

Review

# The Role of Mitochondrial Biogenesis in Ischemic Stroke

Behrouz Shademan<sup>1</sup>, Cigir Biray Avci<sup>1</sup>, Vahidreza Karamad<sup>1</sup>,  
Gholamreza Jahangirzadeh Soureh<sup>2</sup>, Jalhe Bagheri Hamzyan Olia<sup>3</sup>, Farzaneh Esmaily<sup>4</sup>,  
Alireza Nourazarian<sup>3,5,\*</sup>, Masoud Nikanfar<sup>6</sup>

<sup>1</sup>Department of Medical Biology, Faculty of Medicine, Ege University, 35040 Izmir, Turkey

<sup>2</sup>Department of Genetics, Tabriz Branch, Islamic Azad University, 43351-14543 Tabriz, Iran

<sup>3</sup>Department of Basic Medical Sciences, Khoy University of Medical Sciences, 58139-68197 Khoy, Iran

<sup>4</sup>Department of Biology, Sciences and Research Branch, Islamic Azad University, 43351-14543 Tehran, Iran

<sup>5</sup>Neurosciences Research Center (NSRC), Tabriz University of Medical Sciences, 91656-73117 Tabriz, Iran

<sup>6</sup>Department of Neurology, Faculty of Medicine, Tabriz University of Medical Sciences, 91656-73117 Tabriz, Iran

\*Correspondence: [noorazarian\\_a@khoyums.ac.ir](mailto:noorazarian_a@khoyums.ac.ir) (Alireza Nourazarian)

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## Abstract

Ischaemic stroke is a sudden neurological disorder caused by localised cerebral ischaemia and persistent cerebral infarction. Occlusion of large arteries due to atherothrombosis, cerebral embolism (i.e., embolic infarction), no thrombotic occlusion in small, deep cerebral arteries (i.e., lacunar infarction), and stenosis of proximal arteries due to hypotension leading to decreased cerebral blood flow in arterial supply zones are the most common causes of ischemic stroke (i.e., hemodynamic stroke). It is now known that organelles play an important role in various signaling events and cellular functions. The molecular mechanisms of mitochondria are involved in cerebral ischemia by generating and scavenging reactive oxygen species, apoptosis, biogenesis, mitochondrial dynamics, and inflammation are all examples of electron transport chain dysfunction. More knowledge about the involvement of mitochondria in ischemia-induced neuronal death and neuronal protection will contribute to the development of better treatment programs for stroke syndromes such as ischemic stroke.

**Keywords:** ischemic stroke; mitochondrial biogenesis; neuronal death; electron transport chain

## 1. Introduction

Stroke is the most common cause of mental and physical disability and has a high mortality rate in industrialized countries [1]. Rupture or occlusion of a blood vessel causes stroke. Stroke-related impairments always occur at onset (unlike brain tumor symptoms, which gradually worsen) and last longer than 24 hours. They are associated with visible cerebral vascular damage on neurological examination and imaging [2]. The pathophysiology of cerebral ischemic stroke consists of hypoxia and vascular damage, followed by inflammation, apoptosis, etc., resulting in neuronal damage and impaired brain function [3]. According to current guidelines, Intravenous thrombolysis (IVT) and tissue plasminogen activator (tPA) is the first choice of treatment for acute ischemic stroke [4]. Due to the limited time window of therapy, only about 8% of patients are eligible for tPA [5].

Mitochondria are multifunctional organelles involved in many metabolic activities, including energy production and synthesis of biomolecules [6–8]. Shape and distribution of mitochondria within the cell vary with time and reflect the metabolic state of a particular cell type [7]. They organize themselves into a tubular network within the cell that is constantly changing through cell division and fusion.

The network breaks down into parts that spontaneously re-assemble when mitochondria are removed from a cell [8,9]. Isolated mitochondria are capable of respiration and ATP production. They retain the composition, structure, and potential of their membranes, as well as their ability to fuse and import proteins from the environment [10,11]. Dynamic mitochondrial imbalance, free radical formation, calcium accumulation, intrinsic cell death activation, and finally cell death occurs due to negative stimuli as they do in neurodegenerative diseases [12,13].

Mitochondrial biogenesis is a property of mitochondria that generates new mitochondria from existing mitochondria [14]. Therefore, maintaining or improving mitochondrial activity may be a therapeutic strategy for neurodegenerative diseases. Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) co-activator-1alpha (PGC-1) regulates this biogenesis process. PGC-1 activates nuclear respiratory factors 1 and 2 (NRF1 and NRF2) and mitochondrial transcription factor A via phosphorylation or deacetylation (Tfam). When this PGC-1-NRF-Tfam pathway is activated, mitochondrial DNA, proteins and new mitochondria are synthesized [15]. There is evidence of a close association between neuronal cell death and excessive production of reactive oxygen species (ROS)



in some neurological diseases, including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), ischemic stroke, epilepsy, and traumatic brain injury (TBI) [16,17]. Elevated ROS levels damage brain tissue structural and functional integrity, a process critical to the pathophysiology of cerebral ischemia [18–20].

The remarkable role of mitochondrial dysfunction and excessive oxidative stress in the ischaemic cascade has been well established. Recent research has shown that mitochondrial biogenesis and ROS detoxification are two crucial endogenous protective mechanisms in acute cerebral ischemia and chronic neurodegenerative diseases [20–22]. Therefore, preventing the adverse effects of oxidative stress and improving mitochondrial function may be promising strategies for treating ROS-related diseases, including ischaemic stroke.

## 2. Pathophysiology of Ischemic Stroke

According to the National Institute on Aging, stroke is the leading cause of mortality and morbidity in individuals aged >65 [23]. There are two types of strokes: hemorrhagic and ischemic, with ischemic strokes being the most common. Hemorrhagic strokes occur when a blood vessel ruptures and bleeds into the brain, while ischemic strokes occur when a blood clot blocks or severely narrows an artery that supplies blood to the brain. Ischemic stroke causes irreversible damage in the core of the brain, surrounded by reversible damage in the penumbra. The extent of damage varies depending on how long blood flow was obstructed. The longer the reperfusion process takes, the greater the injury [24]. Ischaemia occurs mainly in the infarct core and ischaemic penumbra. Within minutes, blood flow in the infarct core falls below the threshold of energy failure (i.e., 15%–20%), resulting in permanent damage to the infarct core, which may never recover [25]. Functions within the ischaemic penumbra may be impaired, although structural integrity is maintained. The cells in the penumbra can be rescued by vigorous reperfusion, and there is adequate collateral circulation to meet the glucose and oxygen needs of the neurons [24,26].

After stroke, reperfusion occurs in various tissues including the brain, heart, and kidneys. After reperfusion, further damage may occur due to increased  $\text{Ca}^{2+}$  and ROS levels in the tissues. Tissue damage caused by reperfusion is essentially a molecular and cellular process [27]. Excessive ROS production impairs cellular antioxidant defense mechanisms, leading to decreased free radical scavenging and eventual cell death. Reperfusion-induced ROS damage damages DNA, proteins, and lipids and impairs mitochondrial function [28]. After reperfusion injury, impaired mitochondrial function leads to decreased activity and ATP concentrations. Reperfusion-induced oxidative stress is also associated with mitochondrial DNA damage. ROS can damage cells or induce cell death via necrosis or

