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# Research progress of deubiquitinating enzymes in cerebral ischemia-reperfusion injury

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Cerebral ischemia-reperfusion injury (CIRI) is a critical pathological process driving neurological deterioration following ischemic stroke, involving multifaceted mechanisms such as inflammatory cascades, oxidative stress, and programmed cell death (PCD). Deubiquitinases (DUBs), as key regulators of the ubiquitin-proteasome system, dynamically modulate protein stability, signal transduction, and subcellular localization through editing the ubiquitin code, exhibiting dual roles in CIRI—both as drivers of pathogenesis and potential therapeutic targets. This review systematically elucidates the core regulatory mechanisms of DUBs in CIRI: (i) suppression of neuroinflammation via modulation of NLRP6/NF- $\kappa$ B pathways; (ii) mitigation of oxidative stress through the KEAP1-NRF2 axis and mitochondrial quality control; and (iii) neuroprotection by intercepting necroptosis, ferroptosis, and other PCD pathways. We further reveal that CIRI disrupts DUBs functionality through a tripartite mechanism—transcriptional dysregulation, catalytic inactivation, and subcellular mislocalization—transforming DUBs from guardians of homeostasis into mediators of injury. Consequently, DUBs-targeted strategies, including small-molecule inhibitors (e.g., IU1, Vialinin A), genetic editing approaches (e.g., BRCC3 silencing, A20 overexpression), and exosome-based delivery systems (e.g., the KLF3-AS1/miR-206/USP22 axis), demonstrate significant neuroprotective potential. However, challenges persist, such as substrate specificity, ubiquitin chain-type dependency, and barriers to clinical translation. Future research must integrate multi-omics technologies, develop brain-targeted delivery platforms, and explore synergistic effects of DUBs modulation with existing therapies to advance precision medicine in stroke treatment.

## KEYWORDS

cerebral ischemia-reperfusion injury (CIRI), deubiquitinases (DUBs), ischemic stroke, neuroinflammation, oxidative stress, programmed cell death (PCD), ubiquitin-proteasome system, targeted therapy

## 1 Introduction

Stroke is defined as a disease that causes damage to the blood vessels of the brain due to a variety of etiologies, resulting in the necrosis of brain cells and tissues. It ranks as the third leading cause of death in our country, following heart disease and cancer. As of 2023, the incidence of stroke in China stands at  $\sim$ 246.8 per 100,000 population, with a mortality rate of around 149.5 per 100,000. The economic burden of stroke has risen

substantially, with average annual treatment costs exceeding 100,000 yuan (Tu and Wang, 2023). Ischemic stroke (IS), accounting for 75–90% of cerebrovascular events (Jing et al., 2023), is characterized by abrupt blood flow cessation leading to neuronal necrosis within minutes. While reperfusion therapies (thrombolysis/thrombectomy) remain cornerstone treatments, cerebral ischemia-reperfusion injury (CIRI) paradoxically exacerbates damage through inflammatory cascades, oxidative stress, and programmed cell death (PCD; Cheng et al., 2021; Hecht et al., 2021; Campbell and Khatri, 2020).

Ubiquitin is a highly conserved 76-amino acid protein that can covalently conjugate to substrate proteins (a process termed ubiquitination; Hershko and Ciechanover, 1998). Ubiquitin molecules can form functionally distinct chains through linkages via any of their seven lysine residues (K6, K11, K27, K29, K33, K48, K63) or the N-terminal methionine. Among these, K48-linked polyubiquitin chains primarily target proteins for proteasomal degradation, whereas K63-linked chains predominantly regulate signal transduction and protein interactions without directly promoting degradation (Komander and Rape, 2012). Ubiquitin modification, a highly conserved regulatory mechanism across eukaryotes, plays a crucial role in controlling protein stability, activity, and subcellular localization through diverse cellular processes (Muratani and Tansey, 2003; Kim et al., 2011). Deubiquitinases (DUBs) regulate protein degradation and signal transduction by removing ubiquitin modifications from proteins. These enzymes are classified into two main categories: cysteine proteases and metalloproteinases (Amerik and Hochstrasser, 2004). The former can be categorized into several distinct protein families based on sequence conservation and domain structure. These include the ubiquitin C-terminal hydrolase (UCH), ubiquitin-specific protease (USP), ovarian tumor protease (OTU), and

Machado-Josephin disease protease (MJD) families. Additionally, the K48 polyubiquitin-specific MINDY domain family (MINDY) and the recently identified zinc fingers with UFM1-specific peptidase (ZUP1) domains represent emerging classes of DUBs (Li and Reverter, 2021). The latter consists of zinc-dependent enzymes with JAB1/MPN/MOV34 (JAMM/MPN+) domains.

Protein homeostasis collapse represents a hallmark of CIRI, wherein dysregulated ubiquitination drives pathological progression (Liu et al., 2020; Hochrainer, 2018). As critical regulators of the ubiquitin signaling network, DUBs orchestrate essential cellular processes including: ubiquitin recycling and proteostasis (Park and Ryu, 2014; Guo et al., 2024; Vostal et al., 2025), membrane trafficking and receptor regulation (Clague and Urbé, 2017; Cheng and Guggino, 2013), DNA repair and translation (Foot et al., 2017; Kee and Huang, 2016), cell cycle control and autophagy (Park et al., 2019; Jacomin et al., 2018), and mitochondrial quality control under homeostatic conditions (Bingol et al., 2014; Escobar-Henriques, 2014; Marcassa et al., 2018; Gersch et al., 2017). Notably, recent breakthrough research reveals that CIRI subverts DUBs functionality through three synergistic mechanisms: (1) transcriptional chaos, characterized by polarized DUBs dysregulation in ischemic cores (e.g., BRCC3 upsurge vs. USP16 downregulation; Huang X. et al., 2021; Tong et al., 2023); (2) catalytic crippling via ROS-mediated oxidation of essential catalytic cysteine residues (Forman et al., 2017); and (3) spatial disarray exemplified by USP30 displacement from mitochondrial outer membranes (Chen et al., 2021). This pathogenic trilogy transforms DUBs from vigilant guardians of proteostasis into active drivers of neuronal injury—a fundamental paradigm shift underscoring their dual nature as both instigators of CIRI pathogenesis and promising therapeutic targets for intervention.

