Role of mitochondrial Ca^{2+} in stroke: From molecular mechanism to treatment strategy (Review)

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Abstract. Mitochondria serve a pivotal role in the pathological mechanisms of stroke, particularly in the regulation of intracellular calcium homeostasis. Stroke-induced ischemia and reperfusion injury frequently result in disruptions of mitochondrial calcium ion (Ca^{2+}) transport, characterized by Ca^{2+} overload. This imbalance directly impairs mitochondrial function and triggers neuronal death. Mitochondrial Ca^{2+} transport involves calcium influx, primarily mediated by the mitochondrial calcium uniporter (MCU) complex, and efflux, primarily through the sodium-calcium exchanger (NCLX), making this mechanism a critical therapeutic target in stroke. The present review systematically explores the central role of mitochondrial Ca^{2+} transport in ischemia/reperfusion injury, with an in-depth analysis of its pathological mechanisms in cellular energy metabolism, oxidative stress and apoptotic signaling pathways. Additionally, this review summarizes recent advancements in therapeutic strategies targeting mitochondrial Ca^{2+} transport, including MCU inhibitors, NCLX activators, antioxidant therapies and combination treatments. It also highlights the potential of Ca^{2+} signaling for early stroke diagnosis and reviews progress in dynamic monitoring technologies for mitochondrial Ca^{2+}, such as fluorescence probes and super-resolution microscopy. Despite significant progress in basic research, challenges remain in translating these findings into clinical applications. Future efforts should focus on elucidating the regulatory mechanisms of mitochondrial Ca^{2+}, developing diagnostic tools and optimizing therapeutic interventions to improve stroke prognosis and enhance the quality of life of patients.

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1. Introduction

Stroke: From epidemiology to clinical challenges. Stroke, also known as cerebrovascular accident, is an acute neurological disorder caused by the disruption of cerebral blood circulation and is one of the leading causes of mortality and disability worldwide (1). The 2021 global burden of disease (GBD) analysis revealed that stroke ranked first in age-standardized disability-adjusted life years, significantly impacting global health across 19 of the 21 GBD regions (2). The incidence and mortality rates of stroke exhibit marked geographical variation globally (3). In developed countries, improvements in lifestyle and the optimization of emergency care and

secondary prevention measures have led to a steady decline in stroke-related mortality rates (4). However, in low- and middle-income countries, particularly in parts of Asia and Africa, stroke incidence and mortality remain high due to limited early diagnostic capabilities and inadequate medical resources, resulting in significant disability rates (5). Data from 2019 indicate that stroke mortality rates in Asian countries such as China and India continue to rise, closely associated with risk factors such as hypertension, diabetes, smoking and the rapid pace of urbanization (6).

Stroke is primarily classified into two types: Ischemic and hemorrhagic. Ischemic stroke results from cerebral arterial embolism or stenosis, leading to interrupted blood supply to the brain, accounting for the majority of cases (7). Hemorrhagic stroke, on the other hand, is caused by the rupture of cerebral blood vessels, often associated with hypertension and vascular abnormalities (8). This acute neurological disorder can cause severe impairments in the acute phase, such as hemiplegia, speech disorders and dysphagia. Over the long term, it can lead to sequelae including motor, cognitive and other functional deficits, markedly reducing the quality of life of patients while imposing substantial burdens on families and society (9).

In China, stroke is one of the leading causes of disease-associated mortality. Data from the Chinese Center for Disease Control in 2020 indicated that there were ~3.4 million new cases annually, with related deaths reaching ~2.3 million. The direct and indirect economic costs of new cases amount to tens of billions of yuan (10). In conclusion, stroke poses a severe threat to global health and places immense pressure on social and economic systems. With the aging population and the prevalence of unhealthy lifestyles, strengthening stroke prevention, early screening and innovative treatment approaches have become urgent public health priorities (11).

Key role of mitochondrial function in the stroke pathophysiology. Mitochondria are central to cellular energy metabolism and are crucial for the survival and functional maintenance of neurons. In the pathological process of stroke, mitochondria are responsible not only for energy generation but also for regulating cellular calcium homeostasis, redox balance and cell death signaling (12). However, stroke-induced local ischemia, hypoxia and reperfusion injury markedly disrupt mitochondrial structure and function, triggering a series of pathological responses (13).

In the early stages of ischemic stroke, the interruption of blood flow leads to severe hypoxia in brain tissue, inhibiting mitochondrial oxidative phosphorylation and causing a substantial reduction in energy production (14). The lack of energy impairs the ability of neurons to maintain basic physiological functions, leading to cellular dysfunction and death (15). Simultaneously, under hypoxic conditions, mitochondria excessively produce reactive oxygen species (ROS), which not only damage cellular membranes, proteins and DNA but also further damage mitochondrial structure and function, creating a cycle that accelerates the pathological process (16).

