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Research Progress on Mitophagy in the Treatment of Ischemic Stroke

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Abstract: ***Ischemic stroke is a leading cause of mortality and disability globally. Inadequate blood flow results in the deprivation of glucose and oxygen in brain cells, which disrupts cellular homeostasis and initiates a series of molecular reactions. These processes contribute to the accumulation of impaired mitochondria. As a selective type of autophagy, mitophagy plays a central role in mitochondrial quality control and is important for preserving mitochondrial integrity and function. It promotes neuronal survival and improves neuronal function by clearing depolarized mitochondria. Studies have indicated that mitophagy is closely associated with ischemic stroke. In this review, we discuss the mechanisms underlying mitophagy induction, the impact of stroke on mitophagy, and the role of mitophagy in ischemic brain tissue. We further highlight the activation of mitophagy as a potential therapeutic target for ischemic stroke.***

Keywords: Ischemic stroke, Mitophagy, Inflammatory response, Oxidative stress**.**

1. Introduction

Stroke is an acute and critical disease, and has become a major cause of death and a principal source of substantial long-term disability globally [1]. It is classified into ischemic stroke and hemorrhagic stroke, with ischemic stroke being the more common subtype, accounting for approximately 87% of all cases [2]. Currently, the main treatments for ischemic stroke are intravenous thrombolysis and mechanical thrombectomy. These therapies aim to rescue the ischemic penumbra by restoring cerebral blood perfusion [3]. However, despite a success rate of up to 80%, only 33.4% of ischemic stroke survivors achieve favorable functional outcomes, while up to 50% of successfully recanalized patients still face the risk of disability or mortality [4]. Neuroprotective therapy can alleviate neuronal damage, accelerate the process of neurological functional recovery, and significantly extend the effective time window for vascular recanalization, thereby enhancing the overall efficacy of revascularization [5]. Mitochondrial dysfunction is recognized as one of the hallmarks of neuronal death following ischemic stroke. A growing body of research indicates that maintaining mitochondrial structure and function is a critical factor in promoting neuronal survival and optimizing neurological efficacy.

Mitochondria play a central role in critical biological processes like cell proliferation, differentiation, programmed cell death, migration, and autophagy. Together, these functions form a core regulatory network that governs the cellular life cycle [6]. The central nervous system (CNS), being one of the most energy-consuming systems in the human body, relies heavily on proper mitochondrial function for its normal operation. Following the onset of ischemic stroke, compromised mitochondrial respiration and loss of membrane potential initiate a pathological cascade resulting in neuronal demise [7]. Mitophagy, also referred to as selective autophagy of mitochondria, not only clears damaged mitochondria but also suppresses mitochondria-mediated programmed cell death in ischemic neurons. It represents an important mechanism for protecting neurons from ischemic injury [8–11]. Therefore, activating mitophagy may be an important therapeutic strategy for ischemic brain injury.

2. Overview of Mitophagy

Lemasters and his colleagues first described mitophagy, identifying it as a selective autophagic response that targets impaired mitochondria for degradation to preserve mitochondrial homeostasis [12]. Mitophagy relies on multiple proteins and signaling pathways. Impaired mitochondria exhibit reduced membrane potential due to increased permeability, triggering autophagy-related proteins. The damaged organelles are encapsulated into double-membraned mitophagosomes, which fuse with lysosomes for degradation. This process regulates mitochondrial quantity, preserves function, and promotes cell survival [12,13]. In mammalian cells, the mechanisms of mitophagy can be broadly classified into ubiquitin-dependent pathways and ubiquitin-independent pathways [14]. The PINK1-Parkin pathway mediates ubiquitin-dependent mitophagy through three core elements: a damage sensor (PINK1), an amplifier (Parkin), and an effector (ubiquitin chains), forming a coordinated ubiquitination cascade [15], which represents the most extensively studied regulatory mechanism of mitophagy to date. In addition, studies have shown that PINK1 can recruit autophagy receptors OPTN and NDP52 to the mitochondria through ubiquitin phosphorylation without relying on Parkin, thereby promoting the biogenesis of autophagy (Non-Parkin dependent pathway) [16,17].

