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Review

Role of necroptosis in traumatic brain and spinal cord injuries

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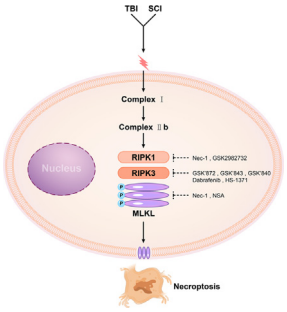
HIGHLIGHTS

- Temporal pattern of RIPK1/RIPK3/MLKL expression is different following CNS trauma.
- Molecular target-mediated necroptosis and potential treatment strategies among cell types are different in CNS trauma.
- MicroRNAs (miRNAs) regulate necroptosis involved in CNS trauma.
- Necroptosis is involved in the regulation of apoptosis and autophagy.
- Targeting necroptosis can be a novel therapeutic strategy for CNS trauma.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Traumatic brain injury (TBI) and spinal cord injury (SCI) are capable of causing severe sensory, motor and autonomic nervous system dysfunctions. However, effective treatments for TBI and SCI are still unavailable, mainly because the death of nerve cells is uncontrollable. Necroptosis is a type of programmed cell death and a critical mechanism in the process of neuronal cell death. However, the role of necroptosis has not been comprehensively defined in TBI and SCI.

Aim of review: This review aimed to summarize the role of necroptosis in central nervous system (CNS) trauma and its therapeutic implications and present important suggestions for researchers conducting in-depth research.

Key scientific concepts of review: Necroptosis is orchestrated by a complex comprising the receptor-interacting protein kinase (RIPK)1, RIPK3 and mixed lineage kinase domain-like protein (MLKL) proteins. Mechanistically, RIPK1 and RIPK3 form a necrosome with MLKL. After MLKL dissociates from the necrosome, it translocates to the plasma membrane to induce pore formation in the membrane and then induces necroptosis. In this review, the necroptosis signalling pathway and the execution of necroptosis

Abbreviations: SCI, Spinal cord injury; TBI, Trauma brain injury; CNS, Central nervous system; RIPK1, Receptor-interacting protein kinase 1; RIPK3, Receptor-interacting protein kinase 3; MLKL, Mixed lineage kinase domain-like protein; TNFR1, Tumour necrosis factor receptor 1; FasR, Fas ligand receptor; TRAIL-R, TNF-related apoptosis-inducing ligand receptor; TLRs, Toll-like receptors; IFNs, Interferons; TRADD, TNFR1-associated death domain protein; TRAF2, TRAF5, TNFR-associated factor 2 and 5; cIAP1/2, Cellular inhibitor of apoptosis 1/2; LUBAC, Linear ubiquitin chain assembly complex; CYLD, Cylindromatosis; NEMO, NF-κB essential modulator; ABIN1, A20-binding inhibitor of NF- κB activation 1; PGAM5, Phosphoglycerate mutase 5; TNF, Tumour necrosis factor; ATG, Autophagy-related gene; DAMPs, Damage-associated molecular patterns; FADD, Fas-associated death domain; LC3, Light chain 3; MiRNAs, MicroRNAs.

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are briefly discussed. In addition, we focus on the existing information on the mechanism by which necroptosis participates in CNS trauma, particularly in the temporal pattern of RIPKs and in different cell types. Furthermore, we describe the association of miRNAs and necroptosis and the relationship between different types of CNS trauma cell death. Finally, this study highlights agents likely capable of curtailing such a type of cell death according to results optimization and CNS trauma and presents important suggestions for researchers conducting in-depth research.

Introduction

Central nervous system (CNS) trauma, including traumatic brain injury (TBI) and spinal cord injury (SCI), continues to be the primary factor of pathogenesis and death rate related to traumatic injuries. The global burden of disease collaborative group study reported 27.08 million new TBI cases and 0.93 million SCI cases worldwide, while their global prevalence reached 55.50 million and 27.04 million, respectively [1]. Neuroinflammation resulting from CNS trauma refers to a major factor causing nerve cell death, scar tissue formation and eventual loss of function [2]. Currently, some treatments can alleviate nerve cell death to some extent, but the recovery of neurological function is still not optimistic. Thus, the exact molecular mechanisms of cell death must be uncovered to develop more effective therapeutic targets.

