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Review

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Review

Regulated necrosis pathways: a potential target for ischemic stroke

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Abstract

Globally, ischemic stroke causes millions of deaths per year. The outcomes of ischemic stroke are largely determined by the amount of ischemia-related and reperfusion-related neuronal death in the infarct region. In the infarct region, cell injuries follow either the regulated pathway involving precise signaling cascades, such as apoptosis and autophagy, or the nonregulated pathway, which is uncontrolled by any molecularly defined effector mechanisms such as necrosis. However, numerous studies have recently found that a certain type of necrosis can be regulated and potentially modified by drugs and is nonapoptotic; this type of necrosis is referred to as regulated necrosis. Depending on the signaling pathway, various elements of regulated necrosis contribute to the development of ischemic stroke, such as necroptosis, pyroptosis, ferroptosis, pathanatos, mitochondrial permeability transition pore-mediated necrosis and oncosis. In this review, we aim to summarize the underlying molecular mechanisms of regulated necrosis in ischemic stroke and explore the crosstalk and interplay among the diverse types of regulated necrosis. We believe that targeting these regulated necrosis pathways both pharmacologically and genetically in ischemia-induced neuronal death and protection could be an efficient strategy to increase neuronal survival and regeneration in ischemic stroke.

Key words: Ischemic stroke, Necroptosis, Pyroptosis, Ferroptosis, Pathanatos, mPTP-mediated necrosis, Oncosis

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Highlights

- This review aims to provide a systematic view of the role of regulated necrosis in neurons, glia cells, platelets and brain microvascular cells in response to ischemia stress.
- We also review recent advances in the underlying molecular mechanisms of regulated necrosis in the pathological process during cerebral ischemic preconditioning, perconditioning and postconditioning.
- In addition, we explore the crosstalk and interplay among the diverse types of regulated necrosis that contribute to ischemic stroke.

Background

Ischemic stroke accounts for 87% of all strokes and is one of the most significant causes of neurological morbidity and mortality globally. This disease causes death and permanent disability in ~10 million people annually, and the risk of ischemic stroke continues to increase in low- and middle-income countries along with the accompanying rising economic and social burden for communities [1]. Ischemic stroke occurs when embolic or thrombotic events interrupt blood circulation to the brain. Although the primary treatment strategies for ischemic stroke are local recanalization and systemic thrombolysis to restore the blood supply as quickly as possible, these therapeutic options have a short therapeutic window within the initial 4.5 h, and only 20% of patients are eligible for these treatments [2]. To make matters worse, the reperfusion of blood could further contribute to neuronal injuries. Generally, the outcome of ischemic stroke is largely determined by the amount of ischemia- and reperfusion-related neuronal death in the infarct region.

The central nervous system (CNS), a major participant in cerebral ischemia–reperfusion, consists of various types of cells, including platelets, endothelial cells, astrocytes, neutrophils, microglia, macrophages and neurons. Due to their structures and functions, CNS cells have extremely high energy demands and are very sensitive to drops in oxygen and glucose due to blood flow interruption. Meanwhile, the cells are susceptible to injuries that are induced by reperfusion [3, 4]. Hence, the loss of CNS cells is the main pathogenesis of ischemic stroke. In recent years, studies have focused on potential neuroprotectants and a more thorough understanding of the mechanisms involved in the ischemic death process. However, a certain number of putative neuroprotectants have been investigated in preclinical models, but none have entered the clinical realm [5]. In the past, three cell death modes were used based on the morphological classification of cell death: apoptosis unassociated with digestion, autophagic cell death associated with autophagy, and heterophagy-related necrosis [6]. However, over the past two decades, numerous studies have focused on regulated necrosis, a type of necrosis that can be tightly regulated and is potentially druggable but nonapoptotic. Based on its morphological features, regulated necrosis is classified into different forms. As reported, almost all forms of regulated necrosis contribute to the development of ischemic stroke, e.g. necroptosis, pyroptosis, ferroptosis, pathanatos, mitochondrial permeability transition pore (mPTP)-mediated necrosis and oncosis [7–11].

As various CNS cells form an extensive communication network, neuronal cell death is a complex process, and regulated necrosis is involved in the death and dysfunction of cells. Except for morphological differences, there are specific molecular pathways for different forms of regulated necrosis. This review summarizes the current studies on the influence of regulated necrosis on the pathological process of ischemia/reperfusion-regulated injuries. The findings of this review may shed light on novel therapeutic targets for ischemic stroke.

Review

Influence of necroptosis on ischemic stroke

An overview of necroptosis in ischemic stroke Necroptosis is the best-characterized form of regulated necrosis. It is mediated by a cascade of kinases: receptor-interacting protein kinase 1 (RIPK1), RIPK3 and mixed-lineage kinase domain-like pseudokinase (MLKL). Upon receiving a necroptosis-inducing stimulus, RIPK1 phosphorylates and activates RIPK3, and the activated RIPK3 phosphorylates and activates MLKL in return, which finally forms a complex termed the necrosome [12]. Necrosome formation initiates necroptosis through plasma membrane disruption and cell lysis [12] (Figure 1). Thus, necroptosis has features of either apoptosis or necrosis. The characteristics of necroptosis are dependent on the kinase domain of RIPK3 that mediates MLKL phosphorylation. This process depends on the oligomerization of active RIPK3 molecules, which is mediated by the RIP homotypic interacting motifs (RHIMs) in RIPK1 and RIPK3 [13].

Death receptors (DRs), such as TNF receptor 1 (TNFR1), TNFR2, FAS (also known as CD95), TNF-related apoptosis-inducing ligand receptor 1 (TRAILR1) and TRAILR2, are well-studied initiators of necroptosis through downstream involvement of RIPK1, RIPK3 and MLKL [14–18]. The intracellular domains of TNFR1, FAS, TRAILR1 and TRAILR2 all include a death domain (DD). With the stimulation of their respective cognate ligands and the homotypic interactions between their DDs, they combine and form membrane-associated complex I with RIPK1, which contains a C-terminal death domain, an intermediate domain and an N-terminal kinase domain [16]. In complex I, RIPK1 is activated by multiple post-translational

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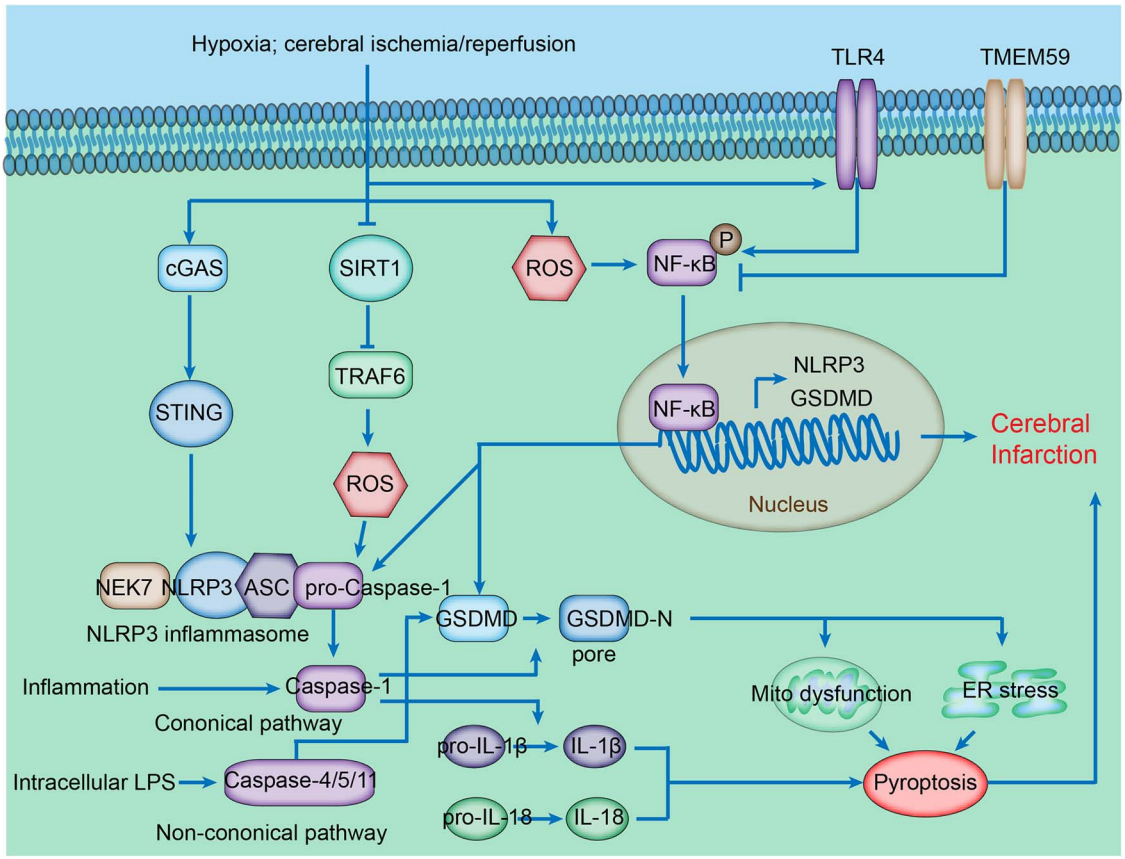

Figure 1. Necroptosis-related signaling pathway in ischemic stroke. Following cerebral ischemia, death ligands bind to death receptors and form complex I through recruiting TNFR1-associated death domain protein (TRADD), cellular inhibitor of apoptosis 1 and 2 (cIAP1/2), receptor-interacting protein kinase1 (RIPK1) and several E3 ubiquitin ligases. The polyubiquitinated RIPK1 in complex I can be deubiquitinated into RIPK1, on the one hand, forming pro-apoptotic complex IIa with TRADD, Fas-associating protein with a novel death domain (FADD) and caspase 8, and on the other hand, forming complex IIb through autophosphorylating itself to recruit and phosphorylate receptor-interacting protein kinase 3 (RIPK3), which subsequently recruits and phosphorylates mixed-lineage kinase domain-like pseudokinase (MLKL). Activated MLKL disrupts the cell membrane and cell lysis of cells and therefore executes necroptosis. Cerebral ischemia can stimulate necroptosis by activating ASIC1a/RIPK1 and TNFR1/RIPK3 signaling pathways. *TNFR* tumor necrosis factor receptor

modifications, including ubiquitylation, deubiquitylation and phosphorylation. Furthermore, active RIPK1 plays an important role in apoptosis and necroptosis. The formation of a RIPK1–TNFR1-associated DD protein–FAS-associated DD protein–caspase 8 complex (complex IIa) leads to RIPK1-dependent apoptosis [19]. Then, activated RIPK1 interacts with and phosphorylates RIPK3, leading to the activation of RIPK3, which subsequently recruits and phosphorylates MLKL. Through forming a RIPK1–RIPK3–MLKL complex (complex IIb), MLKL phosphorylation disrupts the cell membrane, leads to cell lysis and executes necroptosis [20, 21]. Furthermore, due to the lack of cytoplasmic DD in TNFR2, TNFR2 triggers necroptosis by enhancing the cell's sensitivity to endogenous TNF-induced TNFR1 pathways by depletion and/or degradation of TNF receptor associated factor 2 (TRAF2) [22]. Interestingly, in the absence of RIPK1, RIPK3 can be activated by TNF via TNFR1-associated death domain protein (TRADD) [23] and TRAF2. Activated RIPK3 then induces necroptosis through a caspase- and Bax/Bak-independent mechanism [24] (Figure 1).

Both initiators and mediators are widely expressed in various CNS cells, including neurons, microglia and platelets. Several studies have demonstrated that RIPK1, RIPK3 and MLKL participate in the pathogenesis of ischemic stroke after the activation of DRs. For example, mice with various RIPK1 kinase-dead knock-in mutations, RIPK3-knockout mice and MLKL-knockout mice showed normal development and adult life, indicating that necroptosis does not participate in the normal development of cells. Several mutant mice showed certain neuroprotective effects in diverse brain injuries.

RIPK1 inhibitors exert protective effects in ischemic stroke, further revealing the contribution of necroptosis to ischemic stroke [8, 15]. Compared with wild-type mice, $RIPK1^{K45A/K45A}$ and $RIPK1^{D138N/D138N}$ mice demonstrated less neuronal cell death and neurological dysfunction 24 h after intracerebral hemorrhage [25]. Treatment with RIPK1-specific inhibitors such as necrostatin-1 (Nec-1), GSK963 and 5-(3′,5 dimethoxybenzal)-2-thio-imidazole-4-ketone (DTIO) also reduced infarct volume and neurological deficit scores and significantly decreased ischemia-induced neuroinflammation and generation of reactive oxygen species (ROS) [15,25–27]. RIPK3 deficiency attenuated the cognitive dysfunction, inflammation and oxidative stress induced by traumatic brain injury [28]. Based on these results,

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necroptosis can drastically alter neuronal homeostasis, and targeting the RIPK1/RIPK3/MLKL pathway could be a viable treatment strategy against brain injuries.

Since necroptosis was defined in 2005, numerous studies have been dedicated to investigating the activation mechanism and regulation of necrosomes for their extensive expression in almost all cell types after stress stimuli and their close correlation to a series of cell loss-related diseases [15]. By intervening in the RIPK1/RIPK3/MLKL pathway, previous studies have demonstrated that necroptosis contributes to ischemic stroke. The protein levels of RIPK1 MLKL and pMLKL are significantly increased in stroke tissues of both rats [8] and human beings [29]. In the middle cerebral artery occlusion (MCAO) model *in vivo* and the oxygen–glucose deprivation/re-oxygenation (OGD/R) cellular model *in vitro*, blocking the RIPK1/RIPK3/MLKL pathway could effectively alleviate ischemic stroke injuries [15, 30].