During the reperfusion phase, although the restoration of blood flow provides oxygen and nutrients to brain tissue, the rapid influx of oxygen generates excessive ROS, causing reperfusion injury (17). At this stage, excessive calcium ions (Ca^{2+}) rapidly enter the cells and accumulate within the mitochondria, causing mitochondrial Ca^{2+} overload. This impairs energy metabolism, activates calcium signaling and triggers apoptosis and necrosis (18). Furthermore, the interaction between oxidative stress and Ca^{2+} overload creates a cycle, further exacerbating neuronal damage (19). In conclusion, mitochondrial dysfunction is a driver of the pathological onset and progression of stroke. It exacerbates neuronal damage through disruptions in energy metabolism, oxidative stress, Ca^{2+} overload and inflammation signaling networks (20). A deeper understanding of the multiple roles of mitochondria in the pathological process of stroke will help uncover disease mechanisms and provide important directions for the development of innovative therapeutic strategies.

Mitochondrial calcium dysregulation in stroke pathology. Ca^{2+} are vital intracellular signaling molecules involved in regulating physiological processes such as muscle contraction, neurotransmission and cell proliferation (21). In the pathological process of stroke, disruption of intracellular calcium homeostasis is a critical factor leading to neuronal injury. Mitochondria play a central role in maintaining calcium balance by regulating calcium influx and efflux. However, during stroke, particularly in the ischemia/reperfusion phase, mitochondrial Ca^{2+} overload often occurs, resulting in dysfunction and triggering apoptosis or necrosis (22).

Mitochondrial Ca^{2+} transport is mediated by the mitochondrial calcium uniporter (MCU) complex for calcium influx and the sodium-calcium exchanger (NCLX) for calcium efflux. Dysregulation of these calcium channels is closely associated with stroke pathology (23), but their molecular mechanisms and therapeutic potential remain to be elucidated. The present study focuses on the molecular mechanisms of mitochondrial Ca^{2+} transport, with an emphasis on the regulatory properties of the MCU complex and NCLX and their roles in neuronal injury. By elucidating these mechanisms, this research aims to provide new insights into stroke pathology and offer a theoretical foundation for developing innovative therapeutic strategies targeting mitochondrial Ca^{2+} regulation.

2. Molecular mechanisms of mitochondrial calcium transport

Mitochondria are not only the primary energy producers in cells but also play a critical role in regulating intracellular calcium homeostasis. The influx and efflux of Ca^{2+} are essential mechanisms for maintaining mitochondrial function and cellular physiological activities. As a key signaling molecule, Ca^{2+} is indispensable in various physiological processes, including signal transduction, energy metabolism, redox balance and cell survival.

Molecular mechanisms of mitochondrial calcium influx Mitochondrial calcium influx and its function. Mitochondrial Ca^{2+} influx is primarily mediated by the MCU complex, which serves as the core channel for mitochondrial Ca^{2+} transport (24). The influx of Ca^{2+} is driven by the Ca^{2+} concentration gradient between the cytoplasm and mitochondrial matrix, as well as the mitochondrial membrane potential (25). Under normal physiological conditions, the MCU complex maintains the dynamic balance of mitochondrial calcium homeostasis.