Traditionally, basic types of cell death are generally classified into three categories, apoptosis, autophagy, and necrosis, which are characterized by distinct morphological and biochemical changes [3]. Apoptosis is the first cell death type, exhibiting small cells, cell membrane blebbing, retraction of pseudopods and nuclear fragmentation and chromatin condensation [4]. The second type of cell death is autophagy, exhibiting the accumulation of double-membrane covered vacuoles covering the cytosol or cytoplasmic organelles and the redistribution of light chain 3 (LC3) to the autophagosome membrane [5,6]. The third cell death type refers to necrosis, featuring the release of cellular contents, organelle swelling, and plasma membrane rupture [7]. In addition, the biochemical components of the three key cell death pathways are different. Apoptosis has been demonstrated to be regulated by the family of anti- and pro-apoptotic B cell lymphoma-2 (BCL-2) protein family members, and hundreds of caspases are involved in apoptosis [8]. Autophagy is regulated by autophagy-related (ATG) families (such as ATG5 and ATG7), and LC3-I, LC3-II and p62 are involved in autophagy [9]. The process of necrosis includes the accumulation of calpains and cathepsins and the release of damage-associated molecular patterns (DAMPs) and cytokines. The DAMPs and proinflammatory cytokines derived from necrotic cells and subsequently activated immune cells provide feed-forward signals reinforcing programmed necrosis in additional cells [10]. The outcome of the three types of cell death is different. Apoptosis and autophagy do not induce inflammation; however, necrosis often leads to inflammation [11]. The main differences and crosstalk among apoptosis, autophagy, and necrosis are shown in Table 1. Most studies of CNS trauma have concentrated on apoptosis and autophagy because necrosis has long been recognized as irreversible [12,13]. However, necrosis has recently been shown to mediate acute nerve cell loss after CNS trauma. In addition, accumulating evidence has revealed a special form of necrosis termed necroptosis involved in CNS trauma. Necroptosis is similar to cellular necrosis in terms of morphology, but distinct from apoptosis in terms of cell membrane rupture, and considerable intracellular elements are released [14]. However, necroptosis refers to one form of cell death mediated by genetic programming and regulatory processes [15]. Morphologically, necroptosis features mitochondrial swelling and cell plasma membrane loss [16]. Necroptosis is induced by interactions related to death receptor family ligands with agonists, including tumour necrosis factor (TNF), FasL, and TRAIL [17]. In addition, the core necroptotic signalling pathway parts are receptor-interacting protein kinases (RIPKs) and the mixed lineage kinase domain-like protein (MLKL) family [18,19]. The family of RIPKs has seven members that target both tyrosine and serine/threonine residues in substrates [20,21]. Recently, many studies have found that necroptosis vitally impacts TBI and SCI pathogenesis [22,23]. However, few studies have summarized the association of CNS trauma and necroptosis. Thus, the mechanisms of the necroptosis process are briefly summarized, and the accumulating evidence in favour of the role of necroptosis in the progression of CNS trauma is summarized.

Overview of the necroptosis signalling pathway

Necroptosis is induced by various stimuli. According to previous studies, TNF is the classic stimulus [24]. In addition, necroptosis is also capable of stimulating several death receptors, including TNF-related apoptosis-inducing ligand receptor (TRAIL-R), Fas ligand receptor (FasR), tumour necrosis factor receptor 1(TNFR1), Toll-like receptors (TLRs), interferons (IFNs), and other mediators [25]. TNF was employed to elaborate the necroptosis signalling pathway. TNF interacts with the preligand assembly domain in the TNFR1 extracellular portion, which induces TNFR1 trimerization [26]. Next, a transient molecular complex termed complex I starts to be assembled, including TNFR1-associated death domain protein (TRADD), RIPK1, TNFR-associated factor 2 and 5 (TRAF2, TRAF5), cellular inhibitor of apoptosis 1/2 (cIAP1/2), and linear ubiquitin chain assembly complex (LUBAC) [27,28]. In complex I, RIPK1 ubiquitination is catalysed by TRAF-2/5 and cIAP1/2 [29]. RIPK1 ubiquitination critically regulates the active state of the kinase. RIPK1 is essential for initiating cell survival pathways, primarily through ubiquitination by cIAPs and TRAF2/5 [30]. Blockade of RIPK1 ubiquitination based on cIAP1/2 antagonization diminishes the protective effect of nuclear factor kappa-B (NF-κB) and increases cell sensitivity to necroptosis [31,32]. Cylindromatosis (CYLD), an enzyme that deubiquitinates K63 specifically, can mediate the RIPK1 deubiquitinating process to expedite the complex IIb-forming process, covering pro-caspase-8, FADD and TRADD [33]. The formation of complex IIa can activate programmed cell death [34]. Upon the suppression of cIAP, TAK1 or NF-κB essential modulator (NEMO) expression, complex I is internalized and transformed into one death-triggering complex IIb, which consists of FADD, pro-caspase-8, RIPK3 and RIPK1. On the one hand, the formation of complex IIa leads to caspase-8-dependent apoptosis (Fig. 1) [35,36]. On the other hand, when caspase-8 is suppressed, complex IIb undergoes transformation into necrosomes, leading to the execution of necroptosis [37]. For instance, cellular FLICE-like inhibitory protein (c-FLIP), an inactive homologue of caspase-8 from a catalytic perspective, participates in the regulation of necroptosis [38]. If caspase-8 interacts with c-FLIP short , the proteolytic activity of caspase-8 was inhibited. Then, RIPK1 recruits

Table 1
Distinct morphological and biochemical features of apoptosis, autophagy and necrosis.