Next, several studies have revealed that multiple post-translational modification molecules, such as cylindromatosis (CYLD), chromatin immunoprecipitation (CHIP), Triad3A and ribosomal S6 kinase 3 (RSK3), have been implicated in stroke by mediating the activation of RIPK1 and RKPK3 [31–34]. In this paper, we focus on findings about necroptosis in different cell types in ischemic stroke, including neurons, astrocytes, platelets, microglial cells and endothelial cells.

Necroptosis in neurons and microglia following ischemic stroke Due to high energy demands and the inability to perform mitosis, neurons are sensitive to interruptions in blood flow and are the first brain cells to die in ischemic stroke [35]. Neuronal death and loss of interaction between neurons and microglia further induce neuroinflammation [36] and intensify ischemic stroke damage [37]. In previous studies, necroptosis was found to contribute to ischemic stroke by intervening in the RIPK1/RIPK3/MLKL signaling pathway in neurons and microglia. In the MCAO rat model, cerebral ischemia stimulated RIPK1 phosphorylation at the Ser166 residue, RIPK3 phosphorylation at the Ser232 residue and MLKL phosphorylation at the Ser345 residue and significantly increased RIPK1, RIPK3 and MLKL levels, while Nec-1 attenuated these changes by inhibiting phosphorylated RIPK1 kinase [8]. Similarly, a murine endothelin-1 model of ischemia–reperfusion injury exhibited an increase in necroptosis. Furthermore, with Nec-1 treatment, RIPK1 inhibition reduces the activation of both caspase-3 and caspase-7, revealing a new form of interaction between apoptosis and necroptosis [38]. RIPK1 is activated upstream of RIPK3 after ischemic insult, and RIPK3 deficiency reduces long-term ischemic infarcts [39]. Since the expression of TNFR1 and TNFR2 is quite low in normal neurons [40], certain studies have focused on the various activators of the RIPK1/RIPK3/MLKL pathway in addition to TNFα signaling.

Upon TNFα stimulation, RIPK1 is recruited into complex I together with several adaptor proteins, such as TRADD, TRAF2 and cellular inhibitor of apoptosis 1 and 2 (cIAP1/2). TRAF2 is one of the family members of TRAF. It typically shares a common TRAF domain and can bind to TRADD, the adaptor protein. Although TRAF2 has a RING E3 ubiquitin ligase domain, multiple reports have confirmed that TRAF2 participates in the K48- and K63-linked polyubiquitination of RIPKI by binding to cIAP1 and cIAP2 [41, 42] and acts as a necroptosis suppressor through different mechanisms [43]. Ischemic stroke has been shown to induce the expression of TRAF2 in neurons and microglia. Through the formation of a complex with MLKL, TRAF2 protects microglia and neurons against necroptosis, and the knockdown of TRAF2 amplifies infarct volume and increases cell death via the necroptosis mechanism [44]. Triad3A is also involved in ischemic insult. The E3-ubiquitin ligase Triad3A induces the early degradation of necrosomes by promoting the K48-ubiquitin-dependent proteasomal degradation of RIPK1 [45]. Therefore, in the mouse MCAO model, Triad3A was markedly induced, especially in neurons and microglia. Additionally, Triad3A knockdown accelerated cell death, increased microglial activity, enhanced the infarction area and promoted the expression of pro-inflammatory markers, including TNF-α, interleukin (IL)-1β and inducible nitric oxide synthase (iNOS) [33]. Remarkably, transforming growth factor β-activated kinase 1 (TAK1, also called MAP3K7), which is a member of the mitogen-activated protein kinase (MAPK) family, plays an important role in the phosphorylation of RIPK1 at multiple sites [46]. Several studies have proven that TAK1 is rapidly activated in ischemic stroke, and inhibition of TAK1 both via conditional knockout and via treatment with 5Z-7-oxozeaenol can reduce infarct volume and cell death and improve neurological outcomes [47, 48]. Naito *et al*.'s study showed that the conditional knockout of TAK1 in microglia/infiltrated macrophages and neuronal lineages aggravated neuronal death and neuroinflammation after ischemic insult through the transition from early necroptosis to apoptosis [39].

These contradictory results may be related to the inhibition term length of TAK1. Neubert *et al*. demonstrated that the short-term and brief inhibition of TAK1 exerted a neuroprotective effect by interfering with the activation of p38/MAPK and JNK in experimental stroke models. Conversely, prolonged inhibition of TAK1 induced the upregulation of apoptosis signal-regulating kinase-1 (ASK-1), which can offset TAK1 inhibition [49].

In addition to the TNF-associated signaling molecules mentioned above, acidotoxicity, common in ischemic stroke, can induce neuronal necroptosis via acid-sensing ion channel 1a (ASIC1a) [50]. In an experimental stroke model, independent of its ion-conducting function, ASIC1a was significantly induced and participated in the phosphorylation of RIPK1 by recruiting RIPK1 to the ASIC1a C-terminus (CT) and mediated RIPK1 activation. In addition, the N-terminus (NT) of ASIC1a auto-inhibits the CT to prevent RIPK1 recruitment/activation under resting conditions. As a consequence, both expression of mutant ASC1a and

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treatment with N-ethylmaleimide-sensitive fusion ATPase (NSF), which associates with ASIC1a-NT under acidosis, could cause neuronal death, whereas in the presence of a membrane-penetrating synthetic peptide representing the distal 20 ASIC1a NT residues, neuronal damage was reduced in experimental stroke [51]. These results reveal novel strategies for preventing neuronal death and neuroinflammation by targeting the necroptosis pathway.

Necroptosis in astrocytes following ischemic stroke The prominent pathological features of ischemic stroke include reactive astrogliosis and glial scar formation [52]. A glial scar mainly consists of astrocytes, the most abundant cell type in the CNS. The scar is characterized by cellular hypertrophy, proliferation and increased expression of the intermediate filament protein [53].

Increasing evidence attests that depending on brain injury time, reactive astrocytes perform both beneficial and/or detrimental functions. In acute ischemic stroke, reactive astrocytes not only secrete anti-inflammatory factors and take up excess glutamate but also release various toxic molecules, including ROS, cytokines and Vascular endothelial growth factor (VEGF) [54, 55]. In the chronic phase after ischemic stroke, reactive astrocytes express a broad range of inhibitory molecules against axonal regeneration, such as chondroitin sulfate proteoglycans (CSPGs), which inhibit axonal regeneration [52]. In an ischemic stroke study performed by Zhu *et al*., activation of the RIPK1/RIPK3/MLKL pathway contributed to astrogliosis and glial scar formation by enhancing VEGF-D/vascular endothelial growth factor receptor (VEGFR)-3 signaling, and Nec-1 attenuated brain damage by inhibiting the formation of astrogliosis and glial scar via suppression of the RIPK1 and VEGF-D/VEGFR-3 signaling pathways [56]. Similar results were obtained in experimental stroke models; both Nec-1 and SB216763 (a glycogen synthase kinase-3 β inhibitor) attenuated ischemia-induced reactive astrogliosis by reducing the interaction between RIPK1 and glycogen synthase kinase 3β (GSK3β) and inhibiting inflammatory cytokines [57]. The above-mentioned studies support the contribution of necroptosis to ischemic stroke-induced reactive astrogliosis and glial scar formation.

Necroptosis in platelets following ischemic stroke Platelets, which are produced from megakaryocytes, are essential for thrombus formation. As a result, platelets are the primary target of classic anti-platelet agents in ischemic stroke [58]. RIPK3 is known to be a novel modulator of platelet activation through its interaction with Galpha 13 (Gα13) in platelets; thus, RIPK3 deficiency leads to the inhibition of platelet aggregation and thrombus formation [59].

Emerging evidence highlights that platelets contain membrane receptors that detect pathogen- and danger-associated molecular patterns (PAMPs and DAMPs, respectively). Furthermore, platelets mediate heterotypic leukocyte interactions and recruitment, which promote brain injury following ischemic stroke [60]. Recently, necroptosis signaling proteins were suggested to play a role in venous thrombosis based on the effects of RIPK1 and MLKL in modulating neutrophil–platelet aggregation [61]. Reduced neutrophil death and neutrophil–platelet aggregation were evident with Nec-1 and genetic deletion of MLKL; these effects were linked to reduced neutrophil necroptosis induced by activated platelets but not platelet activation itself. In addition, TNFR1 expression in platelets is rather low [62], and both the role and the execution pathway of platelet necroptosis in ischemic stroke still demand more research.

Necroptosis in endothelial cells following ischemic stroke Disruption of the blood–brain barrier (BBB) after ischemic stroke contributes to further development and enlargement of brain injury [63]. Endothelial cells are an important component of both the BBB and the neurovascular unit, and the death of endothelial cells contributes to vascular injury and BBB disruption in ischemic stroke [64]. The role of necroptosis signaling proteins in BBB disruption was demonstrated based on the effects of RIPK1 and MLKL, which were induced by microglia-secreted TNF-α in regulating endothelial cell necroptosis [65]. Decreases in BBB leakage and brain injuries were evident with Nec-1 and anti-TNFα (infliximab) and linked to reduced endothelial necroptosis and microglia-secreted TNF-α. Since endothelial necroptosis is rapidly activated after a transient ischemic insult, more studies are needed to explore the role of endothelial necroptosis in ischemic stroke.

Influence of pyroptosis on ischemic stroke

An overview of pyroptosis in ischemic stroke Pyroptosis, gasdermin-mediated programmed necrosis, is characterized by rapid plasma membrane rupture and extracellular release of inflammatory cytokines. Traditionally defined through its binding with nitric oxide dioxygenase (NOD)-like receptor family pyrin domain-containing 3 (NLRP3) and apoptosis-associated speck-like protein containing a CARD (ASC), caspase-1 cleaves and activates gasdermin-D (GSDMD) and evokes GSDMD-executed pyroptosis cell death [66]. Its lytic and inflammatory type of programmed cell death is characterized by the maturity and release of pro-inflammatory mediators, including IL-1β and IL-18. This progress relies on the activation of pro-inflammatory caspases such as the canonical pathway of caspase-1 and the noncanonical pathway involving caspase-4,5 (in humans), caspase-11 (in mice) and gasdermin protein cleavage (Figure 2). An intense inflammatory response occurs immediately after ischemic stroke. The DAMPs released by dead cells are recognized by pattern recognition receptors (PRRs) and lead to the formation of inflammasomes [67]. Four known inflammasome-forming proteins, namely, NOD-like receptor family pyrin domain-containing 1 (NLRP1), NLRP3, absent in melanoma 2 (AIM2) and NOD-like receptor family caspase recruitment domain protein (CARD) domain-containing 4 (NLRC4), have been confirmed as inflammasome sensors

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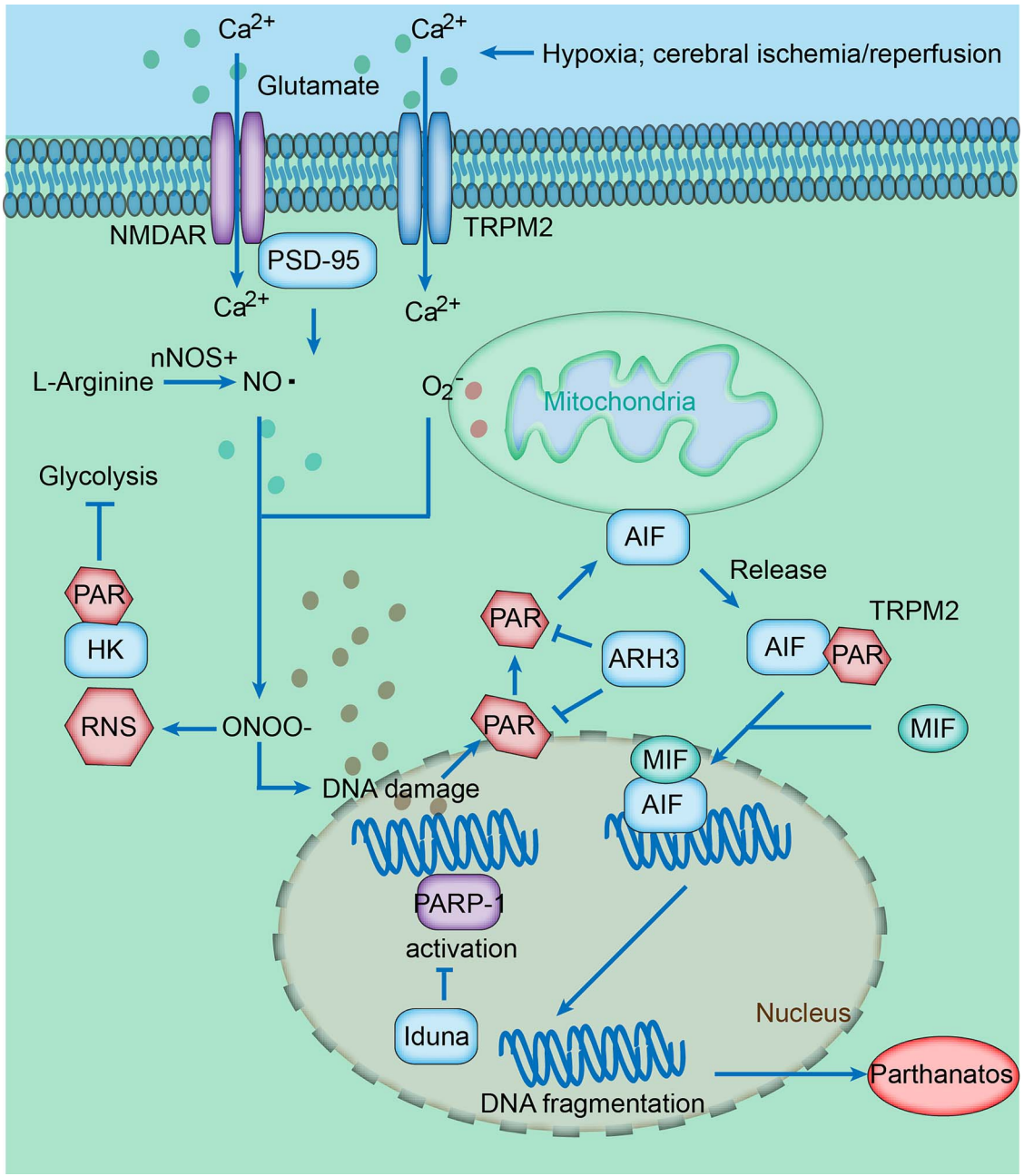