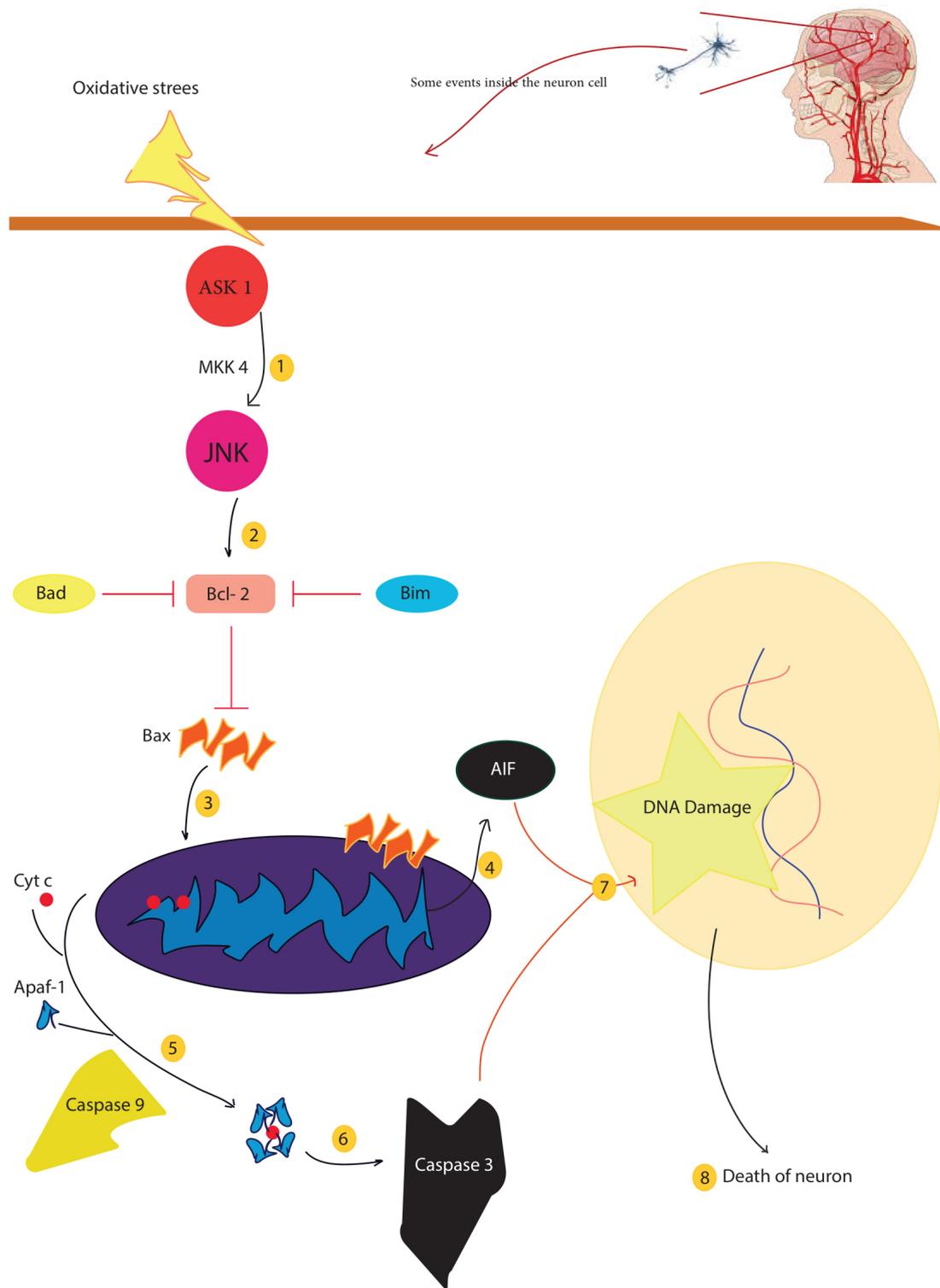
apoptosis (Fig. 1) [29]. Accumulation of reactive oxygen species and calcium ions increases membrane permeability and leads to cell death. Despite their low energy stores, neurons have higher energy requirements than other brain cells. Mitochondrial failure develops minutes after ischemia due to glucose and oxygen deficiency, resulting in decreased ATP synthesis and excess ROS. ATP deficiency is a significant trigger for ischemic cascades, including failure of membrane ion pumps; cellular potassium efflux; sodium, chloride, and water influx; and membrane depolarization [30–32].

Many processes are associated with neuronal loss and cell death after stroke, including mitochondrial dysfunction, excitotoxic cell damage, free radical production, protein misfolding, acid toxicity, and inflammation [33,34].

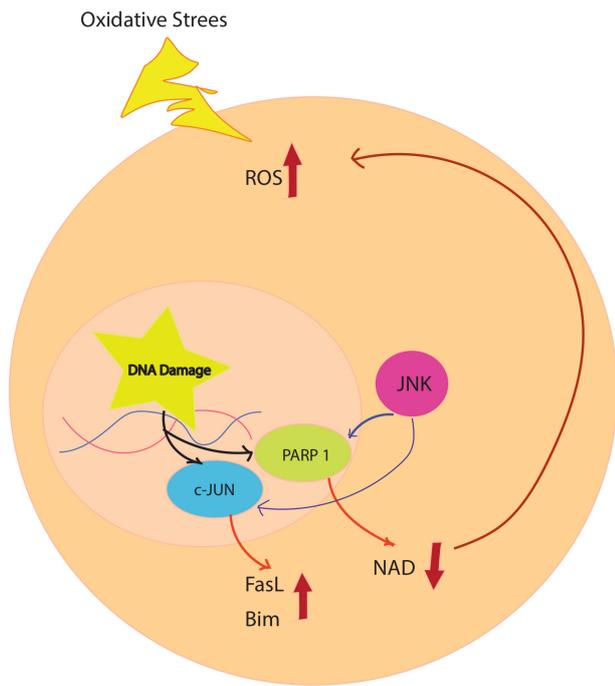
Regulated cell death, such as apoptosis, autophagy, pyroptosis, and necroptosis, is induced by ischemia and contributes to brain damage after stroke. The cell death mechanisms may regulate each other to some extent [35, 36]. Mitochondria are an essential regulator of stress signals [37]. Ischemia impairs mitochondrial respiratory activity and membrane potential, triggering a cascade that ultimately leads to neuronal death (Fig. 2) [38]. Depolarized mitochondria produce an excess of ROS due to their depolarization. The accumulation of ROS and calcium causes the membrane permeability transition to open. This releases cytochrome c, which activates effector caspases and eventually terminates apoptosis [39]. Disruption of signaling pathways may gradually interrupt or delay malignant responses and eventually lead to cell death. Therefore, you should understand mitochondrial biogenesis.

## 3. Structure and Function of Mitochondria

Double-membraned intracellular organelles such as mitochondria are found in all cells and are widely distributed. The outer membrane of the phospholipid bilayer contains protein channel structures that allow molecules with a maximum molecular weight of 10 kDa to pass through, including water, ions, nutrient molecules, ADP, and ATP. The inner membrane layer acts as a reactive apparatus for energy metabolism in the mitochondria. It contains electron transport protein complexes, ATP synthesis complexes, and ATP/ADP transport proteins. Water, oxygen, and carbon dioxide can pass through the inner membrane. The mitochondrial ETC (electron transport chain) plays an essential role in cellular energy production because it most strongly influences the process of oxidative phosphorylation, which involves several enzyme complexes (i.e., complexes I to V) in the inner mitochondrial membrane [40,41]. As biochemically demonstrated, the central part of ATP is used for neuronal electrical activity [42]. For neurons to survive and be excitable, mitochondria must provide sufficient energy. In addition to energy production, mitochondria are an important source of ROS and regulate apoptosis and neurodegenerative diseases [43–45].



**Fig. 1.** There are several factors that lead to mitochondrial dysfunction, including mitochondrial fission and fusion, mitochondrial-induced apoptosis, and mitochondrial phagocytosis. Oxidative stress caused by cerebral ischemia activates mitochondrial death signals, ASK1 activates JNK via MKK4. Activated JNK disrupts the balance of Bcl-2 proteins and mitochondrial membranes, leading to DNA damage and eventually neuronal death. In addition, mitochondrial cytochrome c can induce apoptosis and lead to neuronal cell death. Injury to neurons leads to ischemia, hypoxia, acidosis, and abnormal concentrations of ions, such as calcium and iron. Apaf-1, apoptosis-activating factor-1; ASK1, apoptosis signal-regulating kinase 1; cyt c, cytochrome c; FasL, Fas ligand; JNK, c-Jun–terminal kinase; NAD, nicotinamide adenine dinucleotide; PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species; AIF, Apoptosis-inducing factor.



**Fig. 2. Oxidative stress simultaneously causes DNA damage and activation of ASK1.** DNA damage activates PARP1, which leads to degradation of NAD and an increase in ROS. This cycle repeats because PARP1 activation can activate ASK1, and JNK activation can activate PARP after ischemia. Progressive accumulation of ROS increases JNK activation and damage to multiple mitochondria, leading to neuronal death. ASK1, apoptosis signal-regulating kinase 1; FasL, Fas ligand; JNK, c-Jun-terminal kinase; NAD, nicotinamide adenine dinucleotide; PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species.

Neurons are highly dependent on mitochondria for various functions, including ATP generation and calcium signaling. Therefore, they are sensitive to hypoxia and ischemia during stroke. Therefore, damage to mitochondria has destructive effects on the central nervous system (CNS). Mitochondria have been thought to spend their entire lives inside cells. Recently, there is evidence of intercellular exchange of organelles and membrane vesicles using structures such as microvesicles or tunnel nanotubes (TNT) [46,47]. Several models of cardiovascular injury, including experimental stroke, have shown that transfer of mitochondria occurs [48,49]. Numerous studies have shown that intercellular mitochondrial transfer can act as a protective mechanism that preserves injured cells from the effects of stress-induced mitochondrial dysfunction [48,50,51]. By restoring alveolar bioenergetics via mitochondrial transfer and Connexin 43 (Cx43)-dependent alveolar attachment, Bone marrow (BM)-derived stromal cells protect lungs from acute lung injury [52]. The data show that there is an exchange between healthy and injured cells by mitochondrial transfer. Recipient cells can take up and reprogram the released mitochondria to activate cell survival signals.

