The role of necroptosis in neurosurgical diseases

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Abstract
Programmed necrosis or necroptosis is an alternative form of cell death that is executed through a caspase-independent pathway. Necroptosis has been implicated in many pathological conditions. Genetic or pharmacological inhibition of necrototic signaling has been shown to confer neuroprotection after traumatic and ischemic brain injury. Therefore, the necroptotic pathway represents a potential target for neurological diseases that are managed by neurosurgeons. In this review, we summarize recent advances in the understanding of necroptotic signaling pathways and explore the role of necroptotic cell death in craniocerebral trauma, brain tumors, and cerebrovascular diseases.

Key words: Molecular mechanism; Pathogenesis; Programmed cell death; Therapy

Introduction

The two basic forms of cell death, necrosis and apoptosis, play essential roles in development, homeostasis, and pathogenesis (1). Necrosis has long been considered an uncontrolled form of cell death, with morphological features of loss of plasma membrane integrity, organelle swelling, and leakage of cell contents. In contrast, apoptosis is a tightly regulated form of cell death characterized by nuclear shrinkage and fragmentation, membrane blebbing, and apoptotic body formation. However, growing evidence has described an active and well-orchestrated form of necrosis, termed necroptosis (2). Numerous cellular stimuli (e.g., tumor necrosis factor [TNF], Fas ligand, TNF-related apoptosis inducing ligand [TRAIL], double-stranded RNA, interferon-γ, ATP depletion, ischemia-reperfusion injury, and pathogens) have been shown to induce necroptosis (3). Compared to apoptotic cells, necroptotic cells have distinct features that are not induced by the caspase activation that is typically required for apoptotic death (4). However, there are no specific biochemical markers for necroptosis. It has become clear that the kinase receptor interacting protein 1 (RIP1) participates in regulating both necroptosis and apoptosis (5). When apoptotic cell death is blocked by pan-caspase inhibitors, death signals such as Fas ligand and TNF can trigger necroptosis as an alternative cell death pathway (5). RIP1 kinase activity is also crucial for this alternative death pathway (6). It has been documented that the allosteric RIP1 kinase inhibitor (necrostatin-1) inhibits death receptor-induced necroptosis in various cellular models, indicating the essential role of RIP1 activity in necroptotic signaling (5). In addition to RIP1, RIP3 kinase activity has also been implicated in this caspase-independent mode of cell death (7). Indeed, using genetically engineered mice expressing a kinase-inactive mutant of RIP3, Newton et al. (8) found that necroptosis was blocked in the whole animal. These findings support a mediating role of RIP kinases in necroptotic cell death.

Necroptosis is involved in many pathological processes (9-11). It contributes to ischemia-reperfusion injury in the kidney, heart, and brain (12). Necroptosis has also been shown to participate in pathogen- or injury-induced inflammatory diseases (13). The necroptotic pathway therefore constitutes a potential target for preventing cell death. Indeed, chemical inhibition of necroptosis with necrostatin-1 or necrostatin-5 has been shown to confer cardioprotective effects on the isolated rat heart subjected to global ischemia-reperfusion (14). Similarly, inhibition of necroptosis with necrostatin-1 was found to attenuate myocardial ischemia-reperfusion injury in isolated guinea pig hearts (15). In the pathogenesis of neurosurgical diseases (e.g., craniocerebral trauma, brain tumor, and cerebrovascular diseases), necroptosis also plays a critical role. Li et al. (16) reported that necroptosis contributes to the N-methyl-D-aspartate (NMDA)-induced excitotoxicity in isolated rat cortical neurons. Yamanaka et al. (17) reported that necroptosis mediates 24(S)-hydroxycholesterol-induced neuronal cell death. In vivo animal studies revealed that administration of the necroptosis inhibitor necrostatin-1 delayed mouse ischemic brain injury (18). Injection of necrostatin-1 has protective
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effects against neurovascular injury secondary to intracerebral hemorrhage (ICH) in a mouse model (19). Collectively, these studies suggest that necroptosis represents an attractive therapeutic target for a broad range of diseases. Therefore, understanding necroptotic signaling machinery has clinical relevance.