This review systematically elucidates the regulatory roles of DUBs in CIRI through three pivotal pathological axes: (i) orchestration of inflammatory response and neuroprotection, (ii) modulation of oxidative stress defense systems, and (iii) intervention of PCD pathways. By comprehensively dissecting the molecular mechanisms of key DUBs (e.g., USP16, A20, OTUD1) and their spatiotemporal dynamics in CIRI, we not only advance the mechanistic understanding of stroke pathogenesis but also provide a conceptual framework for developing DUBs-targeted therapeutic strategies.

## 2 General functions of DUBs in cellular homeostasis

### 2.1 Ubiquitin recycling and proteostasis

DUBs play a crucial role in maintaining the cellular ubiquitin pool by recycling and generating free ubiquitin molecules, which are essential for protein degradation and signaling pathways (Park and Ryu, 2014). Protein ubiquitination and deubiquitination are important forms of post-translational modification. Ubiquitination involves the regulation of protein stability or activity through ubiquitin ligases, which attach ubiquitin chains to proteins, marking them for degradation.

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**Abbreviations:** IS, ischemic stroke; CIRI, cerebral ischemia-reperfusion injury; DUBs, deubiquitinases; UCH, ubiquitin C-terminal hydrolase; USP, ubiquitin-specific protease; OTU, ovarian tumor protease; MJD, Machado-Josephin disease protease family; MINDY, K48 polyubiquitin-specific MINDY domain family; ZUP1, zinc fingers with UFM1-specific peptidase; JAMM, JAB1/MOV34/MPN1 metalloproteases; BRCC3, BRCA1-BRCA2-containing complex subunit 3; EGFR, epidermal growth factor receptor; MDM2, murine double minute 2; RTU, redox control of translation by ubiquitin; ROS, reactive oxygen species; E3, ubiquitin-protein ligase; mTOR, mammalian target of rapamycin; DEPTOR, DEP domain-containing mTOR-interacting protein; CYLD, cylindromatosis; NLRP6, NOD-like receptor family pyrin domain containing 6; NRF2, nuclear factor erythroid 2-related factor 2; KEAP1, Kelch-like ECH-associated protein 1; MFN2, mitofusin 2; TRAF6, TNF receptor-associated factor 6; RIP1, receptor-interacting protein kinase 1; TRAF2, TNF receptor-associated factors 2; OTUD1, OTU domain-containing protein 1; RIPK2/RIP2, receptor-interacting serine/threonine-protein kinase 2; TLR, Toll-like receptor; TAB2, TAK1-binding protein 2; TAK1, TGF- $\beta$ -activated kinase 1; ceRNA, competitive endogenous RNA; SIRT1, stabilizes sirtuin 1 SIRT1; REST, RE-1 silencing transcription factor; HO-1, heme oxygenase-1; ARE, antioxidant response element; NOX4, NADPH oxidase 4; RIP3, receptor-interacting protein kinase 3; mTORC1, mammalian target of rapamycin complex 1; TFEB, transcription factor EB; NCOA4, nuclear receptor coactivator 4.

Deubiquitination, a crucial post-translational modification process, functions by either modifying ubiquitin molecules and chains or removing polyubiquitin chains from target proteins, thereby counteracting protein ubiquitination and subsequent degradation (Guo et al., 2024; Vostal et al., 2025).

## 2.2 Membrane trafficking and receptor regulation

At the plasma membrane, ubiquitination serves as a critical regulatory mechanism for protein endocytosis, a cellular process whereby vesicles form to internalize extracellular materials and cell-surface proteins. This regulation can be achieved through the ubiquitination of either cargo proteins directly or their associated adaptor proteins, thereby modulating both the efficiency and specificity of the endocytic pathway (Clague and Urbé, 2017). The ubiquitinated cargo undergoes transport to endosomes, lysosomes, or the endoplasmic reticulum for processing or degradation (Cheng and Guggino, 2013; Fujita et al., 2013; Haglund et al., 2003; Piper et al., 2014). In these organelles, DUBs can remove ubiquitin tags from cargo proteins, preventing their degradation and enabling their recycling to cellular surfaces or other destinations (Balut et al., 2011; Clague and Urbé, 2006). Ubiquitination plays a crucial role in regulating cell surface receptor dynamics through its dual control of receptor endocytosis and signal transduction. For example, epidermal growth factor receptor (EGFR), upon ligand binding, is ubiquitinated and internalized into endosomes, thereby terminating its signaling. USP8 prevents EGFR degradation by removing ubiquitin tags, thus facilitating EGFR recycling to the cell surface and sustaining signal duration (Berlin et al., 2010; Hurley and Stenmark, 2011).

## 2.3 DNA repair and translation

DUBs regulate DNA damage repair mechanisms to ensure their availability during cellular stress and damage responses (Foot et al., 2017; Kee and Huang, 2016). Under normal conditions, phosphorylated USP7 stabilizes MDM2, which then promotes p53 degradation through ubiquitination (Sarkari et al., 2010; Sheng et al., 2006). Upon cellular stress, USP7 dephosphorylation causes murine double minute 2 (MDM2) destabilization, resulting in p53 accumulation (Khoronenkova et al., 2012). DUBs also regulate protein translation by modifying ribosomal ubiquitination (Kapadia and Gartenhaus, 2019). DUBs play a crucial role in regulating global protein synthesis. A newly discovered regulatory mechanism in yeast, called redox control of translation by ubiquitin (RTU), involves ribosomal DUBs (Dougherty et al., 2020). Ubp2 (a ubiquitinating enzyme) at the center of the pathway is reversibly inhibited by reactive oxygen species (ROS) during oxidative stress in RTU. This inhibition results in the accumulation of K63-linked polyubiquitin chains on the ribosome, catalyzed by ubiquitin-conjugating enzymes Rad6 and ubiquitin-protein ligase (E3) Bre1, which subsequently blocks translation elongation (Simões et al., 2022).