#### 4. The Role of Mitochondria in Nerve Cells

Mitochondria are highly active organelles that divide purposefully, fuse, and migrate through axons and dendrites [53]. Mitochondria in neurons arise from a highly dynamic compartment in form and function, including the movement of mitochondria through neuronal segments and the strategic placement of mitochondria at sites where ATP supply and Calcium ion ( $\text{Ca}^{2+}$ ) handling are required. And ions and substrates mediate a highly regulated link between neuronal activity and mitochondrial function. Mitochondria are critical for neuron survival, neurotransmission, and plasticity [54,55]. Neurons are increasingly dependent on mitochondrial activity to establish membrane excitability and to carry out complex neurotransmission and plasticity processes [56]. Mitochondrial dynamics and metabolic changes in human neurogenesis were studied using neural progenitor cell (NPCs) generated from human pluripotent stem cells [57,58].

At different stages of neuronal development, the expression patterns of metabolic genes were studied. As NPCs differentiate, the expression of *mitofusin 2 (MFN2)*, an important component of mitochondrial fusion, increases [57]. MFN2 deficiency inhibits neuron formation in NPCs, whereas overexpression promotes neuronal development, suggesting a crucial function of mitochondrial dynamics in human neurogenesis. During differentiation of NPCs into neurons, expression of the essential glycolytic genes *Hexokinase 2 (HK2)* and *Lactate Dehydrogenase A (LDHA)* is reduced. However, transcript levels of most *oxidative phosphorylation (OXPHOS)* genes remain constant, as during neurogenesis in the adult hippocampus [58]. Mitochondria are concentrated in the pre- and post-synapses of the formed neurons, where there is a substantial energy demand [59,60]. At synaptic terminals, mitochondria supply local ATP to neurons and regulate cytosolic calcium levels. Neuron function would be impaired if mitochondria could not communicate with the center throughout development and beyond [61]. Astrocytes are essential components of the central nervous system as they are involved in neurodevelopment, neurotransmission, cerebral metabolism, and control of blood flow [62]. Normal astrocytes protect neurons from oxidative stress and excitotoxicity [63,64]. Dysfunctional astrocytes can produce harmful substances that cause neuronal damage [65]. Although mitochondria are derived from internal nuclei for energy production and survival, they can also be released into the extracellular environment [66]. The ability of neuroglia to maintain these protective mechanisms may depend on the integrity of their mitochondria, as inhibition of astrocytic mitochondria provides a source of energy to neurons threatened with cell death [65]. Examples include retinal neurons that transport mitochondria to astrocytes for recycling and disposal and bone marrow-derived stromal cells that transport mitochondria to alveoli to protect them from acute lung injury [67,68]. Recently, it was hypothesized that neurons dis-

charge damaged mitochondria and recycle them into astrocytes [67]. Because of this ability to exchange mitochondria, it is conceivable that cells in the CNS communicate with each other.

Additionally, astrocytes can provide functional mitochondria for neurons. A calcium-dependent mechanism involving the cluster of differentiation 38 (CD38)/cyclic ADP-ribose signaling pathway regulates the ability of an astrocyte to release extracellular mitochondrial particles. In mice, transient local cerebral ischaemia causes astrocytic mitochondria to invade neighbouring neurones and enhances cell survival signals. Simultaneous suppression of CD38 signaling decreased extracellular mitochondrial transfer and worsened neurological status [48]. Astrocytes, another subset of glial cells in the brain, perform important physiological roles, including neuroprotective effects in the A2 state and neuroinflammatory functions in the A1 state. In animal models, active microglia rapidly generate the A1 state of astrocytes after brain injury, leading to further neuronal damage and cell death [69]. Drp1/Fis1-triggered enhanced fission of mitochondria in activated microglia results in the production of fragmented and damaged mitochondria that are rejected by these cells. Defective extracellular mitochondria, therefore, contribute to the propagation of neurodegenerative signals from microglia [70]. This study shows that extracellularly damaged mitochondria release more damaged mitochondria from microglia when they lose their immune-privileged state and that damaged extracellular mitochondria play a direct role in disease propagation by acting as effectors of the innate immune response on nearby astrocytes and neurons. Fragmented mitochondria have been detected in clinical and experimental studies of biofluids from individuals with subarachnoid hemorrhage and stroke [48,71]. While extracellularly injured mitochondria are harmful, the mitochondrial transfer is beneficial, as demonstrated in a mouse model of acute lung injury and a stroke model [48,68]. Joshi *et al.* [70] show that the ratio of damaged to functional mitochondria in the extracellular environment, determined by the extent of pathological fission in donor microglia, affects the fate of neurons.

## 5. Mitochondrial Biogenesis in Neurodegeneration

The gradual loss of neurones and tissue damage in the brain is known as neurodegeneration. Because of their high energy requirements, neurons are susceptible to damage and death from dysfunctional mitochondria. Severe damage to mitochondria leads to cell death because cells can no longer generate sufficient energy. Many clinical and physiological studies suggest that mitochondrial dysfunction and dynamics play a critical role in the development of neurodegenerative diseases [72]. Mitochondrial biogenesis is required to maintain mitochondrial homeostasis throughout the mitochondrial life cycle to meet the physiological needs of eukaryotic cells [73].

After long-term local cerebral ischemia, progressive loss of mitochondrial DNA (mtDNA) suggests mitochondrial regeneration. Several studies have shown that creating a dense population of functional mitochondria reduces ROS production [74]. Mitochondrial biogenesis is a precise process that requires both mitochondrial and nuclear genomes. It often occurs in healthy cells that are constantly dividing and fusing, whereas division (fission) after brain trauma leads to fragmentation of the mitochondrial network [75]. Increased biogenesis is associated with increased mitochondrial DNA copy number and increased mitochondrial gene expression. Peroxisome proliferator-activated receptor co-activator 1 alpha (PGC-1 $\alpha$ ), mitochondrial transcription factor A (TFAM), nuclear respiratory factors 1 and 2 (NRF1 and NRF2), and mitochondrial transcription factor B1 (TFB1M) are involved in mitochondrial biogenesis [76]. Several processes appear to be involved in the mitochondrial formation, including (1) outer and inner membrane synthesis, (2) mitochondrial protein synthesis, (3) synthesis and import of proteins encoded by the nuclear genome, (4) lipid import, (5) mitochondrial DNA replication, (6) mitochondrial Fusion and fission, and mitochondrial Fusion and fission [77–80].

Due to altered physiological circumstances, such as increased ATP consumption, the cell's ability to produce ATP is impaired. Cell division and repair, embryonic development, changes in physiological state (e.g., sympathetic stimulation, caloric restriction, exercise, cold stress, and energy deficiency), hormones (thyroid hormone, leptin, and erythropoietin), and mitochondrial disease/damage (e.g., inflammation and hypoxia/ischemia) are all major triggers of mitochondrial biogenesis [80]. Another important trigger of mitochondrial biogenesis is mitochondrial oxidative dysfunction, which contributes to various neurological disorders, as shown by numerous studies [80].

It is emphasized that decreased mitochondrial biogenesis can lead to mitochondrial dysfunction, which is necessary for the development of neurodegenerative diseases. These diseases have been linked to genes that produce proteins associated with mitochondrial biogenesis [81].

AD is a common neurodegenerative disease characterized by memory loss and cognitive impairment and manifested by various neurological, mental, and behavioral symptoms [82,83]. Recent studies have shown that mitochondrial dysfunction is an essential and early feature of AD, with reduced energy metabolism being one of the most visible critical abnormalities of the disease [84]. Mitochondrial intervention may help slow the progression of amyloid plaque (A $\beta$ )-induced degradation and dysfunction. These findings are supported by *in vivo* observations of mitochondrial accumulation in AD patients [85,86]. Degradation of  $\beta$ -secretase controlled by PGC-1 $\alpha$ , a transcriptional co-activator, has been shown to control the formation of A (BACE1). PGC-1 $\alpha$  is a transcription factor that affects energy metabolism by regulating mitochondrial ac-

tivity in various tissues. Amyloid precursor protein (APP) is altered in the brains of patients with AD, with increased a-secretase activity contributing to pathological A $\beta$  formation [87]. PGC-1 expression is reduced in the brains of patients with AD, and this has been linked to the pathological formation by altering the processing of APP through increased a-secretase activity.

