

Pyroptosis in cerebral ischemia-reperfusion injury: Molecular mechanisms and therapeutic implications (Review)

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Received September 5, 2025; Accepted February 13, 2026

DOI: 10.3892/mmr.2026.13835

Abstract. Cardiac arrest is a notable emergency in clinical medicine; following the return of spontaneous circulation, cerebral ischemia-reperfusion (IR) injury remains a primary driver of persistently high mortality and adverse neurological outcomes. The pathophysiology of cerebral IR injury is multifaceted, involving oxidative stress, calcium overload and mitochondrial dysfunction. Pyroptosis has emerged as a focal research point, characterized by inflammasome-dependent activation, membrane pore formation and the release of proinflammatory mediators. The present review highlighted the cell type-specific roles of pyroptosis within the central nervous system during cerebral IR injury. The present review further examined its contribution to neuroinflammatory cascades, underscoring its pivotal role in driving neuronal damage.

Furthermore, the underlying molecular mechanisms, the interplay between pyroptosis and other programmed cell death pathways and recent therapeutic advances targeting these processes were summarized. Collectively, the present review provided novel perspectives for improving post-IR outcomes and advancing the clinical translation of neuroprotective strategies.

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Abbreviations: CA, cardiac arrest; ROSC, return of spontaneous circulation; IR, ischemia reperfusion; GSDM, gasdermin; BBB, blood-brain barrier; NTD, N-terminal domain; PRRs, pattern recognition receptors; CARD, caspase recruitment domain; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; DAMPs, damage-associated molecular patterns; NLR, NOD-like receptor; Gzms, granzymes; OGD/R, oxygen-glucose deprivation/reoxygenation; AD, Alzheimer's disease; STING, stimulator of interferon genes; Drp1, dynamin-related protein 1; PINK1, PTEN-induced putative kinase 1; c-FLIP, cellular FLICE-like inhibitory protein; RIPK1, receptor-interacting serine/threonine-protein kinase 1; MLKL, mixed lineage kinase domain-like protein; TXNIP, thioredoxin-interacting protein; Nrf2, nuclear factor erythroid 2-related factor 2; miR, microRNA; EA, electroacupuncture; ncRNA, non-coding RNA

Key words: pyroptosis, cerebral ischemia reperfusion injury, NLRP3, GSDMD

1. Introduction

Cardiac arrest (CA) is defined by the sudden cessation of cardiac mechanical activity due to various causes. As a life-threatening emergency, which represents a leading global driver of mortality and neurological disability. Each year, >500,000 individuals undergo out-of-hospital resuscitation for CA, with the annual incidence estimated at 30-97 per 100,000 population (1). While cardiopulmonary resuscitation can lead to return of spontaneous circulation (ROSC), patients often suffer from severe neurological dysfunction. This impairment is driven by secondary cerebral ischemia-reperfusion (IR) injury, which remains a marked determinant of poor outcomes (2). Cerebral IR injury is a multifaceted process involving oxidative stress, calcium overload and mitochondrial dysfunction. These pathways culminate in neuroinflammation, neuronal death and lasting neurological deficits (3). Despite progress, mechanistic insights remain incomplete, particularly concerning inflammation-mediated cell death.

Consequently, investigating novel death pathways has become a research priority.

Pyroptosis is a unique programmed cell death pathway, which is molecularly mediated by inflammasomes and gasdermin (GSDM) proteins. The core mechanism involves the activation of inflammatory caspases, including caspase-1/4/5/11. These enzymes cleave GSDM family members to form membrane pores, facilitating the release of intracellular contents (4). In contrast to apoptosis, necroptosis or ferroptosis, pyroptosis is characterized by cellular swelling and membrane rupture; this process leads to the robust release of pro-inflammatory cytokines (5). Evidence suggests that pyroptosis is involved in diverse conditions, including infection, autoimmune disease and cardiovascular disorders. It also figures prominently in neurodegenerative diseases, where it amplifies inflammation and drives tissue injury. As a result, pyroptosis has become a primary focus of translational research (6).

During cerebral IR injury, pyroptosis is excessively activated, which triggers neuroinflammatory cascades, disrupts the blood-brain barrier (BBB) and exacerbates neuronal loss, thereby accelerating brain damage (7). Experimental evidence indicates that inhibiting pyroptosis mitigates tissue injury and confers neuroprotection (8). This offers a promising therapeutic avenue for treating cerebral IR injury. Given its central role in IR-induced brain damage, pyroptosis is now recognized as a compelling therapeutic target (9). Current interventions show promise, further underscoring its translational value (10). In the present review, the molecular mechanisms of pyroptosis and its roles in cerebral IR injury were systematically summarized. The potential therapeutic strategies were also explored to provide a framework for both fundamental research and clinical translation. While the present review primarily focused on brain injury following CA/ROSC, evidence from focal ischemic stroke models (such as middle cerebral artery occlusion) and *in vitro* systems [oxygen-glucose deprivation/reoxygenation (OGD/R)] is integrated as supportive data, particularly in mechanistic areas where CA-specific evidence remains nascent.

Search strategy. To ensure a representative and comprehensive overview of the field, a literature search was conducted across PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Web of Science (<https://www.webofscience.com/>) and Scopus (<https://www.scopus.com/>) databases for articles published through August 2025. The search strategy involved various combinations of keywords, including ‘pyroptosis’, ‘cardiac arrest’, ‘cardiopulmonary resuscitation’, ‘cerebral ischemia-reperfusion injury’ and ‘neuroinflammation’. Selection was limited to peer-reviewed, English-language publication studies that provided notable insights into the molecular cascades and therapeutic potential of pyroptosis within the specific context of post-arrest brain injury were prioritized.

2. Molecular mechanisms of pyroptosis

Overview of pyroptosis. The concept of pyroptosis was first proposed by Cookson and Brennan in 2001 (11). The authors described pyroptosis as a caspase-1-mediated, proinflammatory form of programmed cell death observed in *Salmonella typhimurium*-infected macrophages. This process is characterized by membrane rupture and cellular

swelling, which facilitates the release of intracellular contents, leading to a marked inflammatory response. Morphologically and mechanistically, pyroptosis is distinct from apoptosis and necrosis (12). While apoptosis is characterized by cell shrinkage and non-inflammatory clearance, and necrosis is generally an uncontrolled lytic process, pyroptosis is a form of programmed lytic death defined by cellular swelling, membrane rupture and the robust release of pro-inflammatory cytokines. The mechanistic foundation of pyroptosis dates back to 1992, where Zychlinsky *et al* (13) reported an unconventional form of caspase-1-dependent cell death in a *Shigella* infection model; these findings established the groundwork for subsequent studies.

Further investigations revealed that pyroptosis is not limited to the caspase-1 pathway. In mice, caspase-11 can directly bind lipopolysaccharide (LPS) to induce pyroptosis. The human homologs, caspase-4 and caspase-5, function similarly, collectively constituting the non-canonical pathway (14). In 2015, Shi *et al* (15) and Kayagaki *et al* (16) independently identified GSDMD as the key substrate of inflammatory caspases. Upon cleavage, the N-terminal domain (NTD) of GSDMD inserts into the plasma membrane; this process forms large non-selective pores that drive cellular content release and robust inflammatory responses. This breakthrough established the core execution mechanism of pyroptosis and represented a major advancement in the field (15,16).

Subsequent research has elucidated the crosstalk between pyroptosis and other cell death pathways. In 2017, Wang *et al* (17) demonstrated that caspase-3 can cleave GSDME, thereby converting apoptosis into pyroptosis. As summarized in the 2018 recommendations of the Nomenclature Committee on Cell Death, pyroptosis, which can also be initiated by caspase-8 via GSDMD cleavage, was redefined as a GSDM-dependent form of programmed cell death (18). This pathway is typically triggered by the activation of inflammatory caspases. The GSDM family comprises GSDMA, GSDMB, GSDMC, GSDMD, GSDME and GSDMF; among these members, GSDMD is the most extensively characterized (19).

Canonical inflammasome-dependent pyroptosis. The canonical caspase-1-dependent pyroptotic pathway is driven by the activation of inflammasomes. Inflammasomes are cytosolic multiprotein complexes, which are composed of pattern recognition receptors (PRRs), the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and pro-caspase-1. These complexes detect pathogen-associated molecular patterns and host-derived danger-associated molecular patterns (DAMPs) (20). Common PRRs include members of the NOD-like receptor (NLR) family, such as NLRP1, NLRP3 and NLRC4, and other sensors include AIM2-like receptors and pyrin. Among these, the NLRP3 inflammasome is the most extensively characterized (21). NLRP3 activation typically follows a two-step model: First, a priming signal involving microbial or endogenous factors activates NF- κ B signaling to upregulate NLRP3 and pro-IL-1 β ; second, an activation signal is triggered by cellular events, such as potassium efflux, mitochondrial dysfunction or lysosomal rupture (22). Upon activation, NLRP3 recruits ASC and pro-caspase-1 to orchestrate inflammasome assembly. Pro-caspase-1 then undergoes

autocatalytic cleavage to generate active caspase-1 (23), which subsequently cleaves pro-IL-1 β and pro-IL-18 into their mature forms. These cytokines are then released through membrane pores to propagate the inflammatory response. Simultaneously, caspase-1 cleaves GSDMD to release its NTD. This domain oligomerizes within the plasma membrane to form pores, thereby disrupting osmotic balance and ultimately precipitating cell rupture and pyroptosis (24).