## 2.4 Cell cycle control and autophagy

Not only that, DUBs also play crucial roles in mitosis, as exemplified by USP7, which controls G1-S and G2-M checkpoints through claspin deubiquitination. USP16 controls chromosome segregation and alignment through its deubiquitinating activity on histones H2A and PLK1, thereby regulating G0 and early M phase. Independently of the spindle assembly checkpoint, USP28 modulates the G1-S checkpoint by deubiquitinating p53 (Park et al., 2019).

In addition, DUBs can also participate in the regulation of autophagy. For example, during macroautophagy, double-membrane phagosomes and autophagosomes initially form within the cytoplasm. The mammalian target of rapamycin (mTOR) primarily regulates phagosome formation, while DEP domain-containing mTOR-interacting protein (DEPTOR) acts as its negative regulator by inhibiting mTOR kinase activity. Under normal conditions, DEPTOR undergoes continuous degradation through the ubiquitin-proteasome system. During nutrient deprivation, OTUB1 prevents DEPTOR degradation, which is essential for mTOR inactivation (Jacomin et al., 2018).

## 2.5 Mitochondrial quality control under homeostatic conditions

USP30, a predominantly mitochondria-localized deubiquitinase anchored to the outer membrane, plays pivotal roles in homeostatic mitochondrial quality control through the following mechanisms. (1) Negative Regulation of PINK1/Parkin-Mediated Mitophagy: USP30 counteracts ubiquitination of outer membrane proteins (e.g., TOM20, MFN2), thereby suppressing excessive mitophagy initiation (Bingol et al., 2014). (2) Modulation of Mitochondrial Dynamics: By deubiquitinating the fusion GTPase MFN1, USP30 preserves mitochondrial fusion capacity, ensuring network homeostasis (Escobar-Henriques, 2014; Yue et al., 2014). (3) Oxidative Stress Defense: USP30 stabilizes mitochondrial antioxidant proteins (e.g., SOD2), mitigating ROS accumulation (Marcassa et al., 2018). (4) Energy Metabolism Maintenance: Through deubiquitination of electron transport chain subunits (e.g., NDUF10), USP30 safeguards respiratory chain integrity and oxidative phosphorylation efficiency (Gersch et al., 2017).

While these general functions maintain cellular homeostasis, DUBs exhibit context-specific roles under pathological conditions such as CIRI, as discussed below.

## 3 Regulatory mechanisms of DUBs in IS: from homeostasis to pathology

### 3.1 Physiological regulation of DUBs activity

The activity of DUBs is tightly controlled through three principal regulatory strata: post-translational modifications, protein-protein interactions, and transcriptional networks,

collectively maintaining cellular homeostasis. At the post-translational level, phosphorylation emerges as a dominant regulatory switch; USP14, for example, acquires full catalytic competence only through dual regulation by proteasomal association and AKT-dependent phosphorylation (Wang et al., 2021), whereas cylindromatosis (CYLD, a USP-family deubiquitinase) undergoes functional silencing via Ser418 phosphorylation (Hutti et al., 2009). The regulatory landscape extends to redox control, where ROS induce transient catalytic inactivation of cysteine-dependent DUBs (including USP and OTU family members) through reversible oxidation of the active-site cysteine to disulfide states (Clague, 2013; Cotto-Rios et al., 2012; Kulathu et al., 2013; Lee et al., 2013). Structural orchestration plays an equally critical role—USP14 and UCHL5 achieve functional activation exclusively through incorporation into the 19S proteasomal regulatory complex (de Poot et al., 2017), while WDR48/WDR20 allosterically activates USP46 via precise  $\beta$ -propeller domain engagement (Cohn et al., 2009; Kee et al., 2010; Yin et al., 2015). Complementing these mechanisms, an intricate layer of transcriptional governance exists, featuring miRNA-directed mRNA degradation (notably miR-124-mediated USP14 suppression) and lncRNA-operated competitive endogenous RNA (ceRNA) networks (exemplified by KLF3-AS1's sequestration of miR-206 to derepress USP22 expression; Song et al., 2019; Xie et al., 2023).

### 3.2 Dysregulation of DUBs in CIRI

CIRI disrupts DUBs regulatory networks through three principal mechanisms: (i) Transcriptional dysregulation, where ischemic stress induces BRCA1-BRCA2-containing complex subunit 3 (BRCC3, a JAMN domain-containing  $Zn^{2+}$ -metalloprotease deubiquitinating enzyme) upregulation to activate the NOD-like receptor family pyrin domain containing 6 (NLRP6) inflammasome (Huang X. et al., 2021), while nuclear factor erythroid 2-related factor 2 (NRF2)-mediated negative feedback suppresses USP16 expression, impairing Kelch-like ECH-associated protein 1 (KEAP1) degradation (Tong et al., 2023); (ii) Catalytic impairment, as ROS accumulation causes irreversible oxidation of UCH-family DUBs (Forman et al., 2017; Kahles et al., 2021; Defelipe et al., 2015; Devarie-Baez et al., 2016), and energy depletion reduces AKT-mediated activating phosphorylation of USP14 (Wang et al., 2021); (iii) Subcellular mislocalization, exemplified by USP30 dissociation from mitochondrial membranes, leading to pathological mitofusin 2 (MFN2) hyperubiquitination and mitochondrial fragmentation. These coordinated perturbations collectively compromise the homeostatic regulatory functions of DUBs (Chen et al., 2021).

As reviewed, the functional impact of DUBs in CIRI is governed by three fundamental determinants: substrate specificity, ubiquitin chain selectivity, and spatiotemporal regulation. Under physiological conditions, DUB activity is precisely constrained within homeostatic boundaries. However, CIRI induces three distinct pathological cascades: (i) ROS-mediated oxidation inactivates cysteine-dependent DUBs (e.g., UCHL1); (ii) bioenergetic failure impairs kinase-dependent DUB activation (e.g.,

the AKT-USP14 axis); and (iii) transcriptional reprogramming alters DUB expression profiles (e.g., NF- $\kappa$ B-driven BRCC3 upregulation). This triad of disturbances transforms DUBs from homeostatic guardians to disease modifiers, capable of either exacerbating injury (as demonstrated by USP16/KEAP1-mediated NRF2 suppression) or facilitating recovery (exemplified by OTUD1-RIP2's anti-inflammatory activity). Given their pleiotropic regulation of core CIRI pathways—including inflammatory responses, oxidative stress, and programmed cell death—the mechanistic dissection of DUB networks warrants systematic investigation.