Figure 2. Pyroptosis signaling pathway in ischemic stroke. After ischemic stroke, NLRP3 inflammasome is activated through several mechanisms, including the activation of the cGAS/STING, ROS/NF-κB and TLR4/NF-κB pathways, and the inhibition of the SIRT1/TRAF6 and TMEM59/NF-κB pathways, and contributes to caspase-1 activation. Activated caspase-1 cleaves the hinge region between the active N- and inhibitory C-terminal domains of gasdermin-D (GSDMD) and converts pro-interleukin (IL)-1β and pro-IL-18 into their active forms. GSDMD-N translocates to the plasma membrane and organelle membranes and forms membrane pores, which could induce pyroptosis. In the non-canonical pathway, intracellular lipopolysaccharide causes activation of caspase-4/5/11, leading to GSDMD cleavage. *ROS* reactive oxygen species, *NF-κB* nuclear factor kappa-B

in ischemic stroke with highlighted upregulation [68]. In the canonical pathway of caspase-1-mediated pyroptosis, caspase-1 is recruited to inflammasome sensor proteins via the interaction of the CARD domain with ASC. Caspase-1 is also self-activated by proteolytic cleavage to its catalytically active species, the p10/p20 tetramer [69, 70]. On the one hand, activated caspase-1 recognizes and converts IL-1β and IL-18. In contrast, activated caspase-1 cleaves the hinge region between the N- and C-terminal domains of GSDMD. Then, it oligomerizes GSDMD-N, which contains a pore-forming domain to mediate the formation of membrane pores with a diameter of ~10–14 nm in the plasma membrane [71]. Apart from caspase-1, caspase-4 and caspase-5/11, activated by lipopolysaccharide, cleave GSDMD and initiate pyroptosis in the non-canonical pathway, but without producing IL-1β and IL-18 [72, 73]. Moreover, similar to GSDMD, GSDME

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(originally identified as deafness autosomal dominant 5, DFNA5) contains a pore-forming domain in the N-terminal which is specifically cleaved by caspase-3 to form a pyroptosis pore in the plasma membrane and switches the activation of caspase-3 from driving an apoptotic program to causing pyroptosis [74]. In addition, GSDME is a transcriptional target of p53, indicating its novel cellular function in the stress response [75].

In 2001, pyroptosis was established as pro-inflammatory programmed cell death in the immune, central nervous and cardiovascular systems [76]. As a result, researchers of pyroptosis began to focus on CNS diseases. Pyroptosis contributes to neuroinflammation and neurodegeneration and aggravates brain tissue damage in ischemic stroke by directly promoting the pyroptotic cell death of neurons, endothelial cells and glial cells [77]. Emerging evidence has shown that pyroptosis-related molecules, including NLRP1, NLRP3, NLRC4, AIM2 and caspase-1, are significantly elevated after cerebral ischemic injury [78–80]. In the MCAO rat model, GSDMD, a key executor of pyroptosis, immediately increased after ischemic insult and then peaked at 24–48 h post-ischemia [81]. Accumulated evidence has shown that the inhibition of pyroptosis via pharmacologic intervention or gene knock-down of pyroptosis-related molecules such as multiple inflammasomes, caspase-1/3/4/5/11 and gasdermin family proteins has neuroprotective effects against cerebral ischemic stroke injuries [82–85]. Additionally, a number of studies have revealed that multiple pathways, such as nucleic acid-sensing cyclic GMP-AMP synthase (cGAS), nuclear factor-kappaB (NF-κB) and triggering receptor expressed on myeloid cells-1 (TREM-1), are involved in stroke via the activation of inflammasomes (Figure 2).

Pyroptosis in microglia following ischemic stroke As the primary immune defense cells of the brain, microglia can differentiate into either a classical pro-inflammatory state (M1) or an alternative anti-inflammatory state (M2) in response to ischemic stroke insult [86]. As M1 phenotype microglia release pro-inflammatory cytokines, mounting evidence has verified that cerebral ischemic injury-induced neuroinflammation and pyroptosis can be attenuated by modulating microglial phenotypes.

TREM-1, a transmembrane immune receptor, is expressed in most innate immune cells. It is a potent amplifier of inflammation by playing an important role in microglial pyroptosis [87]. Xu *et al*. found that the activation of TREM-1 was accompanied by microglial M1 polarization following cerebral ischemic injury through interactions with spleen tyrosine kinase (SYK) and elevated levels of GSDMD and GSDMD-N [88]. Remarkably, pharmacologic inhibition of TREM-1 with the synthetic peptide LP17 abrogated microglial M1 polarization and the activation of pyroptosis-related molecules, improving stroke outcomes [88, 89]. Similarly, TMEM59 (also known as dendritic cell-derived factor 1), a type I transmembrane protein, plays a crucial role in neuroinflammation [90]. Liu *et al*. found that TMEM59 could interact with a triggering receptor expressed on myeloid cells 2 (TREM2). In turn, this interaction mediateed the degradation of TMEM59, and the knockdown of TMEM59 promoted microglial activities [91]. In Zhang *et al*.'s study, knockdown of TMEM59 dramatically potentiated microglial activation, pyroptosis and neurological injury after ischemic insult via activation of the NF-κB signaling pathway [92].

In addition, cGAS is a key cytosolic DNA sensor that is significantly upregulated along with microglial activation and pyroptosis by interacting with stimulators of interferon genes (STING) after experimental ischemic stroke, while A151 could effectively attenuate these changes by inhibiting the cGAS–STING pathway [93]. In addition to pharmacologic intervention or gene knockdown strategies, exosomes exhibit protective effects against cerebral ischemia-induced pyroptosis [94, 95]. Overall, these findings reflect the therapeutic potential of the microglial pyroptosis pathway in treating ischemic stroke.

Pyroptosis in neurons following ischemic stroke As mentioned above, the loss of neurons is the core problem in stroke; meanwhile, the death of neurons can lead to the death of other neurons connected to the dying neurons [96]. The evidence for the role of pyroptosis in stroke-induced neuronal death has been well defined in several studies. In MCAO mice, the nicotinamide adenine dinucleotide-dependent deacetylase Sirtuin 1 (Sirt1) reduced ischemic stroke-induced neuronal pyroptosis by inhibiting ROS and tumor necrosis factor receptor associated factor 6 (TRAF6) in hippocampal neurons [9], while valproic acid countered neuronal pyroptosis via apoptosis repressor with caspase recruitment domain (ARC)/caspase-1/GSDMD axis in cerebral ischemic injury [97]. In another study, low-density lipoprotein receptor (LDLR), which is important for cholesterol uptake, suppressed neuronal pyroptosis by inhibiting the NLRP3/ASC/caspase-1 pathway following ischemic stroke in mice. Moreover, administration of CY-09 (a inhibitor of NLRP3) substantially deferred neuronal pyroptosis in $Ldlr^{-/-}$ stroke mice [98]. In addition, the long non-coding RNAs maternally expressed gene 3 (MEG3) was upregulated in experimental stroke *in vivo* and *in vitro* by sponging miR-485 and abrogating the suppression of AIM2. Thus, knockdown of MEG3 could inhibit neuronal pyroptosis by repressing AIM2/ASC/caspase-1 signaling [99]. Furthermore, Zhao *et al*. found that knockdown of platelet-activating factor receptor (PAFR) via XQ-1H (a specific PAFR inhibitor) or small interfering RNA (siRNA) mitigated neuronal pyroptosis by suppressing the NLRP3/ASC/caspase-1 axis following cerebral ischemic stroke [100]. Therefore, targeting these regulatory pathways in neuronal pyroptosis may be a novel therapeutic strategy for treating ischemic stroke.

Pyroptosis in endothelial cells can further contribute to ischemia-induced BBB dysfunction. Liang *et al*. demonstrated that administration of vx-765 (a specific caspase-1 inhibitor) ameliorated the activation of ischemia-induced pyroptosis

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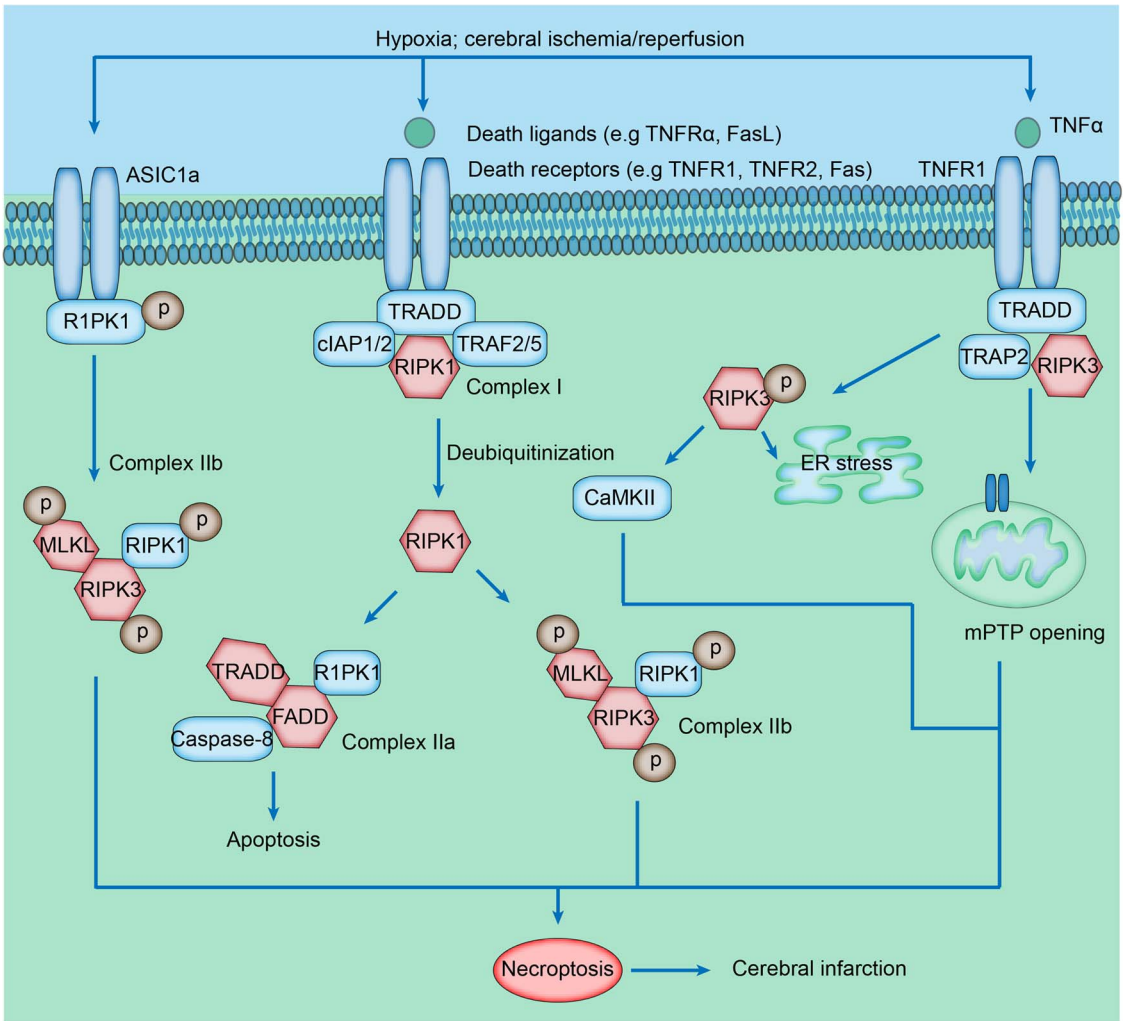

Figure 3. Ferroptosis-related signaling pathway during the progression of ischemic stroke. During the onset of cerebral ischemia ischemic stroke, impaired system x_c- causes suppression of cystine–glutamate exchange and deficiency of glutathione (GSH) and glutathione peroxidase 4 (GPX4), accumulating toxic lipid reactive oxygen species (ROS). Increased intracellular Fe^{3+} is transported to cytoplasm and reduced to Fe^{2+} with the synergy of the six-transmem brane epithelial antigen of prostate 3 (STEAP3). Abundant Fe^{2+} accelerates the generation of lipid ROS through Fenton reaction. In addition, polyunsaturated fatty acids consumption, with the cooperation of acyl-CoA synthetase long-chain family member 4 (ACSL4) and Fenton reaction, results in ferroptosis. Nuclear receptor coactivator 4 (NCOA4) and USP14/NCOA4 pathways also promote ferroptosis by inhibiting ferritinophagy

and restored the permeability of the BBB by inhibiting the receptor for advanced glycation end products/MAPK pathway [101]. Additionally, medioresinol attenuated the pyroptosis of brain microvascular endothelial cells in ischemic stroke as a novel PGC-1α activator by promoting the PPARα/GOT1 axis [102]. Moreover, pyroptosis of astrocytes and microglia is able to trigger the accumulation of amyloid-beta peptide (Aβ) and BBB dysfunction in ischemic stroke by inhibiting the polarization of aquaporin-4 (AQP-4) [103].