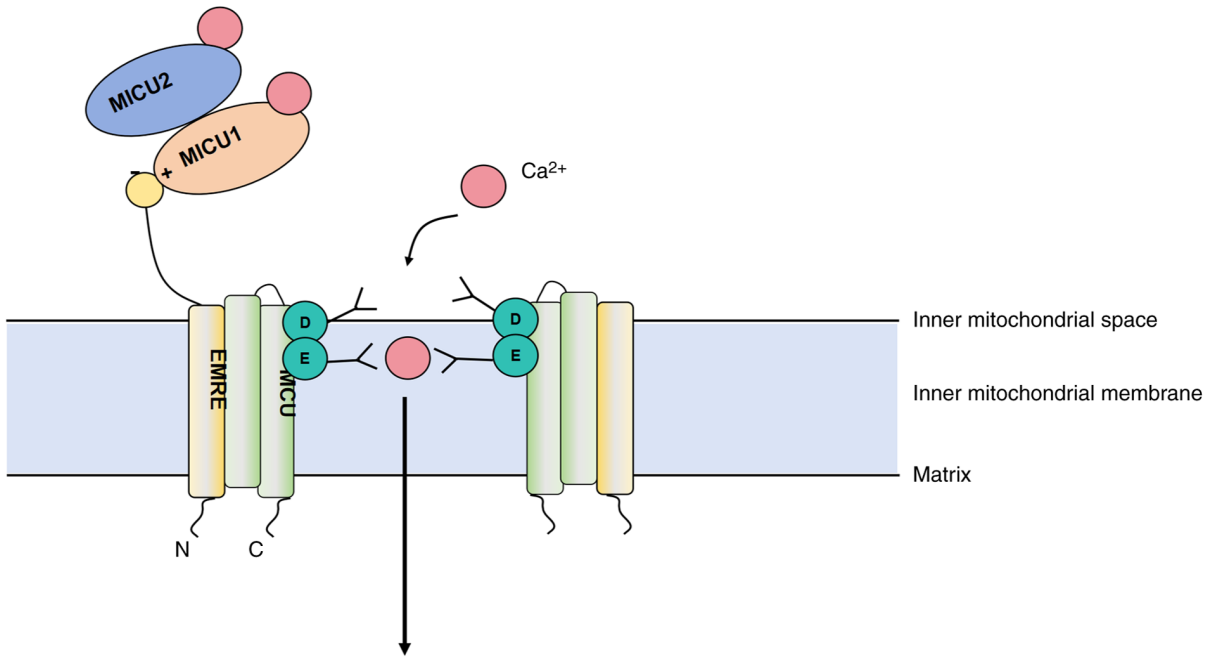

Figure 1. Structure of the MCU complex. The core pore-forming protein MCU mediates Ca^{2+} influx into the matrix. This process is regulated by the EMRE subunit and the MICU1/MICU2 heterodimer, which serve as gatekeepers to maintain calcium homeostasis. MCU, mitochondrial calcium uniporter; EMRE, essential MCU regulator; MICU, mitochondrial calcium uptake; D, Aspartate residue; E, Glutamate residue.

However, under pathological conditions, excessive Ca^{2+} influx can lead to mitochondrial Ca^{2+} overload, disrupting the mitochondrial membrane potential, activating excessive ROS production and ultimately inducing apoptosis or necrosis (26).

Composition and regulation of the MCU complex. The MCU complex is located in the inner mitochondrial membrane and is responsible for the influx of Ca^{2+}. It consists of the core protein MCU and auxiliary proteins such as mitochondrial calcium uptake (MICU)1, MICU2, essential MCU regulator (EMRE), and mitochondrial calcium uniporter regulator 1 (MCUR1) (27), which work together to regulate the amount and rate of Ca^{2+} influx, allowing Ca^{2+} to enter the mitochondrial matrix and participate in the regulation of key cellular functions, including energy metabolism, signal transduction and stress responses (28). MCU is a transmembrane protein with its N-terminus facing the mitochondrial matrix and its C-terminus facing the cytoplasm. The transmembrane region forms the Ca^{2+} channel, and its opening and closing are regulated by factors such as mitochondrial membrane potential, intracellular Ca^{2+} levels and redox status (29). In addition to serving as a Ca^{2+} channel, MCU also plays a role in regulating mitochondrial energy metabolism. Its activation promotes ATP synthesis, enhances the tricarboxylic acid cycle and oxidative phosphorylation. Moreover, by facilitating the influx of Ca^{2+}, MCU activates enzymes involved in fatty acid oxidation and glucose metabolism, thus supporting cellular energy production (29). The structure of the MCU complex is presented in Fig. 1.

The activity of the MCU complex is finely regulated by MICU1, MICU2, EMRE and MCUR1. MICU1 and MICU2 are located on the cytoplasmic side of the MCU and sense the Ca^{2+} concentration in the mitochondrial matrix, regulating the opening of the MCU channel to control Ca^{2+} influx. When the intracellular Ca^{2+} concentration is low, they bind to the MCU complex and inhibit the opening of the channel; when the Ca^{2+} concentration rises, they dissociate or undergo structural changes, promoting the opening of the channel (30). EMRE enhances the Ca^{2+} transport activity of the MCU channel, and its absence leads to a decrease in MCU complex function (31). MCUR1 interacts with the MCU complex to maintain its stability and function; the absence of MCUR1 causes abnormal Ca^{2+} accumulation within the mitochondria, affecting cellular energy metabolism (27). The coordinated action of these regulatory factors ensures that the MCU complex efficiently and accurately regulates mitochondrial Ca^{2+} concentrations under various physiological and pathological conditions, preventing excessive accumulation that could lead to mitochondrial damage and cell death.