Cell death pathway	Morphological features					Key biochemical pathway components	Outcome	Crosstalk between cell deaths
	Plasma membrane	Nucleus	Chromatin	Mitochondria	Cytoplasm			
Apoptosis	blebbing	fragmentation	Margination, condensation	Normal	Shrinkage	Caspase activation, pro-apoptotic BCL-2 family members, cleavage of hundreds of caspase substrates	1. A programmed cell death 2. No inflammation	The crosstalk between apoptosis and autophagy is mediated in part by the functional and structural interaction between Beclin 1 and the anti-apoptotic proteins BCL-2 and BCL-XL.
Autophagy	Rupture in late phase	focal concavity, dilatation of perinuclear space	Minor/mild condensation	Mild dilatation	Massive vacuolation	ATG family of gene encoded proteins, LC3-I to LC3-II conversion and cleavage of p62.	1. Cell survival or autophagic cell death (programmed) 2. No inflammation	The crosstalk between autophagy and necroptosis is the caspase-8 activity
Necrosis (including necroptosis)	Rupture early	Dilatation of nuclear membrane	Mild-moderate condensation	Swelling	Minor	Involvement of calpains and cathepsins, depletion of ATP, release of DAMPs	1. Regulated or accidental necrosis 2. Inflammation	The crosstalk between necrosis and apoptosis is mediated by the p53 and BCL-2 family members

RIPK3 and interacts with RIPK3 via the RIPK homotypic interaction motif (RHIM) to phosphorylate RIPK3 [39,40]. RIPK3 and RIPK1 phosphorylation stabilizes their correlation inside the pro-necrotic complex termed the necrosome, which subsequently activates MLKL and PGAM5 and CaMKII [41,42]. MLKL undergoes phosphorylation by RIPK3 and then forms oligomerized homotrimers via the relevant amino-terminal coiled-coil domain. Cai et al demonstrated that MLKL forms trimers upon necroptosis induction [43]. However, Liu et al reported that phosphorylated MLKL forms tetramers [44]. Moreover, previous studies have also indicated different structures of MLKL [45,46]. Therefore, we hypothesize that the different structures of MLKL may exert different effects to stimulate necroptosis. This possibility needs to be verified in further investigations. Finally, MLKL oligomers translocate to the plasma membrane, forming membrane pores [43]. In addition, PGAM5, which includes PGAM5S and PGAM5L, is downstream of the necrosome. PGAM5S activates the GTPase activity of mitochondrial fission through the dephosphorylation of dynamin-related protein 1 (Drp1) at serine 637, resulting in mitochondrial fragmentation [47]. Another downstream target of the necrosome is CaMKII, which is involved in nerve cell reactive oxygen species (ROS) overproduction, mitochondrial permeability transition pore (MPTP) opening, and subsequent necroptosis [48].

Involvement of necroptosis in CNS trauma

Functions of necroptosis in CNS trauma

CNS trauma, including TBI and SCI, causes death and disability worldwide [49]. According to considerable research, cell death is an integral component of TBI and SCI pathogenesis [50]. Recently, a novel mechanism named programmed necrosis (necroptosis) was found to be a vital element mediating cell death in response to CNS trauma [51,52]. Initial experiments on necroptosis following TBI in mice were conducted in 2008 by You et al, who exploited a regulated cortical impact model [53]. Subsequently, Ni et al reported that TBI trauma elevates the expression levels pertaining to the RIPK3 and RIPK1 proteins, as well as their substrate MLKL [54]. Liu et al tested whether neural cell death is induced by necroptosis and showed that treatment using necrostatin-1, a particular inhibitor of RIPK1, reduces histopathological and functional deficits after TBI in rodents [53,55]. Moreover, RIPK3-knockout mice display a decrease in posttraumatic neuronal loss and even optimized function-related results compared with TBI alone [56]. RIPK1 kinase activity is necessary for TNF-α-induced necroptosis. A study reported that RIPK1 expression was significantly increased in various types of neural cells (neurons, astrocytes, and oligodendrocytes) at the injured site following spinal cord hemisection. The inhibition of RIPK1 activity was shown to reduce necroptosis and have a tissue-protective function in SCI [46]. MLKL is a pivotal regulator of necroptosis. MLKL content increased significantly post-SCI, and inhibition of MLKL improved recovery of neurological function in SCI [57]. In summary, several experimental studies have confirmed the protective influence exerted by necroptosis on TBI and SCI from the perspective of key enzymes, proteins, specific inhibitors and related genes.