Influence of ferroptosis on ischemic stroke Ferroptosis is iron-regulated necrosis characterized by iron overload or lipid peroxidation accumulation. It is launched by four crucial signaling methods: (1) iron overload, (2) lipid peroxidation, (3) impaired system x_c^- and (4) depletion of glutathione (GSH) and inactivation of glutathione peroxidase 4 (Gpx4) [104, 105] (Figure 3). As the basic structure of cell membranes, polyunsaturated lipids are at high risk of lipid peroxidation, especially in the state of abundant Fe(II) and Fe(II)-dependent enzymes, and the membranes turn into a provider of damaging reactive lipid peroxides [106] (Figure 3). Therefore, ferroptosis is defined by the accumulation of iron-dependent lipid-ROS and the consumption of cell membrane polyunsaturated fatty acids (PUFAs) [107, 108].

GSH is necessary to prevent lipid-ROS accumulation by participating in reactions catalyzed by GPX4, GSH-dependent glutathione transferases (GSTs) and GPXs [109, 110]. Moreover, GSH synthesis requires consecutive import of cystine–cystine disulfide (Cys_2) by system x_c^-, which serves as a Cys_2/glutamate antiporter [111]. Hence, as mentioned before, system x_c^- inhibitors (glutamate, erastin and sorafenib), GPX4 inhibitors (RSL3 and FIN56) and GSH inhibitors (buthioninesulfoximine and acetaminophen) can all induce ferroptosis [107, 112, 113]. Interestingly, GSH

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depletion and/or GPX4 inhibition fail to induce ferroptosis without incorporating PUFAs into membrane phospholipids [114].

In the CNS, iron catalyzes the generation of hydroxyl radicals through the Fenton reaction and inducing oxidative stress [115]. As early as the 1990s, it was already known that iron overload accelerates neuronal damage both in clinical and experimental models of ischemic stroke, while iron depletion attenuates ischemia impairment [116, 117]. Tuo *et al*. first confirmed that ferroptosis inhibitors (liproxstatin-1 and ferrostatin-1) attenuated ischemia–reperfusion injury in the MCAO mouse model. Moreover, they found that tau depletion protected against MCAO-induced ferroptosis in young mice and that iron-targeting interventions contributed to restoring the protective effect of tau depletion in an MCAO model of aged mice, indicating that ferroptosis is involved in ischemia-induced neuronal death. The tau–iron interaction can be a multi-effect modulator of ferroptosis and ischemic stroke [118]. In a study performed by Cui *et al*. [119], acyl-CoA synthetase long-chain family member 4 (ACSL4) overexpression exacerbated neuronal ferroptosis and the release of pro-inflammatory cytokines in microglia by catalyzing the esterification of arachidonoyl and adrenoyl into phosphatidylethanolamine. Thus, interventions involving the ACSL4 pathway in defending against ferroptosis might provide a novel therapeutic strategy for ischemic stroke [119, 120].

Another study demonstrated that the downregulation of light-chain subunit solute carrier family 7 member 11 (SLC7A11), a multi-pass transmembrane protein that serves as the glutamate–cystine antiporter in system x_c^-, resulted in neuronal ferroptosis following OGD/R stimuli in primary mouse cortical neurons. Treatment with kaempferol could reverse OGD/R-induced ferroptosis by accelerating the expression of SLC7A11 [121].

In addition, p53 promotes ferroptosis by inhibiting the expression of SLC7A11. Lu *et al*.'s study indicated that miR-214 downregulation was correlated with neuronal ferroptosis in acute ischemic stroke patients by abrogating the suppression of p53, while long non-coding RNA PVT1 promoted ischemic injury by binding to miR-214 [122]. Li *et al*. demonstrated that the upregulation of nuclear receptor coactivator 4 (NCOA4) in the cytoplasm, a selective cargo receptor mediating ferritinophagy, promoted neuronal ferroptosis in an MCAO mouse model, and the knockdown of NCOA4 protected neurons from ferroptosis by blocking ferritinophagy. Moreover, USP14 was identified as the deubiquitinating enzyme (DUB) to cleave ubiquitin from NCOA4, and USP14 inhibition had neuroprotective effects by suppressing NCOA4-induced ferroptosis [123].

As mentioned above, liproxstatin-1 and ferrostatin-1 are the typically acknowledged inhibitors of ferroptosis and have been reported to alleviate cerebral ischemia/reperfusion injury in rats [118]. Multiple iron chelators, such as deferoxamine, deferasirox and dexrazoxane, have shown anti-ferroptotic effects in clinics and experimental stroke models [124–126]. Additionally, certain herbal compounds, such as carvacrol and kaempferol, protect neurons from cerebral ischemia-induced ferroptosis in experimental stroke models by increasing the expression of GPX4 [121, 127]. Electroacupuncture, a common treatment in the clinic to improve the neurological function and quality of life of ischemic stroke patients, can suppress neuronal ferroptosis by accelerating the expression of GPX4 and ferritin heavy chain 1 (FTH1) in MCAO rats [128]. Thus, ferroptosis inhibition could potentially improve ischemic stroke outcomes in the clinic.

Influence of parthanatos on ischemic stroke Parthanatos is a poly-(ADP-ribose) polymerase 1 (PARP1)-dependent necrotic cell death and is triggered by excessive DNA damage. PARP1 overactivation leads to the accumulation of poly-(ADP-ribose) (PAR) polymers in the nucleus and results in the depletion of NAD^+ and ATP. The depletion of NAD^+ and free PAR polymer can cause the release of apoptosis inducing factor (AIF) from mitochondria and nuclear translocation of the AIF/MIF complex and finally generate chromatin condensation and large scale DNA fragmentation (~50 kb) leading to cell death [129, 130] (Figure 4). Intervention in the form of PARP inhibitors and knockdown of PARP1 can effectively suppress AIF release and cell death [131]. Multiple mechanisms, including oxidative stress, nitrosative stress, inflammation, hypoxia, ischemia and DNA-alkylating agents, contribute to the pathological activation of PARP1. Recent studies suggest that parthanatos plays a crucial role in neuronal cell death in ischemic stroke.

In 1994, Zhang *et al*. revealed that activation of PARP by treatment with N-methyl-D-aspartic acid (NMDA) or high levels of NO contributed to cell death in rat cerebral cortical neurons, and suppression of PARP effectively defended against NMDA neurotoxicity [132]. Eliasson *et al*. reported that gene disruption of PARP could abrogate glutamate–NO-mediated neurotoxicity in both cortical neurons and an MCAO rat model, suggesting that inhibition of PARP may provide a novel therapeutic strategy in ischemic stroke [133]. Consistent with these findings, various studies have found that the accumulation of PAR and the expression of PARP1 are significantly upregulated in ischemic brain tissue [134, 135], and PARP1 inhibitors such as 3-amino benzamide, 3,4-dihydro-5-[4–1(1-piperidinyl) butoxy]-1(2H)-isoquinolinone, and thieno[2,3-c]isoquinolin-5-one provide significant neuroprotection in experimental models of ischemic stroke *in vivo* and *in vitro* [136–138]. JPI-289 (also called amelparib), a PARP1 inhibitor, has also shown effective neuroprotection in experimental models of ischemic stroke *in vivo* and *in vitro* [139]. Moreover, the safety, tolerability and pharmacokinetics of JP1–289 were evaluated in healthy Korean male volunteers through single ascending dose and multiple ascending dose studies [140].

By hydrolyzing PAR polymers to free ADP-ribose units by its endo- and exo-glycosidase activities, PAR glycohydrolase (PARG) acts as a crucial enzyme in the

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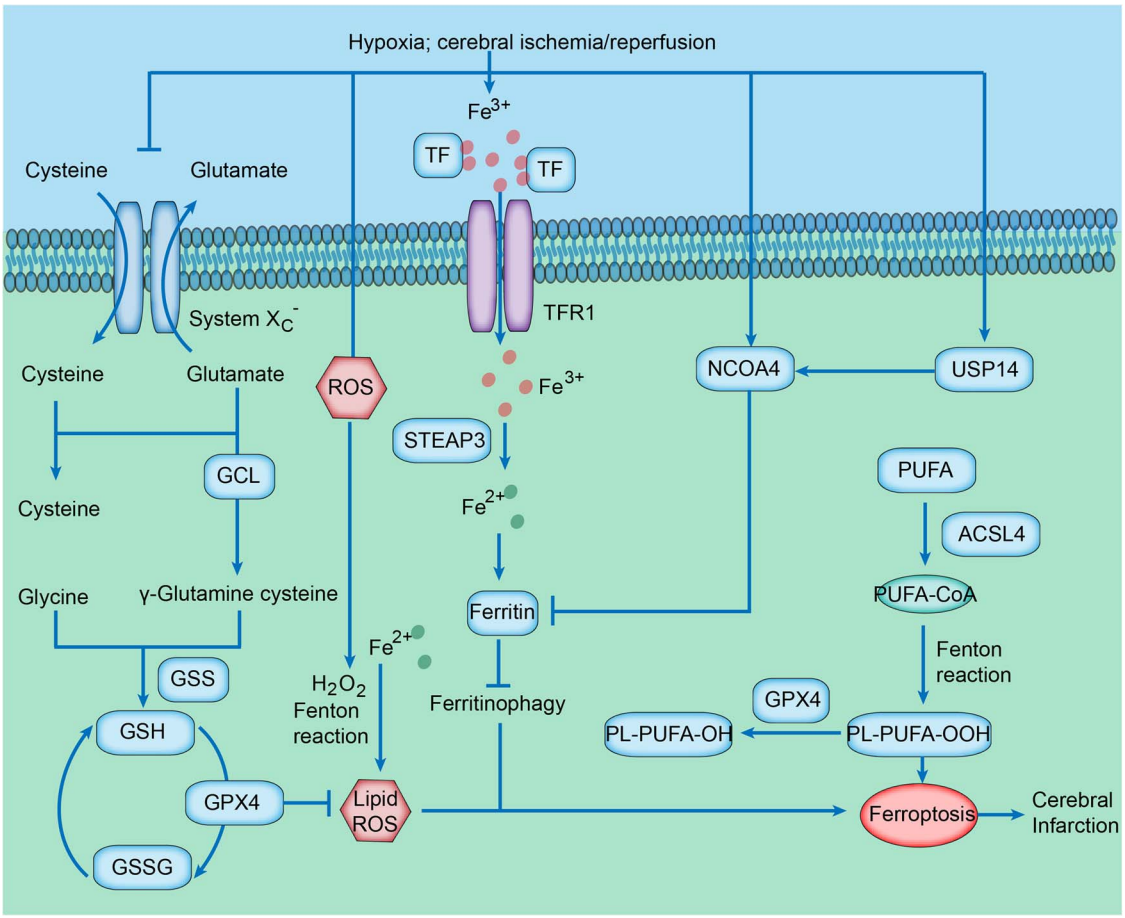

Figure 4. Parthanatos signaling pathway in ischemic stroke. During the progression of ischemic stroke, increased intracellular Ca^{2+} binds to calmodulin and causes abundant $ONOO^{-}$, stimulating DNA damage. Poly-(ADP-ribose) polymerase 1 (PARP1) is over-activated by DNA damage and generates poly-(ADP-ribose) (PAR). The translocation of PAR polymer from the nucleus to mitochondria causes the release of apoptosis inducing factor (AIF) from mitochondria and contributes to the formation of AIF/MIF complex. Subsequently, AIF/MIF complex translocates to the nucleus and generates DNA fragmentation, leading to parthanatos. In addition, PAR polymer can inhibit glycolysis via binding HK. Iduna and ADP-ribose-acceptor hydrolase 3 (ARH3) also inhibit parthanatos by mediating the ubiquitination of PARP1 and decreasing PAR accumulation in both cytoplasm and nucleus

reactivation of automodified PARP1 and plays an important role in hyper-poly(ADP-ribosyl)ation, contributing to neuronal death [141]. Therefore, PARG inhibition could lead to the accumulation of PARP1 auto-poly(ADP-ribosyl)ation and suppression of PARP1 hyperactivation. Multiple PARG inhibitors, such as gallotannin, nobotanin B and *N*-bis-(3-phenyl-propyl)9-oso-fluorene-2,7-diamide, have demonstrated remarkable neuroprotection against PARP1-dependent cell death in ischemic brain damage [141, 142]. However, although ADP-ribose-acceptor hydrolase 3 (ARH3) is crucial in PAR degradation, it contributes to cleaving ADP-ribosylated serine by hydrolyzing PAR and *O*-acetyl-ADP-ribose and decreasing PAR accumulation in the cytoplasm and nucleus [143] (Figure 4). Depletion of ARH3 leads to PAR accumulation in the cytoplasm and AIF translocation to the nucleus and increases sensitivity to parthanatos in cerebral ischemia/reperfusion injury [144, 145]. Moreover, intervention with PARP1 inhibitors assuaged ischemic brain damage through lowering parthanatos in ARH3-deficient mice [145].

Iduna is a PAR-dependent ubiquitin E3 ligase, and it mediates the ubiquitination and proteasomal degradation of PARP1 in turn [146] (Figure 4). Overexpression of Iduna alleviates cerebral ischemic injury by reducing PAR polymer and AIF accumulation [147]. Targeting the PAR/AIF pathway, Danhong injection, astragaloside IV and baicalein inhibited parthanatos, eliminated neurological dysfunction and ameliorated cerebral ischemia injury *in vivo* and *in vitro* [148–150]. Further study also revealed that co-culturing with mesenchymal stem cells protected cortical neurons against OGD-induced parthanatos by inhibiting AIF nuclear translocation [151]. Furthermore, the overproduction of ROS and increasing calcium (Ca^{2+}) are known as essential messengers in cell death, and these can result in PARP1 activation and parthanatos [152, 153]. 14,15-Epoxyeicosatrienoic acid attenuated ischemia-induced parthanatos through reducing the generation of ROS in a MCAO rat model [154]. The endoplasmic reticulum (ER) and mitochondria are the cores of ROS production and Ca^{2+} release and are involved in parthanatos. In Zhong *et al.*'s study, propofol diminished

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infarct volume and enhanced neurological score by restraining ischemia-induced parthanatos via suppression of ROS and Ca^{2+} release in a MCAO mouse model [155]. Therefore, targeting the mediators of parthanatos may be a potential strategy for treating ischemic stroke.

