RIP1/RIP3-regulated necroptosis as a target for multifaceted disease therapy (Review)

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Received December 24, 2018; Accepted June 11, 2019

DOI: 10.3892/ijmm.2019.4244

Abstract. Necroptosis is a type of programmed cell death with necrotic morphology, occurring in a variety of biological processes, including inflammation, immune response, embryonic development and metabolic abnormalities. The current nomenclature defines necroptosis as cell death mediated by signal transduction from receptor-interacting serine/threonine kinase (RIP) 1 to RIP3 (hereafter called RIP1/RIP3). However, RIP3-dependent cell death would be a more precise definition of necroptosis. RIP3 is indispensable for necroptosis, while RIP1 is not consistently involved in the signal transduction. Notably, deletion of RIP1 even promotes RIP3-mediated necroptosis under certain conditions. Necroptosis was previously thought as an alternate process of cell death in case of apoptosis inhibition. Currently, necroptosis is recognized to serve a pivotal role in regulating various physiological processes. Of note, it mediates a variety of human diseases, such as ischemic brain injury, immune system disorders and cancer. Targeting and inhibiting necroptosis, therefore, has the potential to be used for therapeutic purposes. To date, research has elucidated the suppression of RIP1/RIP3 via effective inhibitors and highlighted their potential application in disease therapy. The present review focused on the molecular mechanisms of RIP1/RIP3-mediated necroptosis, explored the functions of RIP1/RIP3 in necroptosis, discussed their potential as a novel therapeutic target for disease therapy, and provided valuable suggestions for further study in this field.

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Key words: necroptosis, receptor-interacting serine/threonine kinase 1, receptor-interacting serine/threonine kinase 3, pathological mechanisms, disease therapy

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1. Introduction

With the rapid development of research in the field of cellular death, it is acknowledged that necrosis can also be regulated in a programmed manner via a specific signal transduction pathway called necroptosis or programmed necrosis (1,2). Necroptosis-mediated cell rupture is morphologically characterized by the loss of cell plasma membrane and the swelling of organelles (particularly mitochondria). Nevertheless, necroptosis, a form of programmed cell death (PCD), and its upstream molecular signaling pathways are under strict control (3,4). The initiation of necroptosis requires several different stimuli, as well as the kinase activity of receptor-interacting serine/threonine kinase 1 (RIP1) and receptor-interacting serine/threonine kinase 3 (RIP3) (5).

The human *RIP* gene, located on chromosome 6p25.2, encodes seven splicing isoforms: RIP1, RIP2, RIP3, RIP4, RIP5, RIP6 and RIP7 (6). RIP1 was initially identified in 1995 as a protein that interacted with the death domain (DD) of receptor Fas (CD95) and elicited a characteristic programmed death response in susceptible cells (7). In 1997, RIP3 was discovered as a protein attenuating both RIP1 and tumor necrosis factor receptor 1 (TNFR1)-induced NF-κB activation (8). RIP1 and RIP3 are critical signaling molecules in necroptosis and regulated by the caspase pathway and ubiquitination (9). Ubiquitination of RIP1 activates NF-κB and mitogen-activated protein kinases (MAPKs), leading to cell survival, while deubiquitinated RIP1 induces the caspase-8-mediated apoptosis pathway (3). When caspase-8 is inhibited or deficient, RIP1 combines with RIP3 via the C-terminal RIP homotypic interaction motif (RHIM) domain to form the RIP1/RIP3 complex (10). The complex initiates downstream signal transduction and triggers necroptosis (10). Excessive necroptosis can cause embryonic lethality (11) and initiate multiple human diseases, including, but not limited to, systemic inflammation, ischemic reperfusion injury and neurodegeneration (12).

The present review examined the functions of RIP1/RIP3-regulated necroptosis on multifaceted pathological mechanisms. Furthermore, the importance of RIP1/RIP3 in determining the cell outcome was highlighted by the interactive molecular pathways noted among cell survival, apoptosis and necroptosis. Then, the pivotal roles of RIP1/RIP3 in disease treatment were discussed, highlighting their application potential as new drug targets.

2. Structural characteristics of RIP1/RIP3

Structurally, RIP1 and RIP3 share nearly half of their amino acid sequences and have very similar topology features. The RIP1 protein consists of 671 amino acids (*Homo sapiens*). It contains a N-terminal serine/threonine kinase domain, an intermediate domain (ID), a RHIM and a C-terminal DD (13) (Fig. 1A). RIP3 is composed of 518 amino acids (*Homo sapiens*), and contains a N-terminal kinase domain similar to that of RIP1, a RHIM domain and a unique C-terminus without a DD (14) (Fig. 1A). RIP1 acts as a multifunctional adaptor protein in response to the activated signal of death receptors (DRs), and its DD binds to the DRs of TNFR1, Fas and TNF-related apoptosis inducing ligand (TRAIL) (15,16). It mediates prosurvival NF-κB activation, caspase-dependent apoptosis and RIP kinase-dependent necroptosis (17). The ID of RIP1 contains the RHIM that enables the protein to combine with RIP3. In contrast to RIP1, RIP3 is not directly required for DR-induced cell survival or death (18). RIP3 binds to RIP1 through its unique C-terminal segment to inhibit RIP1 and TNFR1-mediated NF-κB activation (19) (Fig. 1B). Experiments have revealed that tumor necrosis factor (TNF) induces the formation of an RIP1/RIP3 complex, indicating that RIP1 interacts with RIP3 through the homotypic RHIM domain (20).