## 4 Mechanisms of DUBs in CIRI

Recent studies have shown that DUBs play an important regulatory role in CIRI, affecting the survival, death, and repair of neurons (Liu et al., 2020; Hochrainer, 2018; Chen et al., 2020). DUBs modulate the inflammatory response by suppressing the release of inflammatory mediators, thereby mitigating cellular damage. Additionally, these enzymes regulate oxidative stress responses following CIRI by reducing free radical production, thus protecting cells from oxidative damage. Furthermore, DUBs contribute to cellular protection through their involvement in PCD, specifically by regulating processes such as ferroptosis and mitophagy (Zhang et al., 2023; Marques et al., 2019; Lu et al., 2021; Jayaraj et al., 2019).

### 4.1 Inflammatory responses modulation and neuroprotection

The inflammatory response in CIRI is a complex process involving the activation and regulation of multiple signaling pathways. The NLRP6 inflammasome, NF- $\kappa$ B, and JAK/STAT signaling pathways are critical in mediating inflammatory responses during cerebral ischemia-reperfusion injury (He et al., 2024; Nazarinia et al., 2021; Zhao et al., 2022; Zhu et al., 2021). In recent years, it has been found that DUBs have an important impact on the activation and inhibition of some important signaling pathways.

NLRP6, a recently identified member of the NLP family, exacerbates neuronal damage following oxygen-glucose deprivation through inflammasome activation and apoptosis induction (Wang et al., 2015; Zhang et al., 2020). This pathway is significantly affected by DUBs during ischemia-reperfusion. For example, BRCC3 regulates multiple cellular processes, including cell cycle control, DNA repair, and immune responses (Sun et al., 2016; Wang et al., 2019). In the context of CIRI, an augmentation in BRCC3 expression was observed, which concomitantly exhibited co-localization with NLRP6 (Huang X. et al., 2021). Concurrently, the BRCC3 influence on the ubiquitination of NLRP6 and the promotion of NLRP6 inflammasome assembly was also evident (Huang et al., 2024).

DUBs also have been shown to play a pivotal role in the NF- $\kappa$ B-related signaling pathway. NF- $\kappa$ B, functioning as a transcription factor, has the capacity to regulate the expression of a multitude of inflammation-related genes (Lawrence, 2009).

Harhaj and Dixit systematically elucidated the regulatory mechanisms of A20 and CYLD on NF- $\kappa$ B signaling (Harhaj and Dixit, 2012). As an OTU-family member, A20 (TNFAIP3) employs its distinctive OTU domain to cleave K63-linked ubiquitin chains while utilizing zinc finger domains (ZnF4-7) to catalyze K48-linked polyubiquitination, thereby facilitating proteasomal degradation of key signaling proteins including TNF receptor-associated factor 6 (TRAF6) and receptor-interacting protein kinase 1 (RIP1; Wertz et al., 2004). This dual enzymatic activity establishes a negative feedback loop for NF- $\kappa$ B signaling (Wertz et al., 2004)—a mechanism corroborated by sustained NF- $\kappa$ B activation and pronounced inflammatory phenotypes in A20-knockout mice (Lee et al., 2000). Zhao and colleagues further demonstrated that mesencephalic astrocyte-derived neurotrophic factor promotes microglial polarization toward an anti-inflammatory phenotype via the A20/NF- $\kappa$ B axis, significantly mitigating cerebral ischemia-reperfusion injury in murine models (Zhao et al., 2024). Complementary work by Zhan's group revealed that neuron-specific A20 upregulation effectively suppresses NF- $\kappa$ B-mediated neuroinflammatory damage in rat ischemia-reperfusion models (Zhan et al., 2016). Collectively, these studies establish A20 as a master regulator of neuroinflammatory responses. CYLD, negatively regulates NF- $\kappa$ B signaling by removing K63-linked polyubiquitin chains from key mediators including TNF receptor-associated factors 2 and 6 (TRAF2/6; Trompouki et al., 2003). Emerging evidence demonstrates that CYLD upregulation attenuates inflammatory damage in CIRI through dual mechanisms: (1) direct suppression of NF- $\kappa$ B transcriptional activity (Jiang et al., 2017), and (2) inhibition of NLRP3 inflammasome activation, thereby preventing pathological microglial overactivation and subsequent neuroinflammatory injury in the ischemic cortex (Lin et al., 2021).

OTU domain-containing protein 1 (OTUD1), a crucial member of the OTU protease family, plays a pivotal role in modulating the ubiquitination status of receptor-interacting serine/threonine-protein kinase 2 (RIPK2/RIP2). As a downstream effector of both nucleotide-binding oligomerization domain-containing proteins 1/2 and Toll-like receptor (TLR) signaling pathways (Honjo et al., 2021; Liu et al., 2015), RIP2 undergoes ubiquitination that potently enhances NF- $\kappa$ B-mediated inflammatory signaling (Annibaldi and Meier, 2018). Mechanistic studies demonstrate that during CIRI, OTUD1 directly interacts with either the kinase domain or caspase activation and recruitment domain of RIP2, selectively cleaving its K63-linked polyubiquitin chains. This deubiquitination event effectively suppresses NF- $\kappa$ B pathway activation, thereby attenuating neuroinflammation and ischemic brain damage. Importantly, genetic ablation of OTUD1 exacerbates RIP2 ubiquitination, leading to aggravated cerebral ischemic injury (Zheng et al., 2023). These findings establish the OTUD1-RIP2 axis as a critical regulatory mechanism in CIRI pathogenesis.

TAK1-binding protein 2 (TAB2), a pivotal scaffold protein, orchestrates innate immune responses, inflammatory signaling, and cellular stress reactions by facilitating the assembly of the TGF- $\beta$ -activated kinase 1 (TAK1) complex, thereby precisely controlling NF- $\kappa$ B pathway activation (Wang et al., 2001; Takaesu

et al., 2000). Recent studies demonstrate that the USP25 exerts anti-inflammatory effects during stroke by selectively cleaving K63-linked ubiquitin chains from TAB2, consequently attenuating NF- $\kappa$ B activation and mitigating microglial inflammation (Li Z. et al., 2023).

