

Review

Mitochondria Beyond Bioenergetics: Canonical and Non-Canonical Functions in Ischemic Stroke and Aging

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ABSTRACT: Ischemic stroke remains a leading cause of death and long-term disability worldwide, yet effective neuroprotective therapies beyond reperfusion strategies are still lacking. Emerging evidence highlights mitochondria as central regulators of neuronal fate, expanding their traditional role in cellular energy homeostasis through ATP production by oxidative phosphorylation (OXPHOS). Together with calcium buffering and redox regulation, these represent canonical mitochondrial functions. Mitochondria also regulate cellular adaptation to stress through mitochondrial quality control (MQC), mitochondrial ferritin-dependent iron buffering, signaling mediated by mitochondrial metabolites and mitochondrial DNA (mtDNA), and intercellular organelle transfer. These non-canonical functions link mitochondrial state to ferroptotic cell death, innate immune activation, and epigenetic regulation of nuclear responses. During cerebral ischemia, mitochondrial dysfunction involves impaired OXPHOS, the generation of reactive oxygen species (ROS), opening of the permeability transition pore, disruption of organelle dynamics, and dysregulation of non-canonical functions, collectively promoting neuronal injury, inflammation, and impaired recovery. These disturbances are further exacerbated by aging, which compromises MQC mechanisms, including fusion/fission balance, mitophagy, and biogenesis, and increases neuronal vulnerability. Mitochondrial signals, including mtDNA, cardiolipin, and ROS, also contribute to inflammasome activation and glial metabolic reprogramming, linking mitochondrial injury to neuroinflammation. Moreover, mitochondrial ferritin and iron buffering may influence oxidative damage and ferroptosis. Therefore, integrating canonical and non-canonical mitochondrial functions provide a broader framework for understanding ischemic injury and underscore the potential of MQC, redox regulation, iron homeostasis, and mitochondrial transfer as therapeutic targets and strategies for stroke recovery.

Keywords: Aging, Ischemic stroke, Mitochondrial dysfunction, Neuroinflammation, Mitochondrial quality control

Introduction

Ischemic stroke remains one of the leading causes of death and long-term disability worldwide, with a significant socioeconomic burden and limited therapeutic options beyond thrombolysis or thrombectomy [1]. Despite decades of research, neuroprotective strategies aimed at preserving neuronal viability have largely failed to translate into clinical applications [2–6]. This discrepancy

between preclinical and clinical efficacy has prompted a re-evaluation of the underlying mechanisms contributing to ischemic brain injury, with growing attention directed toward cellular organelles that govern not only energy production but also stress signaling, cell death, and neuroimmune crosstalk [7–14]. Mitochondria are traditionally known as the “powerhouses of the cell” because of their role in oxidative phosphorylation (OXPHOS) and ATP production [15]. Here, these bioenergetic functions, together with substrate oxidation

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through β -oxidation and the tricarboxylic acid (TCA) cycle, calcium uptake and buffering, redox homeostasis and reactive oxygen species (ROS) detoxification, heme and iron–sulfur cluster biosynthesis, amino acid and intermediary metabolism, are referred to as canonical mitochondrial functions. Collectively, these processes sustain cellular metabolic, ionic, and redox homeostasis. Alongside these direct homeostatic functions, mitochondria engage in regulatory, signaling, and adaptive processes referred to here as non-canonical. These include mitochondrial quality control (MQC), encompassing mitochondrial biogenesis, fusion/fission dynamics, and mitophagy, as well as the regulation of cell-fate and cell-death pathways, including apoptosis and the modulation of ferroptosis and pyroptosis. Non-

canonical functions also encompass mitochondria-to-nucleus retrograde signaling involving redox, metabolic, stress, and epigenetic responses; innate immune signaling driven by mitochondria-derived danger signals, including released mitochondrial DNA (mtDNA) and externalized or oxidized cardiolipin and intercellular mitochondrial transfer (Fig.1) [9, 16–24]. These processes are dynamically remodeled in response to injury, as reflected by changes in mitochondrial morphology, abundance, and contacts with other organelles [14, 25–28]. During cerebral ischemia, their disruption may influence cell death, inflammatory, and reparative responses, making the mechanisms governing mitochondrial plasticity and signaling potential therapeutic targets (Fig.1).

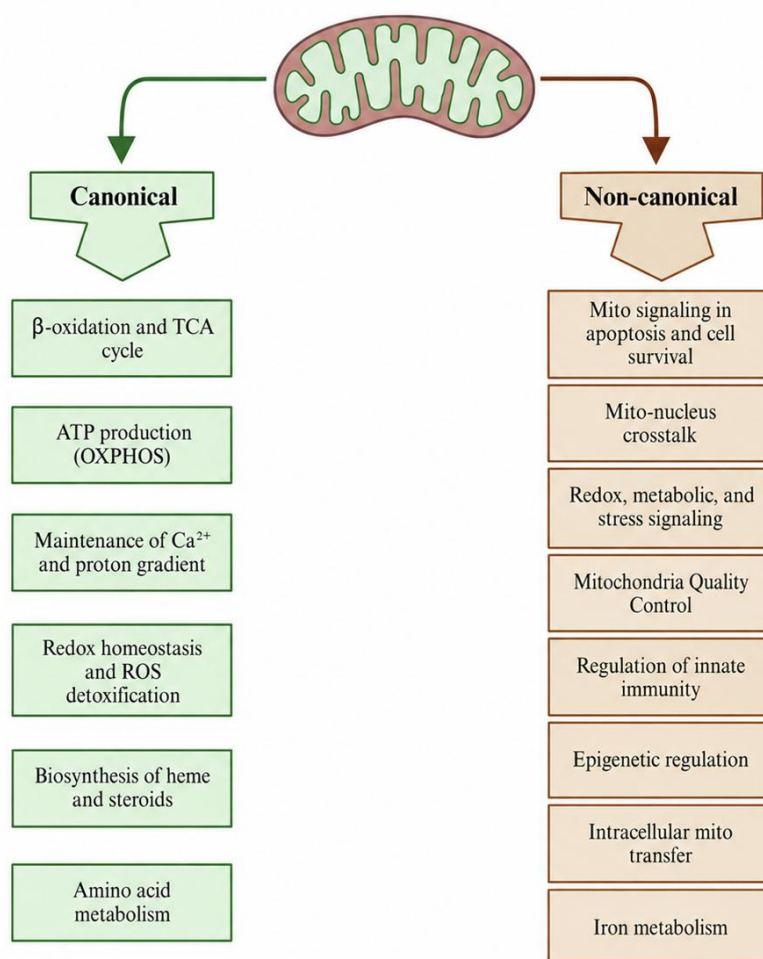


Figure 1. Canonical and non-canonical mitochondrial functions. Mitochondria play fundamental canonical functions in cellular bioenergetics and metabolism, including β -oxidation, the tricarboxylic acid (TCA) cycle, oxidative phosphorylation (OXPHOS), ATP production, redox homeostasis, and biosynthetic processes. Moreover, mitochondria perform non-canonical functions, including serving as signaling and regulatory centers involved in apoptosis and cell survival, mitochondrial quality control (MQC; dynamics and mitophagy), interactions between mitochondria and the cell nucleus, stress signaling, innate immunity, epigenetic regulation, intercellular mitochondrial transfer, and iron metabolism.

Ischemia induces a rapid collapse of the mitochondrial membrane potential, the generation of ROS, and the opening of the mitochondrial permeability transition pore (mPTP), leading to the release of pro-apoptotic factors [8]. Beyond these acute events, mitochondria play central roles in shaping long-term neuroinflammatory cascades, cell fate decisions, and supporting regenerative processes [29–36]. Particularly relevant are the mechanisms of MQC, including mitophagy, dynamics (fusion and fission), and biogenesis, which become progressively impaired with aging and under ischemic stress [10, 13, 14, 37–41]. Additionally, mitochondria act as key modulators of intercellular communication, enabling transfer of intact mitochondria between cells, notably from astrocytes to neurons, in a potentially reparative mechanism following stroke [25, 27, 42, 43].

This narrative review first outlines how ischemia-induced failure of canonical mitochondrial functions disrupts metabolic, ionic, and redox homeostasis, thereby engaging non-canonical responses. It then examines MQC, with particular emphasis on mitophagy, alongside

immunometabolism, mitochondrial iron handling and ferroptosis, mitochondria-nucleus crosstalk, and intercellular mitochondrial transfer. Building on these mechanisms, the review considers how aging modifies mitochondrial responses to ischemia and influences post-stroke injury, repair, and therapeutic responsiveness. Finally, it addresses the potential therapeutic relevance and translational limitations of targeting these pathways.

2. Mitochondrial dysfunction in ischemic stroke, pathophysiology

The pathophysiology of ischemic stroke involves a complex cascade of events triggered by the sudden disruption of cerebral blood flow [8, 44]. At the cellular level, mitochondria are among the first organelles affected, experiencing a rapid collapse in OXPHOS, loss of membrane potential ($\Delta\psi_m$), and deficiency of ATP production [45–50]. While this energetic crisis is central to early neuronal injury, mitochondria are also involved in amplifying oxidative and inflammatory damage (Fig. 2) [9, 20, 51, 52].

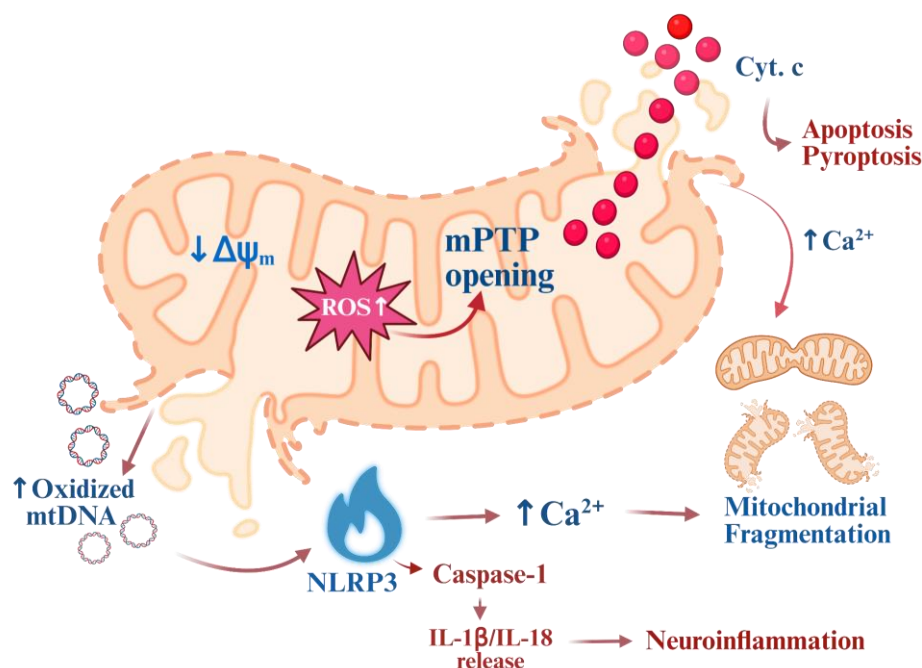


Figure 2. Mitochondrial dysfunction and inflammatory signaling in cerebral ischemia. Cerebral ischemia disrupts mitochondrial homeostasis through a cascade of related events, including the loss of mitochondrial membrane potential ($\Delta\psi_m$), reactive oxygen species (ROS) generation, intracellular Ca^{2+} accumulation, and opening of the mitochondrial permeability transition pore (mPTP). These changes impair oxidative phosphorylation and mitochondrial bioenergetics and promote mitochondrial dysfunction. Mitochondrial membrane permeabilization facilitates the release of cytochrome c (Cyt. c) and mitochondrial DNA (mtDNA) into the cytosol. While Cyt. c contributes to the activation of apoptotic signaling, oxidized mtDNA and ROS overload promote the activation of the NLRP3 inflammasome, which in turn, by activating caspase-1, leads to the maturation and release of interleukin-1 β (IL-1 β) and interleukin-18 (IL-18), thereby contributing to neuroinflammatory signaling. These interrelated processes exacerbate mitochondrial dysfunction and inflammatory responses following cerebral ischemia.

Within seconds-minutes of oxygen and glucose deprivation, neurons switch from efficient mitochondrial respiration to anaerobic glycolysis, leading to intracellular acidification and lactate (LA) accumulation. Simultaneously, the electron transport chain (ETC) becomes uncoupled, and mitochondrial ROS generation rapidly increases [53–57]. In particular, reperfusion after ischemia initiates a burst of superoxide ions (O_2^-) and hydrogen peroxide (H_2O_2) production, for example, at complex I and III of the ETC, a phenomenon largely attributed to reverse electron transport (RET) and succinate accumulation during ischemia [20, 48, 58, 59].

Among the ROS, the hydroxyl radical ($\cdot OH$) is one of the most toxic species, as it damages mtDNA, lipids, and proteins, and also activates signaling pathways that prompt cell death and neuroinflammation [60–65].