3. Molecular mechanisms of RIP1/RIP3-regulated necroptosis

Necroptosis can provide a substitute suicide mechanism in case of malfunction of the classical apoptosis machinery (21). Most work on necroptosis concerns studies of TNF signaling. TNF is a pleiotropic cytokine that has an essential role in inflammation, tissue injury and cell death (22). In the TNF receptor superfamily, researchers have found six human DRs, including TNFR1, Fas (also known as CD95 or APO-1), DR3 (also known as TRAMP or APO-3), TRAIL receptor 1 (TRAILR1, also known as DR4), TRAIL receptor 2 (TRAILR2, also known as DR5, TRICK or KILLER) and DR6 (also known as CD358) (23-26). However, the most prevalent pathway is the TNFR1-mediated signal transduction, which can propel cell survival, apoptosis and necroptosis (27). The present review focused on the three most dominant of those TNF-mediated pathways.

Different modifications of RIP1 can induce distinct outcomes of cell survival, apoptosis and necroptosis. Following binding of TNF-α to TNFR1 at the plasma membrane, TNF-receptor-associated death domain (TRADD) recruits downstream proteins, namely RIP1, the E3 ubiquitin ligases TNF-receptor-associated factor (TRAF) 2, TRAF5, and the cellular inhibitor of apoptosis (cIAP) 1 and cIAP2, to form the complex I (28,29). Then, the complex I mediates NF-κB and MAPK signaling, contributing to cell survival or other non-death functions (4,30,31). The K63-linked ubiquitination of RIP1 by cIAP1/2 promotes both the formation and activation of the transforming growth factor-activated kinase 1 (TAK1)-binding protein (TAB) complex and the inhibitor of NF-κB kinase (IKK) complex (consisting of NF-κB essential modulator, IKKα and IKKβ), supporting the NF-κB pathway activation, and ultimately leading to cell survival (1) (Fig. 2).

Complex I internalizes and transforms into a death-inducing complex II following caspase-8 activation (29). Two distinct types of complex II (IIa and IIb) can be distinguished based on their composition and the activity of their proteins. After dissociating from TNFR1, TRADD recruits Fas-associated protein with death domain (FADD) and further promotes recruitment and activation of caspase-8 to form the complex IIa (29,32). Activation of caspase-8 subsequently induces apoptosis independently of RIP1 or its kinase activity (32). In certain circumstances, including upon the absence of cIAP1/2, upon inhibition of IAP mediated by a small molecule mimic of diablo IAP-protein mitochondrial protein (33) and upon auto-degradation of cIAP1/2, RIP1 is released from complex I to form a caspase-8-activating complex (complex IIb) which mediates apoptosis (32). Complex IIb consists of RIP1, RIP3, FADD and the FLICE-like inhibitory protein long form (FLIPL)/caspase-8 heterodimer, and it promotes RIP1- and caspase-8-dependent apoptosis (22). Hence, RIP1 is not an indispensable factor in apoptosis, although the process is favored by its presence. Under physiological levels, the caspase-8/FLIPL heterodimer facilitates caspase-8 oligomer assembly to trigger apoptosis. By contrast, high levels of FLIPL and FLICE-like inhibitory protein short form (FLIPS) restrict caspase-8/c-FLIPL/S heterodimer activity, leading to inhibition of apoptosis (34-36). Inactivation of RIP1 and RIP3, mediated by cleavage via caspase-8/FLIPL heterodimer in Complex IIb, inhibits necroptosis (37,38) (Fig. 2).

When caspase-8 is inhibited by zVAD, RIP1 and RIP3 are combined via the RHIM, and form complex IIc, also known as the necrosome (39). Complex IIc is a crucial cytoplasmic signaling complex, which does not appear in the TNF-induced