Non-canonical inflammasome-dependent pyroptosis. The non-canonical pyroptotic pathway operates independently of canonical inflammasomes (25). In humans, this pathway is mediated by caspase-4 and caspase-5, whereas caspase-11 serves as the mediator in mice. In this pathway, LPS directly binds the caspase recruitment domain (CARD) of caspase-4/5/11. This binding event leads to their activation, and activated caspases subsequently cleave GSDMD. This cleavage releases the pore-forming NTD, which executes pyroptosis (26). Notably, caspase-4/5/11 do not directly process pro-IL-1 β or pro-IL-18, but instead, GSDMD-mediated pore formation promotes potassium efflux. This ion shift secondarily activates the NLRP3 inflammasome and caspase-1, thereby driving the maturation and secretion of IL-1 β and IL-18 (27).

Caspase-3/8-mediated pyroptosis. Caspase-3 and caspase-8 are traditionally regarded as key executioners of apoptosis; however, recent evidence implicates them in pyroptosis via the cleavage of specific GSDM family members (28). In cells with high GSDME expression, caspase-3 cleaves the GSDME linker region, which releases the pore-forming NTD and shifts the mode of death from apoptosis to pyroptosis (29). Notably, GSDME expression levels dictate the cell death outcome, where high expression favors caspase-3-mediated pyroptosis and low expression favors apoptosis (30). Additionally, during *Yersinia* infection, the effector YopJ inhibits TAK1 signaling, which subsequently activates caspase-8. Activated caspase-8 then cleaves both GSDMD and GSDME. This process liberates their NTDs to form membrane pores and trigger pyroptosis (31). This pathway demonstrates that caspase-8 can initiate pyroptosis independently of canonical inflammasomes.

Granzyme (Gzm)-mediated pyroptosis. Gzms are the key effector molecules of cytotoxic T lymphocytes and natural killer cells. They enter target cells via perforin-mediated pores to induce cell death (32). Studies have elucidated their direct role in pyroptosis regulation; GzmB can induce pyroptosis indirectly through caspase-3 activation. Furthermore, it directly cleaves GSDME at the same site to release the pore-forming NTD and drive cell lysis (33). In addition, GzmA was found to specifically cleave GSDMB at Lys229/Lys244. This cleavage liberates the NTD to form pores, establishing a caspase-independent pyroptotic pathway. Notably, GSDMB is constitutively expressed in epithelial cells and certain brain tissues, and its expression is markedly upregulated within inflammatory microenvironments (34). These findings provide new mechanistic insights and a theoretical basis for targeting pyroptosis in cerebral IR injury. Fig. 1 illustrates the key molecules and pathways involved in pyroptosis.

Moreover, the mitochondrial-GSDMD axis functions as a pivotal amplifier of neuroinflammation during cerebral IR (35). Specifically, the N-terminal fragment of GSDMD (GSDMD-N) facilitates mitochondrial membrane permeabilization, triggering the release of mitochondrial DNA (mtDNA) into the cytosol (36). This translocation subsequently engages the cyclin GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway, markedly exacerbating secondary neuroinjury and inflammatory cascades following ROSC.

Hierarchical framework for validating pyroptosis stages. To ensure scientific rigor and distinguish between pro-inflammatory signaling and terminal cell lysis, the following three-stage experimental validation framework is proposed.

Stage 1 (priming). Focuses on transcriptional readiness with key readouts of NF- κ B nuclear translocation; mRNA and protein induction of NLRP3 and pro-IL-1 β .

Stage 2 (inflammasome activation). Focuses on proteolytic processing with key readouts: ASC speck formation; detection of cleaved caspase-1 (p20); and quantification of mature IL-1 β /IL-18 secretion.

Stage 3 (execution). Focuses on membrane perforation and cytolysis with key readouts; Identification of GSDMD-N or GSDME-N fragments; LDH release assays; and propidium iodide or ethidium homodimer staining.

Methodological note. While Stage 2 markers indicate an active inflammatory response, terminal pyroptosis must be confirmed by Stage 3 markers (GSDMD cleavage and membrane rupture), which represent the definitive hallmarks of lytic cell death.

3. Improving outcomes after post-CA brain injury

Neuronal pyroptosis. Neurons are the most vulnerable cell type during cerebral IR injury. Their heightened sensitivity to ischemia-hypoxia largely determines the severity of neurological deficits (37). Pyroptosis has emerged as a distinct form of inflammatory programmed cell death, which amplifies neuroinflammation through inflammasome activation and cytokine release; this mechanism has been validated across multiple neurological disorders, including Alzheimer's disease, Parkinson's disease and traumatic brain injury (38). At the molecular level, NLRP3 inflammasome activation triggers caspase-1 activation and GSDMD cleavage. These events lead to pore formation, neuronal swelling and lysis. Such findings are well-demonstrated in both cerebral IR injury and OGD/R models (39). Moreover, genetic deletion or pharmacological inhibition of NLRP3, caspase-1 or GSDMD markedly reduces neuronal death and attenuates inflammation. These interventions also improve motor and behavioral outcomes, supporting pyroptosis as a potential neuroprotective target (40). Notably, neuronal pyroptosis is not restricted to ischemic injury; it has also been implicated in Parkinson's disease and Alzheimer's disease (AD), suggesting it may represent a common neuronal response to diverse insults (41). In summary, elucidating the molecular mechanisms of neuronal pyroptosis in cerebral IR injury deepens the understanding of pathology. It also provides novel avenues for neuroprotective intervention (42).

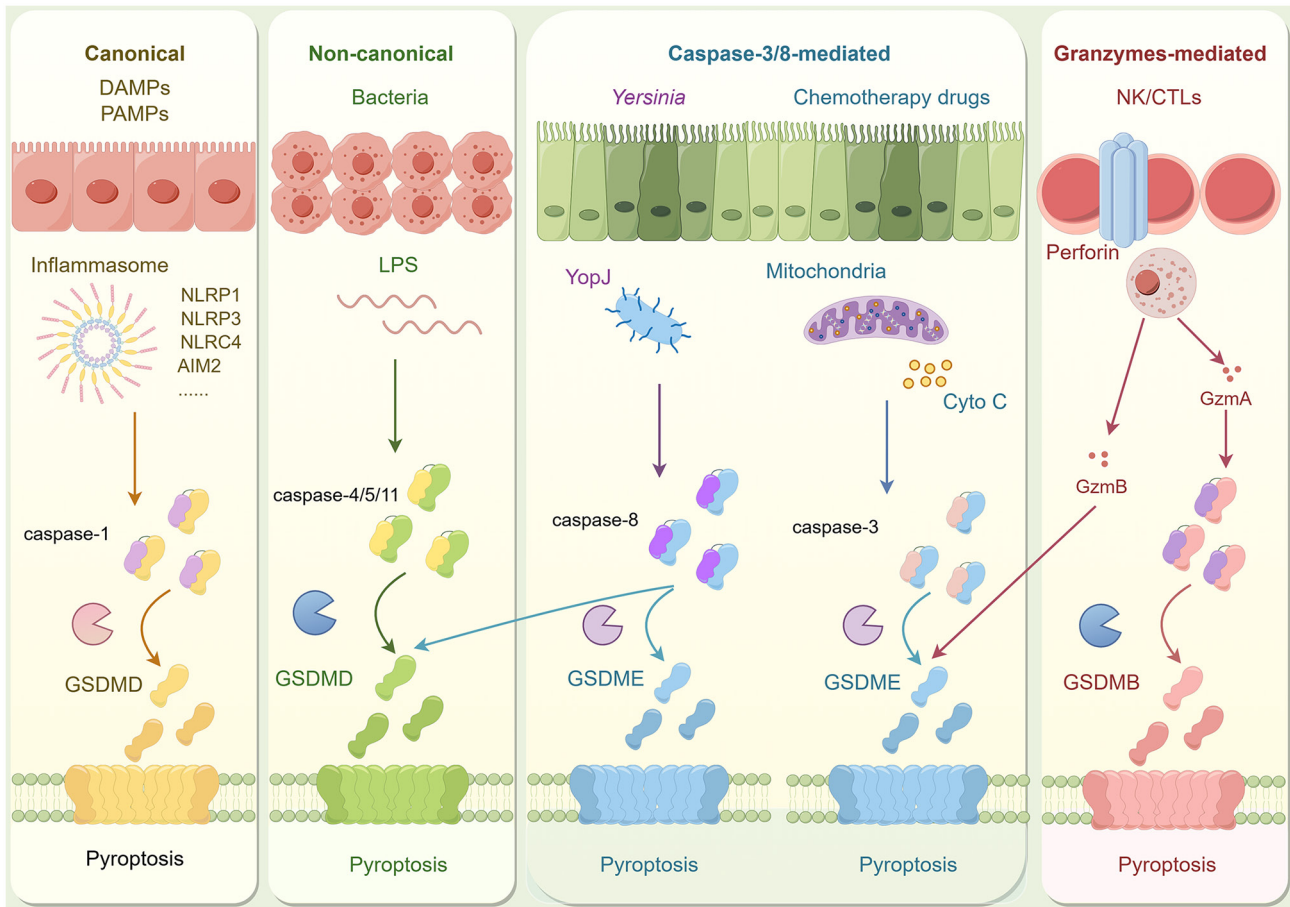


Figure 1. Key molecules and pathways in pyroptosis. This figure illustrates the canonical, non-canonical and alternative pathways of pyroptosis, including those mediated by caspase-3/8 and granzymes. The figure was created using Figdraw (figdraw.com). DAMPs, danger-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; LPS, lipopolysaccharide; GSDMB/D/E, gasdermin B/D/E; Gzm, granzyme; NK, natural killer; CTLs, cytotoxic T lymphocytes.