Furthermore, a pivotal study has elucidated the dual regulatory mechanism of USP8 in neuroinflammatory modulation: in LPS-induced neuroinflammation models, USP8 suppresses the TLR4/MyD88/NF- $\kappa$ B signaling cascade, concurrently inhibiting pro-inflammatory (M1) microglial activation while promoting anti-inflammatory (M2) polarization (Zhao et al., 2020). Functional validation in murine models shows that USP8 overexpression rescues LPS-induced cognitive deficits and motor coordination impairment, whereas USP8 depletion exacerbates neuroinflammatory damage (Zhao et al., 2020). These discoveries not only elucidate USP8's master regulatory role in neuroinflammation but also identify novel therapeutic targets for neurodegenerative disorders.

While USP22 expression is upregulated following CIRI, its biological functions and underlying mechanisms remain incompletely characterized. A groundbreaking study reveals that bone marrow mesenchymal stem cell-derived exosomes deliver long non-coding RNA KLF3-AS1, which functions as a ceRNA to sequester miR-206, thereby relieving miR-206-mediated post-transcriptional repression of USP22 (Xie et al., 2023). The upregulated USP22 in turn stabilizes sirtuin 1 (SIRT1) protein levels through deubiquitination—this NAD<sup>+</sup>-dependent deacetylase executes multifaceted neuroprotective effects, including anti-inflammatory, antioxidant, and anti-apoptotic activities, ultimately improving CIRI outcomes (Xie et al., 2023). This work establishes the KLF3-AS1/miR-206/USP22/SIRT1 axis as a previously unrecognized regulatory pathway in ischemic brain injury.

Emerging evidence indicates that USP14 exerts detrimental effects in ischemic brain injury. A seminal study by Hou et al. (2023) delineates the dual neuroprotective mechanisms of USP14 inhibition in CIRI: (i) At the vascular level, it stabilizes tight junction proteins (occludin and claudin-5) to maintain blood-brain barrier integrity, effectively mitigating vasogenic edema; (ii) In inflammatory modulation, it suppresses microglial hyperactivation and the NF- $\kappa$ B signaling pathway, significantly reducing the release of pro-inflammatory cytokines including TNF- $\alpha$  and IL-6. These findings validate USP14-specific inhibitors (e.g., IU1) as promising therapeutic agents for IS. Notably, follow-up research reveals that microRNA-124 (miR-124) binds the 3'-untranslated region (3'-UTR) of Usp14 mRNA to suppress its expression, thereby attenuating USP14-mediated deubiquitination of RE-1 silencing transcription factor (REST). This promotes REST degradation via the ubiquitin-proteasome system, ultimately relieving REST-mediated transcriptional repression of neuroprotective genes. In focal cerebral ischemia models, this miR-124/Usp14/REST axis demonstrates remarkable neuroprotection, characterized by reduced infarct volume and improved neurological function (Doepfner et al., 2013). This work not only elucidates a novel regulatory axis in ischemic stroke pathogenesis but also provides a theoretical foundation for developing miRNA-based neuroprotective strategies.

Convergent studies have established the pivotal role of ubiquitin C-terminal hydrolase L1 (UCHL1) in axonal preservation and functional recovery following cerebral ischemia, though distinct research groups have elucidated complementary facets of its activity. The Liu group demonstrated that UCHL1 maintains axonal integrity and synaptic plasticity through deubiquitination of structural proteins (e.g., neurofilaments) and synaptic scaffolds (e.g., postsynaptic density protein 95, PSD-95)—genetic ablation exacerbates post-ischemic axonal degeneration and motor deficits (Liu et al., 2019). Complementarily, the Mi group identified the hydrolase activity of UCHL1 as indispensable for neuroprotection: the C90S active-site mutation disrupts ubiquitin homeostasis, precipitating mitochondrial dysfunction and impaired axonal transport, thereby aggravating ischemic injury—a finding that underscores the therapeutic relevance of targeting UCHL1's enzymatic function (Mi et al., 2024). Collectively, UCHL1 orchestrates dual neuroprotective roles in CIRI by modulating the ubiquitin-proteasome system: (1) structural preservation of axonal architecture and (2) functional restoration of neural circuits. These insights posit UCHL1 activators as promising candidates for IS intervention. Notably, whether deubiquitinases engage the JAK/STAT signaling pathway to regulate inflammatory responses in CIRI remains an open question, warranting systematic investigation.

Collectively, these findings position DUBs as master regulators of neuroinflammatory and neuroprotective responses in CIRI, operating through: (i) direct ubiquitin code editing of inflammasome components (BRCC3-NLRP6, CYLD-NLRP3), (ii) multi-layered control of NF- $\kappa$ B signaling (A20/TRAF6, CYLD-TRAF2/6, OTUD1/RIP2, USP25/TAB2), (iii) dynamic modulation of microglial polarization states (USP8-TLR4, USP14-REST), and critically, (iv) axonal integrity preservation via UCHL1-mediated stabilization of synaptic scaffolds (PSD-95/neurofilaments), offering a compelling therapeutic paradigm for targeted immunomodulation in IS. While DUBs modulate inflammatory responses and neuroprotection, their impact on oxidative stress is equally critical, as discussed below.