In summary, mitochondrial Ca^{2+} influx is primarily mediated by the finely regulated MCU complex, which plays a central role in maintaining cellular calcium homeostasis and energy metabolism (26). Under ischemic conditions, dysregulation of MCU-mediated Ca^{2+} uptake contributes to mitochondrial calcium overload, oxidative stress and neuronal injury, highlighting its significance in the pathophysiology of stroke (32). Future research should focus on elucidating the fine regulatory mechanisms of MCU complex components, particularly their spatiotemporal interactions, and developing precise therapeutic strategies, such as selective MCU inhibitors, to mitigate Ca^{2+} overload while preserving physiological Ca^{2+} signaling.

Molecular mechanisms of mitochondrial calcium efflux Function and regulation of NCLX. NCLX is the primary channel for mitochondrial Ca^{2+} efflux. It facilitates the extrusion of Ca^{2+} from the mitochondrial matrix by exchanging it with cytosolic Na^{+}, relying on the concentration gradients

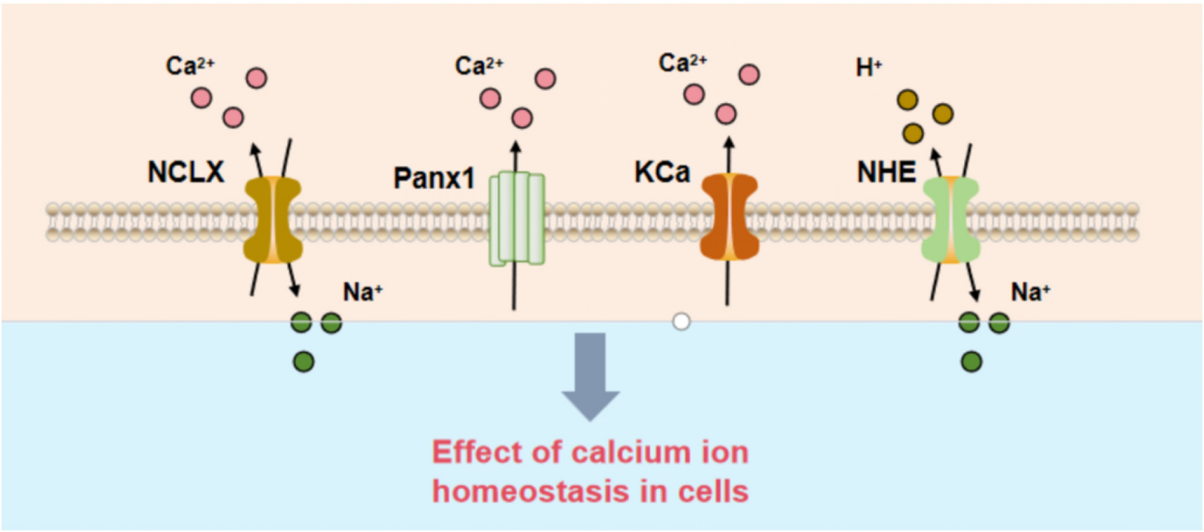

Figure 2. Calcium efflux pathways. Mitochondrial calcium efflux mechanisms, including the NCLX, Panx1, KCa, and NHE, which cooperatively regulate intracellular calcium homeostasis. NCLX, sodium-calcium exchanger; Panx1, pannexin 1; KCa, calcium-activated potassium channels; NHE, sodium ion/hydrogen ion exchanger; Ca^{2+}, calcium ion; Na^{+}, sodium ion; H^{+}, hydrogen ion.

of sodium and calcium (33). This mechanism is crucial for maintaining cellular Ca^{2+} homeostasis and plays a vital role in regulating mitochondrial membrane potential, cellular stress responses and energy metabolism (34). The activity of NCLX is regulated by the sodium gradient and sodium pump function. Under pathological conditions such as ischemia/reperfusion injury and neurodegenerative diseases, NCLX prevents calcium overload, thereby protecting mitochondrial and cellular function (34).

Other potential calcium efflux pathways. While NCLX is the primary efflux pathway, other mechanisms may contribute to mitochondrial Ca^{2+} efflux under specific conditions: i) Tricarboxylic acid transporter (TST) and Na^{+}/H^{+} exchanger (NHE): TST primarily mediates metabolite translocation but may facilitate Ca^{2+} efflux during changes in calcium signaling. Similarly, NHE can regulate mitochondrial Ca^{2+} efflux through exchange mechanisms under pathological conditions (35). ii) Pannexin 1 channels: Pannexin 1, a membrane channel protein, may regulate Ca^{2+} movement during cellular stress or injury. It is hypothesized to play a role in mitochondrial Ca^{2+} efflux, particularly under conditions such as inflammation or hypoxia (36). iii) Calcium-activated potassium channels (KCa channels): KCa channels are activated by elevated Ca^{2+} levels and may indirectly modulate Ca^{2+} efflux, especially during ionic imbalances or stress conditions (36). Fig. 2 illustrates the pathways of Ca^{2+} efflux.