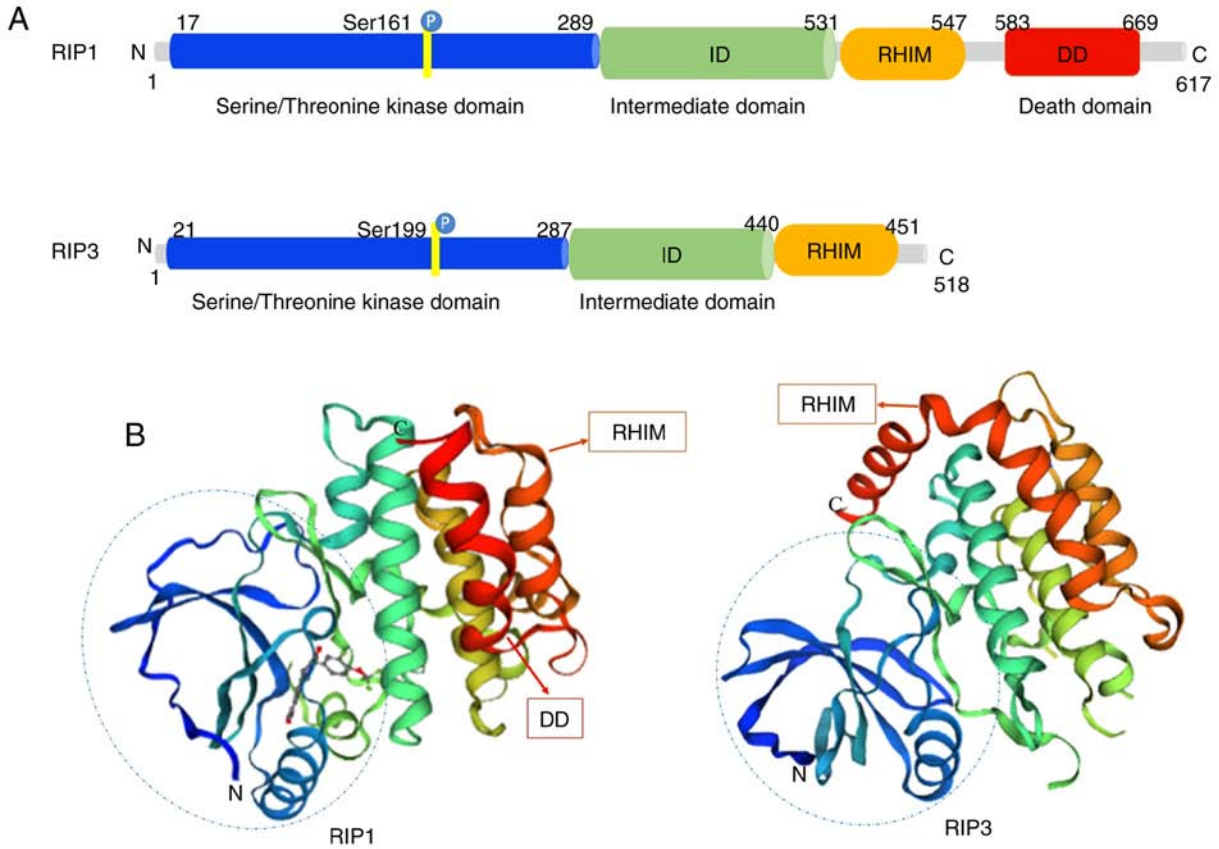

Figure 1. Structural diagrams of RIP1 and RIP3. (A) Schematic of functional domains of RIP1 and RIP3. (B) Protein tertiary structures of RIP1 and RIP3. RIP, receptor-interacting serine/threonine kinase; ID, intermediate domain; RHIM, RIP homotypic interaction motif; DD, death domain.

cell survival or apoptosis (10,40). Mitochondrial reactive oxygen species (ROS) oxidize RIP1 at three crucial cysteine sites (C257, C268 and C586), and promote autophosphorylation of RIP1 at Ser161. RIP1 autophosphorylation is pivotal for the recruitment of RIP3 (41). In addition, CYLD lysine 63 deubiquitinase (CYLD), as a deubiquitinase removing polyubiquitin chains from RIP1, facilitates the formation and activation of RIP1/RIP3 necrosomes by deubiquitylating RIP1. When CYLD is deficient, necrosomes promote a high level of ubiquitinated RIP1 and block phosphorylation of RIP1 and RIP3 (32,42). Furthermore, the RHIM is also required for the RIP1/RIP3 complex formation (19). After RIP1 and RIP3 are combined, RIP1 gets phosphorylated by RIP3 (10). Intramolecular auto- and trans-phosphorylation of RIP1/RIP3 promotes recruitment of another key necroptosis-signaling protein, the mixed lineage kinase domain like protein (MLKL). MLKL is then phosphorylated by RIP3 to initiate necroptosis (43). The phosphorylated MLKL is transferred from the cytosol to the plasma and intracellular membranes via the four helical bundle-brace (4HBD-BR) regions of MLKL (44). The oligomerization of MLKL causes membrane pore formation, resulting in the destruction of membrane integrity and eventually leading to necrotic death (45) (Fig. 2).

In addition to the classic TNFR1-induced necroptosis pathway described above, toll-like receptors (TLR) can also mediate necroptosis. The TLR signaling pathway is generally triggered by pathogen-associated molecular patterns during viral or microbial infection (46,47). TLR-mediated necroptosis results in the destruction of infected cells and is thus beneficial to the host. The downstream MLKL signaling pathway of RIP3 is indispensable for both TNFR1- and TLR-induced signaling (46), and caspase-8 can block necroptosis that is directly initiated by the TIR domain-containing interferon-β (TRIF)/RIP3/MLKL pathway (48,49). TLR4 and TLR3 are respectively activated by lipopolysaccharide (LPS) (50) and polyinosine-polycytidylic acid (I:C), a synthetic double stranded RNA (dsRNA) mimic (51). Thereafter, TLR3 and TLR4 activate RIP3 and participate in ensuing necroptosis via TRIF or MyD88 (52-54). The C-terminal RHIM motif is required for RIP3 to interact with TRIF or MyD88. The RIP3/TRIF signaling complex recruits and phosphorylates MLKL, inducing ROS accumulation and mediating TLR3- and TLR4-induced necroptosis (46,47) (Fig. 3).

Increasing numbers of necroptotic stimuli have been identified and divided into two groups: RIP1-dependent and RIP1-independent (Fig. 3). RIP1-dependent stimuli include TNF-α, Fas, TRAIL, interferon (IFN)-α and IFN-β. The primary death-inducing signaling complex (DISC) is assembled by stimulation of Fas or TRAILR at the plasma membrane, thereby activating caspase-8 and triggering apoptosis independently of RIP1 (55). cIAP deficiency promotes the recruitment of RIP1 and Fas when caspase-8 is blocked, and enhances the formation of the cytosolic ripoptosome complex which induces necroptosis (56). In bone-marrow-derived macrophages, type I IFNα and IFNβ bind to their cognate receptor IFNα/β receptor subunit 1 (IFNAR1) to activate Janus kinase 1 and form the IFN-stimulated gene factor 3 (ISGF3) complex (consisting of STAT1, STAT and IFN-regulatory factor 9). The