## 4.2 Oxidative stress modulation

In addition to the inflammatory responses and neuroprotection, DUBs also have been demonstrated to play a very important role in the oxidative stress response after ischemia-reperfusion injury. In IS, heme oxygenase-1 (HO-1) has been found to be highly expressed in the injured brain area, indicating the activation of anti-oxidative stress (Beschorner et al., 2000). Previous studies have shown that KEAP1-NRF2-antioxidant response element (ARE) signaling pathway also plays a very important role in regulating oxidative stress. Under physiological conditions, KEAP1 forms ubiquitin E3 ligase complexes (KEAP1-CUL3) with CULLIN3 (CUL3) and makes NRF2 poly-ubiquitination (K48-linked), resulting in the rapid degradation of NRF2 by the proteasome system. Under oxidative stress (e.g., CIRI), ROS modify critical cysteine residues in KEAP1 (e.g., Cys151), disrupting the KEAP1-CUL3 complex and inhibiting NRF2 ubiquitination. This stabilizes NRF2, allowing

its nuclear translocation to activate antioxidant genes (e.g., HO-1, NQO1; Chan et al., 2021; Rao et al., 2022; Zhao et al., 2018; Lu et al., 2016; Yamamoto et al., 2018). In this process, the study by Tong et al. (2023) reveals that USP16 specifically recognizes the double-glycine repeat domain of KEAP1, selectively cleaving its K48-linked ubiquitin chains to prevent proteasomal degradation and consequently enhance KEAP1 protein stability. Genetic ablation of USP16 significantly ameliorates ischemia-reperfusion-induced cellular damage and inflammatory responses. Mechanistically, nuclear-translocated NRF2 directly binds to the USP16 promoter, establishing a KEAP1-NRF2-USP16 negative feedback loop that refines our understanding of the KEAP1-NRF2-ARE signaling network. In a parallel regulatory mechanism, Mao et al. (2024) demonstrate that USP4 similarly modulates KEAP1 deubiquitination: USP4 overexpression reduces KEAP1 ubiquitination, whereas USP4 knockdown promotes KEAP1 ubiquitination and subsequent degradation. Importantly, pharmacological inhibition of USP4 activity enhances KEAP1 ubiquitination, triggering NRF2-dependent antioxidant responses and conferring robust neuroprotection in stroke models—evidenced by reduced neuronal apoptosis and improved functional recovery.

Accumulating evidence highlights the pivotal role of DUBs in modulating oxidative stress responses in diverse tissues. In hepatic ischemia-reperfusion injury, CYLD physically interacts with NADPH oxidase 4 (NOX4) and enhances NRF2 signaling through NOX4 deubiquitination, thereby mitigating stress-induced hepatic inflammation and programmed cell death (Zhan et al., 2022). Parallel studies demonstrate that USP25 directly binds KEAP1 and impedes its ubiquitin-dependent degradation (Cai et al., 2023), mirroring the regulatory mechanism employed by USP16.

Furthermore, structural analyses reveal that OTUD1 orchestrates cellular responses to oxidative stress and apoptosis via its dedicated KEAP1-binding domain in a ROS-sensitive manner. Of particular note, USP13 overexpression in osteoarthritis models exerts dual antioxidant effects by simultaneously stabilizing NRF2 protein levels and suppressing caspase-3 activation, resulting in markedly reduced ROS accumulation (Huang J. et al., 2021).

Collectively, the identification of the USP16/KEAP1 and USP4/KEAP1 regulatory axes demonstrates that targeted inhibition of specific DUBs can potentiate the NRF2-mediated endogenous antioxidant defense system. This discovery suggests that selective USP16/USP4 inhibitors may synergize with existing ROS scavengers to develop novel combinatorial therapeutic strategies for stroke. Importantly, the spatiotemporal regulation of distinct DUBs in stroke-associated oxidative stress requires systematic elucidation to inform the development of precision therapeutic interventions.

## 4.3 Programmed cell death control

Cerebral ischemia-reperfusion triggers extensive neuronal death, resulting in neurological dysfunction and cognitive impairment following vascular recanalization (Feigin et al., 2017). Central to this pathological cascade is PCD—a process wherein

cells actively initiate self-elimination through tightly regulated signaling pathways (e.g., apoptosis, necroptosis, autophagy, pyroptosis, and ferroptosis) upon specific stimuli. Crucially, these PCD modalities exhibit dual regulatory roles in CIRI: while their controlled activation clears damaged cells and mitigates secondary necrosis/inflammation, dysregulated execution exacerbates neuronal injury and functional deficits (Lu et al., 2021; Mashimo et al., 2019; Yang et al., 2014, 2017; Zille et al., 2017).

Recent studies have established that DUBs critically modulate PCD pathways in stroke pathophysiology. The anti-inflammatory protein A20 confers cerebral ischemic tolerance by selectively removing K63-linked ubiquitination from receptor-interacting protein kinase 3 (RIP3), thereby suppressing necroptotic signaling and bidirectionally regulating microglial/macrophage polarization—inhibiting pro-inflammatory M1 phenotype while promoting anti-inflammatory M2 transition (Onizawa et al., 2015; Qiu et al., 2024). These findings not only elucidate the pivotal role of the A20-RIP3 axis in neuroinflammation and cell death but also propose novel therapeutic strategies targeting necroptosis and immune microenvironment remodeling.

In mitochondrial quality control, the mitochondria-localized USP30 maintains network integrity by stabilizing key proteins like MFN2 through deubiquitination. During CIRI, USP30 prevents excessive mitochondrial fission and fragmentation by inhibiting ubiquitin-mediated MFN2 degradation. Functional studies demonstrate that USP30 overexpression preserves mitochondrial function and reduces neuronal apoptosis, whereas its knockout exacerbates injury (Chen et al., 2021), positioning the USP30-MFN2 axis as a promising target for mitochondrial therapy.

Beyond its established anti-inflammatory and antioxidant roles, USP22 orchestrates autophagy through the newly identified USP22-PTEN-mTOR-transcription factor EB (TFEB) axis: USP22 inhibition accelerates PTEN degradation, relieving its suppression on mammalian target of rapamycin complex 1 (mTORC1), which subsequently activates TFEB to enhance lysosomal biogenesis and autophagic flux, ultimately improving neurological recovery (Li Y. et al., 2023). Parallely, OTUB1 maintains metabolic homeostasis by stabilizing DEPTOR, an endogenous mTORC1 inhibitor. Intriguingly, pathogens like Salmonella hijack host OTUB1 (via bacterial E3 ligase SopA) to manipulate the DEPTOR-mTORC1 axis for autophagy induction and immune evasion (Ma et al., 2024)—though whether this pathway mediates autophagy in CIRI awaits investigation.