3. Mitochondrial calcium transport in ischemia/reperfusion injury

Ischemia/reperfusion injury is a core pathological process in stroke, characterized by the interplay of energy metabolism dysfunction, oxidative stress and calcium homeostasis imbalance (37,38). During the ischemic phase, blood flow interruption leads to neuronal energy depletion and Ca^{2+} signaling disruption. In the reperfusion phase, although blood flow restoration provides oxygen and nutrients, it is also accompanied by a massive influx of Ca^{2+} into the cells, and Ca^{2+} overload further exacerbates mitochondrial dysfunction (39). The pathological mechanisms of mitochondrial Ca^{2+} transport in stroke are shown in Fig. 3.

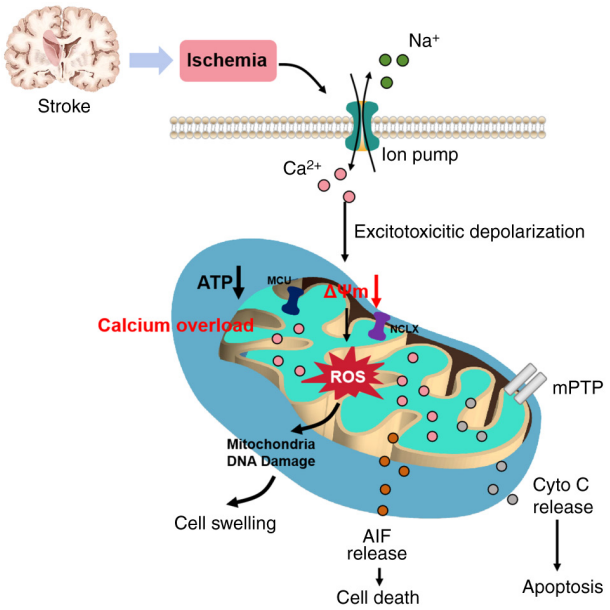

Figure 3. Pathological mechanisms of mitochondrial calcium transport in stroke. Schematic overview of stroke-induced calcium overload leading to mitochondrial dysfunction. Excessive Ca^{2+} influx via the MCU and impaired efflux disrupt mitochondrial membrane potential, generate ROS, trigger mPTP opening, and release apoptotic factors such as AIF and Cyt c, resulting in neuronal death. MCU, mitochondrial calcium uniporter; NCLX, sodium-calcium exchanger; ROS, reactive oxygen species; AIF, apoptosis-inducing factor; Cyto C, cytochrome c; mPTP, mitochondrial permeability transition pore.

Mitochondrial calcium homeostasis imbalance during ischemia. During ischemia, blood flow interruption leads to

severe hypoxia in brain tissue, inhibiting mitochondrial oxidative phosphorylation and resulting in energy depletion that weakens membrane ion pumps (such as Na^+/K^+ ATPase) (32). Elevated extracellular Ca^{2+} concentrations drive a large influx of Ca^{2+} into cells. The accumulation of Ca^{2+} further activates calcium-dependent enzymes (such as calpains and phospholipase A2), which exacerbates membrane structural damage and energy metabolism dysfunction, ultimately impairing mitochondrial function (40).

Furthermore, under the context of calcium homeostasis disruption, mitochondrial membrane potential decreases, and Ca^{2+} transport mechanisms become dysregulated, leading to excessive calcium influx and mitochondrial Ca^{2+} overload, further impairing mitochondrial function (41).

Fluctuations in calcium signaling during reperfusion. Although reperfusion restores blood flow and provides oxygen and glucose, the rapid reintroduction of oxygen triggers a burst of ROS, exacerbating oxidative damage to the mitochondrial membrane and transport proteins. At the same time, the fluctuations in calcium signaling further disrupt membrane permeability, causing a rapid imbalance of Ca^{2+} between the mitochondrial matrix and the cytoplasm (42).

Notably, a feedback loop exists between ROS accumulation and Ca^{2+} overload: ROS oxidize the MCU complex and NCLX, impairing Ca^{2+} transport regulation and further exacerbating Ca^{2+} accumulation, while Ca^{2+} overload enhances ROS production, continuously amplifying mitochondrial damage and increasing the risk of neuronal death (43).

Cellular apoptosis triggered by abnormal calcium transport. Ca^{2+} overload activates apoptosis and necrosis through multiple signaling pathways. For example, mitochondrial Ca^{2+} overload induces the opening of the mitochondrial permeability transition pore, leading to the release of apoptotic factors such as cytochrome c (Cyt c) and apoptosis-inducing factor (AIF). Cyt c activates the intracellular apoptotic signaling pathway and promotes apoptosis through the caspase cascade reaction (32,44), while AIF triggers DNA fragmentation and nuclear membrane collapse, resulting in irreversible damage (45).