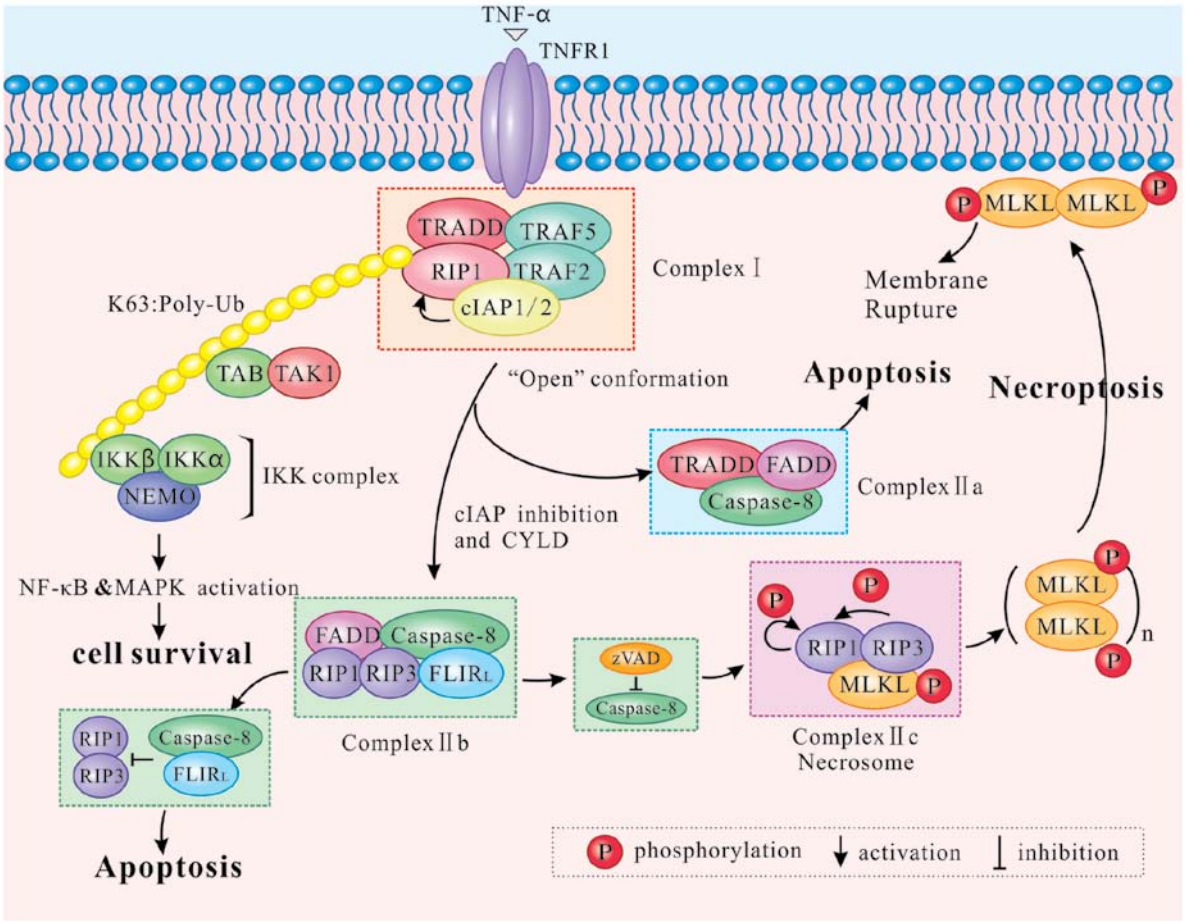

Figure 2. TNFR1-mediated signaling pathways. After the binding of TNF to its receptor, TNFR1 undergoes a conformational change and recruits multiple proteins to form complex I, consisting of TRADD, TRAF2/5, RIP1 and cIAP1/2. The K63-linked ubiquitination of RIP1 by cIAP1/2 promotes the formation and activation of the TAK1/TAB complex and the IKKα/IKKβ/NEMO complex, which induced the NF-κB pathway and cell survival. Destabilization of complex I results in the formation of complex IIa, that contains TRADD, FADD and caspase-8. When cIAPs are blocked and RIP1 deubiquitylated by CYLD, complex IIb is formed. This consists of RIP1, RIP3, FADD, caspase-8 and FLIPL. Both IIa and IIb can initiate apoptosis. When caspase-8 is inhibited by chemical caspase inhibitors, RIP1 binds to RIP3, resulting in the formation of RIP1/RIP3 by intramolecular auto- and trans-phosphorylation. Then, RIP3 recruits and phosphorylates MLKL to form complex IIc, conventionally referred to as the necrosome. The phosphorylated MLKL then translocates from the cytosol to the plasma and intracellular membranes. The oligomerization of MLKL results in membrane pore formation, causing membrane rupture and eventually necroptosis. TNFR1, TNF receptor 1; TNF, tumor necrosis factor; TRADD, TNF-receptor-associated death domain; TRAF, TNF-receptor-associated factor; RIP, receptor-interacting serine/threonine kinase; cIAP, cellular inhibitor of apoptosis 1; TAK1, transforming growth factor-activated kinase 1; TAB, TAK1-binding protein; IKK, inhibitor of NF-κB kinase; NEMO, NF-κB essential modulator; FADD, Fas-associated protein with death domain; CYLD, CYLD lysine 63 deubiquitinase; FLIPL, FLICE-like inhibitory protein long form; MLKL, mixed lineage kinase domain like protein.

ISGF3 complex promotes induction and activation of necrosomes, and triggers necroptosis in a transcription-dependent pathway (57). RIP1-independent stimuli generally refer to LPS, dsRNA and viruses. DNA-dependent activator of IFN regulatory factors (DAI) can identify viral dsRNA, promote the recruitment of RIP3 to form necrosomes without RIP1, and induce RIP3-dependent necroptosis (58). Illuminating the molecular mechanisms involved in necroptosis will elucidate further the molecular biology underlying the pathology.