Temporal pattern of RIPK1/RIPK3/MLKL expression following CNS trauma

Currently, an increasing number of researchers have analysed the temporal pattern of necroptosis activation in CNS trauma [53]. RIPK1, RIPK3 and MLKL expression increases in the hours to days after TBI. Liu et al performed Western blot analyses and confirmed markedly increased levels of the RIPK3 and MLKL proteins

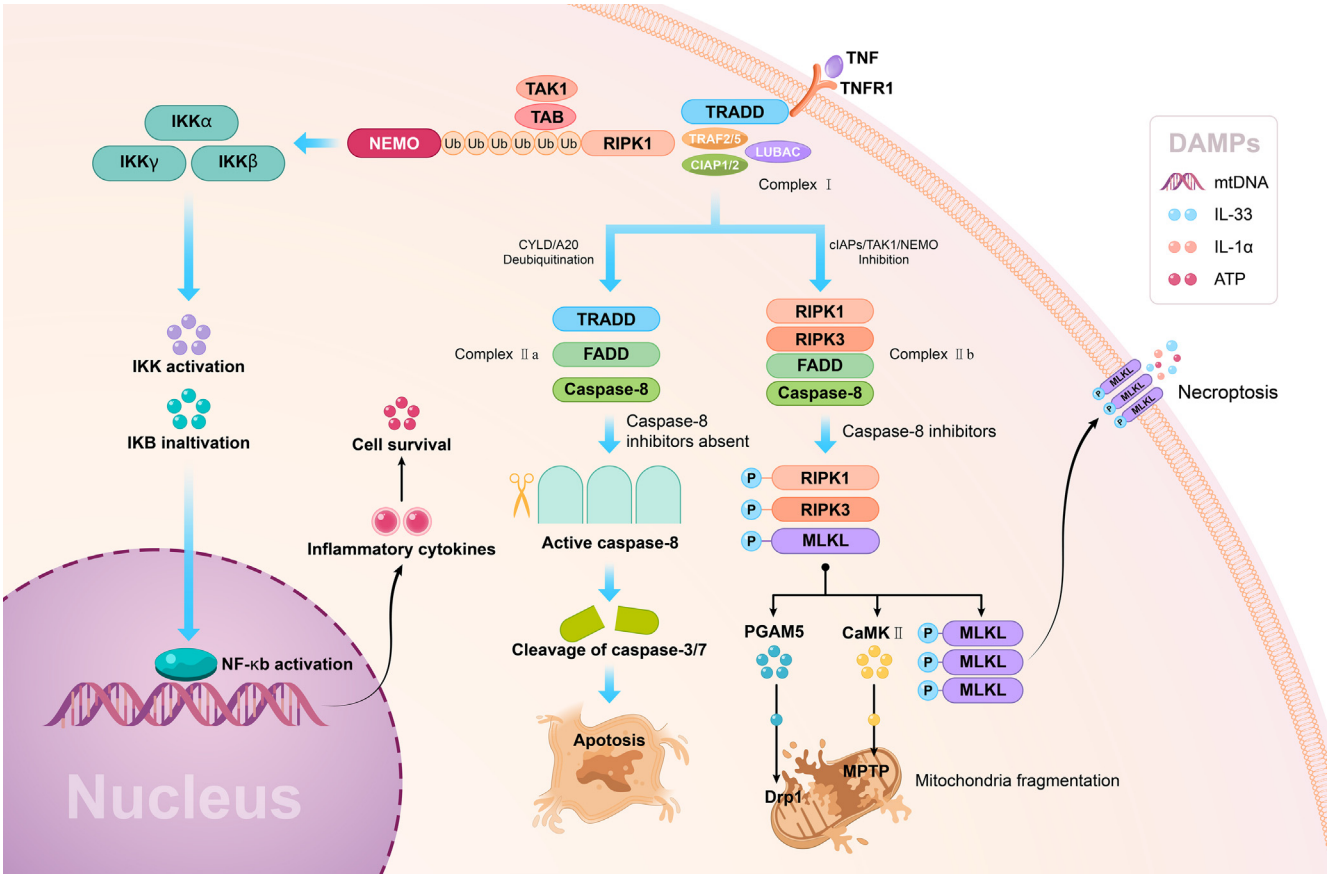

Fig. 1. Caspase crosstalk pathways in necroptosis. The assembly of complex I, composed of TRADD, RIPK1, TRAF2/5, cIAP1/2 and LUBAC, is triggered by TNFR1 ligation. cIAP recruits the LUBAC complex, causing the M1 ubiquitination of RIPK1. The binding of NEMO is a significant modulator of NF-kB, and the polyubiquitin chain of RIPK1 acts as a scaffold. NEMO functions as a regulatory subunit inside the IkB kinase (IKK) complex, which is needed to activate IKK. Activated IKK subsequently inactivates IKB, thus activating NF-kB and its transcription of pro-survival and pro-inflammatory genes. Deubiquitination of RIPK1 by CYLD and A20 can result in RIPK1 dissociating from complex I; then, the complex recruits TRADD, FADD and pro-caspase-8 and forms complex IIa, which activates apoptosis. When the expression of cIAP, TAK1 or NEMO is inhibited, complex I transformes into complex IIb to induce necroptosis, which consists of RIPK1, RIPK3, Fas-associated death domain (FADD), and caspase-8. The change in cells from survival to death is suggested by the conversion from complex I to complex II. Complex IIa is composed of TRADD, FADD, RIPK1 and caspase-8. Caspase-8 cleaves downstream caspases as caspase-3/7 are activated inside complex IIa, thus leading to apoptosis, while RIPK1 and RIPK3 are cleaved and inactivated to terminate necrosis. For complex IIb, in the case of caspase-8 inhibition, the RIPK homotypic interaction motif (RHIM) of RIPK3 allows it to bind to RIPK1 before phosphorylation. Consequently, MLKL is recruited and phosphorylated to generate necrosomes. Then, phosphorylated MLKL moves from the cytosol to the plasma and intracellular membranes. Membrane pores develop due to MLKL oligomerization, which leads to membrane fracture. Ultimately, necroptosis occurs.