Further studies have linked the expression of genes encoding proteins involved in mitochondrial biogenesis, such as *PGC-1a*, *NRF1*, *NRF2*, and *TFAM*, to the development of neurodegenerative diseases such as AD [74]. Significant downregulation of these genes is observed in AD and hippocampal cells and tissues, indicating a decrease in mitochondrial biogenesis [74]. However, due to the pleiotropic nature of PGC-1a, it is unclear how AD affects mitochondrial biogenesis signaling and whether these changes lead to mitochondrial dysfunction [74]. Parkinson's disease (PD) is a degenerative neurological movement disorder of unknown etiology influenced by hereditary and environmental factors [88]. In the early and late stages of Parkinson's disease (PD), significant reductions in putamen ATP and phosphocreatine levels and midbrain ATP levels as high-energy metabolites indicate mitochondrial dysfunction in mesostriatal dopaminergic neurons [89]. Genome-wide analysis of normal subjects and patients with PD revealed that PGC-1a, a key regulator of mitochondrial biogenesis, was reduced in patients with PD [90], suggesting that mitochondrial dysfunction plays a critical role in the cause of PD [91] has found evidence that the deficiency in PD is at or above the level of PGC-1a expression, which controls mitochondrial complex I function. Recent research has linked several proteins involved in familial PD, including  $\alpha$ -syn, Parkin, PTEN-induced putative kinase 1 (PINK1), protein deglycase DJ-1 (Parkinson protein 7), and Leucine-Rich repeat kinase 2 (LRRK2 or Dardarin), to quality control and regulation of mitochondrial dynamics [92,93]. Amyotrophic lateral sclerosis (ALS) is a neurological disorder that manifests as gradual paralysis in adults and is a genetic disorder. In humans, the disease is associated with degeneration of motor neurons and selective loss in the spinal cord and brain, resulting in denervation of muscles and muscle wasting after denervation [94]. Postmortem spinal cord specimens from individuals with Lou Gehrig disease (ALS) show morphological and biochemical mitochondrial abnormalities, including decreased mitochondrial DNA copy number and decreased respiratory chain activity [95]. Mutations/deletions in mitochondrial genes have been associated with specific muscle and neuronal diseases [96]. Mitochondrial dysfunction can lead to certain damage to muscles or neurons.

PGC-1a is thought to have beneficial effects on muscles, including increased mitochondrial activity and muscle hypertrophy. Mitochondrial activity and biogenesis are preserved in late-stage disease, as is muscle function, delayed muscle wasting, and improved muscle tolerance. Although

lifespan could not be extended, drugs that increase PGC-1a activity in muscles are beneficial for preserving muscle functionality throughout ALS [97]. Activation of peroxisome proliferator-activated receptors (PPARs) that interact with PGC-1a and control mitochondrial biogenesis in combination with this transcriptional co-activator is another strategy to increase PGC-1a signaling and improve mitochondrial function [98]. Another study conducted in cell cultures showed that Sirtuin 3 (SIRT3) protects against mitochondrial fragmentation and neuronal cell death during developing AD [99]. The regulating PPARc transcriptional activity in the central nervous system represents a novel approach to slowing ALS progression [100]. Mitochondria, important intracellular organelles that control cell life and death, are being investigated as potential therapeutic targets in pharmaceutical research.

## 6. The Role of Mitochondria in Ischemic Stroke

Mitochondria are critical for cell survival and death. Pathophysiological abnormalities transform mitochondria from essential organelles into activators of cell death [101, 102]. Mitochondria play a crucial role in cell death by ischemia by impairing ATP synthesis and participating in apoptotic and necrotic pathways [103]. The main goal of stroke research is to elucidate the process of neuronal ischemic injury to improve functional outcomes. Neurons are more susceptible to ischemic damage than other cells because they are constantly dependent on an external energy supply. As excitable cells, neurons express many receptors for excitatory transmitters, especially at synapses [104]. In stroke, oxygen and glucose deprivation are the main components of tissue damage. OGD (oxygen-glucose deprivation) models are the most commonly used stroke *in vitro* models. Hydrogen peroxidase exposure or glutamate-induced excitotoxicity is another *in vitro* stroke model. Studies on brain slices or cultured neurons have contributed significantly to our knowledge of neuroprotective and ischemic injury processes [105]. Mitochondria produce energy and are very numerous in brain cells because neurons need a lot of ATP to function properly [106].

Mitochondria use an electron transport chain (ETC) to convert electron donor molecules such as reduced (NADH) nicotinamide adenine dinucleotide into energy through various oxidation/reduction processes. The compounds discussed here transfer electrons and support transmembrane proton transport, creating an electrochemical gradient that favors mitochondrial ATP synthesis [107]. Five enzyme complexes consisting of integral internal membrane proteins are found in ETC: NADH-CoQ reductase, Coenzyme Q (CoQ) cytochrome c reductase, succinate CoQ reductase, cytochrome c oxidase, and ATP synthase (complexes I–V) [108]. Cytochrome c and ubiquinone (CoQ)<sup>++</sup> are two molecules that circulate freely in the complexes and control electron transport. Oxygen is the final electron acceptor

**Table 1. Some recent studies that by increasing biogenesis and improving mitochondrial function, protect against brain injuries caused by cerebral ischemia.**

Cell/Animal model	Conditions of research	Treat wht	Mechanism	Results	Mitochondrial biogenesis	Improves mitochondrial function	Ref
OGD/R-induced SH-SY5Y cells	<i>In vitro</i>	PNGL	Mitochondrial protective effects of PNGL, mediated via the NAMPT-NAD <sup>+</sup>	Inhibited mitochondrial damage, raised neuronal survival under ischemia		√	[130]
MCAO-rat model	<i>In vivo</i>	Selenium	Protects neurons from cerebral I/R damage by mitochondrial biogenesis	Ameliorates cerebral I/R injury	√	√	[131]
NSCs injured by OGD	<i>In vitro</i>	Amlodipine camsylate	Protection against mitochondria cause raised neuronal survival	Increased the viability and proliferation of NSCs	√	√	[132]
MCAO-rat model	<i>In vivo</i>	Sovateltide	ETBR mediated neural regeneration and repair	Activates neuronal differentiation and prevents mitochondrial dysfunction	√		[3]
C57BL/6 mice	<i>In vivo</i>	Taurine	Axonal Sprouting Through Mitochondrial Improvement	Recovered the motor function of focal cerebral cortical ischemic mice	√		[133]
HPPNCs	<i>In vitro</i>	CTRP3	CTRP3 prevented mitochondria from OGD/R injury by activating the AMPK/SIRT1-PGC-1 $\alpha$ pathway	Angiogenesis and enhancement of mitochondrial biogenesis	√	√	[134]
Human neuroblastoma SK-N-SH cells, subjected to OGD	<i>In vitro</i>	Ginsenoside Rg1	Rg1 improves mitochondrial dysfunction by regulating autophagy in mitochondria	Improves mitochondrial dysfunction, protection from brain injuries		√	[135]
Bb2 KO-mice	<i>In vivo</i>	Drp1	Bb2, a mitochondria-localized PP2A regulatory subunit, is a neuron-specific Drp1 activator <i>in vivo</i>	Mitochondrial biogenesis, maintenance of mitochondria	√	√	[136]

OGD/R, oxygen and glucose deprivation and reperfusion; PNGL, Notoginseng leaf triterpenes; MCAO, Middle cerebral artery occlusion; I/R, ischemia-reperfusion; NSCs, neural stem cells; OGD, oxygen glucose deprivation; AC, amlodipine camsylate; ETBR, endothelin B receptors; HPPNCs hippocampal neuronal cells; CTRP3, C1q/tumor necrosis factor-related protein-3; Drp1, dynamin-related protein 1; PP2A, protein phosphatase 2A; Ref, Reference.

in the inner mitochondrial membrane and generates an electrochemical proton gradient (IMM) [109]. ROS are mainly generated in this ETC, especially after reperfusion. Numerous findings suggest that most ROS generated during reperfusion is due to mitochondrial ETC, which exacerbates ischemic cell death [110]. After ischemia, the significant lack of ATP generation and the elevated levels of ROS trigger the mitochondrial death pathway by apoptosis [111]. After ischemia, brain cells produce less ATP, compromising membrane-bound channels required for normal cell function. Mitochondria generate significant ROS.

The superoxide anion radical is a precursor of several other potentially dangerous ROS, including hydroxyl, hydrogen peroxide, and peroxynitrite. Mitochondrial dysfunction is caused by oxidative stress, energy deficiency, and impaired calcium homeostasis in the cell. Reperfusion increases the production of ROS in mitochondria, and  $\text{Ca}^{2+}$  activates caspase-independent and caspase-dependent death pathways [112].

Mitochondria, as receptors for a variety of stimuli, trigger both caspase-independent (AIF, Apoptosis-inducing factor) and caspase-dependent (cytochrome c) cell death [113]. Ischemia promotes these processes and leads to necrotic and apoptotic death of brain cells. Inflammation is another critical factor in the pathophysiology of cerebral ischemia. Glial cell activation, infiltration by peripheral leukocytes, and damage-associated chemicals such as high mobility group protein 1, nucleotides, nucleic acid fragments, and purines trigger the inflammatory response after ischemia [114,115]. Acute systemic inflammatory stimuli increase the outcome of ischemic stroke, with the pro-inflammatory cytokine IL-1 playing a crucial role [116]. Recent research has shown that mitochondria alter their function in controlling the inflammatory response [117–119]. Mitochondria are the primary regulators of Neutrophil-Lymphocyte Ratio (NLR) Family Pyrin-Domain-Containing Protein 3 (NLRP3) inflammasome activation [120]: the outer mitochondrial membrane serves as an assembly platform for the inflammasome. It also induces pyroptosis and innate immune defence through caspase-1 and various pro-inflammatory cytokines [121,122]. Recent studies indicate that the NLRP3 inflammasome contributes to renal and cardiac ischemia [123–126]. This review focuses on the rapidly evolving and complex function of mitochondria in ischemic cerebral stroke. Understanding the fundamental processes behind the potentially protective activities of mitochondria may offer insights into developing new treatments.