The mPTP, a high-conductance channel formed under pathological conditions, plays a critical role in ischemic injury. Its opening triggers mitochondrial matrix swelling, rupture of the outer membrane, and release of cytochrome c and other pro-apoptotic factors into the cytosol, activating caspase-dependent and independent cell death pathways (Fig. 3) [66–69].

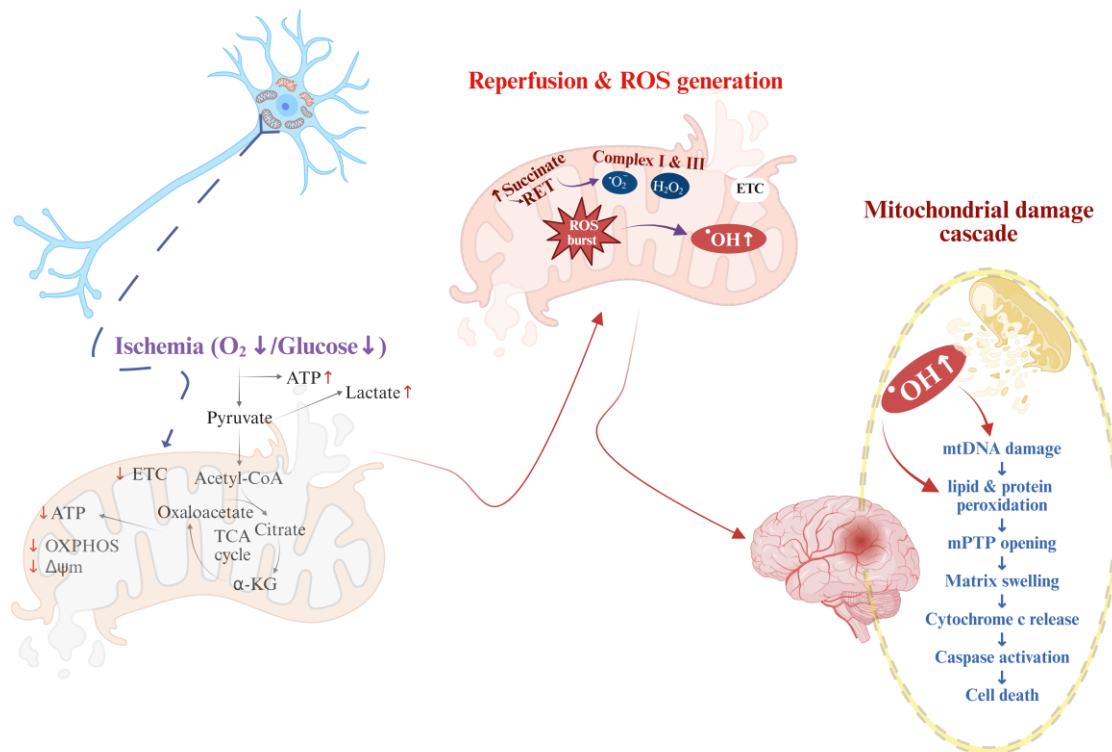


Figure 3. Mitochondrial dysfunction and ROS generation during ischemia-reperfusion. Ischemia impairs mitochondrial respiration, resulting in reduced electron transport chain (ETC) activity, decreased oxidative phosphorylation (OXPHOS), ATP depletion, and a metabolic shift toward increased glycolysis and lactate (LA) production. Reperfusion restores oxygen availability but promotes a rapid increase mitochondrial ROS generation, particularly at ETC complexes I and III, including through reverse electron transport (RET). Overproduction of ROS, including hydroxyl radicals ($\cdot OH$), contributes to oxidative damage to mitochondrial DNA (mtDNA), lipids, and proteins. These changes are associated with mitochondrial permeability transition pore (mPTP) opening, matrix swelling, and cytochrome c (Cyt. c) release, followed by caspase activation and cell death.

Mitochondrial function is further compromised by dysregulation of calcium homeostasis. Excess glutamate release and N-methyl-D-aspartate (NMDA) receptor activation promote massive Ca^{2+} influx into neurons, overloading mitochondria and triggering ROS generation through enhanced TCA cycle activity. This vicious cycle of oxidative stress and mitochondrial damage contributes to the expansion of the ischemic core and impairs recovery in the penumbra [8]. Importantly, ischemic stroke alters not only mitochondrial function but also the

distribution and morphology of the mitochondrial network [45, 70–73]. Fragmentation of mitochondria due to excessive fission, primarily mediated by dynamin-related protein 1 (Drp1), reduces interconnectivity and impairs metabolic efficiency [74–78]. Mitochondrial swelling, disruption of cristae, and altered contact with the endoplasmic reticulum (ER) have also been observed in experimental models of cerebral ischemia [7, 79]. Moreover, ischemic mitochondria release damage-associated molecular patterns (DAMPs), including

mtDNA and cardiolipin fragments, which may activate innate immune sensors such as the NLR family pyrin domain-containing 3 (NLRP3) inflammasome. This contributes to a self-propagating inflammatory response that amplifies tissue damage above the initial ischemic insult [31, 32, 80–84].

Mitochondrial-derived stress also engages retrograde signaling to the nucleus. The mitochondrial unfolded protein response (UPR_{mt}), although less well characterized in the brain than in other tissues, is believed to play a role in the transcriptional adaptation to mitochondrial dysfunction, involving activating transcription factor 4 (ATF4) and activating transcription factor 5 (ATF5), and C/EBP homologous protein (CHOP) [85–90]. This may serve both protective and maladaptive roles, depending on context and duration of activation.

Mitochondria also serve as a central regulator of intracellular iron metabolism [91]. Mitochondrial ferritin (FtMt), an iron-storage protein localized within the mitochondrial matrix, has been linked to buffering of redox-active iron and reduction of ROS-driven lipid peroxidation [92].

Notably, mitochondrial susceptibility to ischemic stress is modulated by age, sex, and metabolic

comorbidities [93–97]. Aging is characterized by a decline in mitochondrial density, reduced respiratory reserve, and impaired antioxidant defense, all of which may exacerbate injury and limit endogenous repair. Furthermore, age-related loss of mitochondrial membrane fluidity and cardiolipin integrity disrupts cristae structure and compromises the activity of key respiratory complexes [98–101].

Taken together, these findings show that ischemic stroke disrupts canonical mitochondrial functions, as manifested by bioenergetic failure, dysregulated calcium handling, and loss of redox balance. These homeostatic disturbances are closely intertwined with changes in non-canonical mitochondrial processes, including MQC, iron buffering, stress and immune signaling, and cell-death regulation, which are discussed in the following sections. Table 1 summarizes the key mitochondrial functions, molecular pathways, aging-related changes, and potential therapeutic relevance. Age-related evidence is limited for several mechanisms and may derive from non-stroke models or studies without direct young-aged comparisons.

Table 1. Canonical and non-canonical mitochondrial functions in ischemic stroke and their modification by aging.

Class/function	Key mediators/pathways	Affected cell types	Aging-related changes	Therapeutic relevance	References
Canonical: Bioenergetics	OXPHOS, β -oxidation, TCA cycle, ETC and substrates: CoQ and cyt. c	Primarily neurons; also reported in astrocytes, microglia/macrophages, oligodendrocytes, and endothelial cells.	Reduced respiratory capacity and metabolic flexibility limit ATP recovery after ischemia; aged animals show persistent acylcarnitine accumulation and glycolytic remodeling.	Preservation of substrate oxidation, ETC function, mitochondrial $\Delta\psi_m$, and NAD ⁺ availability can sustain ATP production and metabolic recovery.	[45–50, 93–101, 108]
Canonical: Ca²⁺/mPTP	Ca ²⁺ uptake, mPTP, OPA1 and cyt. c	Primarily neurons; also reported in astrocytes, microglia and endothelial cells.	Reduced cardiolipin and OPA1 content with increased cardiolipin peroxidation; direct evidence for age-related changes in mitochondrial Ca ²⁺ handling and mPTP sensitivity remains limited.	Preventing mitochondrial Ca ²⁺ overload and the permeability transition to preserve $\Delta\psi_m$, cristae architecture, and ATP production, and to limit infarct expansion.	[8, 66–69, 98–101]
Canonical: Redox	ROS generation: ETC/RET Antioxidant systems: NRF2 signaling, GSH	Neurons and oligodendrocytes are primary targets of mitochondrial oxidative injury; glial, vascular, and infiltrating immune cells modulate ROS production and redox signaling.	Reduced antioxidant capacity and increased oxidative damage to macromolecules with lower cardiolipin content and complex I activity, and higher cardiolipin peroxidation.	Restraining excessive ETC/RET-derived ROS and reinforcing antioxidant systems can limit cardiolipin, mtDNA, protein, and lipid oxidation; preserve ETC and reduce oxidative injury in neurons and the neurovascular unit.	[20, 48, 58–65, 98–101, 120, 121]

Canonical: Iron metabolism	FtMt, heme/Fe-S, ETC, MPs, and mitochondrial labile Fe ²⁺ pool	Astrocytes transfer mitochondria/mitochondrial cargo to neurons, endothelial cells, and pericytes; activated microglia transfer mitochondria to neurons.	Aging promotes cortical cytosolic and mitochondrial non-heme iron accumulation, associated with increased HAMP and decreased FTM-1, suggesting impaired iron export. Evidence for age-related changes in heme or Fe-S cluster synthesis is lacking.	Restoring balanced mitochondrial iron availability can sustain heme and Fe-S cluster synthesis, aconitase, ETC activity, and ATP production, while limiting the labile Fe ²⁺ pool that promotes Fenton reaction and lipid peroxidation.	[91, 214, 218, 219, 225, 228]
Non-canonical: MQC	AMPK/SIRT1/PGC-1 α /NRF1/TFAM; MFN1/2, OPA1, Drp1; PINK1/Parkin	Primarily neurons; also reported in astrocytes, microglia/macrophages, oligodendrocytes, and endothelial cells.	Reduced OPA1 and PGC-1 α signaling, increased mitochondrial fragmentation, altered dynamics and mitophagy (region- and cell type-specific).	Restoring MQC promotes biogenesis, preserves mitochondrial integrity, enhances mitophagy, and improves neuronal survival and functional recovery.	[10, 13, 14, 37–41, 93, 104–116, 164–180]
Non-canonical: Immune signaling	mtDNA, cardiolipin, ROS, NLRP3/cGAS-STING/TLR9/TXNIP signaling	Neurons; microglia and monocyte-derived macrophages (major responders); astrocytes and endothelial cells (additional responders).	Age-related mitochondrial disruption and cytosolic mtDNA accumulation are associated with cGAS–STING activation. TXNIP-dependent inflammasome activity also increases with brain aging.	Suppress mtDAMP/NLRP3-cGAS-STING-TLR9-TXNIP signaling to reduce sterile inflammation and secondary injury; promote reparative microglial/macrophage metabolism to enhance debris clearance, remyelination, and tissue repair.	[31, 60, 80–84, 122, 123, 183–201]
Non-canonical: Iron buffering and ferroptosis regulation	Ferritin, labile Fe ²⁺ /Fe ³⁺ , Fenton reaction, GSH/GPX4, ACSL4	Primarily neurons; also reported in oligodendrocytes and endothelial cells.	Increased non-heme iron, higher HAMP and reduced FPN1; altered FtMt regulation/function in the aged ischemic brain remains unknown.	Enhancing FtMt-dependent buffering of labile mitochondrial Fe ²⁺ can restrict Fenton reaction and lipid peroxidation, preserve GSH/GPX4-dependent antioxidant defense, and reduce ferroptotic injury in neurons and brain endothelial cells.	[18, 19, 214–230]
Non-canonical: Mitochondria-nucleus signaling	NAD ⁺ /sirtuins, AMPK, PGC-1 α , UPRmt	Primarily neurons and microglia/macrophages, also reported in astrocytes and endothelial cells.	Aging is associated with declining NAD ⁺ availability, altered sirtuin signaling, decreased PGC-1 α signaling, reduced mtDNA content/maintenance; age-related changes in UPRmt, metabolite-dependent chromatin signaling, and HDACs remain unclear.	Enhancing communication between mitochondria and the nucleus supports proteostasis, antioxidant defense, biogenesis, and metabolic adaptation, improving neuronal resilience and recovery.	[85–90, 117–119, 231–282]
Non-canonical: Mitochondrial transfer	CD38/cADPR; LRP1/ARF1 lactylation; RHOT1/Miro1/PGC-1 α	Astrocytes to neurons, endothelial cells, and pericytes; pro-inflammatory microglia to neurons; MSCs to neurons and cerebral endothelial cells.	Limited evidence; increased astrocyte-derived mitochondrial signal in cerebral capillaries in young adult mice (7 vs. 3 months).	Enhancing mitochondrial transfer restores bioenergetics, reduces oxidative stress and apoptosis, stabilizes the BBB, and promotes repair; limiting damaged-mitochondria transfer may prevent dysfunction spread.	[25, 27, 42, 43, 283–310]

Abbreviations: AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; BBB, blood–brain barrier; cADPR, cyclic ADP-ribose; DAMP, damage-associated molecular pattern; Drp1, dynamin-related protein 1; ER, endoplasmic reticulum; ETC, electron transport chain; FPN1, ferroportin; FtMt, mitochondrial ferritin; GSH, glutathione; HAMP, hepcidin; HDAC, histone deacetylase; IMT, intercellular mitochondrial transfer; MCAO, middle cerebral artery occlusion; MQC, mitochondrial quality control; mtDNA, mitochondrial DNA; mPTP, mitochondrial permeability transition pore; NAD⁺, nicotinamide adenine dinucleotide; NLRP3, NLR family pyrin domain-containing 3; NRF2, nuclear factor erythroid 2-related factor 2; OPA1, optic atrophy protein 1; OXPHOS, oxidative phosphorylation; PGC-1 α , peroxisome proliferator-activated receptor- γ coactivator 1 α ; PINK1, PTEN-induced kinase 1; RET, reverse electron transport; ROS, reactive oxygen species; STING, stimulator of interferon genes; TCA, tricarboxylic acid; TFAM, mitochondrial transcription factor A; TNT, tunneling nanotube; UPRmt, mitochondrial unfolded protein response; $\Delta\psi_m$, membrane potential.