Astrocytic pyroptosis. Astrocytes are essential for maintaining ion homeostasis, neurotransmitter metabolism and BBB integrity. They provide notable neuronal support in the central nervous system. Consequently, astrocyte dysfunction or death is linked to multiple neurological disorders (43). During cerebral IR injury, astrocytic pyroptosis occurs via two primary pathways. These include inflammasome-mediated caspase-1-dependent GSDMD cleavage and caspase-4/5/11 signaling. Overexpression of CD73 inhibits astrocytic pyroptosis via the adenosine A2B receptor/NF- κ B signaling axis; this modulation effectively mitigates brain injury (44). Following pyroptosis, astrocytes release pro-inflammatory cytokines such as IL-1 β and IL-18. This release exacerbates local inflammation and neuronal damage. In AD brains, astrocytic and neuronal pyroptosis have been linked to amyloid- β deposition and neurodegeneration (45). Specifically, amyloid- β aggregates act as DAMPs that trigger NLRP3 inflammasome activation; in turn, the resulting pyroptosis and chronic neuroinflammation can further promote amyloid- β production and aggregation, creating a feed-forward pathological loop. Furthermore, astrocytic inflammasome activation induces cell death and disrupts hippocampal synaptic plasticity. It also impairs glutamate homeostasis through IL-1 and IL-18 signaling, thereby aggravating excitotoxicity (46). Astrocytic pyroptosis is associated with early neurological deficits

following brain injury. Conversely, TNF-stimulated gene-6 alleviates this process by suppressing NLRP3 inflammasome activation. This reduction in pyroptosis alleviates brain edema and improves neurological function (47). Astrocytic pyroptosis is broadly involved in diverse neurological diseases; therefore, targeting inflammasome, caspase or GSDM pathways offers therapeutic promise. However, challenges remain in achieving selectivity and clinical translation (48).

Microglial pyroptosis. Microglia are the primary immune effector cells of the central nervous system, where they activate rapidly in response to cerebral IR injury. In this context, microglia mediate inflammation and exacerbate neuronal damage. Furthermore, their activation can impede long-term recovery (49). Beyond conventional immune activation, microglia can undergo pyroptosis. This process amplifies neuroinflammation through pore formation and cytokine release. The interaction between interferon-inducible protein 204 and SUMO-specific protease 7 activates STING signaling. This pathway induces both pyroptosis and mitochondrial dysfunction. Notably, silencing these factors alleviates neurological deficits (50). Mechanistically, microglial pyroptosis is largely dependent on the NLRP3/caspase-1/GSDMD axis, and ASC specks further amplify NLRP3 activity to promote pyroptosis. This process accelerates α -synuclein aggregation

and neuronal degeneration, suggesting relevance to diverse neurodegenerative diseases (51). The pyroptotic release of cytosolic contents and inflammatory mediators exacerbates neuroinflammation and secondary injury. cGAS-STING activation drives NLRP3-mediated microglial pyroptosis, which exacerbates pathology and motor deficits. Conversely, pharmacological or genetic inhibition of this axis markedly alleviates tissue damage (52). In cerebral IR models, delayed administration [24 h post-transient middle cerebral artery occlusion (tMCAO)] of the selective NLRP3 inhibitor MCC950 still reduces infarct size and improves neurological outcomes. Although MCC950 is not cell-type specific, its neuroprotective effects in cerebral IR models are largely characterized by the suppression of microglial-mediated neuroinflammation and cytokine release (53). In summary, microglial pyroptosis represents a notable driver of cerebral IR injury and a promising therapeutic target; however, its complex regulation and clinical feasibility warrant further investigation (54).

Pyroptosis and BBB disruption. Disruption of the BBB is a central pathological event in cerebral IR injury; this breach further exacerbates neuronal damage (55). As an inflammasome-dependent death pathway, pyroptosis aggravates BBB permeability via GSDM-mediated pore formation (56). The canonical NLRP3 inflammasome pathway is a validated driver of IR pathology; caspase-1 activation and IL-1 β release promote inflammatory signaling. These events directly injure endothelial cells and compromise BBB integrity (57). Simultaneously, the non-canonical pathway contributes to this dysfunction; caspase-11-mediated GSDMD activation induces endothelial rupture and the release of pro-inflammatory cytokines. This process spreads inflammation and exacerbates BBB impairment (58), and oxidative stress further amplifies BBB disruption during pyroptosis. It induces lipid peroxidation and mitochondrial dysfunction, which enhance inflammatory signaling and membrane injury (59). Astrocytes also actively participate in this process; astrocytic secretion of CXCL10 promotes endothelial pyroptosis via the CXCR3/cGAS/AIM2 pathway. This mechanism leads to BBB breakdown, intensifying neuroinflammation and aggravating brain injury (60). Together, these findings demonstrate that pyroptosis contributes to BBB disruption through multiple mechanisms. These insights highlight pyroptosis as a notable therapeutic target in cerebral IR injury.

Summary and perspectives

Core synthesis. Pyroptosis in cerebral IR involves the synchronized activation of both canonical (caspase-1) and non-canonical (caspase-4/5/11) pathways. These cascades converge on GSDMD-mediated membrane perforation, which serves as the definitive execution step of lytic cell death.

Critical specificity. Cellular heterogeneity within the neurovascular unit. A key distinction exists within the neurovascular unit, while neurons are the primary victims of terminal lytic death, microglia and astrocytes function as 'inflammatory amplifiers'. This glial activation sustains the neuroimmune response and exacerbates BBB instability, creating a feed-forward cycle of secondary neuroinjury.

Future perspectives. Future research should shift toward the development of cell-specific modulators. The ultimate aim is to selectively inhibit neuronal loss without compromising

the essential trophic and phagocytic support provided by glial cells during the recovery phase.

4. Relationship between cerebral IR injury and pyroptosis

Oxidative stress. Oxidative stress is a key driver of cell death and tissue damage in cerebral IR injury. Together with mitochondrial dysfunction and excitotoxicity, it forms the pathological basis of injury in cerebral IR (61). In cerebral IR models, reperfusion is characterized by a sharp rise in reactive oxygen species (ROS) levels. These levels positively associate with infarct volume and neurological deficits. Notably, inhibition of NADPH oxidase 2 effectively reduces ROS production and tissue injury (62). Mechanistically, ROS promote NLRP3 inflammasome assembly by inducing the dissociation of thioredoxin-interacting protein from thioredoxin, which subsequently binds to the leucine-rich repeat domain of NLRP3 to trigger its activation, and caspase-1 activation; this cascade leads to GSDMD cleavage and pyroptosis (63). Conversely, ROS scavengers or inflammasome inhibitors can partially reverse this injury. Beyond direct effects, oxidative stress indirectly facilitates inflammasome activation via mitochondrial dysfunction and calcium dysregulation. These interactions form complex signaling networks that represent potential therapeutic targets (64). In animal IR models, nano-material-based antioxidant delivery effectively scavenges ROS. This intervention suppresses the NLRP3/caspase-1/GSDMD pathway, reducing neuronal pyroptosis and alleviating brain damage (65). These findings support antioxidant interventions as viable neuroprotective strategies. Collectively, oxidative stress drives neuronal pyroptosis via both direct and indirect mechanisms (66).

Calcium dyshomeostasis. Calcium homeostasis is essential for neuronal survival and function. Its disruption triggers cell death via mechanisms such as endoplasmic reticulum stress and mitochondrial dysfunction (67). In cerebral IR injury, excessive glutamate receptor activation and abnormal calcium channel opening contribute to Ca²⁺ overload; the reversal of Na⁺/Ca²⁺ exchange also plays a role. These events collectively drive excitotoxicity and neuronal injury (68). Calcium overload activates the calcium-dependent cysteine protease calpain, which subsequently liberates caspase-1 from the cytoskeleton to promote its activation. This process leads to GSDMD cleavage and the formation of pore-forming N-terminal fragments that drive canonical pyroptosis (69). Aberrant activation of the endoplasmic reticulum Ca²⁺ channel inositol 1,4,5-triphosphate receptor 1 has been shown to enhance Ca²⁺ efflux and induce pyroptosis via the NLRP3/caspase-1 pathway (70). In cerebral IR models, xanthine has been identified as a key metabolite; it triggers endothelial Ca²⁺ overload and induces GSDME-dependent pyroptosis, thereby exacerbating BBB disruption and brain injury (71). Thus, calcium dyshomeostasis acts as both a potent driver of pyroptosis and a central pathological mechanism of IR injury. Elucidating the cross-talk between Ca²⁺ imbalance and pyroptosis will provide opportunities for identifying novel therapeutic targets (72).