4. Functional features of RIP1/RIP3 in necroptosis

RIP1 and RIP3 were recently delineated as two important effectors in the cell death network, as they are cascade proteins responding to complex TNFR signaling and regulating cellular survival, apoptosis and necroptosis. It is important to note that RIP3 is indispensable for necroptosis, whereas RIP1 is not. TNF-α-induced RIP1/RIP3 interaction engages RIP3 recruitment, leading to RIP3/RIP3 homo-oligomerization and RIP3 autophosphorylation. Phosphorylated RIP3 recruits and phosphorylates MLKL, which promotes MLKL oligomer-executed necroptosis (59). However, the directorial functions of RIP1 are not always positive for RIP3. Researchers have constructed RIP3 dimers and RIP3 oligomers to analyze how RIP1 induces the activation of RIP3 (60). The results demonstrated that the dimerization of RIP3 alone was insufficient to induce the cell death. The RIP3 dimer seeded a RHIM-dependent complex controlled by both caspase-8 and RIP1. On the other hand, without TNF stimulation and RIP1 activity, the oligomerization of RIP3 was sufficient to induce necroptosis (60). These results indicated that RIP1 not only activates RIP3 in response to TNF signaling, but also participates in cytoprotection. TNFR1 promotes the activation of the NF-κB pathway, p38α and its downstream effector MAPK-activated protein kinase 2 kinase (MK2), thereby promoting cell survival (61). Recently, Jaco *et al* (62) reported