4. Therapeutic strategies targeting mitochondrial calcium transport

Current therapeutic strategies primarily focus on regulating mitochondrial Ca^{2+} influx via the MCU complex, enhancing Ca^{2+} efflux through the NCLX channel and employing multi-target combination therapies to comprehensively restore mitochondrial calcium homeostasis. These approaches demonstrate significant therapeutic potential and will be discussed in detail below.

Intervention strategies targeting the MCU complex

Research progress on MCU inhibitors. The MCU complex is the primary Ca^{2+} influx channel on the mitochondrial inner membrane. However, excessive activation of the MCU complex can lead to mitochondrial Ca^{2+} overload, triggering oxidative stress, apoptosis and tissue damage (44). Therefore, MCU inhibitors effectively prevent mitochondrial dysfunction and cell death caused by Ca^{2+} overload by limiting mitochondrial Ca^{2+} influx.

Ru360, a classic MCU inhibitor, markedly reduces oxidative stress and apoptotic responses during ischemia/reperfusion injury, thereby improving neurological recovery. However, its clinical application is limited by poor water solubility and insufficient specificity (46). By contrast, DS16570511 demonstrates improved selectivity and pharmacokinetic properties in animal models. It effectively inhibits mitochondrial Ca^{2+} accumulation, reduces oxidative stress levels in brain tissue and enhances neuronal survival. These findings lay a foundation for further optimization of MCU inhibitors (47).

Potential benefits of moderate MCU activation in stroke recovery. Although MCU inhibition is the primary focus of current research, the potential benefits of moderate MCU activation during the recovery phase of stroke also warrant attention. During the subacute and recovery stages of stroke, a moderate increase in mitochondrial calcium may play a positive role by promoting neuronal metabolic recovery and signal transmission. A study has shown that moderate MCU activation enhances mitochondrial responsiveness to calcium signaling, stimulates ATP production and improves energy metabolism in damaged neurons, thereby facilitating functional recovery (48). However, the precise molecular mechanisms of MCU activation, its optimal activation range, and its safety and efficacy in clinical applications require further investigation.

Therapeutic strategies to regulate mitochondrial calcium efflux

Development and application of NCLX regulators. The development of NCLX activators aims to promote mitochondrial Ca^{2+} efflux, thereby preventing Ca^{2+} overload, mitigating damage to mitochondrial membrane potential and energy metabolism and enhancing neuronal survival (49). A recent study demonstrated the critical role of NCLX in regulating glycolysis in astrocytes (50). In adult mice downregulation or loss of NCLX function reduces lactate output, thereby impairing neuronal function and synaptic plasticity, ultimately leading to deficits in learning and memory. These findings suggest that NCLX may serve as a potential therapeutic target for Alzheimer's disease and other cognitive impairment-related disorders (51,52).

However, although NCLX-targeting therapeutic strategies have shown potential in basic research, their translation from the laboratory to clinical application still faces numerous challenges (53). Future research should prioritize the development of novel, highly specific NCLX-targeted strategies to overcome the limitations of existing compounds. Additionally, efforts must focus on elucidating the mechanisms of action and targeting specificity while rigorously evaluating their safety and efficacy in clinical settings, facilitating their translation from laboratory research to therapeutic application.

Target screening for protecting mitochondrial calcium efflux pathways. In addition to directly activating NCLX, protecting the function of proteins related to mitochondrial Ca^{2+} efflux is also an important research direction. For example, enhancing the expression of calcium-binding proteins or modulating

their function can indirectly promote the efficiency of calcium efflux (54). Furthermore, a structural-functional study of NCLX have shown that some small molecules can bind to key sites on NCLX to prevent functional impairment caused by oxidative stress or pathological environments (55). A deeper understanding of the regulatory mechanisms of the NCLX pathway could help to identify more potential therapeutic targets.

Multi-target combination therapy for comprehensive regulation of mitochondrial calcium homeostasis

Antioxidant combination therapy targeting calcium homeostasis. During stroke, oxidative stress is closely related to calcium homeostasis imbalance. Antioxidants combined with drugs that regulate mitochondrial Ca^{2+} transport may have a synergistic effect. For instance, antioxidants such as vitamin C, resveratrol and N-acetylcysteine can reduce ROS production, thereby mitigating oxidative stress-induced damage to MCU and NCLX (56). When these antioxidants are used in combination with MCU inhibitors or NCLX activators, they can provide dual protection by reducing calcium overload and lowering oxidative stress, which effectively alleviates stroke-induced damage (57).