The death receptor pathway for necroptosis

Necroptosis is generally activated by ligation of death receptors including Fas, TNF-receptor 1 (TNFR1), TNFR2, TRAIL receptor 1 (TRAILR1), and TRAILR2 (6). These death receptors were originally recognized to initiate apoptotic death. TNF is the inducer of necroptosis that has been most studied (20). Depending on cell type, TNF administration may trigger apoptosis or necroptosis (21), which suggests a complex signal mechanism in cell death regulation. TNFR1 can be activated by TNF through the pre-ligand assembly domain of the Cys-rich domain 1 in the extracellular portion of TNFR1. The activated TNFR1 subsequently forms a trimer that enables its intracellular part to recruit various proteins, such as TNFR-associated death domain (TRADD), RIP1, cellular inhibitor of apoptosis 1 (cIAP1), cIAP2, TNFR-associated factor 2 (TRAF2), and TRAF5 (22). As E3 ubiquitin ligases, cIAPs catalyze the addition of Lys63-linked polyubiquitin moieties of RIP1, which subsequently provide three docking sites for transforming growth factor-β-activated kinase 1 (TAK1), TAK1-binding protein 2 (TAB2), and TAB3 to constitute the TAK1-TAB2-TAB3 complex, leading to the activation of nuclear factor (NF)-κB and transactivation of cytoprotective genes such as cellular FLICE-like inhibitory protein (c-FLIP). cFLIP-mediated ubiquitination of RIP1 prevents RIP1 from integrating and activating death complexes, impairing caspase activation (23). TAK1 knock-out leads to the propensity of cells to undergo necroptosis (24). However, several lines of evidence demonstrate that prolonged and excessive activation of TAK1 induces phosphorylation and activation of RIP3, leading to necroptosis even without TNF stimulation, whereas ablation of TAK1 causes caspase-dependent apoptosis (25). Therefore, TAK1 may act as a common upstream regulator of both apoptosis and necroptosis. RIP1 can be deubiquitylated by the Lys63-deubiquitylating enzyme cylindromatosis (CYLD) (26) and form a complex with TRADD, FAS-associated death domain protein (FADD), and caspase-8 (i.e., the death-inducing signaling complex [DISC]) (27). Activation of caspase-8 in the DISC is believed to initiate the proapoptotic caspase cascade (28). However, it remains unclear whether FADD and TRADD are required to assemble the necroptosis-signaling complex, or “necroosome.” When caspase-8 activity is genetically or pharmacologically inhibited, the RIP1-RIP3 complex activates necroptotic signaling (29), and necosome assembly is required for necroptosis. A recent study has demonstrated that the guanine nucleotide-binding protein γ 10 gene is involved in TNF-induced necroptosis via promotion of necrosome translocation (29). RIP1, a 74-kDa protein, contains an N-terminal Ser/Thr kinase, an intermediate domain containing a RIP homotypic interaction motif (RHIM) that binds to the RHIM in RIP3 and a C-terminal death domain (30). The C-terminal domain of RIP1 mediates the interaction of RIP1 with Fas and other DD-containing proteins. RIP1 is a pivotal component of the DISC complex and plays a pivotal role in cell-fate decision (30). Since necrostatin-1 was identified as a small-molecule inhibitor of RIP1 by Degterev et al. (18), it has been widely used to study the molecular mechanisms of necroptosis. Shulga and Pastorino identified RIP1 as the mediator of signal transducer and activator of transcription 3 (STAT3) Ser727 phosphorylation, which leads to the translocation of STAT3 into mitochondria (31). This finding provides insight into how RIP1 inhibits apoptosis but promotes necroptosis in the event of stroke (31). The RIP1 cognate kinase RIP3 can trigger necroptosis irrespective of the presence of RIP1, at least in some experimental scenarios. Infection with murine cytomegalovirus has also been observed to induce RIP3-dependent but RIP1-independent necroptosis (32), suggesting the importance of RIP3.

Narayan et al. (33) reported that SIRT2, one of the sirtuin family of proteins, plays a significant role in the initiation of necroptosis. They found that SIRT2 binds constitutively to RIP3 and forms the RIP3-SIRT2 complex, whereas SIRT2 deletion or knockdown prevents TNF-induced necroptosis in L929 cells. The suppression of necroptosis can also be detected after the administration of a specific SIRT2 deacetylase inhibitor (34). In contrast, addition of recombinant SIRT2 protein is sufficient to constitute the RIP1-RIP3 complex for deacetylation of RIP1 (33).