Influence of mPTP-mediated necrosis on ischemic stroke The mitochondrial permeability transition pore (mPTP) is the pore formed in the inner mitochondrial membrane (IMM). Induced by oxidative stress and cytosolic/mitochondrial Ca^{2+} accumulation, the mPTP is responsible for both apoptotic and necrotic cell death. The pores have a diameter of ~1.4–2.3 nm and render the IMM freely permeable to molecules up to 1.5 kDa in size, resulting in loss of mitochondrial membrane potential, expansion of the matrix and rupture of the mitochondrial outer membrane [156].

Cyclophilin D (CypD) is a peptidyl-prolyl *cis–trans* isomerase in the mitochondrial matrix and mediates mPTP opening depending on its sensitivity to Ca^{2+} [157]. CypD is an essential mediator in mPTP-mediated necrosis. In Schinzel *et al*.'s study, CypD knockout produced effective resistance against cerebral ischemia/hypoxia-induced mitochondrial dysfunction, oxidative stress and Ca^{2+} overload *in vivo* and *in vitro* [157]. Denorme *et al*. found that mice with CypD-deficient platelets demonstrated fewer neutrophils and platelet–neutrophil aggregates, significant enhancement of cerebral blood flow, improvement of neurological and motor functions and decreased infarct volume in ischemic stroke, indicating that CypD is a crucial mediator in mPTP-mediated necrosis in ischemic stroke [158]. The immunosuppressant drug cyclosporin A (CsA) serves as an inhibitor of cyclophilins. It exhibits neuroprotective effects on cerebral ischemia/reperfusion-treated brains and hypoxia/reoxygenation-treated neurons by inhibiting mPTP opening and interacting with CypD [159–161].

Further studies have found that multiple post-translational modifications, including phosphorylation, deacetylation, ubiquitination and *S*-nitrosylation of CypD, could regulate mPTP opening and cell death. GSK3β translocates from the cytosol to mitochondria to induce mPTP opening by interacting with CypD, and GSK3β inhibition exerts neuroprotective effects that attenuate mitochondrial dysfunction and ameliorate infarct volume in the MCAO mouse model [162, 163]. Sirt3 has been proven to deacetylate CypD in various types of cells, and Sirt3 overexpression protects neurons against metabolic and excitotoxicity stress [164]. Furthermore, several studies have reported that Sirt3 overexpression suppresses cerebral ischemia-induced mPTP opening [165–167], but the connection between its neuroprotective effects on ischemic stroke and the deacetylation of CypD is still unclear. In addition, p53 has been reported to interact with CypD and trigger mPTP-mediated necrosis of neurons in ischemic stroke [168], and p53 deficiency could remarkably suppress mPTP opening in OGD/R-treated neurons [160]. Until now, the structure and components of the mPTP have not been entirely clear; hence, more studies are needed to elucidate the signaling pathways of the mPTP. To summarize, mPTP-mediated necrosis could be a potential therapeutic target in ischemic stroke.

Influence of oncosis on ischemic stroke Oncosis is characterized by cellular swelling, organelle swelling and blebbing, and increased membrane permeability. It is usually triggered by ischemia and toxic agents through interruption of plasma membrane ionic pumps [169]. Various CNS cell types, such as neurons, glia and vascular endothelial cells, suffer oncosis and manifest as cytotoxic oedema and vasogenic oedema in ischemic stroke [170, 171]. The depletion of ATP and ionic dyshomeostasis (Ca^{2+}, Na^{+} and Cl^{-} influx and K^{+} efflux) induced by sudden termination of blood flow have been found to contribute to the oncotic process in ischemic stroke.

Calpain, the calcium-activated neutral protease, has been implicated in OGD-induced astrocytic oncosis. In a previous study, intervention with a calpain inhibitor delayed OGD-induced astrocytic oncosis, suggesting that calpain is an important mediator of oncosis [172]. Transient receptor potential melastatin 4 (TRPM4), a nonselective cation channel of oncosis, is sensitive to intracellular ATP and Ca^{2+} levels, and its expression and activity levels have been reported to be enhanced in ischemic stroke [173–175]. A recent study performed by Wei *et al*. showed that ischemia-induced activation of TRPM4 resulted in Na^{+} influx, and TRPM4 inhibition significantly attenuated ATP depletion and oncotic cell death in hypoxia-treated neurons, astrocytes and vascular endothelial cells [176]. Additionally, various ion channels and transporters that regulate ionic movement across cell membranes are involved in ischemic stroke, while the role of ion channels and transporters in oncosis still requires further investigation.

The interplay of regulated necrosis pathways in ischemic stroke As stated above, ischemic stroke leads to multiple types of regulated necrosis in various CNS cells. Furthermore, mitochondria act as the core mediator of the cross-regulation between regulated necrosis in ischemic stroke. Mitochondria play a central role in ATP generation in eukaryotic cells. As a consequence, mitochondrial dysfunction caused by ischemic stroke manifests as ATP deficiency, mPTP opening, ROS generation, calcium overload and cytochrome c release [177]. CypD-mediated mPTP opening is essential to mPTP-mediated necrosis, disruption of ion homeostasis and ROS generation; therefore, it is deeply involved in necroptosis, ferroptosis, parthanatos and oncosis [178].

In ischemia–reperfusion injury, the upregulation of RIPK1 and RIPK3 results in CypD-mediated mPTP opening, Ca^{2+} influx and ROS generation, and the inhibition of CypD-mediated mPTP opening could alleviate ischemic stroke-induced necroptosis [179, 180]. ACSL4 overexpression, GPX4 inhibition and GSH downregulation in ischemic stroke lead to lipoxygenase activation, Ca^{2+} influx, mitochondrial membrane rupture and inhibition of ferroptosis, effectively attenuating mPTP opening and leading to mitochondrial

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dysfunction [120, 181, 182]. In addition, iron overload contributes to the overproduction of membrane peroxides, mPTP opening and necroptosis by catalyzing ROS generation and damaging the BBB in stroke [183–185]. As one of the mechanisms of parthanatos, AIF showed the ability to interact with RIPK3 in the nucleus and induce necroptosis in intracerebral hemorrhage mice, and intervention with CsA blocked the RIPK3–AIF complex by inhibiting mPTP opening [186].

Notably, ROS, which are mainly generated in mitochondria as a consequence of oxidative phosphorylation (OXPHOS), contribute to various types of regulated necrosis in ischemic stroke and act as the major mechanism of membrane lipid peroxidation, oxidative stress-triggered DNA degradation and oxidative damage to proteins [187–189]. Several studies have indicated that ROS generation leads to PARP activation, AIF release, RIPK1 autophosphorylation, RIPK3 recruitment and NLRP3 inflammasome activation in response to ischemic stroke [187, 190, 191]. In gliomas, ROS upregulation acts as an important mechanism of oncosis [192]. Meanwhile, necrosomes, the NLRP3 inflammasome and iron overload induce ROS accumulation in turn [193, 194]. Additionally, the interplay between ER stress and ROS generation via the activation of the unfolded-protein response also plays an important role in necroptosis, ferroptosis and mPTP-mediated necrosis [195–197].

Hence, as the different types of regulated necrosis are closely interrelated, inhibition of one type of regulated necrosis could effectively block the mechanisms of other regulated necrosis types. Moreover, Tian *et al*. found that the combination of emricasan and ponatinib could inhibit both apoptosis and necroptosis more effectively than inhibition of apoptosis or necroptosis alone in ischemic stroke rats [198]. Hence, compounds that target multiple types of regulated necrosis may be a potential treatment for ischemic stroke.

Conclusions

Due to its high morbidity, mortality and disability rates, ischemic stroke has become an intractable issue for public health, especially in China. In addition to apoptosis, necrosis and autophagy, which are traditionally the three major types of cell death, growing evidence has indicated that several types of regulated necrosis, including necroptosis, pyroptosis, ferroptosis, parthanatos, mPTP-mediated necrosis and oncosis, contribute to CNS cell death in ischemic stroke. Interestingly, a number of studies have found that ischemic stroke-induced regulated necrosis is interconnected and closely related to the functions of organelles such as mitochondria and the ER. Notably, various traditional Chinese medicines have been reported to inhibit ischemic stroke-induced regulated necrosis. For example, kaempferol alleviates OGD/R injury by inhibiting ferroptosis and ROS generation. To alleviate ischemic stroke, inhibiting multiple types of regulated necroptosis has been found to be more effective than blocking a single pathway. Hence, investigating the mechanisms of regulated necrosis and the interplay among various regulated necrosis types could shed light on a novel direction to treat ischemic stroke injury. However, many unknown factors need to be uncovered before these strategies can be applied in the clinical treatment of ischemic stroke.

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Data availability

All data generated or analyzed in this study are available from the corresponding author on reasonable request.

Authors' contributions

KR, JP, YG, YJ, QF and YY conceptualized and wrote the manuscript and created the figures. KR, HX, YX and QF contributed to writing the manuscript. KR, YY, QF and JY reviewed and modified the manuscript. All authors approved the final version of the manuscript.

Conflicts of interests

All the authors in this study declare that there are no competing interests.

References

1. Collaborators GBDS. Global, regional, and national burden of stroke, 1990-2016: a systematic analysis for the global burden of disease study 2016. *Lancet Neurol*. 2019;18:439–58.
2. Catanese L, Tarsia J, Fisher M. Acute ischemic stroke therapy overview. *Circ Res*. 2017;120:541–58.
3. Cheon SY, Kim EJ, Kim JM, Koo BN. Cell type-specific mechanisms in the pathogenesis of ischemic stroke: the role of apoptosis signal-regulating kinase 1. *Oxidative Med Cell Longev*. 2018;2018:1–9.
4. Jayaraj RL, Azimullah S, Beiram R, Jalal FY, Rosenberg GA. Neuroinflammation: friend and foe for ischemic stroke. *J Neuroinflammation*. 2019;16:142.
5. O'Collins VE, Macleod MR, Cox SF, Van Raay L, Aleksoska E, Donnan GA, *et al*. Preclinical drug evaluation for combination therapy in acute stroke using systematic review, meta-analysis, and subsequent experimental testing. *J Cereb Blood Flow Metab*. 2011;31:962–75.
6. Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke EH, *et al*. Classification of cell death: recommendations of the nomenclature committee on cell death 2009. *Cell Death Differ*. 2009;16:3–11.