Gene therapy and mitochondrial-targeted drug delivery systems. Gene therapy holds potential for the treatment of mitochondrial calcium homeostasis. By using gene editing technologies, such as CRISPR-Cas9 (58), to regulate the expression of key proteins such as MCU and NCLX, mitochondrial calcium dynamics can be precisely controlled. Moreover, mitochondrial-targeted drug delivery systems (such as the MITO-Porter nano-delivery system) developed in previous years can deliver drugs specifically to mitochondria, increasing drug concentrations at the target site and enhancing therapeutic effects (59). The combination of targeted delivery technology and gene therapy is expected to enable precise regulation of mitochondrial Ca^{2+} homeostasis.

Comparative analysis of mitochondrial calcium-targeted strategies in stroke. Combination therapies offer a holistic approach but require careful optimization to balance efficacy and safety. Mechanistically, MCU inhibitors act rapidly to block Ca^{2+} influx, making them vital during the ischemic phase when Ca^{2+} surges are most damaging (46,47), but their broad inhibition may impair beneficial Ca^{2+} signaling needed for recovery (48). Conversely, NCLX activators fine-tune Ca^{2+} efflux, supporting mitochondrial resilience and neuronal repair (49,50), yet the scarcity of specific activators hinders their immediate clinical use (53). Combination therapies, integrating antioxidants or targeted delivery, provide a multifaceted approach, addressing both Ca^{2+} dysregulation and oxidative stress (56,59), but their complexity poses challenges in dosing and patient compliance. Clinically, MCU inhibitors are prioritized in emergency settings (46), NCLX activators hold promise for rehabilitation (50) and combination therapies suit complex cases with comorbidities such as cardiovascular disease (60). Patient-specific factors, such as stroke severity and concurrent conditions, further dictate strategy selection, underscoring the need for personalized diagnostics and scalable delivery systems to enhance translation from bench to bedside (58,59).

In summary, MCU inhibitors excel in acute neuroprotection, NCLX activators support long-term recovery and combination therapies, including antioxidants and gene-based approaches, holistically address Ca^{2+} and oxidative stress, particularly in complex cases. Current research has advanced MCU inhibitor specificity (e.g., DS16570511) and explored novel delivery systems (e.g., MITO-Porter), but challenges persist, including limited NCLX activator development, off-target effects and clinical translation barriers due to stroke heterogeneity and comorbidity interactions. Future efforts should prioritize specific NCLX activators, optimize combination therapy regimens and develop biomarkers for personalized treatment to overcome these hurdles and improve stroke outcomes.

5. Diagnosis and dynamic monitoring of mitochondrial calcium transport

Identifying specific biomarkers to monitor abnormal mitochondrial Ca^{2+} transport during stroke has become a hotspot in neuroscience research. Changes in mitochondrial Ca^{2+} transport proteins, calcium signaling abnormalities and dynamic monitoring technologies provide new directions for early diagnosis and real-time monitoring (61).

Early diagnostic calcium signaling biomarkers. Key proteins involved in mitochondrial Ca^{2+} transport, including the MCU complex, NCLX and other regulatory factors, play a crucial role in the pathogenesis of stroke. Therefore, detecting their expression and activity levels is an important approach to exploring stroke pathophysiology and identifying diagnostic biomarkers (62). For instance, methods such as Western blotting, immunohistochemical staining, fluorescence staining and reverse transcription-quantitative PCR can be used to assess changes in the expression of these proteins in stroke models (63). Furthermore, flow cytometry and electrophysiological techniques allow for real-time monitoring of calcium signaling changes in cellular and animal models, providing potential methods for the early detection of mitochondrial Ca^{2+} transport abnormalities (64).

Development of dynamic monitoring technologies for mitochondrial calcium transport. In previous years, real-time monitoring techniques based on fluorescent probes and calcium imaging have made notable progress in the biomedical field, becoming essential tools for studying mitochondrial Ca^{2+} transport and Ca^{2+} signaling changes (65). Compared with traditional methods, these technologies offer unique advantages in accurately capturing the temporal changes of mitochondrial Ca^{2+} transport. For example, a study developed a copper nanocluster-based fluorescent probe for real-time imaging and ratio detection of calcium ions in neurons. This probe demonstrates a strong linear response to Ca^{2+} concentrations in the range of 2-350 μM, enabling the rapid detection of dynamic calcium signal changes in neurons (66). Additionally, Thiabaud *et al* (67) developed an innovative texaphyrin-based calcium sensor designed for dynamic monitoring and detection of intracellular calcium signals using multimodal imaging techniques. This technology shows promising potential applications in neuroscience, cardiovascular disease research

and the exploration of calcium signaling-related pathological mechanisms.