The interaction of RIP1 and RIP3 has been suggested to contribute to necosome assembly via the RHIM, a hydrophobic patch of β sheet (IQIGXXN for RIP1 [amino acids 539-542] and VQVGXXN for RIP3 [amino acids 458-461]) flanked by unstructured coiled-like residues (35). Mutations of the core RHIM residues of RIP1 (IQIG) or RIP3 (VQVG) impair necrosome formation and necroptotic cell death. These results suggest that the RIP kinase activities and necosome assembly might function in a feed-forward manner to amplify the necroptotic signal. Mixed lineage kinase domain-like protein (MLKL) has been identified as a mediator of RIP3-dependent necroptosis (36). MLKL can be phosphorylated by RIP3 at its threonine 357 and serine 358 residues, and these phosphorylation events are essential for necroptosis. Treating cells with necrosulfonamide or knocking down MLKL expression was found to arrest necroptosis (36). MLKL-deficient cells are resistant to TNF-induced necroptosis unless MLKL expression is restored, confirming the key role of MLKL in TNF-induced necroptosis (37). Structurally, MLKL comprises a four-helical bundle tethered to the pseudokinase domain that contains an unusual pseudoactive site. Mutation of the MLKL pseudoactive site leads to constitutive, RIPK3-independent necroptosis, indicating the importance of MLKL modification in the necroptosis pathway downstream of RIPK3 (37).
The toll-like receptor (TLR) pathway for necroptosis

TLR signaling is triggered by pathogen-associated molecular patterns and plays a critical role in cytokine production. TLRs were found to induce RIP3-dependent necroptosis through either TIR domain-containing adapter induction of IFN-beta (TRIF) or myeloid differentiation primary response protein 88 (MyD88) signal transduction (38). In fibroblasts, activation of this pathway depends on MLKL downstream of RIP3 kinase. Kim and Li (39) reported that there is a close link between TLR signaling and RIP3-dependent necroptosis in microglia. The deubiquitinase CYLD has been identified to be involved in TLR-induced necroptosis in macrophages from wild-derived MOLF mice (40).

Execution of necroptosis

Caspases are the agents of apoptosis; however, these proteases are not essential for necroptosis. Several downstream mediators of necroptosis have been identified. Reactive oxygen species (ROS) have been identified as agents of necroptosis. TNF stimulation leads to ROS production in multiple cell types (41). ROS activity is also linked to the oxidation of MAP kinase phosphatases (MKPs) whose normal function is to downregulate c-Jun N terminal kinase (JNK) pathway signaling (42). Excessive ROS results in prolonged JNK activation and subsequent cell death. Treatment with an antioxidant such as butylated hydroxyanisole (BHA) is able to reduce ROS levels and also to inhibit cell death in some cell types (41). However, there are conflicting reports on the effect of ROS inhibition on necroptosis (18), suggesting that the ability of ROS to act as agents of necroptosis is likely to be a cell-type dependent phenomenon.

During apoptosis, ATP-consuming processes such as gene translation and proteasome-mediated degradation are rapidly shut off by caspases. In contrast, these processes persist during TNF-induced necroptosis, thereby leading to the lethal decline in intracellular ATP (43). Overactivation of poly-ADP-ribose polymerase 1 (PARP1), a nuclear enzyme involved in DNA repair and transcriptional regulation, may cause ATP depletion. PARP1 has been implicated in the necrotic response of L929 fibrosarcoma cells to TNF (44). Mechanistically, PARP1 activation promotes the translocation of apoptosis-inducing factor (AIF) from the mitochondrial intermembrane space to the nuclear compartment, where it mediates large-scale, caspase-independent DNA fragmentation (45). Pharmacological and genetic inhibition of PARP1 or blockade of AIF expression has been found to protect cells against necrototic stimuli (46). It has been documented that RIP1-deficient mouse embryonic fibroblast cells are resistant to PARP1-induced cell death in response to DNA alkylating agents (47), suggesting that RIP1 activation occurs downstream of PARP1. Adenine nucleotide translocase (ANT) and cyclophilin D (CypD), which participate in the mitochondrial permeability transition, have been proposed to be involved in necroptosis. CypD ablation protects mice from ischemic renal injury via inhibition of ATP depletion (48). ANT has been suggested to interact with CypD and the voltage-dependent anion channel (VDAC) to form the permeability transition pore complex (PTPC) (49). Pharmacological and genetic inhibition of the backbone components of the PTPC, including VDAC, ANT, and CYPD, exerts consistent cytoprotective effects against numerous insults in vitro and in vivo (50). These results underscore the importance of mitochondrial events in necroptosis.