## 7. Mitochondrial Biogenesis in Ischemic Stroke

The function of mitochondrial biogenesis in neurons is poorly understood. First, researchers investigated the transcriptional control of mitochondrial biogenesis in mice lacking the mitochondrial transcription factor peroxisome

proliferator-activated receptor co-activator (PGC) 1. A ROS-mediated mechanism is involved in the progression of oxidative stress-induced neurodegeneration [127]. Hypoxic preconditioning increases *PGC-1* expression in response to neuronal nitric oxide synthase (nNOS) activity. Oxidative stress is associated with widespread mitochondrial fission and neuronal death throughout the body. It has been shown that mitofusin 2, a mitochondrial Fusion protein, overexpression can reverse neuronal death [81]. For neuronal survival to occur, precise regulation of Fusion and fission must be maintained. Recent studies have also shown that hypoxia-ischemia stimulates mitochondrial production. Under hypoxia, mitochondrial DNA increases, as does the number of mitochondrial cells [128]. Hypoxia also increases the production of PGC-1, downstream mitochondrial transcription factors (mitochondrial transcription factor A and nuclear respiratory factor 1), and the mitochondrial protein heat shock protein 60 (HSP60). This breakthrough finding suggests that mitochondrial biogenesis is a novel endogenous neuroprotective response that can be activated.

Mitochondria are damaged after a fatal ischemic insult, and autophagy is triggered to remove damaged organelles from the cell. The maintenance of energy balance and calcium and zinc homeostasis is essential for neuronal function. Microsomal biogenesis is a crucial endogenous neuroprotective process that produces new functional mitochondria when the body is exposed to high-stress levels. A novel strategy for neuroprotection is to increase or accelerate mitochondrial biogenesis, a process unknown. Further studies are needed to understand the molecular basis of mitochondrial biogenesis after stroke. Other studies on the role of mitochondrial turnover, mitochondrial Fusion and fission mechanisms, and alternative transcriptional pathways after ischemia are warranted [129]. Identifying critical mediators of autophagy and mitochondrial biogenesis will lead to the identification of new therapeutic targets to enhance the beneficial effects of mitochondrial maintenance (Table 1, Ref. [3,130–136]).

## 8. Conclusions

The lack of ATP and nutrients in ischemia impairs the leading role of mitochondria in stroke pathology. The rapid degeneration of penumbral neurovascular units essentially requires a new effective therapeutic strategy for stroke. Recent evidence of mitochondrial transfer through the formation of TNT, cell Fusion or extracellular vesicles (EVs) and the potential restoration of mitochondrial function in promoting cell survival in numerous preclinical studies argues for stem cell-mediated mitochondrial transfer therapies in stroke. Mitochondria are mainly in the presynaptic nerve terminals of neurons. When ischemia occurs, neurons are likely to shift mitochondria for both disposal and recycling to respond quickly to the lack of energy caused by the condition. After ischemia, mitochondria cannot be re-

plenished promptly because they must be transported long distances. Therefore, extracellular mitochondrial translocation may provide a new opportunity for neuroprotective strategies. Further research is needed to understand better the impact of mitochondrial biogenesis and function on ischemic stroke.

### Author Contributions

BS, CBA, MN, and AN developed the concept and designed the study. VK, GJS, FE, and JBHO did a systematic search and prepared the first draft. All authors participated in the revising of the manuscript before submission. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

### Ethics Approval and Consent to Participate

Not applicable.

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### Conflict of Interest

The authors declare no conflict of interest.

### References

- [1] Shademan B, Nourazarian A, Laghousi D, Karamad V, Nikanfar M. Exploring potential serum levels of Homocysteine, interleukin-1 beta, and apolipoprotein B 48 as new biomarkers for patients with ischemic stroke. *Journal of Clinical Laboratory Analysis*. 2021; 35: e23996. (Online ahead of print)
- [2] Ajoolabady A, Shademan B, Avci CB, Nikanfar M, Nourazarian A, Laghousi D. Diagnostic Potential of Autophagy-5 Protein, Apolipoprotein B-48, and Oxidative Stress Markers in Serum of Patients with Early-Stage Ischemic Stroke. *World Neurosurgery*. 2022; 167: e656–e663.
- [3] Gol S, Pena RN, Rothschild MF, Tor M, Estany J. A polymorphism in the fatty acid desaturase-2 gene is associated with the arachidonic acid metabolism in pigs. *Scientific Reports*. 2018; 8: 14336.
- [4] Ferrigno M, Bricout N, Leys D, Estrade L, Cordonnier C, Peronnin T, *et al*. Intravenous Recombinant Tissue-Type Plasminogen Activator: Influence on Outcome in Anterior Circulation Ischemic Stroke Treated by Mechanical Thrombectomy. *Stroke*. 2018; 49: 1377–1385.
- [5] Peña ID, Borlongan C, Shen G, Davis W. Strategies to Extend Thrombolytic Time Window for Ischemic Stroke Treatment: An Unmet Clinical Need. *Journal of Stroke*. 2017; 19: 50–60.
- [6] Naia L, Pinho CM, Dentoni G, Liu J, Leal NS, Ferreira DMS, *et al*. Neuronal cell-based high-throughput screen for enhancers of mitochondrial function reveals luteolin as a modulator of mitochondria-endoplasmic reticulum coupling. *BMC Biology*. 2021; 19: 57.
- [7] Lackner LL. Shaping the dynamic mitochondrial network. *BMC Biology*. 2014; 12: 35.
- [8] Alexandre A, Reynafarje B, Lehninger AL. Stoichiometry of vectorial H<sup>+</sup> movements coupled to electron transport and to ATP synthesis in mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*. 1978; 75: 5296–5300.
- [9] Viana MP, Brown AI, Mueller IA, Goul C, Koslover EF, Rafelski SM. Mitochondrial Fission and Fusion Dynamics Generate Efficient, Robust, and Evenly Distributed Network Topologies in Budding Yeast Cells. *Cell Systems*. 2020; 10: 287–297.e5.
- [10] Meeusen S, McCaffery JM, Nunnari J. Mitochondrial fusion intermediates revealed in vitro. *Science (New York, N.Y.)*. 2004; 305: 1747–1752.
- [11] Schmidt O, Pfanner N, Meisinger C. Mitochondrial protein import: from proteomics to functional mechanisms. *Nature Reviews. Molecular Cell Biology*. 2010; 11: 655–667.
- [12] Misrani A, Tabassum S, Yang L. Mitochondrial Dysfunction and Oxidative Stress in Alzheimer’s Disease. *Frontiers in Aging Neuroscience*. 2021; 13: 617588.
- [13] Cenini G, Lloret A, Cascella R. Oxidative Stress in Neurodegenerative Diseases: From a Mitochondrial Point of View. *Oxidative Medicine and Cellular Longevity*. 2019; 2019: 2105607.
- [14] Simmons EC, Scholpa NE, Schnellmann RG. Mitochondrial biogenesis as a therapeutic target for traumatic and neurodegenerative CNS diseases. *Experimental Neurology*. 2020; 329: 113309.
- [15] Chattopadhyay E, Roy B. Altered Mitochondrial Signalling and Metabolism in Cancer. *Frontiers in Oncology*. 2017; 7: 43.
- [16] Song K, Li Y, Zhang H, An N, Wei Y, Wang L, *et al*. Oxidative stress-mediated blood-brain barrier (BBB) disruption in neurological diseases. *Oxidative Medicine and Cellular Longevity*. 2020; 2020: 1–27.
- [17] Stephenson J, Nutma E, van der Valk P, Amor S. Inflammation in CNS neurodegenerative diseases. *Immunology*. 2018; 154: 204–219.
- [18] Popa-Wagner A, Mitran S, Sivanesan S, Chang E, Buga AM. ROS and brain diseases: the good, the bad, and the ugly. *Oxidative Medicine and Cellular Longevity*. 2013; 2013: 963520.
- [19] Chen SD, Lin TK, Yang DI, Lee SY, Shaw FZ, Liou CW, *et al*. Protective effects of peroxisome proliferator-activated receptors gamma coactivator-1alpha against neuronal cell death in the hippocampal CA1 subfield after transient global ischemia. *Journal of Neuroscience Research*. 2010; 88: 605–613.
- [20] Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, *et al*. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Frontiers in Physiology*. 2020; 11: 694.
- [21] Oscai LB, Caruso RA, Wergeles AC. Lipoprotein lipase hydrolyzes endogenous triacylglycerols in muscle of exercised rats. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*. 1982; 52: 1059–1063.
- [22] Guo JD, Zhao X, Li Y, Li GR, Liu XL. Damage to dopaminergic neurons by oxidative stress in Parkinson’s disease (Review). *International Journal of Molecular Medicine*. 2018; 41: 1817–1825.
- [23] Deuschl G, Beghi E, Fazekas F, Varga T, Christoforidi KA, Sipido E, *et al*. The burden of neurological diseases in Europe: an analysis for the Global Burden of Disease Study 2017. *The Lancet. Public Health*. 2020; 5: e551–e567.
- [24] Karsy M, Brock A, Guan J, Taussky P, Kalani MYS, Park MS. Neuroprotective strategies and the underlying molecular basis of cerebrovascular stroke. *Neurosurgical Focus*. 2017; 42: E3.
- [25] Ahnstedt H, Sweet J, Cruden P, Bishop N, Cipolla MJ. Effects of Early Post-Ischemic Reperfusion and tPA on Cerebrovascular Function and Nitrosative Stress in Female Rats. *Translational Stroke Research*. 2016; 7: 228–238.
- [26] Reegenhardt RW, Das AS, Stapleton CJ, Chandra RV, Rabinov JD, Patel AB, *et al*. Blood Pressure and Penumbral Sustenance