Mitochondrial dysfunction. Mitochondrial dysfunction during cerebral IR leads to marked energy failure, and also serves as

an inflammatory signaling hub that drives pyroptosis. In this context, the NLRP3 inflammasome acts as a notable bridge in stroke-associated inflammation (73). Mitochondria-derived danger signals include excessive mitochondrial ROS, oxidized mtDNA leakage and abnormal mitochondrial permeability transition pore opening. These signals induce or amplify NLRP3 inflammasome activation to promote pyroptotic cascades (74). Previous evidence demonstrates that GSDMD forms pores in both the plasma and mitochondrial membranes; this process facilitates mtDNA release and activates the cGAS-STING pathway. This mechanism establishes a positive feedback loop between inflammation and pyroptosis, thereby exacerbating tissue damage (75). In cerebral IR models, hyperactivation of dynamin-related protein 1 (Drp1) induces mitochondrial dysfunction and GSDMD-mediated pore formation. This aggravates neuronal pyroptosis and tissue injury. Conversely, Apelin receptor early endogenous ligand mitigates this process by suppressing Drp1 hyperactivation and NLRP3 signaling (76). Furthermore, enhancing PTEN-induced putative kinase 1 (PINK1)/Parkin-dependent mitophagy markedly inhibits pyroptosis. This suggests that the coupling of mitochondrial quality control and pyroptosis represents a conserved regulatory axis (77). Overall, targeting mitochondrial function effectively suppresses inflammation and pyroptosis, underscoring its central role in the pathophysiology of cerebral IR injury (78).

Summary and perspectives

Core synthesis. The mitochondrial-pyroptotic triad: Oxidative stress, calcium overload and mitochondrial dysfunction form a self-perpetuating ‘triad’ that drives NLRP3 inflammasome activation. This triad represents the most validated common pathway across diverse cerebral IR models.

Critical specificity. GSDMD-mediated feed-forward loops. Evidence identifies mitochondrial GSDMD pores as a critical amplifier. These pores allow mtDNA leakage, which directly amplifies intracellular inflammatory signaling beyond the initial insult.

Future perspectives. Identifying the ‘point of no return’. Mapping the precise spatiotemporal sequence of these drivers is vital. Determining the transition from reversible stress to irreversible lysis will help identify the optimal therapeutic window for interventions.

5. Pyroptosis-induced neuroinflammatory cascade

Pyroptosis serves as a pivotal driver and amplifier of the neuro-inflammatory response following cerebral IR injury. Pyroptotic cells recognize endogenous danger signals and release IL-1 β , IL-18 and DAMPs. These events activate local immune responses and trigger neuroinflammation (79). During cerebral IR, released DAMPs activate the TLR4 signaling pathway, which amplifies inflammasome activity and promotes pro-inflammatory cytokine production, further propagating the inflammatory cascade (80). Moreover, GSDMD cleavage drives pore formation in the plasma membrane to facilitate the release of intracellular contents. The liberation of DAMPs and subsequent membrane rupture are key steps in amplifying the inflammatory response (81).

Evidence indicates that pyroptosis activates microglial NLRP3 inflammasomes. This activation subsequently

induces A1-type astrocyte activation, generating a neurotoxic response (82). The interaction between NLRP3 and NF- κ B is a marked amplification mechanism; initial NLRP3 activation is mediated by κ B kinase β . This kinase recruits inflammasome components to the trans-Golgi network to sustain inflammatory progression (83). These inflammatory signals exacerbate pyroptosis in endothelial cells and pericytes, intensifies BBB disruption and facilitates leukocyte infiltration (84). In mice IR models, endothelial pyroptosis upregulates IL-1 β , TNF- α and vascular cell adhesion molecule-1. This promotes immune cell extravasation and increases BBB permeability; conversely, inhibiting pyroptosis reduces these factors and partially restores BBB integrity (85).

Beyond localized cell death, pyroptosis orchestrates the broader neuroimmune landscape by releasing DAMPs and pro-inflammatory cytokines. These signals actively recruit peripheral immune cells to the site of injury. For instance, mediators such as high-mobility group box 1 (HMGB1) facilitate BBB breakdown, allowing T cells to infiltrate the brain parenchyma (86). This persistent neuroimmune crosstalk drives the shift from acute phase inflammation to chronic neuroinflammation. Ultimately, such chronic activation hinders long-term neurorepair and functional recovery (87).

The contribution of pyroptosis to neuroinflammation extends beyond amplification to include resolution. If pyroptotic cells are not promptly cleared, persistent DAMP release drives chronic inflammation and glial scar formation; these events exacerbate neuronal injury (88). Membrane repair mechanisms, including endosomal sorting complexes required for transport-III (ESCRT-III) and nerve injury-induced protein 1 (NINJ1), regulate the outcome of pyroptosis. These systems determine whether inflammation resolves, thereby influencing tissue repair and functional recovery (89). In summary, pyroptosis orchestrates the neuroinflammatory cascade across the initiation, amplification and resolution phases. This process aggravates cerebral IR injury while providing a theoretical basis for novel therapeutic interventions (90) (Fig. 2).

Apart from their fundamental roles in disease pathogenesis, pyroptosis-associated molecules, particularly N-terminal GSDMD-N and IL-18, are increasingly recognized as viable biofluid biomarkers for quantifying the severity of ischemic cerebral insults. Growing evidence indicates that increased concentrations of these proteins in the serum or cerebrospinal fluid reflect the degree of BBB permeability. Furthermore, these biochemical signatures may function as reliable D indicators for neurological outcomes, providing an objective measure of the extent of brain tissue damage (91).

Summary and perspectives

Core synthesis. The acute-to-chronic transition: Pyroptosis dictates the transition from acute injury to chronic neuroinflammation. This pathological progression is balanced by membrane repair systems, such as ESCRT-III and molecular executioners such as NINJ1.

Critical specificity. Neuroimmune crosstalk and regeneration: Beyond localized cell damage, pyroptotic DAMPs (such as HMGB1) facilitate T-cell infiltration into the brain parenchyma. This sustained neuroimmune crosstalk may create a hostile microenvironment that markedly hinders long-term axonal regeneration.