3. Aging as a modifier of mitochondrial responses to ischemic stroke

Aging is the strongest non-modifiable risk factor for ischemic stroke. It is associated with profound changes in mitochondrial homeostasis that may influence both susceptibility to ischemic damage and recovery from stroke. This part of our review focuses on how aging modifies both canonical and non-canonical mitochondrial functions and thereby modifies the response to ischemic stroke. Age-related mitochondrial dysfunction extends beyond impaired bioenergetics and includes changes in MQC, dynamics, redox regulation, metabolism, nicotinamide adenine dinucleotide (NAD⁺)-dependent signaling, immunometabolism, iron handling, proteostasis, and intercellular mitochondrial transfer (IMT). The accumulation of mtDNA damage, reduced OXPHOS efficiency, impaired mitochondrial turnover, and diminished antioxidant capacity may progressively decrease the mitochondrial reserve of the aging brain even before ischemic damage occurs. Consequently, mitochondria in aging cells may have a limited capacity to maintain homeostasis and adapt to the additional metabolic and oxidative stress induced by ischemia and reperfusion. Therefore, mitochondrial aging may represent an important link between increased susceptibility to ischemic injury and decreased capacity for post-stroke repair and recovery. However, the strength of evidence varies substantially across mechanisms. Direct comparisons between young and aged animals in experimental stroke are available for only a subset of these non-canonical mitochondrial processes.

Moreover, aging may impair MQC by disrupting the coordination of mitochondrial biogenesis, dynamics, and mitophagy. Reduced turnover and inefficient removal of damaged mitochondria may impede the restoration of mitochondrial integrity and function following ischemia/reperfusion, thereby reducing mitochondrial resilience to ischemic stress [102]. Recent evidence showed that expression of optic atrophy 1 (OPA1), a mitochondrial and cristae-organizing protein declined with age and was further reduced after stroke. In aged mice subjected to distal middle cerebral artery occlusion (dMCAO), neuron-specific OPA1 deletion exacerbated mitochondrial cristae disruption, infarct expansion, and neuronal loss, whereas translocase of inner mitochondrial membrane 8B (TIMM8B) overexpression partially restored OPA1-dependent cristae organization and reduced injury [103]. In a separate study of aged female mice subjected to cerebral ischemia/reperfusion, mitofusin 2 (MFN2) and Drp1 protein content were reduced in the ischemic cortex, while exercise preconditioning increased both proteins and improved neurological outcomes [104]. Supporting evidence

outside ischemia further shows age-related increases of fission 1 protein (Fis1) and Drp1, higher mitochondrial fragmentation in astrocytes, and reduced OPA1 and translocase of outer mitochondrial membrane 20 (TOMM20) with increased Drp1 immunoreactivity in human motor-cortex neurons [105–107]. Collectively, these findings suggest that aging alters mitochondrial dynamics in a region- and cell-dependent manner, potentially limiting structural recovery after cerebral ischemia, however, direct age-comparative measurements of fusion and fission after stroke remain limited.

Direct studies evaluating whether and how aging modifies mitochondrial biogenesis after ischemic stroke remain sparse. In 18–20-month-old male mice subjected to permanent distal MCAO (pdMCAO) combined with 45 min of hypoxia, formoterol initiated 24 h after stroke increased mtDNA copy number and mitochondrial transcription factor A (TFAM) in the infarct region. These changes were accompanied by reduced acylcarnitine and lipid accumulation, consistent with enhanced mitochondrial biogenesis and lipid processing [108]. However, the absence of a young comparison group precludes direct assessment of age-dependent impairment, although the findings suggest that mitochondrial biogenesis remains pharmacologically inducible in the aged post-stroke brain. Complementary evidence from non-ischemic models indicates age-related changes in mitochondrial biogenesis that may influence mitochondrial resilience to ischemic stress. In male mice, aging from 10 to 30 months was accompanied by a progressive decline in the cortical expression of multiple peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α) isoforms, including neuron-specific brain-derived variants [109]. Consistent with this pattern, separate rodent studies identified age-related reductions in PGC-1 α protein across the cortex, substantia nigra, and hippocampus, variably accompanied by lower nuclear respiratory factor 1 (NRF1) and TFAM content, citrate synthase activity, and mtDNA content [110–112]. In the human post-mortem brain, mtDNA copy number declined with age in the frontal cortex but not the cerebellum, whereas mtDNA deletions accumulated in both regions [113]. Collectively, these findings indicate region and context-dependent remodeling of mitochondrial biogenesis and mtDNA maintenance with age, with converging evidence for a gradual decline in key biogenesis-related pathways across multiple brain regions. Evidence regarding the impact of aging on the mitophagy response after ischemic stroke remains limited, as available studies largely examine interventions in aged animals rather than directly comparing post-ischemic mitophagy across age groups. In aged female mice subjected to cerebral ischemia/reperfusion, nine weeks of exercise

preconditioning increased cortical protein content of BCL2-interacting protein 3-like (BNIP3L/NIX), Parkin, autophagy-related protein 7 (ATG7), microtubule-associated protein 1 light chain 3 (LC3) II and sequestosome 1 (p62/SQSTM1), as well as the LC3 II/I ratio, at 12 h after injury. These changes were accompanied by reduced infarct volume and neurological deficits [104]. However, the single-time-point measurements cannot establish enhanced mitophagy flux or a causal contribution to neuroprotection. Complementary evidence from non-stroke models indicates that aging alters mitophagy in region and cell-type-specific trajectories. For example, mt-Keima (mitochondria-targeted, pH-sensitive reporter of mitochondrial delivery to acidic lysosomes) imaging showed lower lysosomal mitophagy signal in cortical neurons and astrocytes of 18–20-month-old male mice compared with young animals, although astrocytes maintained higher mitophagy than neurons in both ages [114]. Other studies similarly indicate region-specific and age-dependent changes in basal and inducible mitophagy, including altered PTEN-induced kinase 1 (PINK1)/Parkin signaling and mitochondrial clearance [93, 115, 116]. Collectively, aging appears to differentially affect mitochondrial damage recognition and clearance across brain regions and cell types, whereas how these changes modify the mitophagy response to ischemic stroke remains unresolved.

Age-related changes in NAD⁺ metabolism and sirtuin signaling pathways are key factors determining mitochondrial resilience. NAD⁺ concentration decreases with aging, which may restrict the activity of NAD⁺-dependent sirtuins that link cellular energy status to mitochondrial metabolism, antioxidant defense, and stress adaptation. Moreover, NAD⁺-dependent pathways are also closely linked to the regulation of inflammation and epigenetics, serving as a mechanistic link between mitochondrial aging and broader cellular dysfunction [117]. In particular, sirtuin 1 (SIRT1) regulates transcription pathways associated with mitochondrial biogenesis and cellular stress response, while the mitochondrial sirtuin 3 (SIRT3) regulates protein acetylation, oxidative metabolism, and redox homeostasis [118]. Reduced NAD⁺ availability and altered sirtuin activity may consequently impair the ability of aging mitochondria to adapt to the energetic and oxidative challenges associated with ischemia/reperfusion. This may be particularly meaningful during reperfusion, when the restoration of oxygen availability is accompanied by intensified mitochondrial ROS generation. A study on cerebral ischemia has shown that ischemia itself depletes mitochondrial NAD⁺ and that restoration of NAD⁺ availability may diminish mitochondrial protein hyperacetylation, ROS generation, and mitochondrial

fragmentation through SIRT3-dependent mechanisms [119]. Therefore, age-related impairment of NAD⁺-sirtuin signaling may reduce mitochondrial resilience to ischemic stress and shift the balance from adaptive redox signaling toward oxidative damage. These alterations may also promote inflammatory signaling, providing a mechanistic link between mitochondrial dysfunction and the development of inflammaging.

It is also worth noting that the redox environment of the aged brain further contributes to mitochondrial vulnerability. Thus, aging is associated with aggravated oxidative damage, accumulation of oxidized macromolecules, and reduced efficiency of endogenous antioxidant systems [120]. At the same time, overproduction of ROS not only generates harmful byproducts but also participates in redox signaling and the regulation of cellular adaptation [121]. The age-related shift toward a less effective redox control system may therefore alter both the magnitude and duration of mitochondrial responses to ischemia/reperfusion. Additionally, when oxidative burden generated during reperfusion is imposed on this pre-existing redox imbalance, mitochondrial proteins, lipids, and mtDNA may become more susceptible to oxidative modifications [120]. Therefore, aging may lower the threshold above which physiological mitochondrial ROS signaling shifts toward chronic oxidative stress and pathophysiological dysfunction of mitochondria.

Aging is associated with chronic low-grade inflammation, oxidative stress, and dysregulation of immune response, collectively contributing to the state of inflammaging. In the aged brain, altered basal activity of microglia, astrocytes, and endothelial cells, as well as peripheral immune pathways, may create an inflammatory environment even before a stroke occurs [122]. In this way, mitochondria may both respond to and contribute to this inflammatory state through ROS generation and the release of mitochondrial components, including mtDNA [123]. Aging may therefore influence not only the magnitude of mitochondrial dysfunction following ischemia but also the inflammatory response that develops in parallel. Moreover, the interaction between mitochondrial stress and inflammaging may contribute to prolonged inflammatory signaling and delayed restoration of cellular homeostasis after stroke.

Another important non-canonical role of mitochondria is related to age-associated alterations in iron homeostasis, which may provide an additional link between mitochondrial dysfunction and ischemic insult. Iron serves as an essential cofactor for numerous enzymes and is involved in numerous physiological processes fundamental to cellular function, including oxygen transport, DNA synthesis and repair, and mitochondrial energy metabolism [124]. Mitochondria play a central

role in cellular iron utilization, heme synthesis, and the biogenesis of iron-sulfur clusters, making mitochondrial function highly dependent on adequate iron availability and regulation. Age-related alterations in iron homeostasis may increase the availability of redox-active iron and the susceptibility of cells to oxidative damage. An expanded labile iron pool may promote ROS generation through iron-catalyzed reactions, including the Fenton reaction, thereby increasing oxidative damage to cellular components and potentially contributing to cellular senescence. When imposed on the metabolic and redox disturbances induced by ischemia–reperfusion, impaired iron homeostasis may further exacerbate lipid peroxidation and increase susceptibility to iron-dependent cell death, including ferroptosis. Moreover, disruption of mitochondrial iron homeostasis may impair mitochondrial function and contribute to age-related mitochondrial dysfunction [125]. Thus, age-related alterations in iron homeostasis may further reduce mitochondrial resilience and contribute to the vulnerability of the aged brain to oxidative and metabolic injury.

Importantly, the effects of mitochondrial aging extend beyond neurons to the entire neurovascular unit. Mitochondrial dysfunction in endothelial cells, pericytes, astrocytes, and vascular smooth muscle cells may influence vascular tone, endothelial function, blood–brain barrier (BBB) integrity, cerebral blood flow, and metabolic coupling between vascular and neural cells. Since aging itself is associated with progressive impairment of neurovascular function, age-related mitochondrial dysfunction may further reduce the neurovascular unit's capacity to respond to ischemia and restore homeostasis after reperfusion [126]. This vascular aspect is particularly relevant to ischemic stroke, as mitochondrial dysfunction may affect both the metabolic support of neural tissue and the vascular processes required for cerebral perfusion and neurovascular repair [127].

The consequences of age-related mitochondrial dysfunction likely extend beyond the acute phase of ischemic damage and affect the ability to recover after a stroke. Recovery requires significant metabolic flexibility and coordinated mitochondrial function to support synaptic remodeling, axonal plasticity, angiogenesis, neurogenesis, and the restoration of neurovascular interactions [128]. Aging is accompanied by reduced regenerative capacity and impaired neuroplasticity [129], while persistent mitochondrial dysfunction, oxidative stress, impaired MQC, and chronic inflammatory signaling may further limit the cellular energy and signaling necessary for these processes [130].