- in Stroke from Large Vessel Occlusion. *Frontiers in Neurology*. 2017; 8: 317.
- [27] Sims NR, Yew WP. Reactive astrogliosis in stroke: Contributions of astrocytes to recovery of neurological function. *Neurochemistry International*. 2017; 107: 88–103.
- [28] Xu H, Zheng YW, Liu Q, Liu LP, Luo FL, Zhou HC, *et al.* Reactive oxygen species in skin repair, regeneration, aging, and inflammation. *Reactive Oxygen Species (ROS) in Living Cells*. 2018; 8: 69–88.
- [29] Kiselyov K, Muallem S. ROS and intracellular ion channels. *Cell Calcium*. 2016; 60: 108–114.
- [30] Forrester SJ, Kikuchi DS, Hernandez MS, Xu Q, Griending KK. Reactive Oxygen Species in Metabolic and Inflammatory Signaling. *Circulation Research*. 2018; 122: 877–902.
- [31] Steiner P. Brain Fuel Utilization in the Developing Brain. *Annals of Nutrition & Metabolism*. 2019; 75 Suppl 1: 8–18.
- [32] Dharmasaroja PA. Fluid Intake Related to Brain Edema in Acute Middle Cerebral Artery Infarction. *Translational Stroke Research*. 2016; 7: 49–53.
- [33] Li PY, Wang X, Stetler RA, Chen J, Yu WF. Anti-inflammatory signaling: the point of convergence for medical gases in neuroprotection against ischemic stroke. *Medical Gas Research*. 2016; 6: 227–231.
- [34] Sekerdag E, Solaroglu I, Gursoy-Ozdemir Y. Cell Death Mechanisms in Stroke and Novel Molecular and Cellular Treatment Options. *Current Neuropharmacology*. 2018; 16: 1396–1415.
- [35] Duris K, Splichal Z, Jurajda M. The Role of Inflammatory Response in Stroke Associated Programmed Cell Death. *Current Neuropharmacology*. 2018; 16: 1365–1374.
- [36] Wei H, Li Y, Han S, Liu S, Zhang N, Zhao L, *et al.* cPKC $\gamma$ -Modulated Autophagy in Neurons Alleviates Ischemic Injury in Brain of Mice with Ischemic Stroke Through Akt-mTOR Pathway. *Translational Stroke Research*. 2016; 7: 497–511.
- [37] Suliman HB, Piantadosi CA. Mitochondrial Quality Control as a Therapeutic Target. *Pharmacological Reviews*. 2016; 68: 20–48.
- [38] Choi TG, Kim SS. Physiological functions of mitochondrial reactive oxygen species. *Free Radical Biology and Medicine*. 2019. Available at: <https://www.intechopen.com/chapters/69153> (Accessed: 18 April 2022).
- [39] Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica et Biophysica Acta*. 2016; 1863: 2977–2992.
- [40] Razak S, Afsar T, Almajwal A, Alam I, Jahan S. Growth inhibition and apoptosis in colorectal cancer cells induced by Vitamin D-Nanoemulsion (NVD): involvement of Wnt/ $\beta$ -catenin and other signal transduction pathways. *Cell & Bioscience*. 2019; 9: 15.
- [41] Ramsay RR. Electron carriers and energy conservation in mitochondrial respiration. *ChemTexts*. 2019; 5: 9.
- [42] Diemel GA. Brain Glucose Metabolism: Integration of Energetics with Function. *Physiological Reviews*. 2019; 99: 949–1045.
- [43] Tang JX, Thompson K, Taylor RW, Oláhová M. Mitochondrial OXPHOS Biogenesis: Co-Regulation of Protein Synthesis, Import, and Assembly Pathways. *International Journal of Molecular Sciences*. 2020; 21: 3820.
- [44] Borrás C, Gambini J, López-Gruoso R, Pallardó FV, Viña J. Direct antioxidant and protective effect of estradiol on isolated mitochondria. *Biochimica et Biophysica Acta*. 2010; 1802: 205–211.
- [45] Kausar S, Wang F, Cui H. The Role of Mitochondria in Reactive Oxygen Species Generation and Its Implications for Neurodegenerative Diseases. *Cells*. 2018; 7: 274.
- [46] Nawaz M, Fatima F. Extracellular Vesicles, Tunneling Nanotubes, and Cellular Interplay: Synergies and Missing Links. *Frontiers in Molecular Biosciences*. 2017; 4: 50.
- [47] Torralba D, Baixauli F, Sánchez-Madrid F. Mitochondria Know No Boundaries: Mechanisms and Functions of Intercellular Mitochondrial Transfer. *Frontiers in Cell and Developmental Biology*. 2016; 4: 107.
- [48] Hayakawa K, Esposito E, Wang X, Terasaki Y, Liu Y, Xing C, *et al.* Transfer of mitochondria from astrocytes to neurons after stroke. *Nature*. 2016; 535: 551–555.
- [49] Liu K, Ji K, Guo L, Wu W, Lu H, Shan P, *et al.* Mesenchymal stem cells rescue injured endothelial cells in an in vitro ischemia-reperfusion model via tunneling nanotube like structure-mediated mitochondrial transfer. *Microvascular Research*. 2014; 92: 10–18.
- [50] Melentijevic I, Toth ML, Arnold ML, Guasp RJ, Harinath G, Nguyen KC, *et al.* *C. elegans* neurons jettison protein aggregates and mitochondria under neurotoxic stress. *Nature*. 2017; 542: 367–371.
- [51] Jiang D, Gao F, Zhang Y, Wong DSH, Li Q, Tse HF, *et al.* Mitochondrial transfer of mesenchymal stem cells effectively protects corneal epithelial cells from mitochondrial damage. *Cell Death & Disease*. 2016; 7: e2467.
- [52] Rodriguez AM, Nakhle J, Griessinger E, Vignais ML. Intercellular mitochondria trafficking highlighting the dual role of mesenchymal stem cells as both sensors and rescuers of tissue injury. *Cell Cycle (Georgetown, Tex.)*. 2018; 17: 712–721.
- [53] Mattson MP, Gleichmann M, Cheng A. Mitochondria in neuroplasticity and neurological disorders. *Neuron*. 2008; 60: 748–766.
- [54] Sheng ZH, Cai Q. Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. *Nature Reviews Neuroscience*. 2012; 13: 77–93.
- [55] Pallafacchina G, Zanin S, Rizzuto R. From the Identification to the Dissection of the Physiological Role of the Mitochondrial Calcium Uniporter: An Ongoing Story. *Biomolecules*. 2021; 11: 786.
- [56] Kann O, Kovács R. Mitochondria and neuronal activity. *American Journal of Physiology. Cell Physiology*. 2007; 292: C641–57.
- [57] Fang D, Yan S, Yu Q, Chen D, Yan SS. Mfn2 is Required for Mitochondrial Development and Synapse Formation in Human Induced Pluripotent Stem Cells/hiPSC Derived Cortical Neurons. *Scientific Reports*. 2016; 6: 31462.
- [58] Zheng X, Boyer L, Jin M, Mertens J, Kim Y, Ma L, *et al.* Metabolic reprogramming during neuronal differentiation from aerobic glycolysis to neuronal oxidative phosphorylation. *ELife*. 2016; 5: e13374.
- [59] Kang JS, Tian JH, Pan PY, Zald P, Li C, Deng C, *et al.* Docking of axonal mitochondria by syntaphilin controls their mobility and affects short-term facilitation. *Cell*. 2008; 132: 137–148.
- [60] Li Z, Okamoto KI, Hayashi Y, Sheng M. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell*. 2004; 119: 873–887.
- [61] Calkins MJ, Manczak M, Mao P, Shirendeb U, Reddy PH. Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease. *Human Molecular Genetics*. 2011; 20: 4515–4529.
- [62] Kim Y, Park J, Choi YK. The Role of Astrocytes in the Central Nervous System Focused on BK Channel and Heme Oxygenase Metabolites: A Review. *Antioxidants (Basel, Switzerland)*. 2019; 8: 121.
- [63] Chen Y, Qin C, Huang J, Tang X, Liu C, Huang K, *et al.* The role of astrocytes in oxidative stress of central nervous system: A mixed blessing. *Cell Proliferation*. 2020; 53: e12781.
- [64] Wang XF, Cynader MS. Pyruvate released by astrocytes protects neurons from copper-catalyzed cysteine neurotoxicity. *The Journal of Neuroscience: the Official Journal of the Society for*

Neuroscience. 2001; 21: 3322–3331.