Lyosomal membrane permeabilization (LMP) has been suggested as an early step in TNF-induced apoptosis (51). LMP is a potentially lethal event because the release of lysosomal proteases into the cytosol may cause digestion of vital proteins and activation of other hydrolases, especially caspases. LMP can be stimulated by a variety of distinct stimuli, including ROS, lysosomotropic compounds with detergent activity, and some endogenous cell death effectors. Extensive LMP often results in cell death without caspase activation. Indeed, LMP has been detected in necroptosis (52). Cytosolic phospholipase A2 (cPLA2) and ceramide act upstream of lipid peroxidation to stimulate LMP. It has been documented that cPLA2 overexpression sensitizes TNF-resistant L929 cells to necroptosis (53). Ceramide is capable of triggering either apoptosis or necroptosis, depending on the specific experimental setting (54). cPLA2-deficient L929 cells fail to accumulate ceramide after TNF exposure and are protected against TNF-induced necroptosis, suggesting an essential role for cPLA2 in ceramide generation and consequent necrotic cell death (55).

Involvement of necroptosis in the pathogenesis of traumatic brain injury (TBI)

TBI is a leading cause of death and disability worldwide. Emergency treatment of TBI is critical for reducing secondary insults. Decompressive craniectomy can immediately reduce intracranial pressure and has been used to treat patients with severe TBI and refractory intracranial hypertension (56). Hypothermia therapy has also been used to manage intracranial hypertension in patients with TBI (57). Cell death after TBI is regarded as a significant cause of disability and death worldwide. There is evidence showing that necroptosis plays a significant role in the pathogenices of tissue damage and functional deficits after TBI (58). The necroptosis inhibitor necrostatin-1 has been shown to improve functional outcome after controlled cortical impact in mice (58). Previous studies have demonstrated that both Akt and mammalian target of rapamycin (mTOR) signaling are activated after TBI (59,60). Akt is known as an inhibitor of apoptotic neuronal cell death, while mTOR is a downstream effector of Akt. A recent study reported that the combined inhibition of Akt and mTOR signaling reduces...
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Necroptosis is an alternative form of cell death that can be triggered by a variety of external stimuli, especially in the absence of apoptosis signaling. The MLKL-RIP1-RIP3 necosome complex plays a critical role in the initiation of necrototic cell death. Necroptosis is implicated in the pathogenesis of many diseases including neurosurgical diseases, which is the second leading cause of death worldwide (71). Stroke commonly results in irreversible neuronal death and subsequent poor prognosis. Ischemic brain injury is associated with induction of necroptosis in the absence of intracellular apoptotic signaling (15). Necroptosis is an important cell death pathway involved in cerebral ischemia/reperfusion injury. The administration of the necroptosis inhibitor necrostatin-1 alone or in combination with the apoptosis inhibitor Gly(14)-humanin was shown to have protective effects on oxygen-glucose deprivation-induced cell death (72). Moreover, combined treatment with the two inhibitors improved neurological scores and decreased infarct volume in mice after cerebral artery occlusion and reperfusion (72). 3-Methyladenine (3-MA), a widely used autophagy inhibitor, was observed to prevent severe global cerebral ischemia-induced programmed necrosis of hippocampal rat CA1 neurons (73).

**Induction of necroptosis in neurons by 24(S)-hydroxycholesterol**

The brain is a cholesterol-rich organ and contains about 25% of the total amount of cholesterol in the body (76). Normally, brain cholesterol is prevented from entering into systemic circulation by the blood-brain barrier. Hydroxylation of cholesterol to generate 24(S)-hydroxycholesterol is critical to maintain brain cholesterol homeostasis, as 24(S)-hydroxycholesterol can cross the blood-brain barrier and be delivered to the liver (77). It has been reported that 24(S)-hydroxycholesterol induces necroptosis in neuronal cells that do not express caspase-8 (17). Yamanaka et al. (78) further reported that 24(S)-hydroxycholesterol-induced necroptosis is dependent on the activity of acyl-CoA:cholesterol acyltransferase 1. Caspase-8 has shown to inhibit necroptosis mediated by RIPK1 and RIPK3 (79). Therefore, necroptosis represents an important cell death mechanism in caspase-8-deficient cells.

**Conclusions**

Necroptosis is an alternative form of cell death that can be triggered by a variety of external stimuli, especially in the absence of apoptosis signaling. The MLKL-RIP1-RIP3 necosome complex plays a critical role in the initiation of necrototic cell death. Necroptosis is implicated in the pathogenesis of many diseases including neurosurgical diseases.
conditions. Accumulating evidence shows that genetic and pharmacological inhibition of necroptotic signaling can confer neuroprotection after brain injury, therefore representing a promising therapeutic target. The development of small molecules that can control necroptosis is of clinical significance for preventing and treating neurosurgical diseases.

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