Taken together, aging should be considered a fundamental modifier of canonical and non-canonical

mitochondrial responses to ischemic stroke rather than simply an additional risk factor. The aged brain enters ischemia with reduced mitochondrial energy reserve, disturbed MQC, altered NAD⁺-sirtuin and redox signaling, increased inflammatory tone, and disturbed metabolic and iron homeostasis. Ischemia/reperfusion imposes an additional stress on this already vulnerable system, potentially resulting in a more persistent state of mitochondrial dysfunction and impaired cellular adaptation. These age-dependent changes provide a mechanistic framework linking mitochondrial aging with increased susceptibility to ischemic injury and reduced post-stroke repair. Understanding how biological age modifies mitochondrial responses may therefore be essential for developing mitochondrial-targeted interventions that are effective across different stages of the lifespan.

4. Mitochondrial quality control systems and ischemic sensitivity

Mitochondria are highly dynamic organelles that maintain their function through continuous remodeling, repair, and degradation processes collectively referred to as MQC [131]. These include mitochondrial biogenesis, dynamics, and selective autophagic degradation (mitophagy). In the context of ischemic stroke, MQC mechanisms are disrupted, compromising the ability of cells to restore mitochondrial integrity and metabolic homeostasis [40].

One of the central regulators of mitochondrial biogenesis is PGC-1 α , which coordinates the transcription of nuclear-encoded mitochondrial proteins, antioxidant enzymes, and components of oxidative phosphorylation. PGC-1 α activity is modulated by AMP-activated protein kinase (AMPK) and NAD⁺-dependent SIRT1, forming a metabolic-sensing axis that is sensitive to cellular energy status and redox balance [132, 133]. While mitochondrial biogenesis replenishes the organelle pool, fusion and fission continuously remodel the resulting network by redistributing contents and segregating damaged components for mitophagy. Fusion and fission events regulate mitochondrial morphology and facilitate the distribution of mtDNA, proteins, and lipids across the mitochondrial network. Under physiological conditions, mitochondrial fusion promotes complementation between disrupted and functional mitochondria, stabilizes cristae structure, and supports metabolic efficiency [134]. This process is coordinated by mitofusin 1 (MFN1) and MFN2 on the outer mitochondrial membrane, and OPA1 on the inner membrane. MFN1 is essential for the initial tethering and merging of outer membranes, while MFN2 contributes to ER-mitochondria tethering and calcium homeostasis [135]. OPA1 not only mediates inner membrane fusion but also maintains cristae integrity,

thereby protecting against cytochrome c release and apoptosis [136]. Downregulation of these fusion proteins has been associated with impaired mitochondrial connectivity and increased vulnerability to neuronal death following ischemia [137].

Conversely, mitochondrial fission is mediated primarily by Drp1, a cytosolic GTPase that translocates to the outer membrane upon activation [138]. There, Drp1 is recruited to fission sites through interactions with outer mitochondrial membrane receptors, primarily mitochondrial fission factor (MFF), Fis1, mitochondrial dynamics proteins 49 and 51 (MiD49, and MiD51), which regulate its recruitment and assembly, whereas the contribution of Fis1 to basal mitochondrial fission is less clearly defined and appears to become more relevant under cellular stress [139, 140]. Ischemic stress induces hyperactivation of Drp1 *via* phosphorylation at Ser616 and dephosphorylation at Ser637, resulting in excessive fragmentation, mitochondrial depolarization, and ROS generation [141, 142].

The balance between fusion and fission is critical for maintaining mitochondrial structure and function [143]. Disruption of this stability during ischemic stroke not only impairs mitochondrial bioenergetics but also interferes with mitophagy, calcium and iron buffering, and cell death signaling [7, 22, 73, 77, 144, 145]. Moreover, imbalanced MQC may trigger a sterile innate immune response, contributing to inflammation during both the acute and chronic phases of ischemic stroke [31, 60, 146]. A-kinase anchoring protein 1 (AKAP1) anchors protein kinase A (PKA) at the outer mitochondrial membrane, where it phosphorylates Drp1 at Ser637 to inhibit its fission activity [147, 148]. Loss of AKAP1 leads to mitochondrial fragmentation, ROS generation, decreased membrane potential, and impaired complex II of the ETC [78, 149]. In models of transient middle cerebral artery occlusion (tMCAO), AKAP1 knockout mice show larger infarct volumes and reduced neuronal survival [75, 150]. Conversely, deletion of the mitochondria-localized Drp1 activator neuron-specific B β 2 regulatory subunit of protein phosphatase 2A (PP2A/B β 2) maintains inhibitory Drp1 phosphorylation at Ser637, reduces infarct volume, and improves neuronal survival [150]. Together, these findings identify phosphorylation-dependent control of Drp1 as an important determinant of ischemic sensitivity. Despite this evidence from genetically modified mouse models, no selective pharmacological modulator of AKAP1 has been evaluated in cerebral ischemia.

In the absence of direct AKAP1 modulators, pharmacological approaches have focused on limiting excessive mitochondrial fission by targeting Drp1. A mitochondrial division inhibitor 1 (Mdivi-1) reduced infarct volume and neurological injury in rat MCAO models when administered either before ischemia or

following ischemia immediately before reperfusion [151, 152]. However, Mdivi-1 can reversibly inhibit mitochondrial complex I and alter ROS production independently of Drp1, preventing unambiguous attribution of its neuroprotective effects to inhibition of mitochondrial fission [153]. A more interaction-selective approach is provided by P110, which disrupts the stress-induced interaction between Drp1 and Fis1 [76]. Systemic P110 administered before tMCAO reduced acute infarction and neurological deficits, whereas exosome-mediated delivery at reperfusion increased carrier accumulation in ischemic brain tissue and improved acute outcomes compared with the free peptide [154, 155]. SC9, a small-molecule mimetic developed to reproduce the inhibitory action of P110, reduced Fis1-associated Drp1 dysfunction in cellular and endotoxemia models but has not yet been evaluated in experimental stroke models [141].

Translational evidence remains limited. Studies with Mdivi-1 and P110 were conducted exclusively in otherwise healthy male rodents, without evaluation of aged animals, females, or common stroke comorbidities. Treatment was either preventive or administered in close temporal association with controlled experimental reperfusion, and most outcomes were assessed during the acute phase. Interactions with thrombolysis or thrombectomy were not examined, and quantitative brain pharmacokinetics were not reported for Mdivi-1 or free P110. Free P110 is also susceptible to proteolysis and lacks oral bioavailability, while SC9, although developed to address these limitations, showed a brain-to-plasma ratio of approximately 2% and a plasma half-life below 0.5 h in non-ischemic mice [141]. None of these approaches has entered clinical evaluation in ischemic stroke. Therefore, current findings support excessive Drp1-dependent fission as a pharmacological proof of concept, but its therapeutic efficiency and clinical translatability remain to be established.

Another mechanism through which MQC prevents the accumulation of dysfunctional organelles and the release of pro-apoptotic and pro-inflammatory signals is mitophagy, the selective elimination of dysfunctional mitochondria *via* autophagosomes. The PINK1 pathway, among others, is a well-characterized mitophagy pathway that becomes activated upon mitochondrial depolarization [156].

Altogether, the MQC system represents a critical interface between mitochondrial integrity and cellular homeostasis during cerebral ischemia. In this context, mitochondrial dysfunction in ischemic stroke should not be viewed solely as a source of oxidative damage, but rather as a dynamic hub of redox signaling. ROS, particularly those generated *via* RET during reperfusion, may modulate MQC, inflammasome activation, and

metabolic reprogramming. In this way, the mitochondrial redox landscape integrates bioenergetic failure with immune and epigenetic responses, thereby shaping both acute neuronal injury and long-term regenerative responses. These observations further support the concept that disruption of MQC pathways represents a central mechanism linking mitochondrial dysfunction with ischemic sensitivity and post-stroke neurodegeneration.

5. Mitophagy in stroke: A double-edged process

Mitophagy is a specialized form of autophagy that selectively removes dysfunctional mitochondria to maintain cellular homeostasis. This MQC mechanism is crucial in neurons, which rely heavily on mitochondrial integrity to meet high metabolic demands and maintain calcium and iron homeostasis. In ischemic stroke, mitophagy emerges as a context-dependent modulator of injury and recovery and may become detrimental if clearance is insufficient, excessive, or uncoupled from mitochondrial biogenesis [22, 40, 143, 157, 158]. The best-characterized pathway of mitophagy involves PINK1 and the E3 ubiquitin ligase Parkin. Under physiological conditions, PINK1 is constitutively imported into mitochondria and degraded [156, 157, 159]. Upon mitochondrial depolarization, PINK1 accumulates on the outer mitochondrial membrane and recruits cytosolic Parkin, which ubiquitinates outer membrane proteins such as voltage-dependent anion channel 1 (VDAC1) and MFN2, targeting the mitochondrion for autophagic engulfment [160–162]. This pathway is rapidly activated in neurons following ischemic injury, particularly during reperfusion when ROS generation peaks and mitochondrial membrane potential collapses [163].

Several pharmacological agents have been shown to modulate PINK/parkin-dependent mitophagy after cerebral ischemia. Rapamycin reduced infarct size and preserved mitochondrial function in tMCAO, accompanied by increased recruitment of autophagy-related proteins and ultrastructural evidence of mitochondrial sequestration [164]. However, its broad activation of macroautophagy precludes attributing protection to selective mitophagy. Methylene blue, clinically approved for non-stroke indications and capable of crossing the BBB, demonstrated stronger translational relevance by reducing infarct volume and increasing mitophagy-associated markers, when administered immediately after permanent middle cerebral artery occlusion [165].

More pathway-focused evidence comes from ligustilide and tetrahydroxy stilbene glucoside. Ligustilide enhanced PINK1/Parkin-mediated mitophagy in tMCAO and oxygen-glucose deprivation/reoxygenation (OGD/R) models, and its

mitochondrial protection was attenuated by PINK1 deficiency [166]. Tetrahydroxy stilbene glucoside administered four hours after reperfusion and continued for seven days reduced injury in adult male rats through ubiquitin-specific protease 10 (USP10)-dependent activation of PINK1/Parkin signaling [167].

Sex and metabolic status further shape these responses. In male mice, AP39 administered acutely after photothrombotic stroke improved motor performance, cerebral blood flow, and lesion volume, accompanied by increased PINK1 and Parkin expression, whereas females showed only minimal benefit [168]. In diabetic rats, metformin attenuated hyperglycemia-exacerbated ischemia/reperfusion injury through AMPK/UNC-51-like kinase 1 (ULK1)/PINK1/Parkin signaling, and its protective effects *in vitro* were suppressed by pharmacological inhibition of autophagy or AMPK [169]. Collectively, these studies broaden mechanistic insight into mitophagy modulation after ischemic stroke while also highlighting key limitations; AP39's effects were inferred from pathway-associated markers rather than direct mitophagy flux measurements, and metformin's pleiotropic metabolic actions preclude attributing its protection solely to mitophagy. However, mitophagy is not uniformly protective [170]. Consistent with its context-dependent nature, rehmapicroside reduced ischemic injury after tMCAO by limiting peroxynitrite-driven excessive mitophagy [171]. Sustained mitochondrial degradation can deplete the mitochondrial pool and impair cellular bioenergetics, particularly in neurons with limited capacity for mitochondrial biogenesis [93, 172]. In this setting, mitophagy may act as a “double-edged sword”, mitigating damage through the selective elimination of dysfunctional mitochondria but contributing to delayed energy failure and neuronal death when clearance exceeds replacement [173].

