardiac dysfunction due to doxorubicin: an in vivo study. Biomed Pharmacother 2023;168:115651. doi: https://doi.org/10.1016/j.biopha.2023.115651.
[190] Riou A, Broeglin A, Grimm A. Mitochondrial transplantation in brain disorders: Achievements, methods, and challenges. Neurosci Biobehav Rev 2025;169:105971. doi: https://doi.org/10.1016/j.neubiorev.2024.105971.
[191] Bodenstein DF, Siebiger G, Zhao Y, Clasky AJ, Mukkala AN, Beroncal EL, et al. Bridging the gap between in vitro and in vivo models: a way forward to clinical translation of mitochondrial transplantation in acute disease states. Stem Cell Res Ther 2024;15:157. doi: https://doi.org/10.1186/s13287-024-03771-8.
[192] Chang JC, Lin CH, Yeh CY, Cheng MF, Chen YC, Wu CH, et al. Intranasal Mitochondrial Transplantation Restores Mitochondrial Function and Modulates Glial-Neuronal Interactions in a Genetic Parkinson's Disease Model of UQCRC1 Mutation. Cells 2025;14. doi: https://doi.org/10.3390/cells14151148.
[193] Liao J, He W, Li L, Wang J, Gong L, Zhang Q, et al. Mitochondria in brain diseases: bridging structural-mechanistic insights into precision-targeted therapies. Cell Biomaterials 2025;1:100016. doi: https://doi.org/10.1016/j.celbio.2025.100016.
[194] Liao J, He W, Zhang S, Wang J, Cheng H, Gong L, et al. Magnetic field driven ceria nanosystems for mitochondria targeted therapy of ischemic stroke. Adv Funct Mater 2025;35:2423291. doi: https://doi.org/10.1002/adfm.202423291.
[195] Liao J, Huang Q, Li R, Gong L, Li T, Lin Z. Long-acting nanomedicine for brain diseases. J Control Release 2026;392:114665. doi: https://doi.org/10.1016/j.jconrel.2026.114665.
[196] Liao J, Li Y, Fan L, Sun Y, Gu Z, Xu Q-Q, et al. Bioactive ceria nanoenzymes target mitochondria in reperfusion injury to treat ischemic stroke. ACS Nano 2024;18:5510–29. doi: https://doi.org/10.1021/acsnano.3c10982.
[197] Garré JM, Bukauskas FF, Bennett MVL. Single channel properties of pannexin-1 and connexin-43 hemichannels and P2X7 receptors in astrocytes cultured from rodent spinal cords. Glia 2022;70:2260–75. doi: https://doi.org/10.1002/glia.24250.
[198] Scheiblich H, Eikens F, Wischhof L, Opitz S, Jüngling K, Cserép C, et al. Microglia rescue neurons from aggregate-induced neuronal dysfunction and death through tunneling nanotubes. Neuron 2024;112:3106–3125.e3108. doi: https://doi.org/10.1016/j.neuron.2024.06.029.
[199] Zhao Y, Jiang H, Yu J, Wang L, Du J. Engineered Histidine-Rich Peptides Enhance Endosomal Escape for Antibody-Targeted Intracellular delivery of Functional Proteins. Angew Chem Int Ed Engl 2023;62:e202304692. doi: https://doi.org/10.1002/anie.202304692.
[200] Du Rietz H, Hedlund H, Wilhelmson S, Nordenfelt P, Wittrup A. Imaging small molecule-induced endosomal escape of siRNA. Nat Commun 2020;11:1809. doi: https://doi.org/10.1038/s41467-020-15300-1.
[201] Chatterjee S, Kon E, Sharma P, Peer D. Endosomal escape: a bottleneck for LNP-mediated therapeutics. PNAS 2024;121:e2307800120. doi: https://doi.org/10.1073/pnas.2307800120.

[202] Hoffmann M, Gerlach S, Takamiya M, Tarazi S, Hersch N, Csiszár A, et al. Smuggling on the Nanoscale-Fusogenic Liposomes Enable Efficient RNA-Transfer with Negligible Immune Response In Vitro and In Vivo. Pharmaceutics 2023;15. doi: https://doi.org/10.3390/pharmaceutics15041210.

Rong Fu is a postgraduate student majoring in Pathophysiology at Shenyang Medical College, P.R. of China, under the supervision of professor Ming-Sheng Zhou. In June 2022, she completed her undergraduate studies in Xuzhou Medical University. Her research interests focus on mitochondrial transfer from neurons to microglia in acute ischemic stroke.

Yang Zhao is a PhD candidate at Shenyang Pharmaceutical University, under the supervision of professor Jingyu Yang. She obtained her undergraduate degree from Shenyang Pharmaceutical University in 2019, and at present, she is engaged in a combined master's and doctoral program. Her research focuses on the functional mitochondrial transfer between astrocyte and neuron following cerebral ischemic injury.



Yayi Li is a postgraduate student majoring in Pathophysiology at Shenyang Medical College, P.R. of China, under the supervision of associate professor Yueyang Liu. In June 2023, she completed her undergraduate studies in Sanquan College of Xinxiang Medical College. Her research interests focus on mitochondrial transfer from neurons to microglia in acute ischemic stroke.

Yuliang Zhang is a postgraduate student majoring in Pathophysiology at Shenyang Medical College, P.R. of China, under the supervision of professor Ming-Sheng Zhou. In June 2023, she completed her undergraduate studies in Sanquan College of Xinxiang Medical College. Her research interests focus on mitochondrial transfer from neurons to microglia in acute ischemic stroke.

Meidan Wang, Msc, a doctor in Department of Vasculocardiology, The Second Hospital of Shenyang Medical College, Shenyang, China. Her research interests focus on mitochondrial transfer from neurons to microglia in acute ischemic stroke.

Zhuo Wang is a postgraduate student majoring in Pathophysiology at Shenyang Medical College, P.R. of China. Her research interests focus on mitochondrial transfer from neurons to microglia in acute ischemic stroke.

Ming-Sheng Zhou, MD & Ph D, FAHA, Professor in Department of Physiology & Chief Scientist and Director in Science and Experimental Research Center in Shenyang Medical College, P.R. of China. Dr. Zhou has published over 70 articles in high-circulating journals and over 90 abstracts in international scientific conferences. His research interests focus on vascular biology and the molecular and cellular mechanisms of cardiovascular and cerebrovascular diseases. His research works have been supported by Chinese National Natural Science Foundation & American Heart Association. He currently serves as an associate editor in Cardiovascular Therapeutic Section, Frontier in Cardiovascular Medicine; reviewer editors in Renal Pharmacology Section of Frontier in Pharmacology & Inflammatory Section of Frontier in Immunology. In this review, he was the corresponding author and revised the article, and approved the final manuscript.

Yueyang Liu, PhD, Associate professor in Department of Pharmacology of Shenyang Medical College, P.R. of China. Dr. Liu has published numerous articles in outstanding journals, such as PNAS, Autophagy, Stroke, Acta Pharmaceutica Sinica B, and Pharmacology & Therapeutics and has received funding for various projects such as the National Natural Science Foundation of China. Her research focuses on the molecular mechanisms and targeted therapies for cerebrovascular diseases, with a particular emphasis on ischemic stroke. Currently, she holds positions as a junior editorial board member of Acta Materia Medica and a scientific editor of Military Medical Research. In this review, she was the corresponding author and revised the article, and approved the final manuscript.