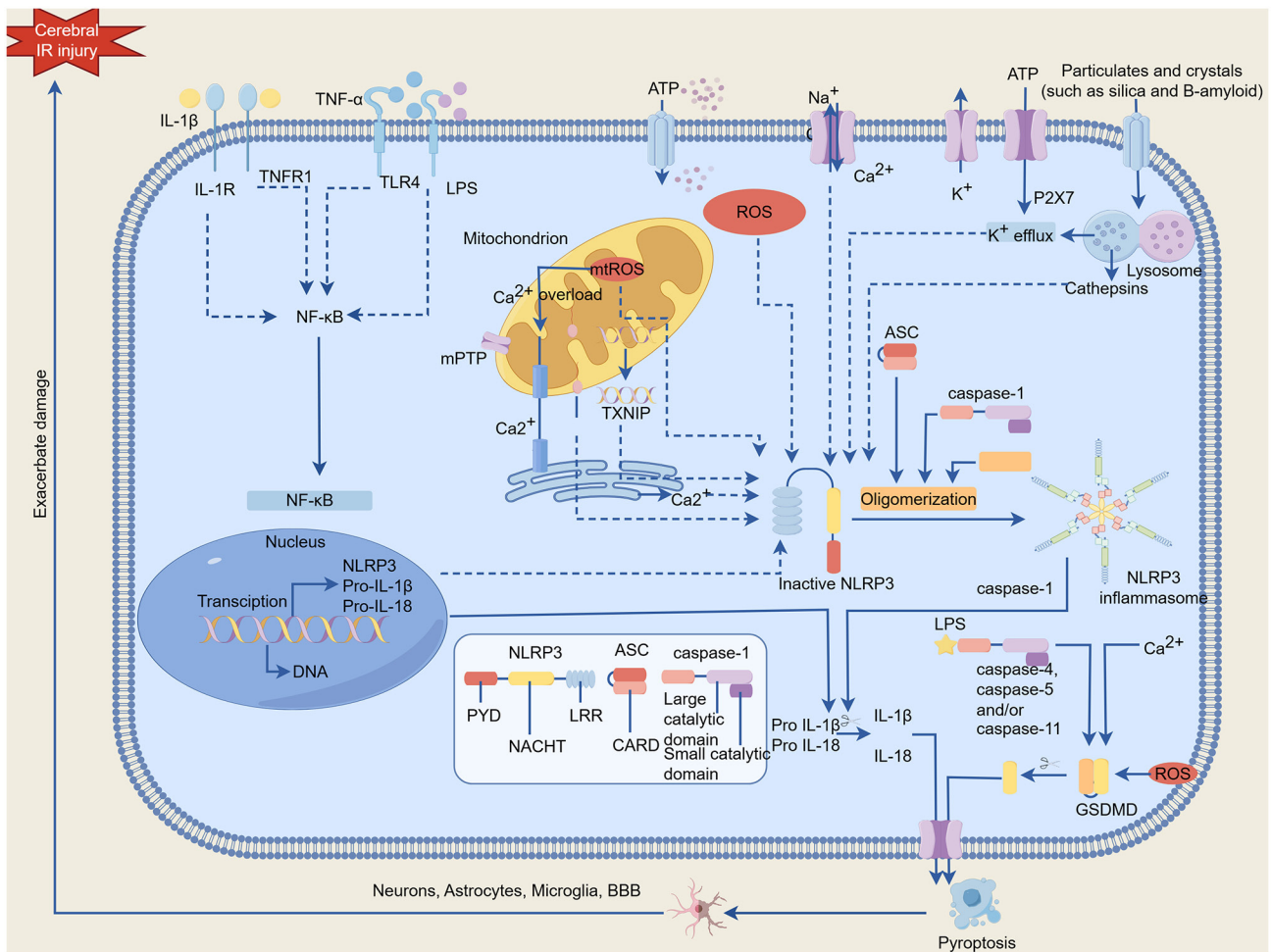


Figure 2. Role and mechanism of pyroptosis in cerebral IR injury. The schematic diagram summarizes the priming and activation of the NLRP3 inflammasome during IR injury, leading to pyroptosis in neurons, astrocytes and microglia. The figure was created using Figdraw (figdraw.com). LPS, lipopolysaccharide; ROS, reactive oxygen species; mtROS, mitochondrial reactive oxygen species; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; GSDMD, gasdermin D; TXNIP, thioredoxin-interacting protein; PYD, pyrin domain; LRR, leucine-rich repeat; CARD, caspase recruitment domain; BBB, blood-brain barrier; IR, ischemia-reperfusion.

Future perspectives. Validation of biofluid biomarkers: A primary goal for clinical translation is the systematic validation of biofluid markers, specifically GSDMD-N and IL-18. Establishing the prognostic value of these markers in serum or cerebrospinal fluid is essential for the real-time monitoring of patients following cardiac arrest.

6. Crosstalk between pyroptosis and other forms of programmed cell death

Pyroptosis and ferroptosis. In cerebral IR injury, pyroptosis and ferroptosis are primary pathological forms of programmed cell death. These pathways independently regulate neuronal damage but also interact synergistically, and this crosstalk further exacerbates tissue injury (92). Mechanistically, iron overload and lipid peroxidation directly activate the NLRP3 inflammasome. This activation triggers caspase-1-mediated GSDMD cleavage and pyroptosis (93). Conversely, GSDMD pore formation facilitates Ca^{2+} influx and ROS accumulation. These events amplify lipid peroxidation and enhance ferroptotic susceptibility, thereby establishing a vicious cycle (94). In cerebral IR models, inhibiting ferroptosis suppresses lipid

peroxidation via the SLC7A11/GPX4/ACSL4 axis. This inhibition also downregulates the caspase-1/GSDMD pathway, thereby attenuating pyroptosis and improving neurological outcomes (95). Moreover, NLRP3-deficient mice exhibit smaller infarct volumes and improved neurological scores. These benefits are attributed to both pyroptosis suppression and GPX4 upregulation. Reduced iron deposition and diminished lipid peroxidation collectively mitigate ferroptosis in these models (96). Autophagy, particularly ferritinophagy, has emerged as a notable node linking ferroptosis and pyroptosis by integrating iron homeostasis and inflammasome activity (97). These insights suggest that combined targeting of ferroptosis and pyroptosis may provide a promising therapeutic strategy for cerebral IR injury.

Pyroptosis and autophagy. Autophagy is an essential degradative pathway which maintains cellular homeostasis by clearing misfolded proteins and damaged organelles to prevent the accumulation of cytotoxic factors (98). In cerebral IR injury, autophagy exerts a dual effect on pyroptosis. Moderate autophagy activation alleviates mitochondrial dysfunction and oxidative stress; this suppresses excessive inflammasome

activation, thereby reducing pyroptosis and conferring neuroprotection (99). For instance, an Astragalus-Angelica herbal formulation activates autophagy via the AMPK/mTOR pathway. This activation suppresses NLRP3-dependent pyroptosis and attenuates cerebral IR injury (100). By contrast, excessive or sustained autophagy can promote GSDMD cleavage and pro-inflammatory cytokine release, which amplifies pyroptosis (101). In such settings, autophagy inhibition mitigates inflammasome-mediated damage and improves neurological function. Thus, autophagy exerts context-dependent effects, acting as either protective or detrimental. This paradox highlights its complexity as a therapeutic target. It underscores the need to dissect the spatiotemporal dynamics of autophagy-pyroptosis crosstalk across diverse cell types and disease stages.

Pyroptosis and apoptosis. Apoptosis and pyroptosis frequently coexist and interact during cerebral IR injury, and their molecular signatures are defined by distinct caspase family members (102). Specifically, caspase-3 and caspase-9 drive classical apoptosis, whereas caspase-1, -4/5 and -11 mediate pyroptosis through GSDMD or GSDME cleavage. Notably, caspase-3-mediated cleavage of GSDME can convert apoptosis into secondary pyroptosis, a lytic transition where cells initially undergoing programmed apoptosis switch to a pro-inflammatory phenotype due to GSDME-mediated membrane pore formation (103). Caspase-8 exhibits dual functions; it triggers apoptosis through canonical pathways while also directly cleaving GSDMD or promoting inflammasome activation. This signaling bifurcation has been validated in TNF- α -induced cell death and infection responses (104). Mitochondrial pathways further integrate apoptosis and pyroptosis; BAX/BAK-mediated mitochondrial outer membrane permeabilization induces Cytochrome c release to drive apoptosis. Simultaneously, this process releases mtDNA to activate the NLRP3 inflammasome and promote pyroptosis (105). Regulatory molecules such as cellular FLICE-like inhibitory protein (c-FLIP), inhibitors of apoptosis proteins and receptor-interacting serine/threonine-protein kinase 1 (RIPK1) act as pivotal nodes in this crosstalk. For example, c-FLIPL deficiency facilitates complex II formation and promotes LPS-induced pyroptosis; this underscores its role in dual-pathway regulation (106). Based on these insights, pharmacological inhibition of caspases or GSDMs has been proposed to attenuate both apoptosis- and pyroptosis-related injury. However, efficacy remains context-dependent, warranting further refinement using multi-omics approaches (107).

Pyroptosis and necroptosis. Both pyroptosis and necroptosis are pro-inflammatory death pathways that often coexist in cerebral IR injury. These processes are tightly interconnected through the PANoptosome complex (108). Key molecular convergence points include RIPK1, RIPK3, MLKL, caspase-8 and GSDMD, which collectively determine cell fate. RIPK1 and RIPK3 activate MLKL to initiate necroptosis; simultaneously, caspase-8 cleaves GSDMD to induce pyroptosis (109). MLKL-mediated K⁺ efflux has been shown to activate the NLRP3 inflammasome, which induces GSDMD pore formation and couples necroptosis with pyroptosis (110). Caspase-8 further promotes IL-1 β maturation, linking the two pathways in

inflammatory cell death. The emerging concept of PANoptosis (pyroptosis, apoptosis, and necroptosis) emphasizes that these pathways are co-regulated within the PANoptosome complex (111). Consequently, the inhibition of a single pathway may be compensated by alternative mechanisms. In cerebral IR models, pyroptosis and necroptosis jointly exacerbate sterile inflammation and neuronal injury. Conversely, neural stem cell transplantation mitigates these effects by down-regulating GSDMD and MLKL expression. This intervention ultimately improves functional recovery (112,113). These findings highlight the therapeutic potential of simultaneously targeting pyroptotic and necroptotic pathways.

Summary and perspectives

Core synthesis. The PANoptosome and programmed cell death crosstalk: Pyroptosis is no longer viewed in isolation but as part of the 'PANoptosome' complex, where it intersects with apoptosis, necroptosis and ferroptosis. This integrated view reflects the complex molecular interplay that dictates neuronal fate following cerebral IR injury.

Critical specificity. Autophagy as a cellular rheostat: Autophagy acts as a complex rheostat in the context of ischemic injury. While physiological autophagy confers neuroprotection by degrading inflammasomes, excessive 'autophagic stress' can facilitate GSDMD cleavage and accelerate lytic cell death.

Future perspectives. Toward multi-node therapeutic inhibition: Future therapies should transition toward 'multi-node' inhibition strategies. Targeting the shared molecular hubs of the PANoptosome may offer improved neuroprotection compared with inhibiting a single death pathway alone, particularly given the compensatory mechanisms inherent in programmed cell death.