In addition to the canonical PINK1/Parkin pathway, receptor-mediated mitophagy also contributes to mitochondrial turnover in stroke. BCL2-interacting protein 3 (BNIP3), BNIP3L/NIX, and FUN14 domain-containing protein 1 (FUNDC1) possess LC3-interacting regions that directly recruit autophagosomes to mitochondria [145, 174–176]. Hypoxia-inducible factor 1- α (HIF-1 α), rapidly stabilized during ischemia, upregulates BNIP3 and NIX while FUNDC1 is regulated through stress-responsive post-translational modifications, including phosphorylation by casein kinase 2 and dephosphorylation by phosphoglycerate mutase family member 5 (PGAM5) [177–179]. Recent evidence showed that Src-dependent phosphorylation contributes to the delayed FUNDC1 inactivation after ischemia. Pharmacological Src inhibition restored FUNDC1-dependent mitophagy in cultured neurons, although this remains an *in vitro* mechanistic observation

rather than a therapeutic strategy [158]. These receptor-mediated pathways provide additional flexibility of mitophagy regulation under different injury contexts. In aged mice, exosome-delivered 3-n-butylphthalide enhanced drug accumulation in ischemic tissue, restored microglial mitochondrial function and phagocytosis, reduced neuroinflammation, and attenuated excessive mitophagy [180]. Clinical evidence remains indirect. In a cohort of 280 patients with acute ischemic stroke, higher serum autophagy-related protein 5 (ATG5) concentration was associated with greater neurological impairment, stroke recurrence, and mortality. However, circulating ATG5 does not specifically reflect mitophagy flux in the ischemic brain [181]. Collectively, the available studies

indicate that therapeutic benefit of mitophagy modulation depends on timing, magnitude, pathway, specificity, and cellular context. No study has yet demonstrated mitophagy-dependent efficacy in humans, and most pharmacological evidence derives from young or adult male rodents, with limited data in females, aged animals, and comorbid stroke models. Brain exposure, mitophagy flux, and causal pathway dependence are rarely assessed, leaving temporal and cell-type-specific therapeutic windows unresolved. Mitophagy is a dynamic, tightly regulated process balancing survival and death in ischemic stroke. An overview of MQC mechanism in ischemic stroke is provided in Fig. 4

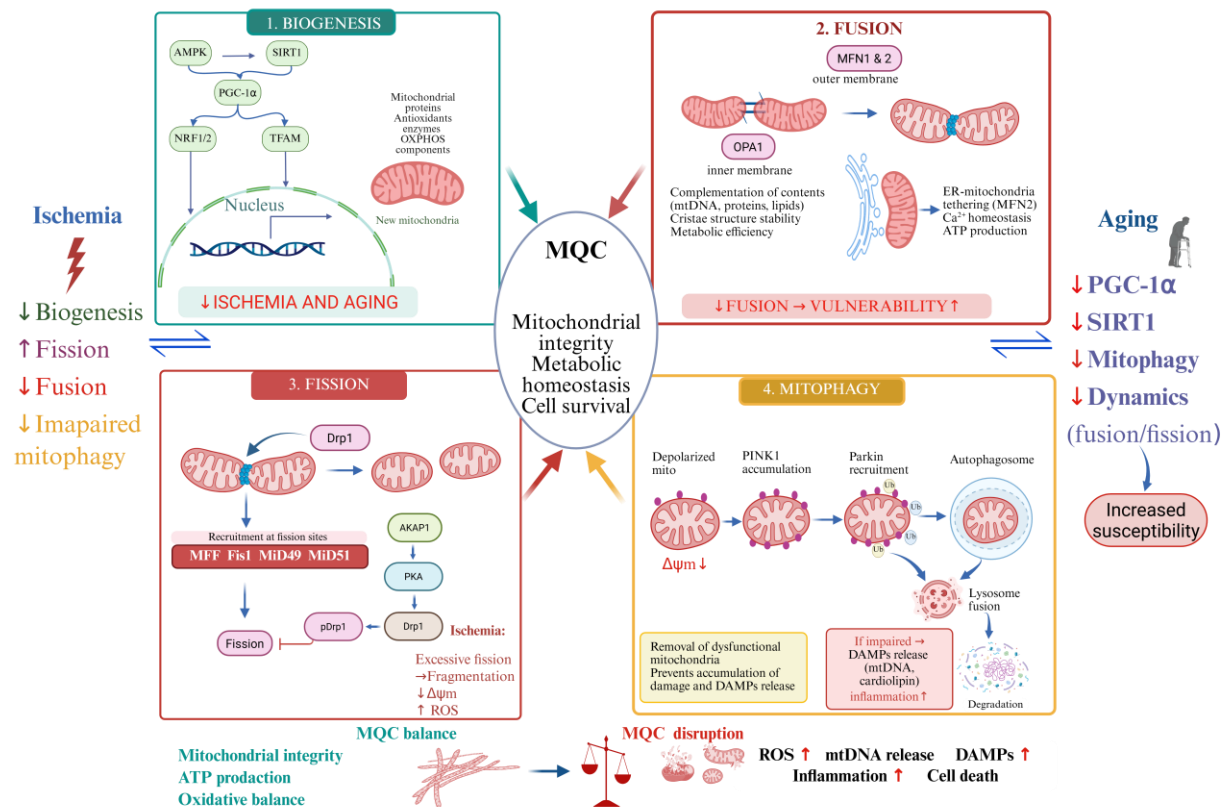


Figure 4. Mitochondrial quality control (MQC) in ischemic stroke. MQC comprises mitochondrial biogenesis, fusion, fission, and mitophagy, which together regulate mitochondrial integrity and metabolic homeostasis. Mitochondrial biogenesis is regulated by the AMP-activated protein kinase (AMPK)/sirtuin 1 (SIRT1)/peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) pathway, which promotes the expression of nuclear and mitochondrial factors required for mitochondrial biosynthesis. Mitochondrial fusion is mediated primarily by mitofusin 1 and 2 (MFN1/2) and optic atrophy 1 (OPA1), while fission involves dynamin-related protein 1 (Drp1) and its associated proteins, including mitochondrial fission factor (MFF), fission 1 protein (Fis1), mitochondrial dynamics proteins of 49 and 51 kDa (MiD49 and MiD51). A-kinase anchoring protein 1 (AKAP1) modulates Drp1-dependent fission through protein kinase A (PKA)-mediated phosphorylation of Drp1. Mitophagy, including the PTEN-induced kinase 1 (PINK1)/Parkin pathway, promotes the selective removal and degradation of damaged mitochondria. Ischemia and aging disrupt the balance among these MQC processes, with reduced biogenesis, altered fusion and fission, and impaired mitophagy. This MQC imbalance compromises mitochondrial integrity and contributes to ROS generation, mtDNA release, inflammation, and cell death.

6. Mitochondria in inflammation and immunometabolism

Mitochondria are increasingly recognized not only as central metabolic hubs but also as active regulators of innate immunity [182]. In ischemic stroke, mitochondrial dysfunction contributes to neuroinflammation through the release of DAMPs, metabolic reprogramming of glial cells, and activation of inflammasomes [31, 81, 82, 183, 184]. Among these, NLRP3 is a central effector of sterile inflammation and an important target of mitochondrial stress signals in the injured brain [84]. NLRP3 activation generally involves an initial priming step, commonly mediated by nuclear factor kappa B (NF- κ B), which increases the NLRP3 and pro-IL-1 β expression, followed by inflammasome assembly with apoptosis-associated speck-like protein containing a CARD (ASC) and procaspase-1. During early ischemia/reperfusion, ETC dysfunction and RET-mediated at complex I can generate mitochondrial ROS, which facilitate inflammasome activation, leading to caspase-1-dependent maturation and release pro-inflammatory cytokines interleukin-1 β (IL-1 β) and interleukin-18 (IL-18).

Cytosolic mtDNA released through mitochondrial membrane permeabilization or insufficient mitophagy provides a parallel link between mitochondrial injury and innate immunity [51, 81, 82, 146]. Oxidized mtDNA may promote NLRP3 activation, although direct evidence for this interaction derives predominantly from immune-cell models outside stroke. Cytosolic mtDNA can also activate cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) signaling, and downstream interferon regulatory factor 3 (IRF3)- and NF- κ B-dependent responses, whereas mtDNA delivered to endolysosomal compartments may be recognized by Toll-like receptor 9 (TLR9). Following tMCAO in rats, phosphorylated STING was prominent early in neurons and progressively increased in microglia over the first 4–24 h of reperfusion [60]. In the same study, oxidized mtDNA accumulated in neurons, and experiments in OGD/R-exposed HT22 cells demonstrated cytosolic mtDNA binding to cGAS and attenuation of pathway activation after mtDNA depletion. A separate mouse and BV2-cell study linked microglial cytosolic mtDNA to STING-dependent inflammatory signaling using exogenous mtDNA and pharmacological STING inhibition [31]. Subsequent genetic evidence further supports a causal contribution of microglial STING to ischemic injury [185]. However, neuron and microglial STING signaling has not been directly compared within the same experimental model. In patients with acute ischemic stroke, elevated plasma mtDNA has been associated with the extent of tissue injury [83]. However, circulating mtDNA may reflect cellular damage and does

not identify its cellular source nor confirm activation of NLRP3, cGAS-STING, or TLR9 in the human brain.

Pharmacological evidence indicates that STING remains modifiable beyond the acute phase. SN-011 initiated three days after reperfusion in young male mice promoted remyelination and long-term functional recovery [186]. More recent delivery approaches have demonstrated increased ischemic-brain accumulation of intranasally administered LB244-loaded microglial-membrane nanoparticles and neuronal-mitochondrial localization of a ROS-scavenging nanozyme that limited mtDNA leakage and reduced cGAS-STING activation in microglia [187, 188]. However, these interventions have not been systematically evaluated across sex, age, or stroke-related comorbidities. [31, 60, 189].

Cardiolipin externalization and ER-mitochondria contact sites provide complementary interfaces through which mitochondrial stress may regulate inflammasome activity. Under stress, cardiolipin redistributes from the inner to the outer mitochondrial membrane, where it can participate in mitophagy and provide a binding surface for NLRP3 [190]. Cerebral ischemia/reperfusion also induces cardiolipin oxidation and hydrolysis, however, a direct contribution of these modifications to NLRP3 activation in the ischemic brain has not been established.

Similarly, mitochondria-associated ER membranes facilitate mitochondrial calcium transfer and may provide a structural platform for inflammasome assembly derives mainly from immune-cell models outside the stroke [191]. However, direct evidence linking cardiolipin or mitochondria-associated membranes (MAMs) to inflammasome assembly derives mainly from immune-cell models outside stroke, their contribution in neurons and glia after cerebral ischemia remains unclear.

Mitophagy provides an important regulatory link between mitochondrial damage and inflammation. Clearance of damaged mitochondria through the PINK1/Parkin pathway or receptor-mediated mechanisms limits ROS generation and mtDNA release, thereby restraining inflammasome activation [81, 192–194]. In contrast, insufficient mitophagy leads to the persistence of dysfunctional mitochondria and amplifies inflammation [195–199]. However, the influence of mitophagy after cerebral ischemia depends on its magnitude, timing, and cellular context, as discussed in the previous section. Thioredoxin-interacting protein (TXNIP) may provide an additional redox-sensitive link between mitochondrial ROS and NLRP3 [200, 201]. Under oxidative stress, TXNIP dissociates from thioredoxin-1 (Trx1) and binds directly to NLRP3, promoting inflammasome activation [201]. In addition, mitochondrial NADPH supports thioredoxin-2 (Trx2) reduction and glutathione-dependent antioxidant systems and may therefore indirectly influence TXNIP access for NLRP3, however, direct

interaction of the mitochondrial Trx2-TXNIP complex has not been established.

In rats subjected to tMCAO, NLRP3 expression peaked at 24 h; Trx1 knockdown increased cytosolic TXNIP and NLRP3 expression, whereas pre-ischemic activation of Nrf2 reduced cytosolic TXNIP, caspase-1, IL-1 β , and IL-18 levels. Notably, the Nrf2-mediated reduction in cytosolic TXNIP was attenuated by Trx1 knockdown [202]. More recent work in a mouse model of global cerebral ischemia demonstrated time-dependent increases in hippocampal TXNIP and NLRP3 expression. Adeno-associated virus (AAV)-mediated TXNIP knockdown reduced microglial activation, neuronal apoptosis, pro-inflammatory cytokine levels, and cognitive impairments [203]. Together, these findings support redox-sensitive regulation of the TXNIP/NLRP3 axis after cerebral ischemia. Mitochondrial ROS represent a plausible upstream signal, but their specific contribution has not yet been directly resolved in this setting.

The involvement of NLRP3 in cerebral ischemic injury has provided a rationale for evaluating pharmacological NLRP3 inhibition in experimental stroke. The selective NLRP3 inhibitor, MCC950 reduced subacute infarct progression when administered 24 h after tMCAO in young male mice and attenuated infarct growth in 18-month-old mice subjected to large-vessel occlusion and reperfusion [146, 204]. In diabetic mice, however, MCC950 was administered before ischemia; therefore, this study does not establish its efficacy when initiated after stroke onset [205]. Complementing these experimental findings, the number of NLRP3-positive leukocytes in pial blood collected before recanalization during mechanical thrombectomy was associated with three-month outcome. This association supports the potential clinical relevance of NLRP3 activation but does not demonstrate the therapeutic efficacy of NLRP3 inhibition [204]. Beyond initiating inflammatory signaling, mitochondria shape immune-cell behaviour through immunometabolism. Acute inflammatory activation is frequently accompanied by increased glycolysis, whereas mitochondrial metabolism continues to provide TCA cycle-derived signals and contribute to mitochondrial redox signaling [206–208]. Three days after tMCAO, microglia-selective deletion of the Na⁺/H⁺ exchanger 1 (NHE1) increased OXPHOS and ATP-linked respiration in isolated purinergic receptor P2Y₁₂ (P2RY12)-positive cells and was accompanied by enhanced phagocytosis, subsequent remyelination, and improved cognitive recovery [209]. Because NHE1 deletion also alters intracellular pH and broader transcriptional programs, these findings link oxidative metabolism with reparative microglial functions but do not establish OXPHOS as their sole driver.