in the cortex at 6 h after TBI compared with the sham group, but not at 24 h or 72 h after TBI [56]. Based on these data, RIPK3/MLKL-mediated necroptosis is likely to partially account for neuronal loss in the cortex at 6 h after TBI. However, few studies have investigated the temporal pattern of RIPK1 expression in TBI, which requires further study. Although TBI and SCI are capable of sharing numerous identical pathophysiology-related characteristics, the temporal patterns of RIPK1/RIPK3 and MLKL expression between SCI and TBI differ. Kanno et al investigated the time course of RIPK1 protein expression state inside impaired neural tissue after SCI and reported that the increase in RIPK1 expression was initiated at 24 h, peaked at 3 days, and continued for 7 days after SCI [58]. Interestingly, Liu et al found that unlike RIPK1, the levels of RIPK3 and MLKL protein rose markedly at 1 day but decreased by day 3 after injury [59]. The difference in peak expression of RIPK1, RIPK3, and MLKL has attracted attention. The time course of inflammation triggering secondary damage peaks at approximately 3 days after SCI [60]. RIPK1, at the crossroads of NF-κB signalling, necroptosis and apoptosis, regulates inflammatory responses [61]. This function may explain why peak RIPK1 expression occurs on the third day. In addition, Wang et al reported that while RIPK3, MLKL, and RIPK1 are required for the necrosome-forming and necroptosis-inducing processes, RIPK3 and MLKL do not impact complex I/RIPK1-dependent NF-κB signalling [62]. Thus, their peak expression occurred close to the peak time of cell death, which was on the first day. However, data related to the temporal pattern of RIPK1/RIPK3/MLKL expression in TBI and SCI remain unclear and require further research.

Differences in molecular target-mediated necroptosis and potential treatment strategies among cell types in CNS trauma

The common CNS physiology and responses towards injury comprise a considerable number of cell types, such as neurons, astrocytes, macrophages and microglia [63]. Neurons are the functional units of the brain and spinal cord. Reactive astrocytes, the dominant part of the glial scar, play important roles in CNS trauma [64]. In addition, some scholars have reported that microglia/-macrophages can initiate innate immune reactions, which contribute to spinal cord cavity formation and enlargement [65]. As key proteins involved in the execution of necroptosis, RIPK1 and RIPK3 play important roles in CNS trauma [66]. Therefore, further studies are needed to explore the occurrence of necroptosis in different types of cells and its contributions to various pathological mechanisms in CNS trauma. Shao et al showed that Smurf1, a HECT-domain E3 ubiquitin (Ub) ligase, effectively induces neu-

ronal necroptosis. Using double immunofluorescence labelling, Smurf1 was observed in the rat brain cortex and colocalized with RIPK1 in neurons [67]. In addition, the application of siRNAs to knock down Smurf1 partially downregulated RIPK1 expression in neurons. Furthermore, Smurf1 also exerts an effect on RIPK3, but one that is not as obvious as that on RIPK1 [67]. Thus, Smurf1 may regulate neuronal necroptosis through RIPK1. Unlike neurons, Smurf1 is rarely detected in astrocytes, which are the major component of the glial scar. Therefore, other pathways likely exist in astrocytes that induce necroptosis. According to Fan et al, the inflammatory response-related genes TLR4 and myeloid differentiation primary response gene 88 (MyD88) induce the necroptosis of astrocytes [68]. In addition, after spinal cord injury, ER stress was recently shown to induce necrosis in microglia/macrophages. The expression of glucose-regulated protein 78 (GRP78) is upregulated in MLKL-positive microglia/macrophages after spinal cord injury in rats, suggesting that necrosis might be related to ER stress [69]. These results indicate that the mechanism of necroptosis initiation may be different in the various cell types after CNS trauma.

It is necessary to target necroptosis in different single types of cells to develop potential treatment strategies for CNS trauma. Previous studies have found therapeutic agents that suppress necroptosis in different types of cells following CNS trauma [70,71]. For example, the level of necroptosis in microglia was decreased by charged multivesicular body protein 4b (CHMP4B), which improved neurological function recovery and protected against cell death after TBI [71]. Moreover, quercetin alleviated oligodendrocyte necroptosis and promoted neurofunctional repair following SCI [70]. However, these studies are limited in that the effect of necroptosis on a single type of cell was not determined. Considering agents with multiple pharmacological targets, agents may inhibit necroptosis in various types of cells at the same time. To resolve this problem, the potentially specific mechanism to initiate necroptosis for a single type of cell should be verified in the field of CNS trauma. Currently, it is realistic to develop therapies targeting several cell types that could be a promising strategy for the treatment of CNS trauma.