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7. Sun J, Ren DD, Wan JY, Chen C, Chen D, Yang H, *et al.* Desensitizing mitochondrial permeability transition by ERK-Cyclophilin D Axis contributes to the neuroprotective effect of Gallic acid against cerebral ischemia/reperfusion injury. *Front Pharmacol*. 2017;8:184.
8. Deng XX, Li SS, Sun FY. Necrostatin-1 prevents necroptosis in brains after ischemic stroke via inhibition of RIPK1-mediated RIPK3/MLKL Signaling. *Aging Dis*. 2019;10:807–17.
9. Yan W, Sun W, Fan J, Wang H, Han S, Li J, *et al.* Sirt1-ROS-TRAF6 Signaling-induced Pyroptosis contributes to early injury in ischemic mice. *Neurosci Bull*. 2020;36:845–59.
10. Li Y, Feng D, Wang Z, Zhao Y, Sun R, Tian D, *et al.* Ischemia-induced ACSL4 activation contributes to ferroptosis-mediated tissue injury in intestinal ischemia/reperfusion. *Cell Death Differ*. 2019;26:2284–99.
11. Sairanen T, Szepesi R, Karjalainen-Lindsberg ML, Saksi J, Paetau A, Lindsberg PJ. Neuronal caspase-3 and PARP-1 correlate differentially with apoptosis and necrosis in ischemic human stroke. *Acta Neuropathol*. 2009;118:541–52.
12. Yuan J, Amin P, Ofengeim D. Necroptosis and RIPK1-mediated neuroinflammation in CNS diseases. *Nat Rev Neurosci*. 2019;20:19–33.
13. Mandal P, Berger SB, Pillay S, Moriwaki K, Huang C, Guo H, *et al.* RIP3 induces apoptosis independent of pronecrotic kinase activity. *Mol Cell*. 2014;56:481–95.
14. Vercammen D, Brouckaert G, Denecker G, Van de Craen M, Declercq W, Fiers W, *et al.* Dual signaling of the Fas receptor: initiation of both apoptotic and necrotic cell death pathways. *J Exp Med*. 1998;188:919–30.
15. Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, *et al.* Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nat Chem Biol*. 2005;1:112–9.
16. Hitomi J, Christofferson DE, Ng A, Yao J, Degterev A, Xavier RJ, *et al.* Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. *Cell*. 2008;135:1311–23.
17. Chan FK, Shisler J, Bixby JG, Felices M, Zheng L, Appel M, *et al.* A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses. *J Biol Chem*. 2003;278:51613–21.
18. Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, *et al.* Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. *Nat Immunol*. 2000;1:489–95.
19. Weinlich R, Oberst A, Beere HM, Green DR. Necroptosis in development, inflammation and disease. *Nat Rev Mol Cell Biol*. 2017;18:127–36.
20. Cho YS, Challa S, Moquin D, Genga R, Ray TD, Guildford M, *et al.* Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. *Cell*. 2009;137:1112–23.
21. Dondelinger Y, Declercq W, Montessuit S, Roelandt R, Goncalves A, Bruggeman I, *et al.* MLKL compromises plasma membrane integrity by binding to phosphatidylinositol phosphates. *Cell Rep*. 2014;7:971–81.
22. Siegmund D, Ehrenschwender M, Wajant H. TNFR2 unlocks a RIPK1 kinase activity-dependent mode of proinflammatory TNFR1 signaling. *Cell Death Dis*. 2018;9:921.
23. Wang L, Chang X, Feng J, Yu J, Chen G. TRADD mediates RIPK1-independent necroptosis induced by tumor necrosis factor. *Front Cell Dev Biol*. 2019;7:393.
24. Moujalled DM, Cook WD, Okamoto T, Murphy J, Lawlor KE, Vince JE, *et al.* TNF can activate RIPK3 and cause programmed necrosis in the absence of RIPK1. *Cell Death Dis*. 2013;4:e465.
25. Lule S, Wu L, McAllister LM, Edmiston WJ, 3rd, Chung JY, Levy E, *et al.* Genetic inhibition of receptor interacting protein Kinase-1 reduces cell death and improves functional outcome after intracerebral Hemorrhage in mice. *Stroke*. 2017;48:2549–56.
26. Jiao Y, Wang J, Zhang H, Cao Y, Qu Y, Huang S, *et al.* Inhibition of microglial receptor-interacting protein kinase 1 ameliorates neuroinflammation following cerebral ischaemic stroke. *J Cell Mol Med*. 2020;24:12585–98.
27. Li W, Liu J, Chen JR, Zhu YM, Gao X, Ni Y, *et al.* Neuroprotective effects of DTIO, a novel Analog of Nec-1, in acute and chronic stages after ischemic stroke. *Neuroscience*. 2018;390:12–29.
28. Liu ZM, Chen QX, Chen ZB, Tian DF, Li MC, Wang JM, *et al.* RIP3 deficiency protects against traumatic brain injury (TBI) through suppressing oxidative stress, inflammation and apoptosis: dependent on AMPK pathway. *Biochem Biophys Res Commun*. 2018;499:112–9.
29. Han F, Guan X, Guo W, Lu B. Therapeutic potential of a TrkB agonistic antibody for ischemic brain injury. *Neurobiol Dis*. 2019;127:570–81.
30. Zhang YY, Liu WN, Li YQ, Zhang XJ, Yang J, Luo XJ, *et al.* Ligustroflavone reduces necroptosis in rat brain after ischemic stroke through targeting RIPK1/RIPK3/MLKL pathway. *Naunyn Schmiedeberg's Arch Pharmacol*. 2019;392:1085–95.
31. Zhang S, Hu ZW, Mao CY, Shi CH, Xu YM. CHIP as a therapeutic target for neurological diseases. *Cell Death Dis*. 2020;11:727.
32. Ganjam GK, Terpolilli NA, Diemert S, Eisenbach I, Hoffmann L, Reuther C, *et al.* Cylindromatosis mediates neuronal cell death in vitro and in vivo. *Cell Death Differ*. 2018;25:1394–407.
33. Yuan Z, Yi-Yun S, Hai-Yan Y. Triad3A displays a critical role in suppression of cerebral ischemic/reperfusion (I/R) injury by regulating necroptosis. *Biomed Pharmacother*. 2020;128:110045.
34. Wang M, Wan H, Wang S, Liao L, Huang Y, Guo L, *et al.* RSK3 mediates necroptosis by regulating phosphorylation of RIP3 in rat retinal ganglion cells. *J Anat*. 2020;237:29–47.
35. Lipton P. Ischemic cell death in brain neurons. *Physiol Rev*. 1999;79:1431–568.
36. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med*. 2011;17:796–808.
37. Xiong XY, Liu L, Yang QW. Functions and mechanisms of microglia/macrophages in neuroinflammation and neurogenesis after stroke. *Prog Neurobiol*. 2016;142:23–44.
38. Dojo Soeandy C, Elia AJ, Cao Y, Rodgers C, Huang S, Elia AC, *et al.* Necroptotic-apoptotic regulation in an Endothelin-1 model of cerebral ischemia. *Cell Mol Neurobiol*. 2021;41:1727–42.
39. Naito MG, Xu D, Amin P, Lee J, Wang H, Li W, *et al.* Sequential activation of necroptosis and apoptosis cooperates to mediate vascular and neural pathology in stroke. *Proc Natl Acad Sci U S A*. 2020;117:4959–70.

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40. Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, *et al*. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci*. 2014;34:11929–47.
41. Legler DF, Micheau O, Doucey MA, Tschopp J, Bron C. Recruitment of TNF receptor 1 to lipid rafts is essential for TNFalpha-mediated NF-kappaB activation. *Immunity*. 2003;18:655–64.
42. Park SM, Yoon JB, Lee TH. Receptor interacting protein is ubiquitinated by cellular inhibitor of apoptosis proteins (c-IAP1 and c-IAP2) in vitro. *FEBS Lett*. 2004;566:151–6.
43. Petersen SL, Chen TT, Lawrence DA, Marsters SA, Gonzalvez F, Ashkenazi A. TRAF2 is a biologically important necroptosis suppressor. *Cell Death Differ*. 2015;22:1846–57.
44. Li J, Zhang J, Zhang Y, Wang Z, Song Y, Wei S, *et al*. TRAF2 protects against cerebral ischemia-induced brain injury by suppressing necroptosis. *Cell Death Dis*. 2019;10:328.
45. Alturki NA, McComb S, Ariana A, Rijal D, Korneluk RG, Sun SC, *et al*. Triad3a induces the degradation of early necrosome to limit RipK1-dependent cytokine production and necroptosis. *Cell Death Dis*. 2018;9:592.
46. Geng J, Ito Y, Shi L, Amin P, Chu J, Ouchida AT, *et al*. Regulation of RIPK1 activation by TAK1-mediated phosphorylation dictates apoptosis and necroptosis. *Nat Commun*. 2017;8:359.
47. White BJ, Tarabishy S, Venna VR, Manwani B, Benashski S, McCullough LD, *et al*. Protection from cerebral ischemia by inhibition of TGFbeta-activated kinase. *Exp Neurol*. 2012;237:238–45.
48. Zeyen T, Noristani R, Habib S, Heinisch O, Slowik A, Huber M, *et al*. Microglial-specific depletion of TAK1 is neuroprotective in the acute phase after ischemic stroke. *J Mol Med (Berl)*. 2020;98:833–47.
49. Neubert M, Ridder DA, Bargiotas P, Akira S, Schwaninger M. Acute inhibition of TAK1 protects against neuronal death in cerebral ischemia. *Cell Death Differ*. 2011;18:1521–30.
50. Wang YZ, Wang JJ, Huang Y, Liu F, Zeng WZ, Li Y, *et al*. Tissue acidosis induces neuronal necroptosis via ASIC1a channel independent of its ionic conduction. *elife*. 2015;4:e05682.
51. Wang JJ, Liu F, Yang F, Wang YZ, Qi X, Li Y, *et al*. Disruption of auto-inhibition underlies conformational signaling of ASIC1a to induce neuronal necroptosis. *Nat Commun*. 2020;11:475.
52. Liu Z, Chopp M. Astrocytes, therapeutic targets for neuroprotection and neurorestoration in ischemic stroke. *Prog Neurobiol*. 2016;144:103–20.
53. Linnerbauer M, Rothhammer V. Protective functions of reactive astrocytes following central nervous system insult. *Front Immunol*. 2020;11:573256.
54. Kim JH, Min KJ, Seol W, Jou I, Joe EH. Astrocytes in injury states rapidly produce anti-inflammatory factors and attenuate microglial inflammatory responses. *J Neurochem*. 2010;115:1161–71.
55. Li L, Lundkvist A, Andersson D, Wilhelmsson U, Nagai N, Pardo AC, *et al*. Protective role of reactive astrocytes in brain ischemia. *J Cereb Blood Flow Metab*. 2008;28:468–81.
56. Zhu YM, Lin L, Wei C, Guo Y, Qin Y, Li ZS, *et al*. The key regulator of necroptosis, RIP1 kinase, contributes to the formation of Astrogliosis and glial scar in ischemic stroke. *Transl Stroke Res*. 2021;12:991–1017.
57. Liu J, Zhu YM, Guo Y, Lin L, Wang ZX, Gu F, *et al*. Inhibition of GSK3beta and RIP1K attenuates glial scar formation induced by ischemic stroke via reduction of inflammatory cytokine production. *Front Pharmacol*. 2020;11:812.
58. Holinstat M. Normal platelet function. *Cancer Metastasis Rev*. 2017;36:195–8.
59. Zhang Y, Zhang J, Yan R, Tian J, Zhang Y, Zhang J, *et al*. Receptor-interacting protein kinase 3 promotes platelet activation and thrombosis. *Proc Natl Acad Sci U S A*. 2017; 114:2964–9.
60. Schuhmann MK, Stoll G, Bieber M, Vogtle T, Hofmann S, Klaus V, *et al*. CD84 links T cell and platelet activity in cerebral Thrombo-inflammation in acute stroke. *Circ Res*. 2020;127:1023–35.
61. Nakazawa D, Desai J, Steiger S, Muller S, Devarapu SK, Mulay SR, *et al*. Activated platelets induce MLKL-driven neutrophil necroptosis and release of neutrophil extracellular traps in venous thrombosis. *Cell Death Dis*. 2018;4:6.
62. Cognasse F, Hamzeh H, Chavarin P, Acquart S, Genin C, Garraud O. Evidence of toll-like receptor molecules on human platelets. *Immunol Cell Biol*. 2005;83:196–8.
63. Nian K, Harding IC, Herman IM, Ebong EE. Blood-brain barrier damage in ischemic stroke and its regulation by endothelial Mechanotransduction. *Front Physiol*. 2020;11: 605398.
64. Abdullahi W, Tripathi D, Ronaldson PT. Blood-brain barrier dysfunction in ischemic stroke: targeting tight junctions and transporters for vascular protection. *Am J Physiol Cell Physiol*. 2018;315:C343–56.
65. Chen AQ, Fang Z, Chen XL, Yang S, Zhou YF, Mao L, *et al*. Microglia-derived TNF-alpha mediates endothelial necroptosis aggravating blood brain-barrier disruption after ischemic stroke. *Cell Death Dis*. 2019;10:487.
66. Kovacs SB, Miao EA. Gasdermins: effectors of Pyroptosis. *Trends Cell Biol*. 2017;27:673–84.
67. Jin R, Yang G, Li G. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. *J Leukoc Biol*. 2010;87: 779–89.
68. Barrington J, Lemarchand E, Allan SM. A brain in flame; do inflammasomes and pyroptosis influence stroke pathology? *Brain Pathol*. 2017;27:205–12.
69. Martinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell*. 2004;117:561–74.
70. Boucher D, Monteleone M, Coll RC, Chen KW, Ross CM, Teo JL, *et al*. Caspase-1 self-cleavage is an intrinsic mechanism to terminate inflammasome activity. *J Exp Med*. 2018;215: 827–40.
71. Ding J, Wang K, Liu W, She Y, Sun Q, Shi J, *et al*. Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature*. 2016;535:111–6.
72. Man SM, Karki R, Kanneganti TD. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. *Immunol Rev*. 2017;277: 61–75.
73. Kayagaki N, Warming S, Lamkanfi M, Vande Walle L, Louie S, Dong J, *et al*. Non-canonical inflammasome activation targets caspase-11. *Nature*. 2011;479:117–21.
74. Wang Y, Gao W, Shi X, Ding J, Liu W, He H, *et al*. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. *Nature*. 2017;547:99–103.
75. Masuda Y, Futamura M, Kamino H, Nakamura Y, Kitamura N, Ohnishi S, *et al*. The potential role of DFNA5, a hearing