7. Therapeutic strategies targeting pyroptosis

NLRP3-related therapeutic targets. The NLRP3 inflammasome is a central sensor in the pyroptotic pathway. It recognizes intracellular danger signals to mediate downstream caspase-1 activation and GSDMD cleavage. These events ultimately lead to plasma membrane pore formation and pro-inflammatory cytokine release (114). In a mouse cardiac arrest/cardiopulmonary resuscitation (CA/CPR) model, selective inhibition of NLRP3 using MCC950 administered post-ischemia notably reduces NLRP3/caspase-1 activation to improve survival and neurological deficit scores (115). Similarly, in a mouse model of tMCAO, β -caryophyllene administered post-ischemia alleviates white matter injury, and improves motor and cognitive functions by suppressing NLRP3-mediated pyroptosis (116). In a rat CA/CPR model, chrysophanol administered post-ischemia also mitigates cerebral IR injury via the inhibition of the TRAF6/NLRP3 signaling pathway (117). In a mouse tMCAO model, pre-ischemic inhibition of the lipocalin-2/24p3R/NLRP3 axis attenuates astrocyte pyroptosis and neuroinflammation by reducing GSDMD-N and IL-1 β levels (118). Collectively, these findings establish NLRP3 as a pivotal mediator of ischemic brain injury; however, its activation mechanisms remain complex, with oxidative stress serving as a primary driver.

Oxidative stress is a primary driver of cerebral IR injury, and ROS are recognized as major triggers for NLRP3

activation (119). In a mouse distal middle cerebral artery occlusion model, administration of hydrogen-rich saline, a potent ROS scavenger, both pre- and post-ischemia effectively suppresses the ROS/NLRP3/caspase-1 pathway and reduces IL-1 β levels, thereby improving neurological deficit scores (120). Additionally, in a mouse tMCAO model, OIP5 Antisense RNA 1 administered as a single dose post-ischemia promotes the degradation of thioredoxin-interacting protein, which inhibits oxidative stress-induced neuronal pyroptosis and alleviates cerebral IR injury (121). Likewise, methyl isoeugenol administered post-ischemia in a rat tMCAO model activates the nuclear factor erythroid 2-related factor 2 (Nrf2)/NQO1/HO-1 antioxidant pathway, which suppresses ROS-driven microglial pyroptosis and attenuates neuroinflammation (122). These findings underscore oxidative stress modulation as an important therapeutic strategy.

Calcium overload and mitochondrial dysfunction also trigger aberrant NLRP3 activation (123). Dexmedetomidine reduces microglial pyroptosis and neural injury in a rat permanent middle cerebral artery occlusion (pMCAO) model when administered via post-ischemic continuous infusion, a process mediated by the inhibition of the P2X7R/NLRP3/caspase-1 pathway (124). By suppressing Drp1-mediated mitochondrial fission, a single post-ischemic dose of FK866 in a rat CA/CPR model ameliorates mitochondrial dysfunction, thereby reducing both pyroptosis and neuroinflammation (125). Single post-ischemic administration of Apelin-13-loaded macrophage membrane-encapsulated nanoparticles in a mouse tMCAO model attenuates pyroptosis by enhancing Sirtuin 3 activity and suppressing NLRP3 assembly (126). These approaches highlight novel strategies targeting calcium overload and mitochondrial dysfunction.

Inflammatory propagation represents another notable pathological process mediated by NLRP3-dependent pyroptosis (127). Pre-ischemic administration of indobufen and aspirin, alone or in combination with clopidogrel or ticagrelor, attenuate pyroptosis in a rat tMCAO model by inhibiting NF- κ B/NLRP3 signaling (128). Knockdown of maternally expressed gene 3 administered as a single pre-ischemic dose in a rat tMCAO model inhibits the microRNA (miR)-145-5p/TLR4/NLRP3 axis, thereby suppressing pyroptosis and conferring neuroprotection (129). In a rat tMCAO model, a single pre-ischemic dose of calycosin reduces pyroptosis by suppressing the HMGB1/NLRP3 signaling axis (130). Post-ischemic electroacupuncture (EA) at the ST36 acupoint in a rat CA/CPR model alleviates neuronal injury and inflammation by activating the α -7 nicotinic acetylcholine receptor, which effectively suppresses microglial pyroptosis (131). Furthermore, in a mouse tMCAO model, a single post-ischemic dose of the STING inhibitor C-176 reduces ischemic brain injury by disrupting the STING-NLRP3 interaction and suppressing microglial pyroptosis (132). Taken together, these findings confirm the central role of NLRP3 in pyroptosis induction and inflammatory amplification, underscoring its promise as a translatable therapeutic target.

Caspase-1 and GSDMD-related therapeutic targets. Caspase-1 is a notable component of the inflammasome complex, which is responsible for the maturation of IL-1 β and IL-18 and the initiation of pyroptosis (133). In a rat pMCAO

model, post-ischemic treatment with the caspase-1 inhibitor VX-765 ameliorates BBB disruption and neuroinflammation by inhibiting caspase-1 and reducing pyroptosis, which ultimately leads to improved neurological outcomes (134). AIM2 inflammasome-driven caspase-1 activation exacerbates brain injury and cognitive impairment. Conversely, pre-ischemic treatment with the caspase-1 inhibitor Ac-YVAD-CMK in a mouse tMCAO model improves cognitive function by suppressing caspase-1-mediated pyroptosis (135). Additionally, miR-96-5p reduces cerebral IR injury in a mouse tMCAO model when administered as a single dose pre-ischemia primarily by downregulating caspase-1 and suppressing pyroptosis (136). Thus, targeting caspase-1 is a promising strategy to mitigate IR-induced pyroptosis and neural injury.

GSDMD is the primary executioner of pyroptosis, and cleavage of GSDMD liberates its NTD. This domain subsequently forms pores in the plasma membrane, ultimately leading to cell rupture (137). Administered post-ischemia in a mouse tMCAO model, the GSDMD inhibitor disulfiram reduces neutrophil infiltration and infarct size in cerebral IR injury (91). Similarly, in a mouse tMCAO model, genetic ablation (knockout) of GSDMD decreases microglial pyroptosis in cerebral IR, a reduction that markedly improves neurological function (138). Therefore, targeting the caspase-1/GSDMD axis enables multi-level intervention. This approach spans from inflammatory sensing to cellular rupture, providing a systematic framework for modulating pyroptosis (Table I) (91,115-118,120-122,124-126,128-132,134-136,138).

Biological and molecular regulators of pyroptosis. Beyond canonical pathways, modulating cell-specific functional states and signaling networks represents a promising therapeutic avenue. Microglia are the primary resident immune cells of the brain where they play central roles in cerebral IR injury, and their polarization state (M1 vs. M2) dictates the extent of neuronal damage (139). Intermittent θ -burst stimulation (iTBS), applied twice daily for 7 days post-ischemia in a mouse tMCAO model, reduces neuronal pyroptosis by modulating microglial M1/M2 polarization (140). Via activation of the HACE1/Nrf2 axis, ETS variant transcription factor 5 administered as a single pre-ischemic dose in a rat tMCAO model inhibits microglial M1 polarization and attenuates brain injury (141). Salvianolic acids for injection administered daily post-ischemia in a rat tMCAO model exert neuroprotective effects by promoting microglial M1-to-M2 polarization and inhibiting the NLRP3 inflammasome (142). Autophagy is a marked cellular self-clearance mechanism, and its role in pyroptosis is complex and often described as a double-edged sword. Both excessive and insufficient autophagic activity can exacerbate injury (143). When administered as a single post-ischemic dose in a rat tMCAO model, dexmedetomidine enhances mitochondrial autophagy via the PINK1/Parkin pathway, thereby reducing pyroptosis and protecting cerebral cells (144). Pre-ischemic combination therapy involving EA and tigustilide in a rat tMCAO model activates the PI3K/AKT/mTOR pathway, which inhibits autophagy and subsequently reduces pyroptosis (145). These studies suggest that regulating microglial activation and balancing autophagy could provide new strategies for treating cerebral IR injury.

Table I. Therapeutic interventions targeting the canonical pyroptosis pathway (NLRP3, caspase-1 and GSDMD).