3. The Impact of Ischemic Stroke on Mitophagy

In recent years, the mechanism of mitophagy has garnered significant research interest. Most studies shown the notion that mitophagy enhances neuronal tolerance to hypoxic stress by clearing dysfunctional mitochondria and blocking the transmission of apoptotic signals [18,19]. During ischemic stroke, mitophagy is typically mediated by the Pink1/Parkin pathway. The overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) during cerebral

ischemia leads to oxidative and nitrosative stress, which are central to the pathogenesis of cerebral ischemia-reperfusion injury [20]. Elevated ROS levels induce mitochondrial depolarization, leading to persistent PINK1 accumulation on the mitochondrial outer membrane. This recruits Parkin, resulting in ubiquitination of mitochondrial outer membrane proteins and triggering mitophagy [18,21]. ONOO- promotes the transport of dynamin-related protein 1 (Drp1) to the mitochondria, ultimately initiating the mitochondrial autophagy mechanism regulated by PINK1/Parkin [20].

Mitophagy receptor proteins BNIP3/NIX and FUNDC1 play crucial roles in ischemia/hypoxia-induced mitophagy. BNIP3 is a target gene of hypoxia-inducible factor 1α (HIF1α). During ischemic stroke (IS), HIF1α is activated under hypoxic conditions, leading to the upregulation of BNIP3 and NIX expression. This activation promotes mitophagy and ameliorates brain injury resulting from ischemia/reperfusion (I/R) damage [22]. Meanwhile, studies have shown that while ischemia-reperfusion can activate mitophagy, ischemia alone only induces general autophagy without triggering mitochondrial autophagy. This is because NIX protein in ischemic neurons undergoes proteasomal degradation, resulting in an impaired mitophagic response [23–25]. Tang's group demonstrated that during the early phase of cerebral ischemia-reperfusion injury, FUNDC1 exhibits a transient activation characterized by dephosphorylation at the Tyr18 site, leading to the induction of mitophagy. However, in later stages of injury (beyond 6 hours), FUNDC1 is inactivated by Src kinase and undergoes re-phosphorylation at Tyr18, reaching levels that surpass baseline. This alteration significantly diminishes the binding capacity of FUNDC1 to LC3, resulting in substantial suppression of mitophagy [26].

4. The Role of Mitophagy in Ischemic Stroke

4.1 Mitophagy and Oxidative Stress

During the pathological process of ischemic stroke, ischemia-hypoxia and reperfusion injury significantly impair the mitochondrial capacity for ATP synthesis, consequently disrupting the maintenance of the electrochemical gradient across cell membranes by ion channels. Under these conditions, voltage-dependent channels remain persistently open, leading to a massive influx of calcium ions into the cell [27], which triggers a phenomenon of calcium overload. At the same time, the reduced activity of mitochondrial superoxide dismutase and the dysfunction of the cytochrome oxidase system result in a large conversion of oxygen into superoxide anions, hydrogen peroxide, and other reactive oxygen species during reperfusion [28]. Accumulated succinate during ischemia is rapidly oxidized by succinate dehydrogenase (SDH) upon reperfusion, driving extensive superoxide generation at Complex I via reverse electron transport [29–31]. Yang's group revealed that the neuroprotective mechanism of scutellarin (Scu) against cerebral ischemia is closely associated with mitophagy. Specifically, Scu enhances mitophagic activity by upregulating the expression of LC3, Beclin1, PINK1, and Parkin proteins. This upregulation attenuates the overload of Intracellular and mitochondrial ROS, reverses mitochondrial structural damage, increases mitochondrial membrane potential (MMP) and ATP content, and promotes Na^+/K^+-ATPase activity. More importantly, these effects of Scu were significantly inhibited by the mitophagy inhibitor Mdivi-1 [32]. In summary, enhancing mitophagy effectively reduces ROS levels, alleviates neuronal oxidative stress damage, and exhibits significant neuroprotective effects against hypoxic-ischemic brain injury.