Emerging research establishes DUBs as pivotal modulators of ferroptosis. The Li group demonstrated that in ischemic stroke models, nuclear receptor coactivator 4 (NCOA4) drives ferroptosis by mediating ferritinophagy—a selective autophagic process that degrades ferritin, leading to free iron release and lipid peroxidation (Li et al., 2021). Crucially, USP14 directly interacts with NCOA4 and stabilizes it through deubiquitination, thereby amplifying NCOA4-mediated ferritinophagy and exacerbating ferroptotic neuronal death. Pharmacological inhibition of USP14 reduces NCOA4 protein levels, significantly attenuating both ferroptosis and cerebral ischemic injury (Li et al., 2021). This work not only delineates the NCOA4-ferritinophagy-ferroptosis axis but also identifies the USP14-NCOA4 interaction as a novel therapeutic target for stroke. Remarkably, this regulatory

paradigm exhibits cross-organ conservation: in renal ischemia-reperfusion injury, USP14 inhibition markedly decreases ROS-dependent ferroptosis in HK-2 cells (Pan et al., 2023), while in cardiac models, OTUD5 upregulation diminishes ubiquitination of glutathione peroxidase 4, preventing its degradation and ultimately reversing 4-HNE-induced ferroptosis to mitigate myocardial damage (Liu et al., 2023).

Collectively, DUBs orchestrate a multi-layered defense against programmed neuronal death in CIRI by: (i) intercepting necroptosis (A20-RIP3), (ii) preserving mitochondrial integrity (USP30-MFN2), (iii) modulating autophagic flux (USP22-PTEN/TFEB), and (iv) suppressing ferroptosis (USP14-NCOA4)—positioning targeted DUB modulation as a strategic approach to concurrently address multiple cell death pathways in IS therapy. Table 1 summarizes the key DUBs, substrates, and molecular mechanisms in CIRI.

## 5 The therapeutic potential of DUBs in CIRI

Emerging evidence positions DUBs as promising therapeutic targets for CIRI, with their modulation demonstrating significant neuroprotective effects in preclinical models. The dynamic interplay between DUBs and their substrates offers multiple intervention strategies, including small-molecule inhibitors, genetic regulation, and upstream signaling pathway modulation, which collectively address key pathological axes such as inflammation, oxidative stress, and PCD.

### 5.1 Pharmacological inhibition of DUBs

Targeted inhibition of pro-injury DUBs has shown efficacy in reducing neuronal damage. For instance, the USP14-specific inhibitor IU1 suppresses ferroptosis by blocking USP14-mediated stabilization of NCOA4, thereby attenuating ferritinophagy and lipid peroxidation in ischemic brain tissue (Li et al., 2021). Similarly, Vialinin A, a natural compound, inhibits USP4 activity, preventing KEAP1 deubiquitination and enhancing NRF2-driven antioxidant responses, which significantly reduces infarct volume and improves neurological recovery (Mao et al., 2024). These findings highlight the therapeutic potential of selective DUB inhibitors in mitigating CIRI-associated pathways.

### 5.2 Genetic and epigenetic regulation

Genetic silencing or overexpression of DUBs provides further mechanistic insights. siRNA-mediated BRCC3 knockdown disrupts NLRP6 inflammasome activation, suppressing neuroinflammation and neuronal apoptosis in rodent CIRI models (Huang et al., 2024). Conversely, A20 upregulation via MALT1 inhibitors (e.g., MI-2) stabilizes RIPK3 ubiquitination, inhibiting necroptosis and microglial M1 polarization (Qiu et al., 2024). Epigenetic approaches, such as miRNA-124 delivery, downregulate USP14 expression, alleviating REST-mediated repression of neuroprotective genes (Doepfner et al., 2013). These strategies

TABLE 1 Key DUBs in CIRI and their molecular mechanisms.

DUBs	Families	Substrate	Role in CIRI	References
BRCC3	JAMM	NLRP6	BRCC3-mediated regulation of NLRP6 inflammasome assembly through ubiquitination modulation	Huang X. et al., 2021; Huang et al., 2024
A20	OUT	TRAF6, RIP1	A20 promotes the degradation of TRAF6 and RIP1, exerting a negative feedback effect on the NF- $\kappa$ B signaling pathway, thereby alleviating inflammatory injury in CIRI	Wertz et al., 2004; Lee et al., 2000; Zhao et al., 2024; Zhan et al., 2016
CYLD	USP	TRAF2, TRAF6, NLRP3	CYLD inhibits the NF- $\kappa$ B signaling pathway and NLRP3 activation, thereby alleviating inflammatory injury in CIRI	Trompouki et al., 2003; Jiang et al., 2017; Lin et al., 2021
OTUD1	OUT	RIP2	OTUD1 suppresses RIP2-induced NF- $\kappa$ B activation by cleaving K63-linked ubiquitin chains on RIP2, thereby mitigating CIRI-induced inflammation and injury	Honjo et al., 2021; Liu et al., 2015; Annibaldi and Meier, 2018; Zheng et al., 2023
USP25	USP	TAB2	USP25 exerts anti-inflammatory effects via selective cleavage of K63-linked ubiquitin chains from TAB2 to attenuate NF- $\kappa$ B activation and microglial inflammation	Wang et al., 2001; Takaesu et al., 2000; Li Z. et al., 2023
USP8	USP	TLR4	USP8 mitigates neuroinflammation by suppressing the TLR4/MyD88/NF- $\kappa$ B signaling pathway, thereby reducing pro-inflammatory (M1) microglial activation and promoting anti-inflammatory (M2) phenotypic polarization	Zhao et al., 2020
USP22	USP	SIRT1	USP22 exerts multiple neuroprotective effects, including anti-inflammatory, antioxidant, and anti-apoptotic functions, by stabilizing SIRT1 protein levels through deubiquitination, thereby significantly improving CIRI outcomes	Xie et al., 2023
USP14	USP	REST; occluding, claudin-5	USP14 stabilizes REST protein and suppresses neuroprotective gene expression. The inhibition of USP14 improves CIRI through dual neuroprotective mechanisms: (1) maintaining blood-brain barrier integrity by stabilizing tight junction proteins (occludin and claudin-5), effectively reducing vasogenic brain edema; and (2) significantly suppressing microglial overactivation and the NF- $\kappa$ B signaling pathway, thereby decreasing the release of pro-inflammatory factors such as TNF- $\alpha$ and IL-6	Hou et al., 2023; Doepfner et al., 2013
UCHL1	UCH	Neurofilament, PSD-95	UCHL1 exerts dual neuroprotective effects in CIRI, promoting both axonal protection and neural functional recovery through modulation of the ubiquitin-proteasome system	Liu et al., 2019; Mi et al., 2024
USP16	USP	KEAP1	USP16 stabilizes KEAP1 Through k48-linked deubiquitination to suppress NRF2-mediated antioxidant responses	Tong et al., 2023
USP4	USP	KEAP1	USP4 exerts pro-oxidative effects through KEAP1 deubiquitination and stabilization	Mao et al., 2024
A20	OUT	RIP3	A20 exerts anti-cell death effects by removing K63-linked ubiquitination of RIP3, thereby blocking the necroptosis signaling pathway	Onizawa et al., 2015; Qiu et al., 2024
USP30	USP	MFN2	USP30 effectively reduces mitochondrial hyper-fission and fragmentation by inhibiting the ubiquitination-mediated degradation of MFN2, thereby attenuating neuronal apoptosis	Chen et al., 2021
USP22	USP	PTEN	Inhibition of USP22 promotes PTEN degradation, thereby relieving its suppression on mTORC1 targets and subsequently activating TFEB to enhance lysosomal biogenesis and autophagic flux, ultimately improving neurological functional recovery	Li Y. et al., 2023
USP14	USP	NCOA4	USP14 stabilizes NCOA4 protein through deubiquitination, enhancing its ferritinophagy activity, which consequently exacerbates ferroptosis and aggravates cerebral ischemic injury	Li et al., 2021