First author, year	Intervention	Model (Species)	Dose, route and timing	Evidence level	Target	Main findings	(Refs.)
Jiang <i>et al.</i> , 2020	MCC950	CA/CPR (Mouse)	10 mg/kg, i.p. qd x 3 days Post-ischemia	NLRP3 caspase-1 IL-1 β	NLRP3	Inhibits NLRP3 inflammasome activation, reducing pyroptosis	(115)
Xin <i>et al.</i> , 2024	β -caryophyllene	tMCAO (Mouse) OGD/R (MO3.13 cells)	20 mg/kg, i.p. qd x 28 days Post-ischemia	GSDMD-N NLRP3 IL-18	NLRP3	Mitigates white matter lesions and improves motor/cognitive function by inhibiting NLRP3-mediated pyroptosis	(116)
Xia <i>et al.</i> , 2022	Chrysophanol	CA/CPR (Rat) OGD/R (PC12 cells)	10 mg/kg, i.p. qd x 7 days Post-ischemia	GSDMD-N NLRP3 IL-1 β	TRAF6 NLRP3	Inhibits TRAF6/NLRP3 signaling pathway	(117)
Li <i>et al.</i> , 2023	Targeting LCN2	tMCAO (Mouse) OGD/R (Astrocytes)	1 μ g/ml, i.c.v. qd x 5 days Pre-ischemia	GSDMD-N NLRP3 IL-1 β	24p3R NLRP3	Inhibits NLRP3 activation via 24p3R, reducing astrocyte pyroptosis	(118)
Du <i>et al.</i> , 2023	HS	dMCAO (Mouse) LPS (BV2 cells)	10 ml/kg, i.p. qd x 3 days Pre and post-ischemia	NLRP3 caspase-1 IL-1 β	ROS NLRP3	Inhibits ROS/NLRP3 pathway	(120)
Li <i>et al.</i> , 2024	OIP5-AS1	tMCAO (Mouse) OGD/R (Neurons)	20 μ g (Exo), i.v. Single dose Post-ischemia	NLRP3 caspase-1 IL-1 β	TXNIP ITCH	Promotes TXNIP ubiquitination, reducing pyroptosis	(121)
Liu <i>et al.</i> , 2025	Methyl isoeugenol	tMCAO (Rat) OGD/R (BV2 cells)	100 mg/kg, i.g. qd x 3 days Post-ischemia	GSDMD NLRP3 IL-1 β	Nrf2 NLRP3	Activates Nrf2/NQO1/HO-1 signaling pathway, inhibiting NLRP3 inflammasome	(122)
Sun <i>et al.</i> , 2021	Dexmedetomidine	pMCAO (Rat)	1 μ g/kg + 0.05 μ g/kg/min, i.v. Continuous infusion Post-ischemia	GSDMD NLRP3 IL-1 β	P2X7R NLRP3	Inhibits P2X7R/NLRP3/caspase-1 pathway	(124)
Zou <i>et al.</i> , 2020	FK866	CA/CPR (Rat)	10 mg/kg, i.p. Single dose Post-ischemia	GSDMD-N NLRP3 IL-1 β	Drp1 NLRP3	Inhibits Drp1, improves mitochondrial morphology, and inhibits NLRP3 inflammasome	(125)
Ma <i>et al.</i> , 2024	MM/ANPs	tMCAO (Mouse) OGD/R (HT22 cells)	100 μ g/kg (Apelin-13), i.v. Single dose Post-ischemia	GSDMD-N NLRP3 IL-1 β	SIRT3 NLRP3	Enhances SIRT3 activity, inhibiting NLRP3 inflammasome assembly	(126)
Li <i>et al.</i> , 2021	IACT	tMCAO (Rat) OGD/R (PC12 cells)	Indobufen (20 mg/kg) Aspirin (10 mg/kg) i.g., qd x 5 days Pre-ischemia	GSDMD-N NLRP3 IL-1 β	NF- κ B NLRP3	Inhibits NF- κ B/NLRP3 pathway	(128)

Table I. Continued.

First author, year	Intervention	Model (Species)	Dose, route and timing	Evidence level	Target	Main findings	(Refs.)
Li <i>et al</i> , 2025	si-MEG3	tMCAO (Rat) OGD/R (HT22 cells)	5 nmol, i.c.v. Single dose Pre-ischemia	NLRP3 caspase-1 IL-1 β	miR-145-5p TLR4 NLRP3	Knockdown of MEG3 inhibits miR-145-5p/TLR4/NLRP3 axis, reducing pyroptosis	(129)
Zhang <i>et al</i> , 2025	Calycosin	tMCAO (Rat) OGD/R (HAPI cells)	75, 150, 300 μ g/kg, i.c.v. Single dose Pre-ischemia	GSDMD-N NLRP3 IL-1 β	HMGB1 NLRP3	Inhibits HMGB1/NLRP3 pathway	(130)
Qin <i>et al</i> , 2025	EA-ST36	CA/CPR (Rat) OGD/R (Microglia)	15 Hz, 1 mA 30 min Post-ischemia	GSDMD-N NLRP3 IL-1 β	α 7nAChR	Activates α 7nAChR, inhibiting microglial pyroptosis	(131)
Li <i>et al</i> , 2024	C-176	tMCAO (Mouse) OGD/R (BV2 cells)	13.3 mg/kg, i.p. Single dose Post-ischemia	GSDMD-N NLRP3 IL-1 β	STING NLRP3	Inhibits STING-NLRP3 interaction and reduces microglial pyroptosis	(132)
Liang <i>et al</i> , 2020	Vx765	pMCAO (Rat)	50 mg/kg, i.p. qd x 3 days Post-ischemia	GSDMD-N NLRP3 IL-1 β	caspase-1	Inhibits caspase-1, reducing pyroptosis	(134)
Kim <i>et al</i> , 2020	Ac-YVAD-CMK	tMCAO (Mouse)	5 mg/kg, i.p. qd x 7 days Pre-ischemia	GSDMD-N caspase-1 IL-1 β	caspase-1	Inhibits caspase-1, reducing pyroptosis	(135)
Jin <i>et al</i> , 2024	miR-96-5p	tMCAO (Mouse) OGD/R (N2a cells)	5 nmol, i.c.v. Single dose Pre-ischemia	GSDMD-N caspase-1	caspase-1	Inhibits caspase-1, reducing pyroptosis	(136)
Hu <i>et al</i> , 2023	Disulfiram	tMCAO (Mouse)	50 mg/kg, i.p. qd x 5 days Post-ischemia	GSDMD-N caspase-1 IL-1 β	GSDMD	Inhibits GSDMD, reducing pyroptosis	(91)
Wang <i>et al</i> , 2020	GSDMD-KO	tMCAO (Mouse) OGD/R (BV2 cells)	Genetic ablation N/A Pre-ischemia	GSDMD-N caspase-1 IL-1 β	GSDMD	Blocks classical and non-classical inflammasome-dependent pyroptosis in microglia	(138)

Evidence levels for pyroptosis are categorized into: i) Execution level (GSDMD-N, the gold standard); ii) pathway level (NLRP3/caspase-1 activation); and iii) cytokine level (IL-1 β /18 release). CA/CPR, cardiac arrest/cardiopulmonary resuscitation; tMCAO, transient middle cerebral artery occlusion; pMCAO, permanent middle cerebral artery occlusion; dMCAO, distal middle cerebral artery occlusion; OGD/R, oxygen-glucose deprivation/reoxygenation; i.p., intraperitoneal injection; i.v., intravenous injection; i.c.v., intracerebroventricular injection; i.g., intragastric gavage; qd, once daily; GSDMD-N, N-terminal fragment of gasdermin D; ROSC, return of spontaneous circulation; miR, micro RNA; OIP5-AS1, OIP5 Antisense RNA 1; HS, hydrogen-rich saline; MM/ANPs, Apelein-13-loaded macrophage membrane-encapsulated nanoparticles; si-MEG3, small interfering RNA targeting MEG3.

Table II. Emerging biological regulators and delivery platforms for pyroptosis modulation: Lessons from focal and global ischemia models.