MicroRNAs (miRNAs) regulating necroptosis involved in CNS trauma

MicroRNAs (miRNAs) are endogenous, small noncoding RNAs with a length of approximately 19–22 nucleotides [72]. Since miRNAs were first observed in *Caenorhabditis elegans* [73], scholars have discovered more than 1000 miRNA genes in the human genome through continuous advances in the maturity of sequencing technologies [74,75]. Notably, miRNAs jointly control approximately 30% of the human genome, highlighting their probable significance as regulators of gene expression. Importantly, miRNAs regulate gene expression at the transcriptional level and have the capacity to regulate particular cellular and physiological processes, which has recently been extended to necroptotic cell death [76,77].

Recently, some studies have elucidated the mechanism by which miRNAs regulate necroptosis. Wang et al reported that miR-223-3p is downregulated during H_2O_2-induced spinal neuron necroptosis. In addition, miR-223-3p binds to the 3′-UTR of RIPK3 mRNA and negatively regulates the RIPK3 necroptotic signalling pathway [78]. This evidence supports the hypothesis that miR-223-3p negatively regulates necroptosis pathways. In other systemic diseases, Liu et al reported that miR-155 targets RIPK1 to inhibit cardiomyocyte necroptosis [79]. In addition, miR-181b-1 and miR-19 bind to CLYD to alleviate the necroptosis signalling pathway during tumour progression [80,81]. Furthermore, miR-874 has been suggested to enhance necroptosis by targeting caspase-8, becoming a vital modulator of necroptosis spread [82]. Wang et al reported that miR-103/107 contributes to H_2O_2-induced necrotic cell death by targeting FADD, but it is not involved in TNF-α-induced necrosis in myocardial ischaemia/reperfusion injury [83]. As shown in the study by Jiang et al, hsa-miR-500a-3P inhibits MLKL phosphorylation and membrane translocation by binding to its 3′-UTR and attenuates cisplatin-induced programmed cell death and NF-κB-driven renal inflammation in tubular epithelial cells [84]. However, researchers have not determined how miRNAs regulate other key necroptotic factors, including RIPK3 and PGAM5. Numerous miRNAs are expressed in the mammalian brain and spinal cord [85,86]. To date, only one study has reported miRNAs that regulate necroptotic cell death in CNS trauma. Nevertheless, the role of miRNAs in regulating necroptosis targets has been widely reported in other diseases. Therefore, the role of the miRNAs mentioned above in CNS trauma is worthy of further study and will be a very promising area of research.

Necroptosis involved in the regulation of apoptosis and autophagy

An increasing number of studies have reported a correlation between apoptosis, autophagy and necroptosis. In addition, it has been suggested that it is possible for the same cell to suffer them all. For whether an individual cell would experience survival, apoptosis or necroptosis, the determining factor is the state of caspase-8. Apoptosis can be induced by the interaction between RIPK1 and caspase-8 [87]. In autophagic death, the JNK pathway mediates RIPK-induced autophagic cell death after caspase-8 inhibition. Moreover, zVAD (a short peptide that acts as a general caspase inhibitor) can robustly induce necroptosis and prevent autophagy through its inhibitory effect on lysosomal cathepsins, underscoring the prosurvival function of autophagy against necroptosis [88,89]. However, it has been widely suggested that caspase-8 is a core target to activate necroptosis [31,35]. Based on the aforementioned research, caspase-8 is emerging as a critical link between apoptosis, autophagy and necroptosis [90]. However, further studies are needed to determine whether these phenomena occur in CNS trauma. Recently, inhibition of apoptotic activation was shown to enhance TBI-induced activation of necroptosis and autophagy through a feedback mechanism. In addition, the application of the specific necroptosis inhibitor necrostatin-1 suppresses autophagy and apoptosis [91]. These results suggested complex crosstalk among different types of cell death after CNS trauma. Further research is needed to understand the relationship between different types of cell death in CNS trauma.

Potential treatment strategies for necroptosis in CNS trauma

As a set of special proteins are considered the major molecules involved in the necroptosis pathway, researchers have developed highly effective inhibitors targeting these proteins. Next, the process used to develop various inhibitors targeting necroptosis is presented, with the aim of identifying new and effective therapeutic agents for necroptosis (Fig. 2). These findings of necroptosis inhibitors will provide potential treatment strategies for CNS trauma

RIPK1 inhibitors

A particularly effective small-molecule inhibitor of necroptosis named Nec-1 was confirmed by Degterev et al in 2005 and has been widely applied in various diseases [15]. Subsequently, many studies have confirmed the exact effect of Nec-1 on inhibiting necroptosis and attenuating nerve injury [51,52]. Nec-1 binds to the hydrophobic pocket of the carboxyl and amino lobes of the RIPK1 kinase domain and is externally located at the ATP-binding site [19,92]. In addition, Nec-1 was observed to exert a protective effect on the mitochondria and the endoplasmic reticulum by