A distinct mitochondrial immunometabolic pathway involves aconitate decarboxylase 1 (ACOD1), formerly immune-responsive gene 1 (IRG1), which diverts mitochondrial cis-aconitate from the TCA cycle to produce itaconate. Itaconate has been proposed to restrain inflammatory immunometabolism by limiting succinate dehydrogenase-dependent succinate oxidation and activating NRF2-heme oxygenase 1 (HO-1) antioxidant signaling. Itaconate and its derivatives have also been reported to suppress NLRP3 inflammasome activation, although this mechanism has been characterized predominantly outside the context of cerebral ischemia. Two independent studies demonstrated more severe acute brain injury following focal cerebral ischemia/reperfusion in global Acod1/IRG1-deficient mice [210, 211]. In one study, ischemic-induced IRG1 expression was detected primarily in CD11b-positive cells. IRG1 deficiency was accompanied by lower microglial HO-1 expression, greater microglial activation, and increased immune-cell infiltration, whereas dimethyl itaconate restored HO-1 expression and attenuated brain injury in IRG1-deficient animals [210]. No comparable IRG1 induction was detected in the CD11b-negative fraction, suggesting that these cells are unlikely to be a major acute source of IRG1 in this model of non-myeloid brain cells. In 18–20-month-old male mice subjected to dMCAO, alterations in glycolytic intermediates, acylcarnitines, and itaconate persisted from the acute phase into recovery. At four weeks spatial transcriptomics analysis associated the corresponding chronic metabolic gene expression signature to TMEM119-positive microglia in degenerating thalamic white matter [212]. Together, these findings are consistent with a protective role of ACOD1-associated immunometabolism after cerebral ischemia and indicated marked regional heterogeneity. However, they do not establish cell-specific itaconate production or metabolic flux. Global knockout models cannot definitively distinguish the effects of resident microglia from those of infiltrating monocyte-derived macrophages or exclude systemic contributions. Moreover, dimethyl itaconate is not converted intracellularly to itaconate and exhibits distinct electrophilic activity. Its protective effect therefore likely reflects pharmacological modulation of related stress-response pathways rather than direct replacement of the endogenous metabolite [213]. Cell-resolved metabolic flux studies and post-ischemic pharmacological interventions are needed to define the therapeutic potential of the ACOD1-itaconate axis. In summary, mitochondria are central to the initiation and regulation of innate immune responses in stroke. By integrating redox signaling, DAMP release, and metabolic status, they act as both sensors and amplifiers of inflammation. Mitophagy can restrain inflammatory signaling by removing damaged mitochondria, whereas

metabolic reprogramming shapes the inflammatory, phagocytic, and reparative functions of microglia. Therapeutic modulation of mtDNA-STING signaling, mitochondria-linked inflammasome activation, and the ACOD1-itaconate axis remains preclinical. Establishing

translational relevance will require post-reperfusion interventions with verified brain and cellular exposure, evaluation in both sexes and in aged and comorbid models, and long-term functional assessment.

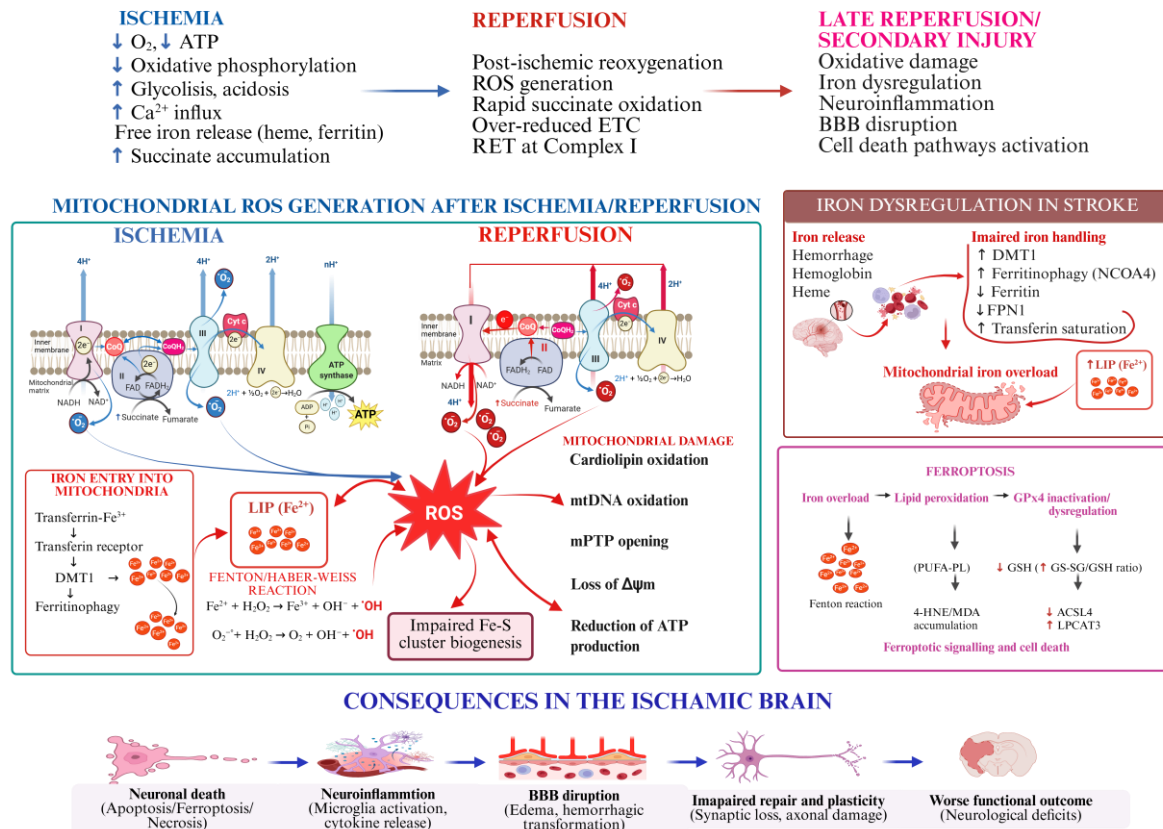


Figure 5. Mitochondrial ROS generation, iron dysregulation, and ferroptosis during ischemia/reperfusion injury in the ischemic brain. The major molecular and mitochondrial mechanisms contributing to oxidative damage following cerebral ischemia/reperfusion. During ischemia, oxygen and ATP depletion impair oxidative phosphorylation, increase glycolysis and acidosis, promote Ca^{2+} overload, and induce succinate accumulation together with iron release from intracellular stores. Upon reperfusion, rapid reoxygenation and accelerated succinate oxidation drive excessive ROS generation, particularly through RET at mitochondrial complex I. Concurrent mitochondrial iron accumulation enhances $\cdot\text{OH}$ generation, elevating lipid peroxidation and mitochondrial dysfunction. These processes lead to cardiolipin oxidation, mtDNA damage, mitochondrial mPTP opening, loss of mitochondrial $\Delta\psi\text{m}$, impaired Fe-S cluster biogenesis, and reduced ATP production. Iron dysregulation, including altered ferritinophagy, increased transferrin saturation, DMT1 upregulation, and reduced ferroportin, further exacerbates mitochondrial iron overload and ferroptotic signaling.

7. Mitochondrial ferritin and neuroprotection

Iron is essential for mitochondrial metabolism as a component of heme- and iron-sulfur-containing proteins required for ETC and other redox reactions [214]. However, excess redox-active Fe^{2+} can catalyze the formation of highly reactive $\cdot\text{OH}$ through the Fenton reaction, thereby aggravating oxidative damage [215]. In the ischemic brain, disruption of iron homeostasis contributes to lipid peroxidation, mitochondrial dysfunction, and ferroptosis. Within this context, FtMt

has emerged as an endogenous modulator of mitochondrial iron handling and resistance to oxidative injury [19, 216, 217].

FtMt is a nuclear-encoded protein selectively targeted to the mitochondrial matrix, where its ferroxidase activity facilitates the conversion of Fe^{2+} to Fe^{3+} and its storage within a ferritin shell [218, 219]. In contrast to cytosolic ferritins, FtMt is not regulated by the classical iron-responsive element (IRE)/iron-regulatory protein (IRP) system. Instead, its expression is controlled through transcriptional and epigenetic mechanisms and can be

induced by oxidative stress or hypoxia [220–222]. FtMt exhibits a restricted tissue and cellular distribution, with prominent expression in the testes and nervous system [223, 224]. In the brain, is detected predominantly in neurons but also in oligodendrocytes and brain microvascular endothelial cells, and its regional abundance appears to correspond more closely to mitochondrial density than to total iron content [18, 223]. During cerebral ischemia/reperfusion, mitochondrial membrane disruption and impaired ETC function promote ROS overproduction, which mobilizes labile mitochondrial iron pools (Fig. 5) [58, 225].

The most direct evidence for a protective role of FtMt comes from a study in three-month-old male mice subjected to 90 min of tMCAO followed by 24 h of reperfusion [217]. FtMt expression increased in the cortical penumbra, whereas FtMt deletion aggravated neurological deficits, infarct volume, BBB leakage, iron accumulation, and lipid peroxidation. These effects were accompanied by glutathione (GSH) and glutathione peroxidase 4 (GPX4) and increased acyl-CoA synthetase long-chain family member 4 (ACSL4) expression, consistent with enhanced ferroptotic injury. Conversely, constitutive FtMt overexpression reduced infarct volume and neurological deficits. Findings from primary cortical neurons and SH-SY5Y cells exposed to OGD/R further implicated a neuronal FtMt in the regulation of labile iron and lipid peroxidation [217].

Subsequent studies extended these observations to other components of ischemic injury. In 12-week-old male mice examined 24 h after tMCAO, FtMt overexpression preserved tight-junction proteins, reduced endothelial apoptosis and oxidative damage, and limited BBB permeability [18]. FtMt deficiency also aggravated impairment of cortical mitochondrial respiration and increased mitochondria-dependent apoptosis after cerebral ischemia/reperfusion [19]. In oxygen-glucose deprivation (OGD) SH-SY5Y cells, FtMt overexpression preserved mitochondrial membrane potential, ATP content, maximal respiration, and spare respiratory capacity. It was also associated with increased glucose utilization and pentose phosphate pathway activity, potentially supporting NADPH and GSH-dependent antioxidant capacity [19]. However, these metabolic effects were demonstrated primarily in a constitutive overexpression cell model and require confirmation in primary neural cells and *in vivo*.

The effects of FtMt expression are nevertheless context-dependent. Although mitochondrial iron is required for heme and iron-sulfur cluster synthesis, excessive sequestration by FtMt may deplete the cytosolic labile iron pool and restrict iron availability for metabolic processes. In tetracycline-inducible H1299 cells, forced FtMt expression for 48–72 h redirected iron from cytosol

to mitochondria, induced a cytosolic iron-deficient phenotype, reduced cytosolic and mitochondrial aconitase activities, and decreased iron incorporation into heme [226]. In a related inducible model, exposure to tert-butyl hydroperoxide, FtMt enhanced ROS accumulation, GSH depletion, apoptosis, and iron-dependent lysosomal destabilization [227]. Because these effects were observed under forced overexpression and acute chemical oxidative stress in non-neural cells, their relevance to endogenous FtMt in the ischemic brain remains uncertain.

Aging provides a clinically relevant but insufficiently investigated model. Increased cytosolic and mitochondrial non-heme iron in the aged mouse cortex has been associated with enhanced local hepcidin (HAMP) expression and reduced ferroportin-1 abundance [228]. Cerebral ischemia can similarly increase astrocytic HAMP, tissue iron accumulation, and ferroptotic injury [229, 230]. These findings establish a plausible interaction between age-related iron dysregulation and post-ischemic injury but do not demonstrate how FtMt expression or function changes in the aged ischemic brain. Therefore, it remains unknown whether stroke-induced FtMt expression is maintained with aging, becomes insufficient relative to iron accumulation, or differs among neurons, endothelial cells, oligodendrocytes, and glial populations.

The translational potential of FtMt modulation remains uncertain. Available *in vivo* stroke studies relied on constitutive FtMt deletion or overexpression in young adult male mice, with outcomes assessed predominantly 24 h after tMCAO [18, 19, 217]. Although these models demonstrate the biological significance of FtMt they do not show that FtMt can be safely increased after stroke within a clinically relevant therapeutic window. No selective pharmacological FtMt modulator has been evaluated in cerebral ischemia, and dose-response relationships, brain delivery, long-term functional effects, and efficacy in females, aged animals, or models incorporating common stroke comorbidities remain undefined. Moreover, the possibility that excessive mitochondrial iron sequestration may compromise heme and iron-sulfur cluster synthesis indicates that therapeutic modulation would require precise control over its magnitude and cellular distribution.

Collectively, current evidence identifies FtMt as an endogenous modulator of mitochondrial iron handling that can limit ferroptotic injury, endothelial dysfunction, and bioenergetic failure in experimental cerebral ischemia. However, its value as a biomarker or therapeutic target remains unestablished, particularly in aged and clinically heterogeneous stroke populations.