4.2 Mitophagy and Neuroinflammation

Neuroinflammation plays an important role in the pathological mechanism of ischemic stroke (IS). Studies have reported that the initiation of neuroinflammatory processes primarily occurs in the penumbra region, where cellular contents and pro-inflammatory molecules released from necrotic cells in the ischemic core exacerbate neuronal apoptosis and tissue damage after stroke [33,34]. Research has revealed a close association between the activation of the NLRP3 inflammasome and mitochondrial dysfunction. The core structure of this complex consists of the NLRP3 cytoplasmic receptor and the ASC adaptor protein. The ASC component contains a CARD domain, which enables specific recognition and binding of procaspase-1 [35]. During activation, procaspase-1 undergoes autocatalysis and is converted into biologically active caspase-1, which subsequently cleaves the precursor forms of IL-1β and IL-18 to generate active inflammatory cytokines. These mature pro-inflammatory cytokines are then secreted into the extracellular space, thereby amplifying the inflammatory response [36]. The activation of the NLRP3 inflammasome is significantly regulated by mitophagy, involving multiple signaling pathways and molecules such as PINK1/Parkin, BNIP3, and PGC-1α. This process facilitates the clearance of intracellular stimuli that trigger NLRP3 activation and promotes the degradation of inflammasome components and cytokines, including NLRP3, ASC, and IL-1β [37]. He's group found that the transcription factor ATF4 significantly upregulates Parkin expression during cerebral ischemia-reperfusion, enhances mitophagic activity, and reduces NLRP3 inflammasome activation via the Parkin-mitophagy axis, accompanied by a notable decrease in inflammatory cytokines such as IL-1β and IL-18. However, siRNA-mediated knockdown of Parkin reversed both the ATF4-induced enhancement of mitophagy and the suppression of NLRP3 inflammasome activation, thereby exacerbating the inflammatory cascade in neurons within the ischemic region and intensifying brain tissue damage [38]. In summary, activation of mitophagy can effectively suppress the inflammatory response triggered by ischemic stroke, thereby ameliorating neuronal damage in the ischemic region and representing a promising therapeutic target for the treatment of ischemic stroke.

4.3 Mitophagy and Apoptosis

Apoptosis in the brain is co-regulated by intrinsic and extrinsic pathways, both characterized by the release of cytochrome c from mitochondria into the cytoplasm. The intrinsic pathway is primarily triggered by mitochondrial damage or permeability changes and depends on Bcl-2 family proteins acting on outer membrane proteins. In contrast, the extrinsic pathway is initiated by the activation of death receptors on the cell membrane and induces apoptosis through death signal transduction cascades [39]. In ischemic stroke,

neuronal death in the core region is predominantly necrotic, whereas delayed neuronal death in the penumbra occurs primarily through apoptosis [40,41]. Timely clearance of dysfunctional mitochondria and reduction of cerebral ischemia-induced apoptosis are crucial for restoring neuronal function in ischemic brain regions. Zhao's group demonstrated that neural stem cell-derived exosomes (NSC-Exo) activate the PINK1/Parkin pathway to promote mitophagy, thereby exerting neuroprotective effects and inhibiting apoptosis. Western blot analysis revealed decreased Bax expression and increased Bcl-2 levels. However, upon PINK1 knockout, the ability to induce mitophagy and attenuate apoptosis was suppressed, accompanied by elevated expression of Bax and caspase-3, as well as a downward trend in Bcl-2 expression [42]. Xu's group reported that RV01 promotes mitophagy through its interaction with CK2α′, thereby effectively reducing damage induced by acute ischemic stroke. It prevents the mitochondrial release of cytochrome c into the cytoplasm and attenuates apoptosis. However, when the mitophagy inhibitor Mdivi-1 was applied, the neuroprotective effects of RV01 were subsequently abolished [43]. In the context of ischemic stroke injury, mitophagy plays a critical role in suppressing neuronal apoptosis and improving neurological function.

5. Conclusions

Mitochondria is important in ischemic stroke. Mitochondrial dysfunction serves as an early initiating event in the pathophysiology of stroke, while aberrant mitochondrial morphology and structure are central to the activation of cell death signaling pathways [8]. Ischemic stroke involves highly complex pathophysiological mechanisms. During its progression, mitophagy may be activated either through the PINK1-Parkin pathway or via mitophagy receptor proteins (such as BNIP3, NIX, and FUNDC1), but it may also be impaired due to the degradation of these receptors. As the condition evolves, mitophagy may even shift from activation to suppression. Nevertheless, it is undeniable that mitophagy effectively clears damaged mitochondria, reduces oxidative stress, improves the inflammatory microenvironment, protects neurons in the ischemic area, and preserves normal neurological function. Therefore, how to enhance mitophagy in ischemic stroke warrants serious consideration. Meanwhile, in-depth research into the molecular mechanisms of mitophagy is expected to open new avenues for the treatment of ischemic stroke and reveal potential therapeutic targets.

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