First author, year	Intervention	Model (Species)	Dose, route and timing	Evidence level	Target	Main findings	(Refs.)
Luo <i>et al.</i> , 2022	iTBS	tMCAO (Mouse)	50 Hz bursts (iTBS) twice daily x 7 days Post-ischemia	GSDMD NLRP3 IL-1 β	TLR4 NF- κ B	Regulates microglial M1/M2 phenotype	(140)
Meng <i>et al.</i> , 2025	ETV5	tMCAO (Rat) OGD/R (Microglia)	1.5x10 ⁶ TU, i.c.v. Single dose Pre-ischemia	GSDMD-N caspase-1	HACE1 Nrf2	Activates HACE1/Nrf2 axis, inhibiting microglial M1 polarization	(141)
Ma <i>et al.</i> , 2021	SAFI	tMCAO (Rat) OGD/R (Neurons/ microglia)	10 mg/kg, i.p. qd (2-6 days) Post-ischemia	GSDMD NLRP3 IL-1 β	NLRP3	Regulates microglial M1/M2 phenotype, inhibiting NLRP3 inflammasome	(142)
Zhang <i>et al.</i> , 2025	Dexmedetomidine	tMCAO (Rat) OGD (Neurons)	9 μ g/kg, i.v. Single dose (30 min) Post-ischemia	GSDMD-N NLRP3 IL-1 β	Mitophagy NLRP3	Promotes mitophagy, inhibiting pyroptosis	(144)
Qiu <i>et al.</i> , 2025	EA + TG	tMCAO (Rat)	TG: 50 mg/kg, i.g., 14 days EA: 2/15 Hz, 1 mA, 5 days Pre-ischemia	GSDMD-N NLRP3 IL-1 β	PI3K	Activates PI3K/AKT/mTOR pathway, inhibiting autophagy and reducing pyroptosis	(145)
Liu <i>et al.</i> , 2025	miR-135a-5p	tMCAO (Mouse) OGD/R (N2a cells)	800 pmol, i.c.v. Single dose Pre-ischemia	GSDMD-N NLRP3 IL-1 β	DDX3X NLRP3	Inhibits DDX3X/NLRP3 pathway	(147)
Zhao <i>et al.</i> , 2025	CircFndc3b	tMCAO (Mouse)	2 μ l (AAV), i.c.v. Single dose Pre-ischemia (4 weeks)	GSDMD-N NLRP3 IL-1 β	KLF2 NLRP3	Inhibits NLRP3 inflammasome via KLF2	(148)
Wang <i>et al.</i> , 2021	si-Fendrr	tMCAO (Diabetic mouse) HG-H/R (BV-2 cells)	2 μ l (AAV), i.c.v. Single dose Pre-ischemia (3 weeks)	NLRC4 caspase-1 IL-1 β	HERC2 NLRC4	Knockdown of Fendrr inhibits NLRC4-mediated pyroptosis	(149)
Sun <i>et al.</i> , 2024	A-Exos (miR-378a-5p)	tMCAO (Rat) OGD (Neurons)	30 μ g/ml (<i>in vitro</i>) 100 μ g (<i>in vivo</i>), i.v. Post-ischemia	GSDMD-N NLRP3 caspase-1	NLRP3	Inhibits NLRP3 inflammasome, reducing pyroptosis	(151)
Liu <i>et al.</i> , 2021	BMSC-Exos	tMCAO (Rat) OGD/R (BV2/PC12 cells)	100 μ g/rat, i.v. Single dose Post-ischemia	GSDMD NLRP3 IL-1 β	NLRP3	Regulates microglial M1/M2 phenotype, inhibiting NLRP3 inflammasome	(152)
Prakash <i>et al.</i> , 2023	M-PMPL@Tf (MCC950)	tMCAO (Wistar rat) OGD (SH-SY5Y cells)	2 mg/kg, i.a. (via ECA) Single dose Post-ischemia	NLRP3 caspase-1 IL-1 β	NLRP3	Targeted delivery of MCC950 inhibits NLRP3 inflammasome activation	(153)

Table II. Continued.

First author, year	Intervention	Model (Species)	Dose, route and timing	Evidence level	Target	Main findings	(Refs.)
Cheng <i>et al.</i> , 2025	MDN-MC	tMCAO (Rat) OGD/R (BV2 cells)	4 mg/kg, i.v. Single dose Post-ischemia	GSDMD-N NLRP3 IL-1 β	ROS NLRP3	Clears ROS, inhibiting NLRP3 inflammasome	(154)

Evidence levels for pyroptosis are categorized into: i) Execution level (GSDMD-N, the gold standard); ii) pathway level (NLRP3/NLRC4/caspase-1 activation); and iii) cytokine level (IL-1 β /18 release). tMCAO, transient middle cerebral artery occlusion; OGD/R, oxygen-glucose deprivation/reoxygenation; HG-H/R, high glucose with hypoxia/reoxygenation; i.p., intraperitoneal injection; i.v., intravenous injection; i.c.v., intracerebroventricular injection; i.g., intragastric gavage; i.a., intra-arterial injection; ECA, external carotid artery; qd, once daily; GSDMD-N, N-terminal fragment of gasdermin D; AAV, adeno-associated virus; iTBS, intermittent θ -burst stimulation; ETV5, ETS variant transcription factor 5; TU, transducing units; SAFI, salvianolic acids for injection; EA, electroacupuncture; TG, tigustilide; miR, microRNA; si-Fendrr, small interfering RNA targeting Fendrr; A-Exos, astrocyte-derived exosomes; BMSC-Exos, bone marrow mesenchymal stem cell-derived exosomes; ROS, reactive oxygen species.

Non-coding RNAs (ncRNAs) are established as marked regulators of pyroptosis (146). For example, targeting the DEAD-box RNA helicase 3X/NLRP3 pathway via a single pre-ischemic dose of miR-135a-5p inhibits pyroptosis in a mouse tMCAO model (147). In a mouse tMCAO model, a single dose of circular RNA Fndc3b administered 4 weeks pre-ischemia suppresses microglial/macrophage pyroptosis via the enolase 1/Kruppel-like factor 2 axis, thereby promoting neurological recovery (148). Similarly, in a diabetic mouse tMCAO model, knockdown of the long ncRNA Fendrr via adeno-associated virus administration 3 weeks pre-ischemia reduces microglial pyroptosis by inhibiting NLRC4 ubiquitination, thereby protecting against cerebral IR injury (149). These findings highlight ncRNAs as potential therapeutic modulators of pyroptosis and neuroprotection.

Advanced delivery platforms and nanotechnology. As advanced drug delivery platforms, exosomes offer promising strategies for the treatment of cerebral IR injury (150). Post-ischemic treatment with astrocyte-derived exosomal miR-378a-5p suppresses NLRP3 activation and alleviates neuronal pyroptosis in a rat tMCAO model (151). Furthermore, a single post-ischemic dose of bone marrow mesenchymal stem cell-derived exosomes in a rat tMCAO model attenuate brain injury by modulating microglial M1/M2 polarization and suppressing NLRP3 inflammasome activation (152). Notably, transferrin receptor-targeted nanomicelles delivering MCC950, administered as a single post-ischemic dose in a Wistar rat model of tMCAO, effectively inhibit NLRP3 activation and ameliorate cerebral IR injury (153). A cascade-type microglial pyroptosis inhibitor was developed to integrate nanotechnology with antioxidative activity; a single post-ischemic administration of this system in a rat tMCAO model regulates the ROS/NLRP3/pyroptosis axis to mitigate neuroinflammation (154). Table II summarizes these emerging biological regulators and delivery platforms, including iTBS, transcription factors, ncRNAs and exosome/nanotechnology-based systems, across various ischemia models (140-142,144,145,147-149,151-154). These studies underscore the therapeutic potential of exosome- and nanotechnology-based interventions in cerebral IR injury.

The success of clinical translation hinges on BBB permeability, optimal administration routes and precise dosing. Small-molecule inhibitors, characterized by their inherent lipophilicity, possess a distinct advantage in the acute CA/ROSC setting due to rapid BBB penetration (155). While nanotechnology and exosome-based platforms offer superior targeting specificity, they must navigate challenges regarding off-target toxicity and potential immunosuppressive risks (156). Biologics, conversely, are often constrained by their large molecular weight, making BBB crossing a notable hurdle (157). Given the narrow therapeutic window in CA/ROSC, optimizing delivery protocols during the hyper-acute phase is imperative to enhance overall pharmacological feasibility.

8. Conclusion and prospects

CA remains a notable clinical challenge in emergency medicine. In this context, cerebral IR injury is a primary driver of high mortality and adverse neurological outcomes (158).

Pyroptosis is central to the pathological progression of cerebral IR injury. Pyroptosis drives neuronal damage by amplifying pro-inflammatory signals and coordinating intercellular interactions. It also orchestrates complex multi-pathway cross-regulation. This pathway integrates various forms of programmed cell death; furthermore, it exhibits cell type-specific roles across different populations in the brain. Targeted interventions against pyroptosis show marked therapeutic potential. These strategies provide novel insights for mitigating cerebral IR injury and enhancing clinical recovery.

Despite these mechanistic advances, the inherent constraints of current preclinical models require careful consideration. The vast majority of the current understanding is derived from rodent studies and *in vitro* cultures, which often struggle to replicate the sophisticated physiological milieu and the complex neurovascular unit of the human brain. Such interspecies discrepancies represent a formidable barrier to clinical translation, likely explaining why numerous neuroprotective candidates with robust preclinical profiles fail to yield therapeutic benefits in survivors of CA/ROSC.

Looking ahead, narrowing the current knowledge gaps in cerebral IR injury requires a shift toward more human-centric research paradigms. The integration of organoids derived from human induced pluripotent stem cells with microfluidic BBB models represents a notable step forward; these high-fidelity systems help circumvent the inherent limitations of animal studies by more accurately reflecting the human neural micro-environment. Parallel to these structural models, the fusion of multiomics and artificial intelligence provides a sophisticated toolkit for mapping the intricate signaling nodes that govern pyroptotic networks, thereby streamlining the identification of novel therapeutic targets (159). Furthermore, evaluating the clinical utility of circulating biomarkers, specifically cleaved GSDMD and IL-18, is essential for establishing reliable diagnostic and prognostic frameworks. Ultimately, the convergence of these advanced biotechnologies is expected to expedite high-throughput drug screening and accelerate the clinical transition of neuroprotective strategies for survivors of cardiac arrest (160).

Acknowledgements

Not applicable.

Funding

The present research was funded by the Henan Provincial Health Commission Provincial-Ministerial Co-Construction Project (grant no. SBJ202102198).

Availability of data and materials

Not applicable.

Authors' contributions

ZLi was responsible for the conceptualization of the present study. ZLi and QM wrote the original draft of the manuscript and created the figures. ZLi made the tables. ZLu acquired the funding. ZLu, TY, ML, LF and JY wrote and edited the review

and, All authors have 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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