- [65] Haidet-Phillips AM, Hester ME, Miranda CJ, Meyer K, Braun L, Frakes A, *et al.* Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. *Nature Biotechnology*. 2011; 29: 824–828.
- [66] Nakamura Y, Park JH, Hayakawa K. Therapeutic use of extracellular mitochondria in CNS injury and disease. *Experimental Neurology*. 2020; 324: 113114.
- [67] Davis CHO, Kim KY, Bushong EA, Mills EA, Boassa D, Shih T, *et al.* Transcellular degradation of axonal mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111: 9633–9638.
- [68] Islam MN, Das SR, Emin MT, Wei M, Sun L, Westphalen K, *et al.* Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nature Medicine*. 2012; 18: 759–765.
- [69] Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, *et al.* Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 2017; 541: 481–487.
- [70] Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn GW, 2nd, *et al.* Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. *Nature Neuroscience*. 2019; 22: 1635–1648.
- [71] Hayakawa K, Chan SJ, Mandeville ET, Park JH, Bruzzese M, Montaner J, *et al.* Protective Effects of Endothelial Progenitor Cell-Derived Extracellular Mitochondria in Brain Endothelium. *Stem Cells (Dayton, Ohio)*. 2018; 36: 1404–1410.
- [72] Benaroya H. Brain energetics, mitochondria, and traumatic brain injury. *Reviews in the Neurosciences*. 2020; 31: 363–390.
- [73] Sheng B, Wang X, Su B, Lee HG, Casadesus G, Perry G, *et al.* Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer’s disease. *Journal of Neurochemistry*. 2012; 120: 419–429.
- [74] Valerio A, Bertolotti P, Delbarba A, Perego C, Dossena M, Ragni M, *et al.* Glycogen synthase kinase-3 inhibition reduces ischemic cerebral damage, restores impaired mitochondrial biogenesis and prevents ROS production. *Journal of Neurochemistry*. 2011; 116: 1148–1159.
- [75] Geto Z, Molla MD, Challa F, Belay Y, Getahun T. Mitochondrial Dynamic Dysfunction as a Main Triggering Factor for Inflammation Associated Chronic Non-Communicable Diseases. *Journal of Inflammation Research*. 2020; 13: 97–107.
- [76] Golpich M, Rahmani B, Mohamed Ibrahim N, Dargahi L, Mohamed Z, Raymond AA, *et al.* Preconditioning as a potential strategy for the prevention of Parkinson’s disease. *Molecular Neurobiology*. 2015; 51: 313–330.
- [77] Okamoto K, Kondo-Okamoto N. Mitochondria and autophagy: critical interplay between the two homeostats. *Biochimica et Biophysica Acta*. 2012; 1820: 595–600.
- [78] Popov LD. Mitochondrial biogenesis: An update. *Journal of Cellular and Molecular Medicine*. 2020; 24: 4892–4899.
- [79] Piantadosi CA, Suliman HB. Redox regulation of mitochondrial biogenesis. *Free Radical Biology & Medicine*. 2012; 53: 2043–2053.
- [80] Zhang Q, Wu Y, Zhang P, Sha H, Jia J, Hu Y, *et al.* Exercise induces mitochondrial biogenesis after brain ischemia in rats. *Neuroscience*. 2012; 205: 10–17.
- [81] Wang W, Zhao F, Ma X, Perry G, Zhu X. Mitochondria dysfunction in the pathogenesis of Alzheimer’s disease: recent advances. *Molecular Neurodegeneration*. 2020; 15: 30.
- [82] Khajenobar NB, Mahboob S, Nourazarian A, Shademan B, Laghousi D, Moayed ZB, *et al.* Comparison between cerebrospinal fluid and serum levels of myelin-associated glycoprotein, total antioxidant capacity, and 8-hydroxy-2'-deoxyguanosine in patients with multiple sclerosis. *Clinical Neurology and Neurosurgery*. 2021; 200: 106377.
- [83] Radaghdam S, Karamad V, Nourazarian A, Shademan B, Khaki-Khatibi F, Nikanfar M. Molecular mechanisms of sex hormones in the development and progression of Alzheimer’s disease. *Neuroscience Letters*. 2021; 764: 136221.
- [84] Ryu WI, Bormann MK, Shen M, Kim D, Forester B, Park Y, *et al.* Brain cells derived from Alzheimer’s disease patients have multiple specific innate abnormalities in energy metabolism. *Molecular Psychiatry*. 2021; 26: 5702–5714.
- [85] Swerdlow RH. Mitochondria and Mitochondrial Cascades in Alzheimer’s Disease. *Journal of Alzheimer’s Disease: JAD*. 2018; 62: 1403–1416.
- [86] Hauptmann S, Scherping I, Dröse S, Brandt U, Schulz KL, Jendrach M, *et al.* Mitochondrial dysfunction: an early event in Alzheimer pathology accumulates with age in AD transgenic mice. *Neurobiology of Aging*. 2009; 30: 1574–1586.
- [87] Gong B, Pan Y, Vempati P, Zhao W, Knable L, Ho L, *et al.* Nicotinamide riboside restores cognition through an upregulation of proliferator-activated receptor- $\gamma$  coactivator 1 $\alpha$  regulated  $\beta$ -secretase 1 degradation and mitochondrial gene expression in Alzheimer’s mouse models. *Neurobiology of Aging*. 2013; 34: 1581–1588.
- [88] Shademan B, Biray Avci C, Nikanfar M, Nourazarian A. Application of Next-Generation Sequencing in Neurodegenerative Diseases: Opportunities and Challenges. *Neuromolecular Medicine*. 2021; 23: 225–235.
- [89] Hattingen E, Magerkurth J, Pilatus U, Mozer A, Seifried C, Steinmetz H, *et al.* Phosphorus and proton magnetic resonance spectroscopy demonstrates mitochondrial dysfunction in early and advanced Parkinson’s disease. *Brain: a Journal of Neurology*. 2009; 132: 3285–3297.
- [90] Su X, Chu Y, Kordower JH, Li B, Cao H, Huang L, *et al.* PGC-1 $\alpha$  Promoter Methylation in Parkinson’s Disease. *PLoS ONE*. 2015; 10: e0134087.
- [91] Thomas RR, Keeney PM, Bennett JP. Impaired complex-I mitochondrial biogenesis in Parkinson disease frontal cortex. *Journal of Parkinson’s Disease*. 2012; 2: 67–76.
- [92] Schon EA, Przedborski S. Mitochondria: the next (neuro)generation. *Neuron*. 2011; 70: 1033–1053.
- [93] Zhu JH, Gusdon AM, Cimen H, Van Houten B, Koc E, Chu CT. Impaired mitochondrial biogenesis contributes to depletion of functional mitochondria in chronic MPP<sup>+</sup> toxicity: dual roles for ERK1/2. *Cell Death & Disease*. 2012; 3: e312.
- [94] Quessada C, Bouscary A, René F, Valle C, Ferri A, Ngo ST, *et al.* Skeletal Muscle Metabolism: Origin or Prognostic Factor for Amyotrophic Lateral Sclerosis (ALS) Development? *Cells*. 2021; 10: 1449.
- [95] Kodavati M, Wang H, Hegde ML. Altered Mitochondrial Dynamics in Motor Neuron Disease: An Emerging Perspective. *Cells*. 2020; 9: 1065.
- [96] Niyazov DM, Kahler SG, Frye RE. Primary Mitochondrial Disease and Secondary Mitochondrial Dysfunction: Importance of Distinction for Diagnosis and Treatment. *Molecular Syndromology*. 2016; 7: 122–137.
- [97] Da Cruz S, Parone PA, Lopes VS, Lillo C, McAlonis-Downes M, Lee SK, *et al.* Elevated PGC-1 $\alpha$  activity sustains mitochondrial biogenesis and muscle function without extending survival in a mouse model of inherited ALS. *Cell Metabolism*. 2012; 15: 778–786.
- [98] Kiaei M. Peroxisome Proliferator-Activated Receptor-gamma in Amyotrophic Lateral Sclerosis and Huntington’s Disease. *PPAR Research*. 2008; 2008: 418765.
- [99] Herskovits AZ, Guarente L. Sirtuin deacetylases in neurodegenerative diseases of aging. *Cell Research*. 2013; 23: 746–758.
- [100] Benedusi V, Martorana F, Brambilla L, Maggi A, Rossi D. The peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) con-

trols natural protective mechanisms against lipid peroxidation in amyotrophic lateral sclerosis. *The Journal of Biological Chemistry*. 2012; 287: 35899–35911.