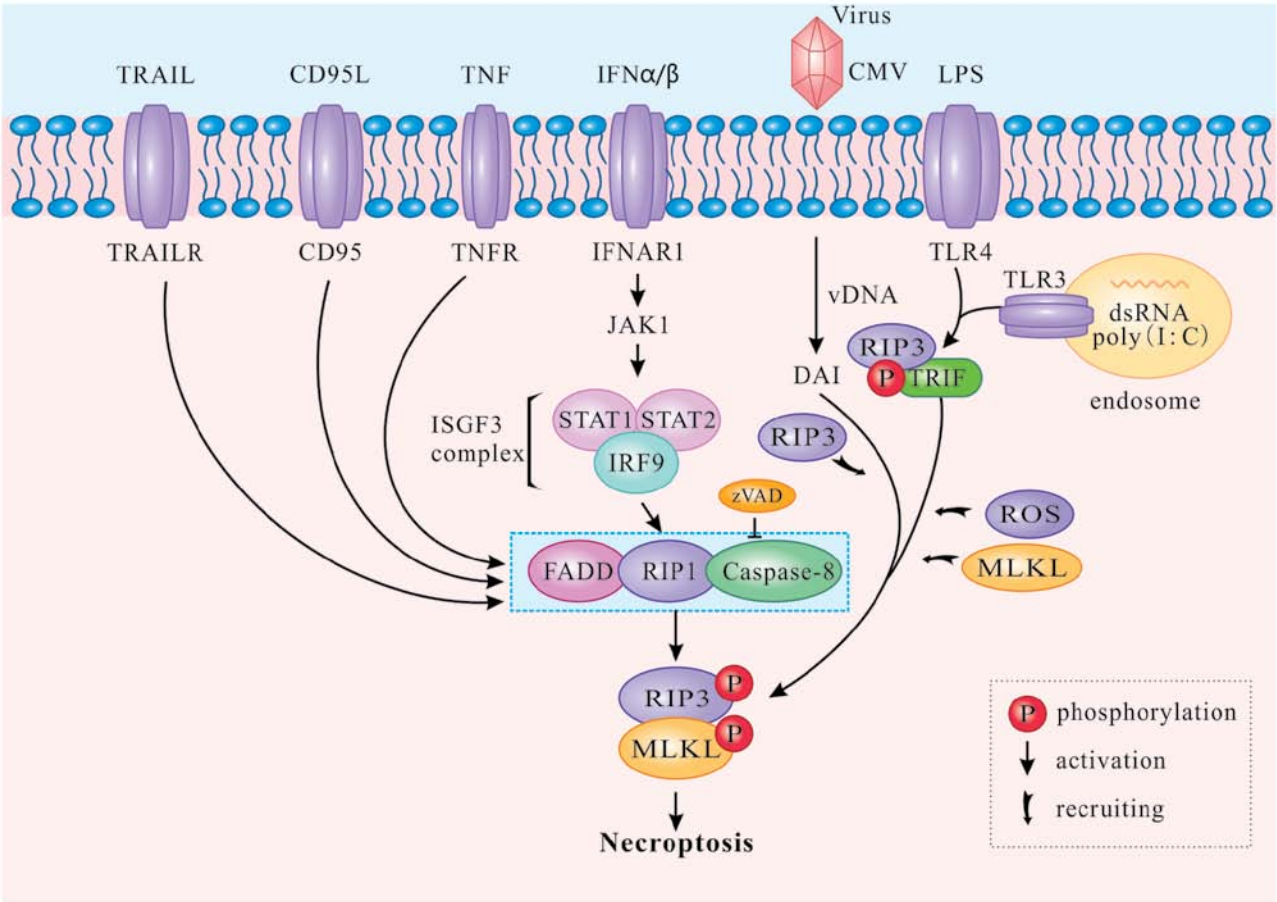

Figure 3. Other stimuli leading to necroptosis. In addition to the TNF-α-mediated necroptosis pathway, multiple other necroptosis triggers have been identified. These involve the canonical pathway that requires RIP1 kinase activity, as well as the non-canonical pathway that is dependent on either the TRIF adaptor or the DAI sensor. RIP1-dependent stimuli include TNF-α, CD95L (also known as FASL), APO-1L, TRAIL (also known as APO-2L) and IFN-α/β. RIP1-independent stimuli include viruses (such as CMV), viral DNA, LPS and polycytidylic acid. After various necroptotic stimuli induce necroptosis, a necrosome is formed. Phosphorylated MLKL by RIP3 transfers from the cytosol to the plasma and intracellular membranes, causing destruction of membrane integrity and eventually necrotic death. TNF, tumor necrosis factor; RIP, receptor-interacting serine/threonine kinase; TRIF, TIR domain-containing interferon-β; DAI, DNA-dependent activator of IFN regulatory factors; FASL, Fas ligand; TRAIL, TNF-related apoptosis inducing ligand; IFN, interferon; CMV, cytomegalovirus; LPS, lipopolysaccharide; MLKL, mixed lineage kinase domain like protein.

that activated MK2 phosphorylated RIP1 at Ser321 to synergize TNF-induced cell rescue. Therefore, these results suggest that RIP1 serves as a positive checkpoint within TNF stimulation that integrates cytokine production and cell survival. RIP1 is therefore thought as an inhibitor rather than an initiator of RIP3-induced necroptosis (63).

Furthermore, ROS participate in the regulation of necroptosis in many cell types, and enhance the formation of necrosomes induced by Smac mimetic bivalent 6 compound (BV6)/TNFα (64). BV6/TNFα-stimulated ROS generation promotes the stabilization of the RIP1/RIP3 feedback loop. A previous study demonstrated that the metabolic enzymes glycogen phosphorylase (PYGL), glutamate dehydrogenase 1 (GLUD1), and glutamate-ammonia ligase (GLUL), are activated by RIP3 (65). Enhancement of aerobic respiration is mediated by TNF-induced ROS. However, the major mechanism of ROS and RIP1/RIP3 remains not completely understood. A recent study demonstrated that mitochondrial ROS activated RIP1 autophosphorylation at Ser161 via oxidation of three crucial cysteines in RIP1 (41). This phosphorylation accelerated RIP1 recruitment of the RIP3 aggregation and formed a necrosome, which then resulted in mitochondrial depolarization and cell necroptosis (66). These studies provided essential insight into the reciprocal regulation between RIP1/RIP3 and ROS in necroptosis.

5. Necroptosis, a timoneer of pathological mechanisms

Role of necroptosis in inflammation. Necroptosis, as a novel pathway of PCD, leads to release of endogenous moldecules from disrupted dying cells, that subsequently triggers inflmmation and immune response (67). Necroptosis-inducing factors include TLR3 and TLR4 agonists [such as interleukin (IL)-1β)], TNF, certain viral infections and T cell receptors (22). TNF-mediated and TLR-mediated signaling pathways are the primary pathways for necroptosis, both regulated by RIP3 (10). RIP1/RIP3 or RIP3/TRIF signaling complexes recruit and phosphorylate downstream MLKL, causing rupture of the plasma membrane, as well as the release of endogenous molecules (45). These endogenous molecules are known as damage-associated molecular patterns (DAMPs). They are also identified as part of the extended IL-1 family (IL-1α, IL-1β, IL-18, IL-33, IL-36α, β and γ) (68). The leakage of DAMPs can activate inflammasomes, facilitate