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impairment gene, in p53-mediated cellular response to DNA damage. *J Hum Genet*. 2006;51:652–64.
76. Cookson BT, Brennan MA. Pro-inflammatory programmed cell death. *Trends Microbiol*. 2001;9:113–4.
77. Mamik MK, Power C. Inflammasomes in neurological diseases: emerging pathogenic and therapeutic concepts. *Brain*. 2017;140:2273–85.
78. Fann DY, Lim YA, Cheng YL, Lok KZ, Chunduri P, Baik SH, *et al*. Evidence that NF-kappaB and MAPK Signaling promotes NLRP Inflammasome activation in neurons following ischemic stroke. *Mol Neurobiol*. 2018;55:1082–96.
79. Kaushal V, Dye R, Pakavathkumar P, Foveau B, Flores J, Hyman B, *et al*. Neuronal NLRP1 inflammasome activation of Caspase-1 coordinately regulates inflammatory interleukin-1-beta production and axonal degeneration-associated Caspase-6 activation. *Cell Death Differ*. 2015;22:1676–86.
80. Heinisch O, Zeyen T, Goldmann T, Prinz M, Huber M, Jung J, *et al*. Erythropoietin abrogates post-ischemic activation of the NLRP3, NLRC4, and AIM2 Inflammasomes in microglia/-macrophages in a TAK1-dependent manner. *Transl Stroke Res*. 2022;13:462–82.
81. Zhang D, Qian J, Zhang P, Li H, Shen H, Li X, *et al*. Gasdermin D serves as a key executioner of pyroptosis in experimental cerebral ischemia and reperfusion model both in vivo and in vitro. *J Neurosci Res*. 2019;97:645–60.
82. Wang K, Sun Z, Ru J, Wang S, Huang L, Ruan L, *et al*. Ablation of GSDMD improves outcome of ischemic stroke through blocking canonical and non-canonical Inflammasomes dependent Pyroptosis in microglia. *Front Neurol*. 2020;11: 577927.
83. Li J, Hao JH, Yao D, Li R, Li XF, Yu ZY, *et al*. Caspase-1 inhibition prevents neuronal death by targeting the canonical inflammasome pathway of pyroptosis in a murine model of cerebral ischemia. *CNS Neurosci Ther*. 2020;26:925–39.
84. Lv Y, Sun B, Lu XX, Liu YL, Li M, Xu LX, *et al*. The role of microglia mediated pyroptosis in neonatal hypoxic-ischemic brain damage. *Biochem Biophys Res Commun*. 2020;521:933–8.
85. Poh L, Kang SW, Baik SH, Ng GYQ, She DT, Balaganapathy P, *et al*. Evidence that NLRC4 inflammasome mediates apoptotic and pyroptotic microglial death following ischemic stroke. *Brain Behav Immun*. 2019;75:34–47.
86. Nakagawa Y, Chiba K. Diversity and plasticity of microglial cells in psychiatric and neurological disorders. *Pharmacol Ther*. 2015;154:21–35.
87. Tammaro A, Derive M, Gibot S, Leemans JC, Florquin S, Dessing MC. TREM-1 and its potential ligands in non-infectious diseases: from biology to clinical perspectives. *Pharmacol Ther*. 2017;177:81–95.
88. Xu P, Zhang X, Liu Q, Xie Y, Shi X, Chen J, *et al*. Microglial TREM-1 receptor mediates neuroinflammatory injury via interaction with SYK in experimental ischemic stroke. *Cell Death Dis*. 2019;10:555.
89. Liang YB, Song PP, Zhu YH, Xu JM, Zhu PZ, Liu RR, *et al*. TREM-1-targeting LP17 attenuates cerebral ischemia-induced neuronal injury by inhibiting oxidative stress and pyroptosis. *Biochem Biophys Res Commun*. 2020;529:554–61.
90. Wang J, Li J, Wang Q, Kong Y, Zhou F, Li Q, *et al*. Dcf1 deficiency attenuates the role of activated microglia during Neuroinflammation. *Front Mol Neurosci*. 2018;11:256.
91. Liu Z, Ning J, Zheng X, Meng J, Han L, Zheng H, *et al*. TMEM59 interacts with TREM2 and modulates TREM2-dependent microglial activities. *Cell Death Dis*. 2020;11:678.
92. Zhang L, Wang T, Chen XF, Xu ZX, Cao JB, Sun H. TMEM59 protects against cerebral ischemic stroke by suppressing pyroptosis and microglial activation. *Biochem Biophys Res Commun*. 2021;543:72–9.
93. Li Q, Cao Y, Dang C, Han B, Han R, Ma H, *et al*. Inhibition of double-strand DNA-sensing cGAS ameliorates brain injury after ischemic stroke. *EMBO Mol Med*. 2020;12:e11002.
94. Liu X, Zhang M, Liu H, Zhu R, He H, Zhou Y, *et al*. Bone marrow mesenchymal stem cell-derived exosomes attenuate cerebral ischemia-reperfusion injury-induced neuroinflammation and pyroptosis by modulating microglia M1/M2 phenotypes. *Exp Neurol*. 2021;341:113700.
95. Wang K, Ru J, Zhang H, Chen J, Lin X, Lin Z, *et al*. Melatonin enhances the therapeutic effect of plasma exosomes against cerebral ischemia-induced Pyroptosis through the TLR4/NF-kappaB pathway. *Front Neurosci*. 2020;14:848.
96. Fricker M, Tolkovsky AM, Borutaite V, Coleman M, Brown GC. Neuronal cell death. *Physiol Rev*. 2018;98:813–80.
97. Zhu S, Zhang Z, Jia LQ, Zhan KX, Wang LJ, Song N, *et al*. Valproic acid attenuates global cerebral ischemia/reperfusion injury in gerbils via anti-pyroptosis pathways. *Neurochem Int*. 2019;124:141–51.
98. Sun R, Peng M, Xu P, Huang F, Xie Y, Li J, *et al*. Low-density lipoprotein receptor (LDLR) regulates NLRP3-mediated neuronal pyroptosis following cerebral ischemia/reperfusion injury. *J Neuroinflammation*. 2020;17:330.
99. Liang J, Wang Q, Li JQ, Guo T, Yu D. Long non-coding RNA MEG3 promotes cerebral ischemia-reperfusion injury through increasing pyroptosis by targeting miR-485/AIM2 axis. *Exp Neurol*. 2020;325:113139.
100. Zhao B, Fei Y, Zhu J, Yin Q, Fang W, Li Y. PAF receptor inhibition attenuates neuronal Pyroptosis in cerebral ischemia/reperfusion injury. *Mol Neurobiol*. 2021;58:6520–39.
101. Liang Y, Song P, Chen W, Xie X, Luo R, Su J, *et al*. Inhibition of Caspase-1 ameliorates ischemia-associated blood-brain barrier dysfunction and integrity by suppressing Pyroptosis activation. *Front Cell Neurosci*. 2020;14:540669.
102. Wang Y, Guan X, Gao CL, Ruan W, Zhao S, Kai G, *et al*. Medioresinol as a novel PGC-1alpha activator prevents pyroptosis of endothelial cells in ischemic stroke through PPARalpha-GOT1 axis. *Pharmacol Res*. 2021;169:105640.
103. Lyu Z, Chan Y, Li Q, Zhang Q, Liu K, Xiang J, *et al*. Destructive effects of Pyroptosis on homeostasis of neuron survival associated with the dysfunctional BBB-Glymphatic system and amyloid-Beta accumulation after cerebral ischemia/reperfusion in rats. *Neural Plast*. 2021;2021:4504363.
104. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, *et al*. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell*. 2017;171:273–85.
105. Liu J, Guo ZN, Yan XL, Huang S, Ren JX, Luo Y, *et al*. Crosstalk between autophagy and Ferroptosis and its putative role in ischemic stroke. *Front Cell Neurosci*. 2020;14:577403.
106. Pratt DA, Tallman KA, Porter NA. Free radical oxidation of polyunsaturated lipids: new mechanistic insights and the development of peroxyl radical clocks. *Acc Chem Res*. 2011;44:458–67.

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107. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, *et al.* Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*. 2012;149:1060–72.
108. Cao JY, Dixon SJ. Mechanisms of ferroptosis. *Cell Mol Life Sci*. 2016;73:2195–209.
109. Ursini F, Maiorino M, Gregolin C. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochim Biophys Acta*. 1985;839:62–70.
110. Seiler A, Schneider M, Forster H, Roth S, Wirth EK, Culmsee C, *et al.* Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab*. 2008;8:237–48.
111. Bannai S, Kitamura E. Transport interaction of L-cystine and L-glutamate in human diploid fibroblasts in culture. *J Biol Chem*. 1980;255:2372–6.
112. Yang WS, Stockwell BR. Ferroptosis: death by lipid peroxidation. *Trends Cell Biol*. 2016;26:165–76.
113. Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, *et al.* Ferroptosis: process and function. *Cell Death Differ*. 2016;23:369–79.
114. Dixon SJ. Ferroptosis: bug or feature? *Immunol Rev*. 2017;277:150–7.
115. Lane DJR, Ayton S, Bush AI. Iron and Alzheimer's disease: an update on emerging mechanisms. *J Alzheimers Dis*. 2018;64:S379–95.
116. Roskams AJ, Connor JR. Iron, transferrin, and ferritin in the rat brain during development and aging. *J Neurochem*. 1994;63:709–16.
117. Patt A, Horesh IR, Berger EM, Harken AH, Repine JE. Iron depletion or chelation reduces ischemia/reperfusion-induced edema in gerbil brains. *J Pediatr Surg*. 1990;25:224–8 discussion 227-8.
118. Tuo QZ, Lei P, Jackman KA, Li XL, Xiong H, Li XL, *et al.* Tau-mediated iron export prevents ferroptotic damage after ischemic stroke. *Mol Psychiatry*. 2017;22:1520–30.
119. Cui Y, Zhang Y, Zhao X, Shao L, Liu G, Sun C, *et al.* ACSL4 exacerbates ischemic stroke by promoting ferroptosis-induced brain injury and neuroinflammation. *Brain Behav Immun*. 2021;93:312–21.
120. Chen J, Yang L, Geng L, He J, Chen L, Sun Q, *et al.* Inhibition of acyl-CoA Synthetase long-chain family member 4 facilitates neurological recovery after stroke by regulation Ferroptosis. *Front Cell Neurosci*. 2021;15:632354.
121. Yuan Y, Zhai Y, Chen J, Xu X, Wang H. Kaempferol ameliorates oxygen-glucose deprivation/Reoxygenation-induced neuronal Ferroptosis by activating Nrf2/SLC7A11/GPX4 Axis. *Biomol Ther*. 2021;11:923.
122. Lu J, Xu F, Lu H. LncRNA PVT1 regulates ferroptosis through miR-214-mediated TFR1 and p53. *Life Sci*. 2020;260:118305.
123. Li C, Sun G, Chen B, Xu L, Ye Y, He J, *et al.* Nuclear receptor coactivator 4-mediated ferritinophagy contributes to cerebral ischemia-induced ferroptosis in ischemic stroke. *Pharmacol Res*. 2021;174:105933.
124. Zhao Y, Rempe DA. Prophylactic neuroprotection against stroke: low-dose, prolonged treatment with deferoxamine or deferasirox establishes prolonged neuroprotection independent of HIF-1 function. *J Cereb Blood Flow Metab*. 2011;31:1412–23.
125. Selim M. Treatment with the iron chelator, deferoxamine mesylate, alters serum markers of oxidative stress in stroke patients. *Transl Stroke Res*. 2010;1:35–9.
126. Chow EJ, Aplenc R, Vrooman LM, Doody DR, Huang YV, Aggarwal S, *et al.* Late health outcomes after dexrazoxane treatment: a report from the Children's oncology group. *Cancer*. 2022;128:788–96.
127. Guan X, Li X, Yang X, Yan J, Shi P, Ba L, *et al.* The neuroprotective effects of carvacrol on ischemia/reperfusion-induced hippocampal neuronal impairment by ferroptosis mitigation. *Life Sci*. 2019;235:116795.
128. Li G, Li X, Dong J, Han Y. Electroacupuncture ameliorates cerebral ischemic injury by inhibiting Ferroptosis. *Front Neurol*. 2021;12:619043.
129. Andrabi SA, Dawson TM, Dawson VL. Mitochondrial and nuclear cross talk in cell death: parthanatos. *Ann N Y Acad Sci*. 2008;1147:233–41.
130. Houl JH, Ye Z, Brosey CA, Balapiti-Modarage LPF, Namjoshi S, Bacolla A, *et al.* Selective small molecule PARG inhibitor causes replication fork stalling and cancer cell death. *Nat Commun*. 2019;10:5654.
131. Yu SW, Andrabi SA, Wang H, Kim NS, Poirier GG, Dawson TM, *et al.* Apoptosis-inducing factor mediates poly(ADP-ribose) (PAR) polymer-induced cell death. *Proc Natl Acad Sci U S A*. 2006;103:18314–9.
132. Zhang J, Dawson VL, Dawson TM, Snyder SH. Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. *Science*. 1994;263:687–9.
133. Eliasson MJ, Sampei K, Mandir AS, Hurn PD, Traystman RJ, Bao J, *et al.* Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. *Nat Med*. 1997;3:1089–95.
134. Joly LM, Benjelloun N, Plotkine M, Charriaut-Marlangue C. Distribution of poly(ADP-ribosyl)ation and cell death after cerebral ischemia in the neonatal rat. *Pediatr Res*. 2003;53:776–82.
135. Love S, Barber R, Wilcock GK. Neuronal death in brain infarcts in man. *Neuropathol Appl Neurobiol*. 2000;26:55–66.
136. Chiarugi A, Meli E, Calvani M, Picca R, Baronti R, Camaioni E, *et al.* Novel isoquinolinone-derived inhibitors of poly(ADP-ribose) polymerase-1: pharmacological characterization and neuroprotective effects in an in vitro model of cerebral ischemia. *J Pharmacol Exp Ther*. 2003;305:943–9.
137. Lo EH, Bosque-Hamilton P, Meng W. Inhibition of poly(ADP-ribose) polymerase: reduction of ischemic injury and attenuation of N-methyl-D-aspartate-induced neurotransmitter dysregulation. *Stroke*. 1998;29:830–6.
138. Chiarugi A. Poly(ADP-ribosyl)ation and stroke. *Pharmacol Res*. 2005;52:15–24.
139. Kim Y, Kim YS, Kim HY, Noh MY, Kim JY, Lee YJ, *et al.* Early treatment with poly(ADP-ribose) Polymerase-1 inhibitor (JPI-289) reduces infarct volume and improves long-term behavior in an animal model of ischemic stroke. *Mol Neurobiol*. 2018;55:7153–63.
140. Han S, Kim YH, Choi HY, Soh DJ, Kim J, Nam J, *et al.* First-in-human evaluation of the safety, tolerability, and pharmacokinetics of a neuroprotective poly (ADP-ribose) Polymerase-1 inhibitor, JPI-289, in healthy volunteers. *Drug Des Devel Ther*. 2020;14:3189–99.
141. Ying W, Sevigny MB, Chen Y, Swanson RA. Poly(ADP-ribose) glycohydrolase mediates oxidative and excitotoxic neuronal death. *Proc Natl Acad Sci U S A*. 2001;98:12227–32.