- [101] Andrabi SS, Ali M, Tabassum H, Parveen S, Parvez S. Pramipexole prevents ischemic cell death via mitochondrial pathways in ischemic stroke. *Disease Models & Mechanisms*. 2019; 12: dmm033860.
- [102] Andrabi SS, Parvez S, Tabassum H. Melatonin and Ischemic Stroke: Mechanistic Roles and Action. *Advances in Pharmacological Sciences*. 2015; 2015: 384750.
- [103] Sun MS, Jin H, Sun X, Huang S, Zhang FL, Guo ZN, *et al*. Free Radical Damage in Ischemia-Reperfusion Injury: An Obstacle in Acute Ischemic Stroke after Revascularization Therapy. *Oxidative Medicine and Cellular Longevity*. 2018; 2018: 3804979.
- [104] Schmidt A, Minnerup J. Promoting recovery from ischemic stroke. *Expert Review of Neurotherapeutics*. 2016; 16: 173–186.
- [105] Skelding KA, Arellano JM, Powis DA, Rostas JA. Excitotoxic stimulation of brain microslices as an in vitro model of stroke. *Journal of Visualized Experiments: JoVE*. 2014; e51291.
- [106] Fidaleo M, Cavallucci V, Pani G. Nutrients, neurogenesis and brain ageing: From disease mechanisms to therapeutic opportunities. *Biochemical Pharmacology*. 2017; 141: 63–76.
- [107] Gollihue JL, Rabchevsky AG. Prospects for therapeutic mitochondrial transplantation. *Mitochondrion*. 2017; 35: 70–79.
- [108] Balasubramaniam S, Yapliito-Lee J. Riboflavin metabolism: role in mitochondrial function. *Journal of Translational Genetics and Genomics*. 2020; 4: 285–306.
- [109] Ponnalagu D, Singh H. Anion Channels of Mitochondria. *Handbook of Experimental Pharmacology*. 2017; 240: 71–101.
- [110] Tang YC, Tian HX, Yi T, Chen HB. The critical roles of mitophagy in cerebral ischemia. *Protein & Cell*. 2016; 7: 699–713.
- [111] Bakthavachalam P, Shanmugam PST. Mitochondrial dysfunction - Silent killer in cerebral ischemia. *Journal of the Neurological Sciences*. 2017; 375: 417–423.
- [112] Rodríguez-Lara SQ, Cardona-Muñoz EG, Ramírez-Lizardo EJ, Totsuka-Sutto SE, Castillo-Romero A, García-Cobián TA, *et al*. Alternative Interventions to Prevent Oxidative Damage following Ischemia/Reperfusion. *Oxidative Medicine and Cellular Longevity*. 2016; 2016: 7190943.
- [113] Thornton C, Hagberg H. Role of mitochondria in apoptotic and necroptotic cell death in the developing brain. *Clinica Chimica Acta; International Journal of Clinical Chemistry*. 2015; 451: 35–38.
- [114] Kawabori M, Yenari MA. Inflammatory responses in brain ischemia. *Current Medicinal Chemistry*. 2015; 22: 1258–1277.
- [115] Mizuma A, Yenari MA. Anti-Inflammatory Targets for the Treatment of Reperfusion Injury in Stroke. *Frontiers in Neurology*. 2017; 8: 467.
- [116] Murray KN, Parry-Jones AR, Allan SM. Interleukin-1 and acute brain injury. *Frontiers in Cellular Neuroscience*. 2015; 9: 18.
- [117] Matheoud D, Sugiura A, Bellemare-Pelletier A, Laplante A, Rondeau C, Chemali M, *et al*. Parkinson's Disease-Related Proteins PINK1 and Parkin Repress Mitochondrial Antigen Presentation. *Cell*. 2016; 166: 314–327.
- [118] Mills EL, O'Neill LA. Reprogramming mitochondrial metabolism in macrophages as an anti-inflammatory signal. *European Journal of Immunology*. 2016; 46: 13–21.
- [119] Zhao J, Mou Y, Bernstock JD, Klimanis D, Wang S, Spatz M, *et al*. Synthetic Oligodeoxynucleotides Containing Multiple Telemetric TTAGGG Motifs Suppress Inflammasome Activity in Macrophages Subjected to Oxygen and Glucose Deprivation and Reduce Ischemic Brain Injury in Stroke-Prone Spontaneously Hypertensive Rats. *PLoS ONE*. 2015; 10: e0140772.
- [120] Barone-Rochette G, Vautrin E, Rodière M, Broisat A, Vanzetto G. First magnetic resonance coronary artery imaging of bioresorbable vascular scaffold in-patient. *European Heart Journal. Cardiovascular Imaging*. 2015; 16: 229.
- [121] Malik A, Kanneganti TD. Inflammasome activation and assembly at a glance. *Journal of Cell Science*. 2017; 130: 3955–3963.
- [122] Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. *International Journal of Molecular Sciences*. 2019; 20: 3328.
- [123] Li H, Zhang S, Li F, Qin L. NLRX1 attenuates apoptosis and inflammatory responses in myocardial ischemia by inhibiting MAVS-dependent NLRP3 inflammasome activation. *Molecular Immunology*. 2016; 76: 90–97.
- [124] Mastrocola R, Collino M, Penna C, Nigro D, Chiazza F, Fracasso V, *et al*. Maladaptive Modulations of NLRP3 Inflammasome and Cardioprotective Pathways Are Involved in Diet-Induced Exacerbation of Myocardial Ischemia/Reperfusion Injury in Mice. *Oxidative Medicine and Cellular Longevity*. 2016; 2016: 3480637.
- [125] Mastrocola R, Penna C, Tullio F, Femminò S, Nigro D, Chiazza F, *et al*. Pharmacological Inhibition of NLRP3 Inflammasome Attenuates Myocardial Ischemia/Reperfusion Injury by Activation of RISK and Mitochondrial Pathways. *Oxidative Medicine and Cellular Longevity*. 2016; 2016: 5271251.
- [126] Szeto HH, Liu S, Soong Y, Seshan SV, Cohen-Gould L, Manichev V, *et al*. Mitochondria Protection after Acute Ischemia Prevents Prolonged Upregulation of IL-1 $\beta$  and IL-18 and Arrests CKD. *Journal of the American Society of Nephrology: JASN*. 2017; 28: 1437–1449.
- [127] Hendrickson MD, Poyton RO. Crosstalk between nitric oxide and hypoxia-inducible factor signaling pathways: An update. *Res. Rep. Biochem*. 2015; 5: 147–161.
- [128] Yin W, Signore AP, Iwai M, Cao G, Gao Y, Chen J. Rapidly increased neuronal mitochondrial biogenesis after hypoxic-ischemic brain injury. *Stroke*. 2008; 39: 3057–3063.
- [129] Diaz F, Moraes CT. Mitochondrial biogenesis and turnover. *Cell Calcium*. 2008; 44: 24–35.
- [130] Xie W, Zhu T, Zhou P, Xu H, Meng X, Ding T, *et al*. Noto Ginseng Leaf Triterpenes Ameliorates OGD/R-Induced Neuronal Injury via SIRT1/2/3-Foxo3a-MnSOD/PGC-1 $\alpha$  Signaling Pathways Mediated by the NAMPT-NAD Pathway. *Oxidative Medicine and Cellular Longevity*. 2020; 2020: 7308386.
- [131] Yang L, Ma YM, Shen XL, Fan YC, Zhang JZ, Li PA, *et al*. The Involvement of Mitochondrial Biogenesis in Selenium Reduced Hyperglycemia-Aggravated Cerebral Ischemia Injury. *Neurochemical Research*. 2020; 45: 1888–1901.
- [132] Park HH, Han MH, Choi H, Lee YJ, Kim JM, Cheong JH, *et al*. Mitochondria damaged by Oxygen Glucose Deprivation can be Restored through Activation of the PI3K/Akt Pathway and Inhibition of Calcium Influx by Amlodipine Camsylate. *Scientific Reports*. 2019; 9: 15717.
- [133] He W, Tian X, Zhang K, Wang H. Taurine Promotes Axonal Sprouting Through Mitochondrial Improvement in Stroke. 2021. (Preprint)
- [134] Gao J, Qian T, Wang W. CTRP3 Activates the AMPK/SIRT1-PGC-1 $\alpha$  Pathway to Protect Mitochondrial Biogenesis and Functions in Cerebral Ischemic Stroke. *Neurochemical Research*. 2020; 45: 3045–3058.
- [135] Tang X, Chen N, Long X. Ginsenoside Rg1 improves ischemic brain injury by balancing mitochondrial biogenesis and mitophagy. *Tropical Journal of Pharmaceutical Research*. 2017; 16: 2469–2475.
- [136] Flippo KH, Lin Z, Dickey AS, Zhou X, Dhanesha NA, Walters GC, *et al*. Deletion of a Neuronal Drp1 Activator Protects against Cerebral Ischemia. *The Journal of Neuroscience: the Official Journal of the Society for Neuroscience*. 2020; 40: 3119–3129.