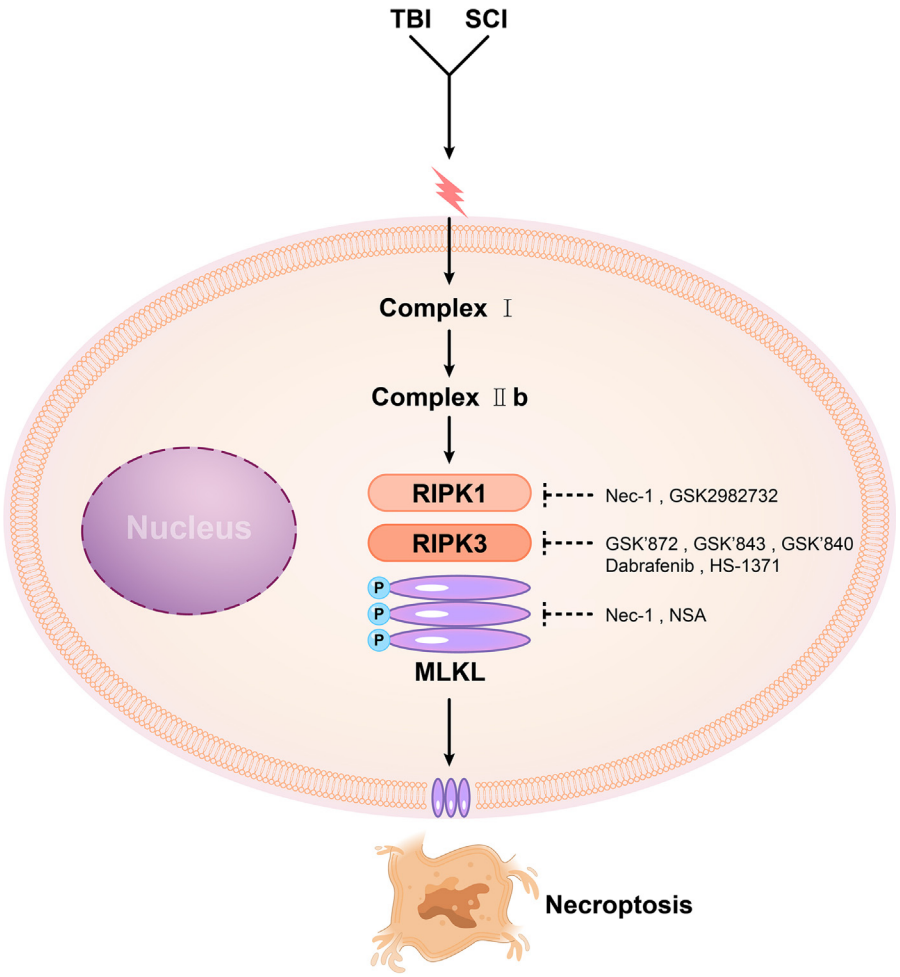

Fig. 2. Targeting necroptosis-regulated cell death for potenticaly therapeutic implications in CNS trauma. Under the stimulation of CNS trauma, complex I forms first and leads to the formation of complex IIb. Then, complex IIb recruits and phosphorylates MLKL to form necrosomes composed of RIPK1, RIPK3, and MLKL. According to the components of necrosomes, drugs targeting various targets have been developed to inhibit necroptosis. Nec-1 combines with RIPK1 and MLKL to inhibit necroptosis. In addition, GSK2982772, as a novel inhibitor of RIPK1, also has a good effect on inhibiting necroptosis. GSK'872, GSK'843 and GSK'840 are the preferable inhibitors for targeting RIPK3. Dabrafenib, another RIPK3 inhibitor, has been approved for clinical use, while the new RIPK3 inhibitor HS-1371 has great potential. There are few reports about small-molecule inhibitors targeting MLKL; Nec-1 and NSA may be the only drugs to target MLKL.

inhibiting the phosphorylation of the MLKL protein [52]. Mitochondrial dysfunction depletes cellular energy stores and subsequently results in death, and high endoplasmic reticulum stress triggers suspended protein synthesis and protein unfolding or misfolding [93,94]. Both processes are basic mechanisms of CNS trauma. Thus, Nec-1 exerts a protective effect on CNS trauma. In addition to Nec-1, GSK2982772 was confirmed as an emerging inhibitor of RIPK1 [95]. GSK2982772 binds to RIPK1, exhibiting high kinase specificity, and displays a highly active state when it blocks TNF-induced necroptosis. Meanwhile, GSK2982772 has advanced to phase IIa clinical trials to treat nonneurological confusion [96]. However, GSK2982772 is not blood–brain barrier permeable [97], which may limit its application in CNS trauma.