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142. Lu XC, Massuda E, Lin Q, Li W, Li JH, Zhang J. Post-treatment with a novel PARG inhibitor reduces infarct in cerebral ischemia in the rat. *Brain Res*. 2003;978:99–103.
143. Burkle A, Virag L. Poly(ADP-ribose): PARadigms and PARadoxes. *Mol Asp Med*. 2013;34:1046–65.
144. Mashimo M, Kato J, Moss J. ADP-ribosyl-acceptor hydrolase 3 regulates poly (ADP-ribose) degradation and cell death during oxidative stress. *Proc Natl Acad Sci U S A*. 2013;110:18964–9.
145. Mashimo M, Bu X, Aoyama K, Kato J, Ishiwata-Endo H, Stevens LA, *et al.* PARP1 inhibition alleviates injury in ARH3-deficient mice and human cells. *JCI Insight*. 2019;4:e124519.
146. Kang HC, Lee YI, Shin JH, Andrabi SA, Chi Z, Gagne JP, *et al.* Iduna is a poly(ADP-ribose) (PAR)-dependent E3 ubiquitin ligase that regulates DNA damage. *Proc Natl Acad Sci U S A*. 2011;108:14103–8.
147. Andrabi SA, Kang HC, Haince JF, Lee YI, Zhang J, Chi Z, *et al.* Iduna protects the brain from glutamate excitotoxicity and stroke by interfering with poly(ADP-ribose) polymer-induced cell death. *Nat Med*. 2011;17:692–9.
148. Zeng M, Zhou H, He Y, Wang Z, Shao C, Yin J, *et al.* Danhong injection alleviates cerebral ischemia/reperfusion injury by improving intracellular energy metabolism coupling in the ischemic penumbra. *Biomed Pharmacother*. 2021;140:111771.
149. Li Y, Yang Y, Zhao Y, Zhang J, Liu B, Jiao S, *et al.* Astragaloside IV reduces neuronal apoptosis and parthanatos in ischemic injury by preserving mitochondrial hexokinase-II. *Free Radic Biol Med*. 2019;131:251–63.
150. Li WH, Yang YL, Cheng X, Liu M, Zhang SS, Wang YH, *et al.* Baicalein attenuates caspase-independent cells death via inhibiting PARP-1 activation and AIF nuclear translocation in cerebral ischemia/reperfusion rats. *Apoptosis*. 2020;25:354–69.
151. Kong D, Zhu J, Liu Q, Jiang Y, Xu L, Luo N, *et al.* Mesenchymal stem cells protect neurons against hypoxic-ischemic injury via inhibiting parthanatos, necroptosis, and apoptosis, but not autophagy. *Cell Mol Neurobiol*. 2017;37:303–13.
152. Chiu LY, Ho FM, Shiah SG, Chang Y, Lin WW. Oxidative stress initiates DNA damager MNNG-induced poly(ADP-ribose)polymerase-1-dependent parthanatos cell death. *Biochem Pharmacol*. 2011;81:459–70.
153. Gerace E, Masi A, Resta F, Felici R, Landucci E, Mello T, *et al.* PARP-1 activation causes neuronal death in the hippocampal CA1 region by increasing the expression of CA(2+)-permeable AMPA receptors. *Neurobiol Dis*. 2014;70:43–52.
154. Zhao H, Tang J, Chen H, Gu W, Geng H, Wang L, *et al.* 14,15-EET reduced brain injury from cerebral ischemia and reperfusion via suppressing neuronal Parthanatos. *Int J Mol Sci*. 2021;22:9660.
155. Zhong H, Song R, Pang Q, Liu Y, Zhuang J, Chen Y, *et al.* Propofol inhibits parthanatos via ROS-ER-calcium-mitochondria signal pathway in vivo and vitro. *Cell Death Dis*. 2018;9:932.
156. Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P. Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. *J Biol Chem*. 2005;280:18558–61.
157. Bernardi P. The mitochondrial permeability transition pore: a mystery solved? *Front Physiol*. 2013;4:95.
158. Denorme F, Manne BK, Portier I, Eustes AS, Kosaka Y, Kile BT, *et al.* Platelet necrosis mediates ischemic stroke outcome in mice. *Blood*. 2020;135:429–40.
159. Kim SY, Shim MS, Kim KY, Weinreb RN, Wheeler LA, Ju WK. Inhibition of cyclophilin D by cyclosporin a promotes retinal ganglion cell survival by preventing mitochondrial alteration in ischemic injury. *Cell Death Dis*. 2014;5:e1105.
160. Zhao LP, Ji C, Lu PH, Li C, Xu B, Gao H. Oxygen glucose deprivation (OGD)/re-oxygenation-induced in vitro neuronal cell death involves mitochondrial cyclophilin-D/P53 signaling axis. *Neurochem Res*. 2013;38:705–13.
161. Ye F, Li X, Li F, Li J, Chang W, Yuan J, *et al.* Cyclosporin a protects against lead neurotoxicity through inhibiting mitochondrial permeability transition pore opening in nerve cells. *Neurotoxicology*. 2016;57:203–13.
162. Xi J, Wang H, Mueller RA, Norfleet EA, Xu Z. Mechanism for resveratrol-induced cardioprotection against reperfusion injury involves glycogen synthase kinase 3beta and mitochondrial permeability transition pore. *Eur J Pharmacol*. 2009;604:111–6.
163. Zhou X, Wang HY, Wu B, Cheng CY, Xiao W, Wang ZZ, *et al.* Ginkgolide K attenuates neuronal injury after ischemic stroke by inhibiting mitochondrial fission and GSK-3beta-dependent increases in mitochondrial membrane permeability. *Oncotarget*. 2017;8:44682–93.
164. Cheng A, Yang Y, Zhou Y, Maharana C, Lu D, Peng W, *et al.* Mitochondrial SIRT3 mediates adaptive responses of neurons to exercise and metabolic and excitatory challenges. *Cell Metab*. 2016;23:128–42.
165. Yang Y, Tian Y, Guo X, Li S, Wang W, Shi J. Ischemia injury induces mPTP opening by reducing Sirt3. *Neuroscience*. 2021;468:68–74.
166. Yang X, Zhang Y, Geng K, Yang K, Shao J, Xia W. Sirt3 protects against ischemic stroke injury by regulating HIF-1alpha/VEGF Signaling and blood-brain barrier integrity. *Cell Mol Neurobiol*. 2021;41:1203–15.
167. Yang X, Geng KY, Zhang YS, Zhang JF, Yang K, Shao JX, *et al.* Sirt3 deficiency impairs neurovascular recovery in ischemic stroke. *CNS Neurosci Ther*. 2018;24:775–83.
168. Vaseva AV, Marchenko ND, Ji K, Tsirka SE, Holzmann S, Moll UM. p53 opens the mitochondrial permeability transition pore to trigger necrosis. *Cell*. 2012;149:1536–48.
169. Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. *Am J Pathol*. 1995;146:3–15.
170. Loh KY, Wang Z, Liao P. Oncotic cell death in stroke. *Rev Physiol Biochem Pharmacol*. 2019;176:37–64.
171. Huang Q, Zhang R, Zou L, Cao X, Chu X. Cell death pathways in astrocytes with a modified model of oxygen-glucose deprivation. *PLoS One*. 2013;8:e61345.
172. Cao X, Zhang Y, Zou L, Xiao H, Chu Y, Chu X. Persistent oxygen-glucose deprivation induces astrocytic death through two different pathways and calpain-mediated proteolysis of cytoskeletal proteins during astrocytic oncosis. *Neurosci Lett*. 2010;479:118–22.
173. Nilius B, Prenen J, Tang J, Wang C, Owsianik G, Janssens A, *et al.* Regulation of the Ca2+ sensitivity of the nonselective cation channel TRPM4. *J Biol Chem*. 2005;280:6423–33.
174. Chen B, Ng G, Gao Y, Low SW, Sandanaraj E, Ramasamy B, *et al.* Non-invasive multimodality imaging directly shows TRPM4 inhibition ameliorates stroke reperfusion injury. *Transl Stroke Res*. 2019;10:91–103.
175. Schattling B, Steinbach K, Thies E, Kruse M, Menigoz A, Ufer F, *et al.* TRPM4 cation channel mediates axonal and neuronal

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degeneration in experimental autoimmune encephalomyelitis and multiple sclerosis. *Nat Med*. 2012;18:1805–11.
176. Wei S, Low SW, Poore CP, Chen B, Gao Y, Nilius B, *et al*. Comparison of anti-oncotic effect of TRPM4 blocking antibody in neuron, astrocyte and vascular endothelial cell under hypoxia. *Front Cell Dev Biol*. 2020;8:562584.
177. Ren KD, Liu WN, Tian J, Zhang YY, Peng JJ, Zhang D, *et al*. Mitochondrial E3 ubiquitin ligase 1 promotes brain injury by disturbing mitochondrial dynamics in a rat model of ischemic stroke. *Eur J Pharmacol*. 2019;861:172617.
178. Li Y, Sun J, Wu R, Bai J, Hou Y, Zeng Y, *et al*. Mitochondrial MPTP: a novel target of Ethnomedicine for stroke treatment by apoptosis inhibition. *Front Pharmacol*. 2020;11:352.
179. Fakharnia F, Khodagholi F, Dargahi L, Ahmadiani A. Prevention of Cyclophilin D-mediated mPTP opening using cyclosporine-a alleviates the elevation of necroptosis, autophagy and apoptosis-related markers following global cerebral ischemia-reperfusion. *J Mol Neurosci*. 2017;61:52–60.
180. Linkermann A, Brasen JH, Darding M, Jin MK, Sanz AB, Heller JO, *et al*. Two independent pathways of regulated necrosis mediate ischemia-reperfusion injury. *Proc Natl Acad Sci U S A*. 2013;110:12024–9.
181. Zhou Y, Liao J, Mei Z, Liu X, Ge J. Insight into crosstalk between Ferroptosis and necroptosis: novel therapeutics in ischemic stroke. *Oxidative Med Cell Longev*. 2021;2021:9991001.
182. Huang Y, Liu J, He J, Hu Z, Tan F, Zhu X, *et al*. UBIAD1 alleviates ferroptotic neuronal death by enhancing antioxidative capacity by cooperatively restoring impaired mitochondria and Golgi apparatus upon cerebral ischemic/reperfusion insult. *Cell Biosci*. 2022;12:42.
183. DeGregorio-Rocasolano N, Marti-Sistac O, Gasull T. Deciphering the iron side of stroke: neurodegeneration at the crossroads between iron Dyshomeostasis, Excitotoxicity, and Ferroptosis. *Front Neurosci*. 2019;13:85.
184. Nakamura T, Naguro I, Ichijo H. Iron homeostasis and iron-regulated ROS in cell death, senescence and human diseases. *Biochim Biophys Acta Gen Subj*. 2019;1863:1398–409.
185. LaRocca TJ, Seeger T, Prado M, Perea-Gil I, Neofytou E, Mecham BH, *et al*. Pharmacological silencing of MicroRNA-152 prevents pressure overload-induced heart failure. *Circ Heart Fail*. 2020;13:e006298.
186. Xu Y, Wu X, Hu W, Yu D, Shao Z, Li W, *et al*. RIP3 facilitates necroptosis through CaMKII and AIF after intracerebral hemorrhage in mice. *Neurosci Lett*. 2021;749:135699.
187. Schriewer JM, Peek CB, Bass J, Schumacker PT. ROS-mediated PARP activity undermines mitochondrial function after permeability transition pore opening during myocardial ischemia-reperfusion. *J Am Heart Assoc*. 2013;2:e000159.
188. Song X, Gong Z, Liu K, Kou J, Liu B, Liu K. Baicalin combats glutamate excitotoxicity via protecting glutamine synthetase from ROS-induced 20S proteasomal degradation. *Redox Biol*. 2020;34:101559.
189. Navarro-Yepes J, Zavala-Flores L, Anandhan A, Wang F, Skotak M, Chandra N, *et al*. Antioxidant gene therapy against neuronal cell death. *Pharmacol Ther*. 2014;142:206–30.
190. He Y, Xu Y, Chen Z, He B, Quan Z, Zhang R, *et al*. Protective effect of Mitochondrially targeted peptide against oxidant injury of cone photoreceptors through preventing necroptosis pathway. *J Biomed Nanotechnol*. 2021;17:279–90.
191. Lu B, Gong X, Wang ZQ, Ding Y, Wang C, Luo TF, *et al*. Shikonin induces glioma cell necroptosis in vitro by ROS overproduction and promoting RIP1/RIP3 necrosome formation. *Acta Pharmacol Sin*. 2017;38:1543–53.
192. Sun Y, Yu J, Liu X, Zhang C, Cao J, Li G, *et al*. Oncosis-like cell death is induced by berberine through ERK1/2-mediated impairment of mitochondrial aerobic respiration in gliomas. *Biomed Pharmacother*. 2018;102:699–710.
193. Tian Q, Qin B, Gu Y, Zhou L, Chen S, Zhang S, *et al*. ROS-mediated necroptosis is involved in iron overload-induced osteoblastic cell death. *Oxidative Med Cell Longev*. 2020;2020:1295382.
194. Gao L, Qin JX, Shi JQ, Jiang T, Wang F, Xie C, *et al*. Fine particulate matter exposure aggravates ischemic injury via NLRP3 inflammasome activation and pyroptosis. *CNS Neurosci Ther*. 2022;28:1045–58.
195. Zhu P, Hu S, Jin Q, Li D, Tian F, Toan S, *et al*. Ripk3 promotes ER stress-induced necroptosis in cardiac IR injury: a mechanism involving calcium overload/XO/ROS/mPTP pathway. *Redox Biol*. 2018;16:157–68.
196. Chen Y, Mi Y, Zhang X, Ma Q, Song Y, Zhang L, *et al*. Dihydroartemisinin-induced unfolded protein response feedback attenuates ferroptosis via PERK/ATF4/HSPA5 pathway in glioma cells. *J Exp Clin Cancer Res*. 2019;38:402.
197. Jacobson J, Duchen MR. Mitochondrial oxidative stress and cell death in astrocytes–requirement for stored Ca2+ and sustained opening of the permeability transition pore. *J Cell Sci*. 2002;115:1175–88.
198. Tian J, Guo S, Chen H, Peng JJ, Jia MM, Li NS, *et al*. Combination of Emricasan with Ponatinib synergistically reduces ischemia/reperfusion injury in rat brain through simultaneous prevention of apoptosis and necroptosis. *Transl Stroke Res*. 2018;9:382–92.

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