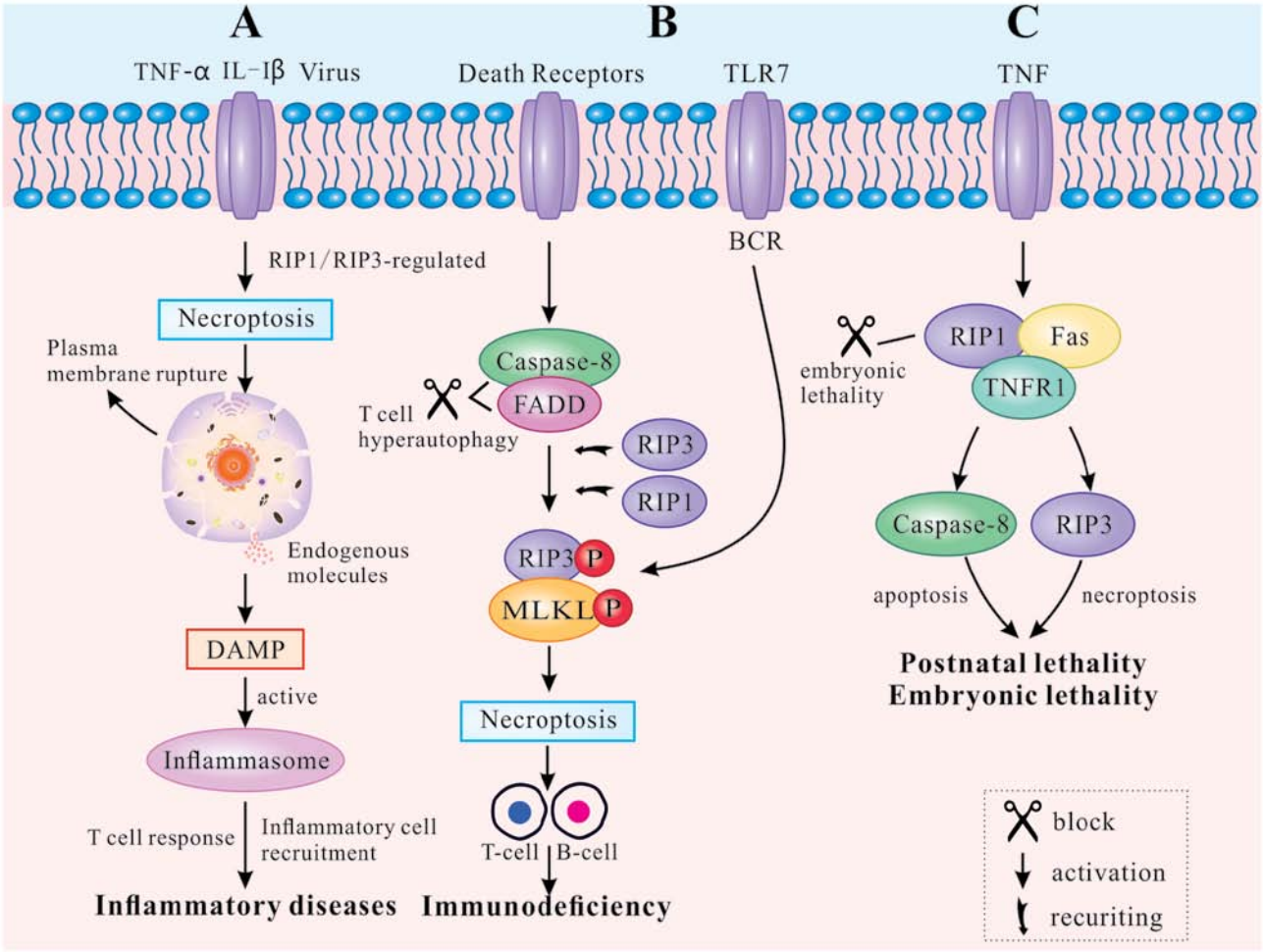

Figure 4. Necroptosis-associated pathological mechanisms. (A) Inflammatory factors include TNF-α and IL-1β. Certain viral infections can induce RIP1/RIP3-mediated necroptosis, which can result in the disruption of the plasma membrane and in the release of endogenous molecules, also known as DAMPs. Inflammasomes are then activated by DAMPs, promoting inflammatory cell recruitment and virus-specific T cell responses to induce inflammatory diseases. (B) DRs can regulate the necroptosis of T and B cells. When either caspase-8 or FADD is deficient, T cells undergo hyper-autophagy and generate a RIP1/RIP3-mediated necrosome, triggering necroptosis. BCR mediates necroptosis via reaction with TLR7. Necroptosis of T lymphocytes and B lymphocytes can cause immunodeficiency. (C) RIP1 prevents embryonic and postnatal lethality by blocking two different cell death pathways: FADD/caspase-8-mediated apoptosis and RIP3-mediated necroptosis. TNF, tumor necrosis factor; IL, interleukin; RIP, receptor-interacting serine/threonine kinase; DAMPs, damage-associated molecular patterns; DR, death receptor; FADD, Fas-associated protein with death domain; BCR, B cell receptor; TLR, toll-like receptor; MLKL, mixed lineage kinase domain like protein; TNFR1, TNF receptor 1.

inflammatory cell recruitment to the site of infection, and promote subsequent virus-specific T cell responses to induce inflammation (69). Thus, regulating the mechanism of necroptosis can result in both inhibition and promotion of the immune response (Fig. 4A).

Role of necroptosis in immunity. DRs regulate necroptosis in various cell types, such as B lymphocytes and T lymphocytes, both of which are essential for immune homeostasis and tolerance (48,70). Blocked DRs fail to clear activated lymphocytes and unbalanced lymphoid homeostasis, ultimately leading to autoimmune lymphoproliferative syndrome (ALPS) (71). FADD, caspase-8 and RIP kinases are indispensable for T cell clonal expansion, contraction and antiviral responses (48). When caspase-8 and FADD are deficient, T cells undergo hyper-autophagy and proliferative inhibition in response to antigenic stimulation. In addition, T cells generate necrosomes, which induce caspase-8-independent necroptosis (72). RIP1 and RIP3 are either recruited by DRs or by other cellular signaling molecules, including DNA damage and antigen receptor ligation (73). Chronic necroptosis may be the basis of human fibrotic and autoimmune disorders (74). Likewise, B cell receptor (BCR) mediates necroptosis via reaction with TLR7 to prevent autoimmune diseases (75,76). The RIP1 inhibitor necrostatin-1 (nec-1) has been demonstrated to suppress B-cell necroptosis, when pretreating B cells from patients with systemic lupus erythematosus (70). Fig. 4B illustrates how programmed death can promote clonal loss of lymphocytes during infection and protect patients against autoimmune disease.

Effects of necroptosis on animal development. Apoptosis and necroptosis are closely associated with embryonic lethality and postnatal development (49). RIP1 determines cell survival or death by associating with TNFR1, TLRs and Fas (17,77). Embryonic lethality of RelA-deficient mice has been demonstrated to be mediated by apoptosis and necroptosis (78). FADD functions as an adaptor to induce apoptosis by