6. Future research directions and challenges

Frontiers in mitochondrial calcium transport mechanisms. Despite notable progress in understanding mitochondrial Ca^{2+} transport mechanisms in recent years, a number of unresolved questions remain (68). For example, the interaction network of mitochondrial Ca^{2+} transport proteins and the regulatory relationships between different Ca^{2+} transport channels within the cell are not fully understood, especially regarding the regulation of the MCU complex. While several key regulatory factors (such as MICU1/2 and EMRE) have been identified, the precise mechanisms by which they fine-tune the spatiotemporal patterns of mitochondrial Ca^{2+} influx remain unclear (69). Furthermore, how to balance Ca^{2+} influx and efflux under various pathological conditions (such as ischemia/reperfusion injury) to prevent mitochondrial calcium overload and cell death remains an urgent issue. Future research will need to explore these regulatory mechanisms in detail through high-throughput screening, proteomics and single-molecule techniques.

Key challenges in translating from laboratory to clinic. Currently, most research on mitochondrial Ca^{2+} transport relies on animal models, particularly mouse models. However, these models face challenges in clinical translation. For instance, the changes in mitochondrial Ca^{2+} transport and underlying pathological mechanisms in animal models may differ from the manifestations of stroke in humans, especially in studies of long-term sequelae and chronic stroke (70). Future research should focus on developing more refined animal models that better simulate the complex pathological processes of human stroke, as well as exploring effective ways to safely and efficiently translate experimental findings into clinical applications.

Challenges of emerging technologies and interdisciplinary integration. With advancements in technology, innovative tools and methods have provided new perspectives for the study of mitochondrial Ca^{2+} transport. Single-cell genomics technology allows for in-depth investigation of the expression and function of Ca^{2+} transport channels at the single-cell level, aiding in the understanding of cellular heterogeneity and individualized responses (63). Super-resolution microscopy techniques, such as STED and SIM microscopes, enable the observation of dynamic changes in mitochondrial calcium at the subcellular level, revealing the mechanisms of Ca^{2+} influx and efflux (71). Despite these advancements driving our understanding of mitochondrial Ca^{2+} transport mechanisms, challenges remain in overcoming issues related to the integration of technologies, interdisciplinary collaboration and extensive data analysis, all of which are crucial for effectively supporting the development of novel therapeutic strategies.

Complex relationship between mitochondrial Ca^{2+} transport and stroke comorbidities (e.g., Alzheimer's and cardiovascular diseases). Mitochondrial Ca^{2+} transport plays a crucial role in the pathophysiology of stroke and is closely linked to other neurodegenerative diseases, such as Alzheimer's disease and cardiovascular diseases. Studies have shown that stroke patients often experience comorbidities, including cognitive impairment and cardiovascular diseases. These comorbidities may interact through mitochondrial Ca^{2+} dysregulation, thereby promoting neurodegenerative processes (72). For example, in Alzheimer's disease, abnormal Ca^{2+} signaling is closely associated with mitochondrial dysfunction, both contributing to neuronal damage (73). Similarly, the increased stroke risk in cardiovascular disease patients is closely linked to the disruption of mitochondrial calcium homeostasis, with Ca^{2+} overload exacerbating cardiovascular injury and worsening the stroke process (60). Therefore, future research should focus on exploring the role of mitochondrial Ca^{2+} transport in stroke and its comorbidities, particularly how targeting Ca^{2+} transport could intervene in the onset and progression of these pathological processes. Interdisciplinary collaborative research will play an increasingly important role in advancing this field.

7. Summary

Mitochondrial Ca^{2+} transport plays a crucial role in the pathophysiology of stroke. During the ischemia/reperfusion injury phase, disruptions in Ca^{2+} homeostasis lead to mitochondrial dysfunction, excessive ROS production and the activation of cell death signals. Dysfunction of the Ca^{2+} influx channel MCU complex and the efflux channel NCLX has been shown to be one of the core drivers of stroke pathogenesis. Targeting the regulation of these channels has become a key direction for stroke therapy, including strategies such as MCU inhibitors, NCLX activators and combined antioxidant treatments. Furthermore, the dynamic monitoring of Ca^{2+} signaling provides new perspectives for early diagnosis and precision treatment. Despite notable progress in basic research, translating these findings into safe and effective clinical treatments remains a challenge. Future research should continue to focus on mitochondrial Ca^{2+} regulatory mechanisms, explore innovative diagnostic tools and develop multidimensional intervention strategies to improve stroke prognosis and enhance patient quality of life.

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Availability of data and materials

Not applicable.

Authors' contributions

YL and WJ performed the literature review and wrote the manuscript. HZ, XD, KL and XY revised the manuscript. XW and HR performed the literature review and contributed to the acquisition and analysis of data. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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