RIPK3 inhibitors

Recently, the phosphorylation of RIPK3 was shown to critically alter the activation of the relevant downstream substrate MLKL [41]. RIPK3 inhibitors have been classified into three types: ATP mimic inhibitors that target active ATP-binding sites in kinases located between the lobes of two catalytic regions, ATP mimic inhibitors for the inactivated state, and unclassified inhibitors. Mocarski and colleagues identified a class of specific RIPK3 inhibitors of necroptosis, such as GSK'872, GSK'843 and GSK'840 [98–100]. Yang et al found that GSK'872 reduces the phosphorylation of MLKL by inhibiting RIPK3 [101]. Furthermore, the B-Raf inhibitor dabrafenib is the only type I RIPK3 inhibitor approved for clinical use [102]. Dabrafenib decreases RIPK3 phosphorylation of MLKL, which leads to the disruption of the interaction between RIPK3 and MLKL [103,104]. Recently, HS-1371 has been reported to be a new type II RIPK3 inhibitor that interacts with the ATP-binding pocket of RIPK3 [105].

MLKL inhibitors

Compared with RIPK1 and RIPK3, few effective inhibitors have been reported to target MLKL, although the phosphorylation of MLKL critically modulates necrosis. Therefore, finding additional small molecules that target MLKL to prevent necroptosis is a future goal [106]. Hildebrand and colleagues developed a small molecule (unnamed) that retards MLKL translocation to membranes and binds at the nucleotide binding site in the MLKL pseudokinase domain to prevent necroptosis [107]. Moreover, necrosulfonamide (NSA) was identified as an inhibitor of human MLKL that was reported to bind MLKL as an affinity probe and block necroptosis

[42]. Given that MLKL plays an essential role in the execution of necroptosis, it may be a potential target for inhibiting necroptosis.

Conclusion and perspective

The cell death pathways responsible for the pathogenesis of CNS trauma are complex and interlinked. According to accumulating evidence, necroptosis inhibition may exert a protective effect on CNS trauma. Necroptosis refers to a novel type of programmed necrosis that is induced by many different extracellular and intracellular stimuli. In this review, we provide an overview of the necroptosis signalling pathway and execution of necroptosis. We discussed the temporal pattern of RIPK1/3 expression following CNS trauma and focused on the peak phase of RIPK1/3 expression during necroptosis. Notably, a slight difference was observed in the peaks of RIPK1/3 and MLKL expression between CNS trauma. In addition, differences in the peaks of RIPK1, RIPK3 and MLKL expression have been detected in spinal cord injury. Thus, additional studies should be conducted to define the effects of RIPK1, RIPK3, and MLKL on SCI based on their temporal expression patterns. The occurrence of necroptosis involves not only temporal expression patterns but also different cell types. Different molecular targets contribute to pathological mechanisms in various cell types. Furthermore, the relationship between miRNAs and necroptosis is also very interesting. We discussed the interactions of different miRNAs and different targets in the necroptosis signalling pathway to inhibit necroptosis. For example, miR-223-3p directly targets the 3′-UTR of RIPK3. However, to date, only a few studies have reported the effects of miRNAs on necroptosis in this field. Thus, studies investigating whether circRNAs or lncRNAs affect necroptosis, particularly in TBI and SCI, are critical. In addition, studies investigating the crosstalk and connection between necroptosis, apoptosis and autophagy will be important. Thus, the relationship between different types of cell death in CNS trauma deserves further exploration. Finally, we highlighted agents that are likely to block this form of cell death.

Several open questions and vital points remain that will likely guide subsequent research. First, the regulatory mechanisms of RIPK1 and MLKL in CNS trauma are not clear. Some epigenetic modifications, such as ubiquitination and phosphorylation, have a primary role in the activation of the necrosome complex and cell death induction [108]. RIPK1 self-phosphorylation at serine 166 (S166) determines the activation of RIPK3, its oligomerization and phosphorylation, which in turn can activate MLKL [109]. In addition, RIPK1 ubiquitination is necessary for RIPK1 to leave the TNFR1 complex to form the necrosome [110]. These findings indicate that epigenetic modifications regulate necroptotic pathways. However, further studies are required to verify the epigenetic modifications regulating necroptosis in CNS trauma. Second, the role of MLKL in nonnecroptotic functions following CNS trauma has not been determined. In a model of sciatic nerve injury, MLKL was reported to be highly expressed by myelin sheath cells to promote breakdown and subsequent nerve regeneration [111]. It was also found that activated MLKL accelerated demyelination in a necroptosis-independent fashion and thereby worsened multiple sclerosis pathology [112]. These results suggested that MLKL-induced nonnecroptotic functions may exert neuroprotective effects following CNS trauma, which needs further investigation. Third, the stoichiometry of necroptotic MLKL oligomers remains contentious, with reports of MLKL trimers [107,113,114], tramers [115,116], hexamers [45], octamers [46], and high-order amyloids [44]. How these findings relate to oligomers formed in cells during necroptosis remains to be determined. The current consensus is that oligomerization results in the formation of human MLKL tetramers and mouse MLKL trimers [117]. Undoubtedly, to fully define the oligomeric species of MLKL during necroptosis, new technologies that resolve oligomers both in vitro and in vivo will be essential. Many studies have been conducted on necroptosis thus far. However, additional efforts are needed to explore necroptotic mechanisms to achieve more efficient CNS trauma therapies.

Compliance with Ethics Requirements

Our article does not contain any studies with human or animal subjects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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