8. Mitochondria–nucleus crosstalk and epigenetic regulation

Mitochondria rely extensively on nuclear-encoded proteins for maintenance, replication, and stress adaptation, and in turn, transmit retrograde signals that reshape nuclear transcription in response to metabolic perturbations, damage, or oxidative stress [85, 88, 231]. This bidirectional communication is highly relevant in ischemic stroke, where mitochondrial dysfunction may alter gene expression, chromatin structure, and cell fate [232, 233].

One of the best studied retrograde pathways is UPRmt, activated by proteostasis disruption, ROS accumulation, and ETC impairment. UPRmt induces chaperones (e.g., HSP60, HSP10), proteases (e.g., LONP1), and antioxidant enzymes through transcription factors such as ATF5, ATF4, and CHOP, which translocate to the nucleus and orchestrate a coordinated gene expression program aimed at restoring mitochondrial integrity [85, 88, 89, 234–236]. In the brain, UPRmt is less well characterized than in other tissues, but recent evidence shows its involvement in stroke pathology [86, 87, 90]. Transient focal cerebral ischemia increased HSP60 and HSP10 transcripts reflecting UPRmt activation [237]. SIRT3 further supports this adaptive response by preserving UPRmt-related gene expression and reducing injury after transient MCAO [238]. Low doses of oligomycin and meclizine also induced UPRmt and protected cortical neurons against OGD; ATF5 knockdown abolishes meclizine protection, and pretreatment reduced infarct volume and neurological outcomes in mice [86]. These findings support an adaptive role of UPRmt in experimental cerebral ischemia, but the boundary between protective and maladaptive signaling remains unclear. Constitutive or excessive UPRmt promotes retention of deleterious mtDNA and non-apoptotic dopaminergic neurodegeneration in *C. elegans* [239, 240] whereas attenuation of overactive signaling improves tissue function during chronic mtDNA damage [241]. In human induced pluripotent stem cell (iPSC)-derived microglia, mitochondrial proteotoxic stress was accompanied by UPRmt activation, a senescence-associated inflammatory phenotype, and disrupted neuron-glia communication [242]. Conversely, ATF5 knockdown increases cytokine release in BV-2 microglia [243]. These observations indicate that UPRmt–inflammation interactions vary across cell types and stress contexts. Although maladaptive UPRmt responses are well documented in other systems, it remains unclear whether UPRmt becomes detrimental after cerebral ischemia or simply reflects unresolved mitochondrial injury.

Beyond proteostasis signaling, mitochondrial stress also influences nuclear transcription through changes in NAD⁺ availability and sirtuin activity. SIRT1 deacetylates histones and transcription factors including PGC-1 α , NF- κ B, and forkhead box O (FOXO) transcription factors [244]. During ischemia, declining NAD⁺ concentrations limit SIRT1 activity, leading to increased acetylation, pro-inflammatory gene expression, and reduced mitochondrial biogenesis [245–248]. Meanwhile, AMPK activation promotes mitochondrial adaptation by increasing PGC-1 α transcription, autophagy, and inhibiting mechanistic target of rapamycin (mTOR) signaling. Together with SIRT1 coordinates metabolic and stress responses, and their dysregulation contributes to impaired mitochondrial recovery and oxidative damage [133, 249, 250]. NAD⁺ precursor studies show context-dependent outcomes. Post-reperfusion nicotinamide riboside (NR) reduces hippocampal injury and improves cognition [251], whereas multiday pretreatment exacerbates ischemic damage [252]. Four-week oral NR supplementation before pdMCAO reduces inflammation and infarct area in both young and aged mice [253]. These divergent outcomes may reflect differences in dosing, timing, age, ischemia model, and metabolic context.

Evidence in females and comorbid models remains limited. Nicotinamide (NAM) shows benefit in female, hypertensive, and diabetic rats [254, 255], but cannot be extrapolated to NR due to distinct salvage pathways and potential inhibition of sirtuins and poly(ADP-ribose) polymerase (PARP) [244, 256]. NAM reduces infarction and BBB leakage but impairs cell maturation [257], indicating central neuroprotection may coexist with peripheral immune suppression. Although oral NR increases cerebral NAD⁺ in humans [258, 259], stroke-specific pharmacokinetics and efficacy remain unresolved.

Mitochondrial dysfunction also alters metabolite concentrations such as α -ketoglutarate, succinate, and fumarate, which modulate dioxygenases and demethylases [20, 232, 233, 260, 261]. Mitochondria-derived metabolites may also influence histone deacetylase (HDAC)-dependent regulation. β -hydroxybutyrate (BHB) crosses the BBB and serves as an alternative oxidative fuel [262, 263]. Initially reported to inhibit class I HDACs [264], subsequent work did not confirm direct inhibition [265]. Post-reperfusion BHB temporarily improves mitochondrial respiration and neurological function [266], but HDAC involvement remains unclear. HDAC inhibition preserves ATP and axonal mitochondrial integrity in white-matter ischemia and activates Nrf2-dependent antioxidant responses [36, 267–269], suggesting mitochondrial bioenergetic and redox processes contribute to HDAC-dependent

neuroprotection. Post-ischemic chromatin remodeling persists beyond the acute phase, but the contribution of mitochondrial metabolites remains unclear [270, 271].

These metabolite-driven mechanisms converge with signaling pathways that sense cellular energy and redox status. Energy and oxidative cues modify class IIa HDAC localization through AMPK-calcium/calmodulin-dependent protein kinase (CaMK), and redox-dependent pathways [272–274]. In neurons, HDAC4 translocates to the nucleus after MCAO and OGD, a response associated with calcium/calmodulin-dependent protein kinase IV (CaMKIV) signaling [275, 276]. NADPH oxidase-derived ROS further reduce HDAC4/5 expression and promote high-mobility group box 1 (HMGB1) mediated inflammatory signaling [277]. Although a direct mitochondrial ROS–HDAC4/5 axis has not been established, ischemia clearly alters class IIa HDAC localization and abundance.

Beyond these epigenetic routes, mitochondria–nucleus communication also involves nuclear receptors, including vitamin D receptor (VDR), which further modulate mitochondrial responses to ischemia. VDR is upregulated in peri-infarct microglia/macrophages, and *Vdr* deletion exacerbates neuroinflammation, BBB disruption, infarct size, and neurological deficits [278]. Vitamin D analogs reduce oxidative stress and apoptosis partly through AMPK/protein kinase B (AKT)/glycogen synthase kinase 3 beta (GSK3 β) and Nrf2/HO1 signaling [279, 280], however, most studies rely on pretreatment and pharmacological inhibition rather than genetic validation. Mitochondrial VDR may regulate mtDNA-encoded OXPHOS transcripts [281], but this mechanism remains unconfirmed. Low vitamin D status in clinical cohorts correlates with poorer prognosis after stroke [282], but causality is unclear.

In summary, mitochondrial dysfunction after cerebral ischemia reshapes nuclear transcription and chromatin regulation by altering proteostasis, cellular energy and NAD⁺ availability, redox state, and metabolite signaling, while nuclear pathways reciprocally modify mitochondrial adaptation. The outcome of this bidirectional crosstalk is not uniformly protective: depending on timing, cell type, and metabolic context, it may restore cellular homeostasis or reinforce inflammation, bioenergetic failure, and neuronal injury. UPRmt, NAD⁺/sirtuin signaling, HDAC-dependent regulation, and VDR-related pathways therefore provide mechanistically distinct points of intervention, but their therapeutic windows and efficacy across sex, aging, and comorbid conditions remain incompletely defined.

9. Intercellular mitochondrial transfer and stroke recovery

Mitochondria can move between cells and thereby provide metabolic support or participate in intercellular signaling [283–285]. IMT occurs without cell division and differs from mitochondrial transplantation, which involves the exogenous administration of isolated organelles [286].

Astrocytes-to-neuron transfer is one of the best-characterized forms of IMT after cerebral ischemia. Relatively hypoxia-resistant astrocytes can release mitochondria into the extracellular space or deliver them directly through tunneling nanotubes (TNTs). CD38 cyclic ADP-ribose signaling promotes mitochondrial release under oxidative and calcium stress, and neuronal acquisition of astrocyte-derived mitochondria has been associated with restored ATP production, improved mitochondrial function, and increased neuronal viability after stroke [27]. Astrocytic low-density lipoprotein receptor-related protein 1 (LRP1) has recently been implicated in metabolic regulation of mitochondrial donation. Mechanistically, LRP1 limits glucose uptake, glycolysis, and the associated production of lactate-derived metabolites that can modify intracellular proteins through lactylation. One critical target of this modification is ADP-ribosylation factor 1 (ARF1), a small GTPase involved in vesicle trafficking. In ischemic conditions, the loss of LRP1 in astrocytes leads to increased LA accumulation and hyper-lactylation of ARF1, which impairs its function and disrupts mitochondrial trafficking toward the astrocytic membrane. As a result, mitochondrial transfer to neurons is severely reduced, compromising neuronal recovery. Restoration of LRP1 function or inhibition of ARF1 lactylation rescues this phenotype and reinstates astrocyte-derived mitochondrial support. In patients with stroke, cerebrospinal fluid LA was inversely associated with the astrocyte-derived mitochondrial signal, although this observational relationship does not demonstrate neuronal uptake or clinical benefit [42].

Astrocyte-derived mitochondrial material may also be transferred to other cells of the neurovascular unit. Using astrocyte-specific genetic mitochondrial labeling, astrocyte-derived mitochondrial signal was detected in brain endothelial cells and pericytes within isolated cerebral capillaries [287]. The signal was greater in capillaries from 7-month-old than from 3-month-old mice. However, these experiments were not conducted after stroke or in advanced-age animals and did not distinguish increased transfer, reduced clearance of transferred material, or demonstrate functional incorporation of intact mitochondria. The effects of aging

on IMT after cerebral ischemia therefore remain insufficiently defined.

The consequences of mitochondrial exchange are not uniformly protective. After experimental ischemia, transfer from pro-inflammatory microglia to neurons was associated with reduced neuronal ATP production and membrane potential, increased ROS generation, and cell death [288]. Ischemic neurons were also reported to release depolarized mitochondria into the cerebrospinal fluid, where they may act as “help-me” signals that stimulate compensatory astrocytic responses [289]. IMT may, therefore, provide metabolic support, propagate mitochondrial dysfunction, or facilitate the disposal and signaling of damaged organelles, depending on the donor and recipient cell types, mitochondrial quality, and post-transfer fate [290].

Mesenchymal stem cells (MSCs) provide another potential source of mitochondria for injured neural and vascular cells. Experimental studies indicate that MSCs can transfer mitochondria to neurons and cerebral endothelial cells through TNTs or extracellular vesicles [42, 291–295]. This transfer has been associated with improved mitochondrial function and recipient-cell survival [292–294]. In experimental stroke, intra-arterial MSC administration reduced infarct size and the severity of neurological deficits and improved mitochondrial function through a mechanism associated with a SIRT1-mitochondrial Rho GTPase 1 (RHOT1/Miro1)-PGC-1 α regulatory loop [296]. However, mitochondrial donation cannot yet be separated from numerous paracrine effects of MSCs.

Beyond TNTs and extracellular vesicle-mediated transfer, cell fusion and endocytic uptake of extracellular mitochondria have been proposed as additional routes of mitochondrial exchange [286, 297–299]. Combined mitochondrial labeling and mtDNA analyses have reported recovery of aerobic respiration and acquisition of donor-derived mtDNA in metabolically deficient recipient cells [300]. However, these findings do not necessarily establish transfer of intact organelles, and evidence for sustained functional integration varies across experimental approaches [286].

The regulation of IMT is influenced by the metabolic status and mitochondrial health of both donor and recipient cells [297, 301, 302]. For instance, activated astrocytes increase mitochondrial biogenesis and export under stress, while mitochondrial dysfunction in recipient cells may promote the uptake of extracellular mitochondria. IMT may therefore act as a compensatory response to mitochondrial damage and energy failure in injured tissue [27, 290, 301–303]. Experimental interventions have attempted to enhance endogenous IMT. In OGD/R models and a photothrombotic stroke model, ginsenoside Rb1 reduced astrocytic ROS

production, preserved extracellular mitochondria, and promoted CD38-associated mitochondrial transfer to neurons [304]. CD38 knockdown attenuated the neuroprotective effect of Rb1, consistent with a contribution of CD38-dependent mitochondrial transfer. Rb1 also influences astrocyte activation and multiple metabolic pathways. Melatonin treatment in an experimental model of cerebral ischemia and mild hypothermia at 33°C in astrocyte-neuron OGD/R co-cultures have each been associated with increased TNT formation and mitochondrial transfer [305]. Because these interventions are pleiotropic, the extent to which mitochondrial transfer is required for their protective effects in vivo remains uncertain.