underscore the versatility of DUBs modulation in restoring cellular homeostasis.

### 5.3 Clinical translation challenges

Despite preclinical success, clinical translation faces hurdles. Most studies rely on rodent models, and human DUB isoforms may

exhibit divergent substrate specificities or regulatory mechanisms. Additionally, systemic DUBs inhibition risks off-target effects due to their pleiotropic roles. Emerging solutions include tissue-specific delivery systems (e.g., exosomal lncRNAs) and isoform-selective inhibitors, as exemplified by the neuroprotective KLF3-AS1/miR-206/USP22/SIRT1 axis delivered via mesenchymal stem cell exosomes (Xie et al., 2023). Future efforts should prioritize optimizing pharmacokinetics and safety profiles of DUBs-targeted agents.

## 6 Prospects and limitations

The exploration of DUBs in CIRI reveals both opportunities and challenges.

### 6.1 Mechanistic complexity

DUBs operate within intricate networks, where their activity is spatiotemporally regulated by post-translational modifications, subcellular localization, and interacting partners. For example, USP30's mitochondrial anchoring is critical for its anti-fission function, yet ischemia-induced displacement disrupts this protective role (Chen et al., 2021). Similarly, redox-sensitive DUBs like A20 and CYLD exhibit context-dependent roles—neuroprotective in CIRI but potentially oncogenic in other settings (Kulathu et al., 2013). Such complexity necessitates precise targeting strategies to avoid unintended consequences.

### 6.2 Technical and translational barriers

Current limitations include: (i) model discrepancies: most findings derive from transient ischemia models, which inadequately mimic chronic human stroke pathologies. (ii) Ubiquitin code specificity: the diversity of ubiquitin chain linkages (e.g., K48 vs. K63) complicates therapeutic targeting. For instance, OTUD1's selective cleavage of RIP2 K63-linked suppresses inflammation (Zheng et al., 2023), but indiscriminate DUBs activation might disrupt proteostasis. (iii) Clinical validation: No DUBs-targeted therapies have entered stroke clinical trials. Promising candidates like IU1 require rigorous evaluation for blood-brain barrier penetration and long-term safety.

### 6.3 Future directions

(i) Multi-omics profiling: integrate proteomics, ubiquitinomics, and single-cell sequencing to map DUBs-substrate interactions in human stroke tissues. (ii) Nanotechnology-driven delivery: develop nanoparticle carriers for brain-specific DUBs inhibitor delivery, leveraging advances in exosome engineering (Song et al., 2019). (iii) Combination therapies: pair DUBs modulation with reperfusion therapies (e.g., thrombectomy) or antioxidants to amplify neuroprotection. For example, USP16 inhibition synergizes with NRF2 activators to enhance oxidative stress defense (Tong et al., 2023).

## 7 Conclusion

DUBs exhibit dualistic and context-dependent roles in CIRI. Key enzymes such as A20, USP30, and OTUD1 confer neuroprotection by suppressing NF- $\kappa$ B-driven inflammation, preserving mitochondrial integrity, and blocking ferroptosis, whereas aberrant activation of BRCC3 or USP14 exacerbates injury. This functional dichotomy hinges on substrate selectivity, ubiquitin chain-type specificity, and spatiotemporal regulation

within ischemic microenvironments. Despite preclinical success in mitigating CIRI through DUB targeting, translational hurdles remain: (i) discrepancies between animal models and human pathophysiology limit mechanistic extrapolation; (ii) the pleiotropic nature of DUB-substrate networks raises concerns about off-target effects; and (iii) blood-brain barrier penetration and long-term safety require rigorous validation. Future efforts should prioritize: (i) mapping DUB-substrate interactions in human ischemic tissues using single-cell sequencing and ubiquitinomics; (ii) engineering nanoparticle or stem cell-derived exosome carriers for brain-specific DUB modulator delivery; and (iii) investigating combinatorial therapies integrating DUB inhibition with thrombolysis or thrombectomy. Through interdisciplinary innovation, DUB-targeted interventions hold transformative potential for post-stroke neurorestoration.

## Author contributions

XQ: Data curation, Methodology, Visualization, Writing – original draft, Writing – review & editing, Project administration, Software, Supervision. JZ: Conceptualization, Formal analysis, Methodology, Resources, Writing – original draft. HL: Conceptualization, Data curation, Methodology, Visualization, Writing – review & editing. MY: Conceptualization, Data curation, Formal analysis, Writing – original draft. ZZ: Data curation, Formal analysis, Methodology, Software, Writing – original draft. WZ: Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft. JC: Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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