Mitochondrial transplantation represents a related but distinct therapeutic approach. In mice, intra-arterial delivery of isolated mitochondria after one hour of pdMCAO reduced infarct volume by 34% at 24 hours, while focused ultrasound increased their extravascular distribution within the ischemic brain [306]. Although genetically labeled MitoTimer mitochondria confirmed donor-derived mitochondrial material, the acute observations do not establish long-term persistence or sustained functional integration. Direct intrastriatal administration of MSC-derived mitochondria 24 hours after reperfusion reduced infarct volume and improved neurological and motor outcomes, with donor mitochondria detected preferentially in neurons and endothelial cells [307]. In a photothrombotic stroke model, intravenous administration of isolated mitochondria within one hour of ischemia suppressed pyroptosis-associated signaling and promoted neurogenesis [23]. Direct intracerebral administration bypasses major delivery barriers but has limited clinical applicability, whereas systemic administration raises questions about brain delivery, organelle integrity, and off-target distribution.

Initial clinical translation has been reported. Pilot trials suggest potential benefits of melatonin and MSC administration after ischemic stroke [308, 309]. More directly, autologous mitochondria were administered intra-arterially during thrombectomy in a study involving four patients, without significant procedure-related adverse events [310]. However, this study establishes feasibility rather than efficacy and cannot determine the influence of sex, age, comorbidities, or stroke subtype. Major translational challenges include preserving mitochondrial stability and functional quality, limiting immunogenicity-particularly for non-autologous preparations-achieving efficient and targeted delivery, and determining the duration of persistence and functional integration [286, 311, 312]. Quantitative longitudinal data on mitochondrial biodistribution, clearance, persistence, and sustained functional competence remain limited [23,

306, 307]. Studies in aged and comorbid stroke models and direct comparisons between sexes are also scarce. Cell-targeted systems such as MitoCatch have improved mitochondrial uptake and intracellular integration outside stroke, but their efficacy and safety in cerebral ischemia remain unknown [313].

In conclusion, IMT represents a context-dependent form of cellular communication in the injured brain. Depending on mitochondrial quality and the donor–recipient context, IMT may support metabolic stabilization, redox homeostasis, and cell survival after stroke. However, transfer of damaged mitochondria may

amplify oxidative stress and cell death or act as a stress signal. Therapeutic exploitation of this process will therefore require control over donor-cell phenotype, mitochondrial quality, recipient-cell targeting, and treatment timing, together with rigorous confirmation that intact mitochondrial transfer is responsible for the observed functional effects. Table 2 summarizes the main experimental findings, treatment characteristics, developmental stage, translational limitations, and evidence from aged or comorbid stroke models for each mitochondria-targeted strategy.

Table 2. Mitochondria-targeted therapeutic strategies in ischemic stroke: experimental evidence, development status, translational limitations, and relevance to aging.

Target/pathway	Intervention	Stroke model/treatment	Main findings	Development stage	Key limitations	Aging/comorbidity evidence	References
Mitochondrial dynamics and biogenesis							
Stress-induced Drp1–Fis1 interaction	P110	Rodent tMCAO; free P110 before ischemia; exosome-P110 at reperfusion.	Reduced infarction and deficits; exosomal delivery improved acute outcomes.	Preclinical only	Proteolysis; limited brain; no reperfusion-therapy studies.	None — healthy male rodents	[76, 154, 155]
Drp1–Fis1 interaction	SC9	Cellular and endotoxemia models; no stroke model.	Reduced Drp1–Fis1 dysfunction in non-stroke model.	Preclinical only	Brain: plasma ratio $\approx 2\%$; plasma half-life < 0.5 h in non-ischemic mice; stroke efficacy unknown.	None	[141]
PGC-1α–NRF1–TFAM biogenesis	Formoterol	Aged male mice (18–20 m old); distal MCAO + hypoxia; 24 h post-stroke.	Increased mtDNA and TFAM; reduced acylcarnitine and lipid accumulation.	Preclinical in stroke; approved for another indication	No young comparator; PK, safety, and reperfusion interaction unknown.	Direct — aged post-stroke model	[108]
Mitophagy and integrated MQC							
mTOR/autophagy-mitophagy	Rapamycin	Adult male rats; tMCAO; acute treatment.	Reduced infarction; preserved mitochondrial function.	Preclinical only	Nonselective autophagy activation; flux not established.	None — adult male	[164]
Mitophagy-associated pathways	Methylene blue	Adult male rats; permanent MCAO; immediately after occlusion.	Reduced infarction; increased mitophagy-associated markers.	Preclinical in stroke; approved for other indications	Pleiotropic effects; nonselective mechanistic evidence.	None — adult male rodents.	[165]
PINK1–Parkin mitophagy	Ligustilide; tetrahydroxystilbene glucoside	Male rat MCAO/R; TSG 4 h post-reperfusion.	Enhanced PINK1/Parkin signaling and mitochondrial protection.	Preclinical only	Limited flux and causal evidence.	None — male rodents.	[166, 167]
PINK1–Parkin mitophagy	AP39	Male/female mice; photothrombotic stroke; 10 min after stroke.	Improved outcomes mainly in males.	Preclinical only	Mitophagy inferred from PINK1/Parkin expression, not flux; sex-dependent effect.	Indirect — both sexes, but no aged animals or comorbidities	[168]

AMPK–ULK1–PINK1–Parkin signaling	Metformin	Diabetic rats; cerebral I/R; schedule as reported.	Attenuated hyperglycemia-aggravated injury.	Early clinical evaluation in ischemic stroke; approved for diabetes	Pleiotropic effects; brain exposure and treatment window unclear.	Direct — diabetic stroke model	[169]
Integrated MQC/excessive mitophagy	3-n-butylphthalide	18-month-old mice; ischemic stroke.	Improved mitochondrial function and reduced neuroinflammation.	Preclinical only	Delivery, PK, and causal mitophagy evidence unresolved.	Direct — aged stroke model	[180]
NAD ⁺ -dependent metabolic adaptation							
NRK1/2-dependent NAD⁺ salvage	Nicotinamide riboside	Post-reperfusion dosing in adult males; or 4-week pretreatment before permanent distal MCAO in young and aged males.	Context- and timing-dependent effects: benefit after reperfusion, but variable outcomes with prolonged pretreatment.	Preclinical only	Direction of effect varies by schedule, dose, route, model, and context; stroke PK, reperfusion interactions, and mechanism unresolved.	Direct — young/aged comparison exists, but mainly male pretreatment data; female/comorbid evidence lacking	[251–253, 258, 259]
NAMPT-dependent NAD⁺ salvage	Nicotinamide	Delayed high-dose IV in female rats and hypertensive/diabetic male rats; dosing at reperfusion in another study.	Reduced infarction; reduced BBB leakage.	Preclinical in stroke; clinical use in other contexts	Pleiotropic effects; possible peripheral immunosuppression.	Direct — female, hypertensive, and diabetic stroke models; aging not directly tested	[254–257]
Mitochondria-linked innate immune and immunometabolic signaling							
mtDNA–cGAS–STING signaling	SN-011	Young male mice; initiated 3 d post-reperfusion.	Promoted remyelination and recovery.	Preclinical only	PK, aging, sex, cell specificity; reperfusion interactions unknown.	None — young male mice	[186]
mtDNA–cGAS–STING signaling	LB244	Experimental ischemia; intranasal/engineered delivery.	Reduced mtDNA leakage and cGAS–STING activation.	Preclinical only	Delivery, biodistribution, PK safety.	None — no systematic aged, sex, or comorbidity testing	[187, 188]
ACOD1–itaconate/NRF2–HO-1	Dimethyl itaconate	Global Acod1/Irg1-deficient focal I/R mice; treatment context in deficient animals.	Restored HO-1 expression and attenuated brain injury in IRG1-deficient animals.	Preclinical proof of mechanism	Not converted intracellularly to itaconate; no established post-stroke window.	Indirect — aged stroke data describe endogenous metabolic signatures	[210–213]
Intercellular mitochondrial transfer and transplantation							
Astrocyte–neuron mitochondrial transfer	Ginsenoside Rb1; melatonin	OGD/R and photothrombotic stroke (Rb1); experimental ischemia or astrocyte–neuron OGD/R (melatonin/hypothermia).	Increased TNT formation, mitochondrial transfer and reduced injury.	Ginsenoside Rb1 - preclinical only,	Pleiotropic effects; mitochondrial transfer/integration not established.	None — aged/comorbid evidence not reported	[304, 305, 308],
MSC-mediated mitochondrial donation	MSC administration or MSC-derived mitochondria	Intra-arterial MSCs after stroke; or direct intrastriatal MSC-derived mitochondria 24 h after reperfusion.	Improved mitochondrial function and neurological/motor outcomes.	Clinical phase II	MSC paracrine effects; persistence/integration unresolved.	None — systematic aged/comorbid and sex comparisons scarce	[42, 291–296, 307, 309],

Mitochondrial replacement	Isolated mitochondria (intra-arterial, IV, or ultrasound-assisted delivery)	Mouse permanent MCAO, 1 h; photothrombotic stroke, within 1 h; Phase 1 thrombectomy, intra-arterial during reperfusion.	Reduced acute infarct volume and altered injury signaling; Phase 1 reperfusion.	Clinical phase I	Mitochondrial quality, immunogenicity, delivery, persistence, and functional integration; limited clinical efficacy data.	None — no systematic aged/comorbid animal studies; n=4 cannot assess age effects	[23, 286, 306–310]
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Evidence classification: Direct, tested as an intervention in an aged and/or comorbid stroke model; Indirect, relevant evidence is available but not as treatment-efficacy evidence in a comparable aged or comorbid stroke model; None, no such evidence was identified. Clinical use or human exposure in another indication does not constitute clinical efficacy in ischemic stroke.

Abbreviations: 3-MA, 3-methyladenine; BBB, blood–brain barrier; Drp1, dynamin-related protein 1; I/R, ischemia/reperfusion; IMT, intercellular mitochondrial transfer; IV, intravenous; MCAO, middle cerebral artery occlusion; MSC, mesenchymal stromal cell; MQC, mitochondrial quality control; NAD⁺, nicotinamide adenine dinucleotide; NR, nicotinamide riboside; PK, pharmacokinetics; ROS, reactive oxygen species; SDH, succinate dehydrogenase; TFAM, mitochondrial transcription factor A; tMCAO, transient middle cerebral artery occlusion; TNT, tunneling nanotube; TSG, tetrahydroxystilbene glucoside; VDR, vitamin D receptor.

10. Conclusions and future perspectives

This narrative review has outlined a diverse array of non-canonical mitochondrial functions, considered in the broader context of canonical roles in bioenergetics, calcium handling, and redox balance. These include MQC, regulation of cell-death pathways, immunometabolic reprogramming and innate immune signaling, mitochondrial ferritin-dependent iron buffering linked to ferroptosis, mitochondria-nucleus and epigenetic signaling, and IMT. Collectively, these processes influence neuronal injury and survival and may also contribute to post-stroke recovery.

Key insights include the understanding that mitochondrial dysfunction is not merely a consequence of ischemia, but an active contributor to secondary injury through the release of damage signals (e.g., ROS, mtDNA), disruption of quality control mechanisms (e.g., mitophagy, dynamics, biogenesis), and loss of metabolic flexibility. Furthermore, mitochondria orchestrate neuroimmune responses, influence gene expression through retrograde signaling, and serve as vehicles for intercellular rescue via mitochondrial transfer. Importantly, these processes are influenced by age, cellular phenotype, and systemic context factors that should be accounted for in both mechanistic studies and therapeutic design.

Therapeutic strategies targeting mitochondria have shown encouraging results in preclinical stroke models. Interventions such as modulators of fission/fusion balance, mitophagy, NAD⁺-elevating strategies, and mitochondrial transplantation have demonstrated the potential to preserve mitochondrial integrity and improve outcomes. However, their clinical translation remains limited, in part due to challenges in delivery, timing, and patient stratification. Therefore, looking forward, several key directions stand out. These include the development of targeted delivery systems, which may modulate mitochondrial function with cell-type specificity, as well as combined therapeutic strategies that integrate mitochondrial interventions with anti-inflammatory, vascular, or metabolic agents. In addition, future studies

should explore mitochondrial roles in long-term stroke recovery, including neuroplasticity, remyelination, and cognitive function, while also accounting for comorbid conditions, sex differences, and age-related metabolic and inflammatory changes that may modify these processes.

In summary, a better understanding of mitochondrial biology in stroke will not only enrich our view of ischemic pathophysiology but also enable the development of novel, mitochondria-focused therapies. Integrating both canonical and non-canonical perspectives highlights mitochondria not only as powerhouses but also as signaling hubs and vehicles of cellular communication. Exploring this duality may hold promise for developing mitochondria-based strategies to sustain neuronal health and enhance post-stroke recovery.

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Author contributions

Conceptualization, “P.B and J.J.K.,” literature analysis and interpretation, “P.B and N.P.,” manuscript preparation, “P.B.,” figure preparation, “J.J.K.,” critical revision of the manuscript, “J.J.K and N.P.,” supervision, “J.J.K.”. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no conflict of interest.

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