recruiting and activating caspase-8 (79). The characteristics of FADD-deficient embryos are high levels of RIP1 production and massive necrosis. RIP1 ablation allows normal embryogenesis in FADD-deficient mice, but these mice usually die around their first postnatal day (80). In addition, different RIP1 kinase inactivating mutations have distinct effects on the embryogenesis of FADD-deficient mice. For example, RIP1K45A has been found not to prevent the embryonic lethality of FADD-deficient mice, while RIP1Δ (with an altered P-loop in the kinase domain) does (81). If RIP1 was necessary for the activation of RIP3, as aforementioned, FADD$^{-/-}$ RIP1$^{-/-}$ and FADD $^{-/-}$ RIP3$^{-/-}$ mice should survive to adulthood (82). Nevertheless, a recent study revealed that RIP1-deficient mice die soon after birth, leading to speculation for the positive role of RIP1 in embryonic development and postnatal life (83). RIP1 deletion enhances primary cell sensitivity to FADD/caspase-8-mediated apoptosis induced by TNF. In addition, RIP1 deletion promotes RIP3/MLKL-mediated necroptosis induced by TLR ligation (via TRIF) or interferon (84). The perinatal lethality of RIP1$^{-/-}$ mice can be rescued by a combination of additional mutations. A recent study indicated that haploid insufficiency of RIP3 improved the survival period of RIP1$^{-/-}$ FADD$^{-/-}$ double knockout mice beyond weaning age, while RIP1$^{-/-}$ FADD$^{-/-}$ RIP3$^{-/-}$ triple knockout (TKO) mice were significantly smaller in size and weight (85). Furthermore, complete ablation of RIP3 further prolonged the life span of TKO mice displaying normal size and weight (85). Therefore, it can be concluded that RIP1 may prevent postnatal lethality by blocking two different cell death pathways: FADD/caspase-8-mediated apoptosis and RIPK3/MLKL-mediated necroptosis. The effect of PCD on animal development is highly complex and has not yet been thoroughly elucidated (Fig. 4C).

6. RIP1/RIP3 inhibition in necroptosis

Inhibitors of RIP1 and RIP3. The increasing discovery of inhibitors and drugs affecting the RIP1/RIP3 cascade pathway already shows promise towards the treatment of various diseases (Table I). Nec-1 has been identified as a specific and potent small-molecule and active inhibitor of RIP1 (2). Nec-1 is widely used in disease models to examine the contribution of RIP1 to cell death and inflammation. Other necrostatins, including Nec-3, Nec-4 and Nec-5, also stabilize RIP1 in an inactive conformation through interactions with hydrophobic pockets and highly conserved amino acids (2,86). However, the strongest inhibition of RIP1 has been observed with Nec-1 stable (Nec-1s) (87). A previous study identified GSK2982772 (compound 5) as a novel inhibitor of RIP1 (88). GSK2982772 potently binds to RIP1 with exquisite kinase specificity and has high activity in blocking TNF-dependent necroptosis, as well as inflammation. Based on this previous study of GSK2982772, Yoshikawa *et al* (27) designed and synthesized a novel class of RIP1 kinase inhibitor, the compound 22 [7-oxo-2,4,5,7-tetrahydro-6H-pyrazolo(3,4-c)pyridine], which possesses moderate RIP1 kinase inhibitory activity and P-gp mediated efflux. Furthermore, using a mouse model of systemic inflammatory response syndrome, it was demonstrated that compound 56 (RIPA-56) targeted RIP1 directly, and reduced TNFα-induced cell mortality and multi-organ damage (89). The pan-Aurora kinase inhibitor Tozasertib (also known as VX-680 and MK-0457) was recently demonstrated as a potent compound in inhibition of RIP1-dependent necroptosis and in the blockage of cytokinesis in cells (90). The food and drug administration-approved anticancer agents ponatinib and pazopanib were demonstrated to be submicromolar inhibitors of necroptosis through the targeting of components upstream of MLKL (91). Ponatinib inhibits both RIP1 and RIP3, while pazopanib preferentially targets RIPK1. Both drugs have potential values for the treatment of pathology caused or aggravated by necroptotic cell death (91). Overall, the aforementioned studies indicated that RIP1 kinase may serve as a novel target for therapeutic drug development in human disease therapy.

RIP3 is a critical regulator of necroptosis, however, very few specific inhibitors have yet been reported. B-Raf (V600E) inhibitors are generally considered as an important anticancer drug in metastatic melanoma therapy (92). To date, the B-Raf inhibitor dabrafenib was demonstrated to be a potent inhibitor of RIP3 (92). Dabrafenib decreased RIP3-mediated Ser358 phosphorylation of MLKL and disrupted the interaction between RIP3 and MLKL (93). Results indicated that dabrafenib could serve as a RIP3 inhibitor and as a potential preventive or therapeutic agent for RIP3-involved necroptosis-related diseases (93). As expected, dabrafenib significantly reduced infarct lesion size and attenuated upregulation of TNF-α in mouse models of ischemic brain injury (94). In addition, murine cytomegalovirus (CMV) M45 contains a RHIM domain, and was confirmed to be a competitive inhibitor of RIP3 (95). Human CMV blocks TNF-induced necroptosis following RIP3 activation and MLKL phosphorylation (95), leading to inhibition of the host defense mechanism.

MicroRNA (miRNA)-associated inhibition by targeting RIP1/RIP3. As diagnostic and therapeutic strategies, miRNAs provide a novel perspective for RIP1 inhibition. As aforementioned, several inhibitors have been demonstrated to suppress the pro-necroptosis function of RIP1 at the protein level; however, these do not function at the mRNA level. miR-155 represses cardiomyocyte progenitor cell necroptosis by targeting RIP1 rather than activating the Akt pro-survival pathway (96), suggesting that miR-155 might be a novel approach in improving cell engraftment. Additionally, in a myocardial ischemia/reperfusion model, it was demonstrated that miR-103/107, as a necrosis-suppressor miRNA, directly targeted FADD (97,98). FADD participates in hydrogen peroxide-induced necroptosis by influencing the formation of RIP1/RIP3 complex, suggesting that FADD-targeting by miR-103/107 might be a new approach for preventing myocardial necrosis. Recent research has indicated that miRNA dysregulation is involved in triple-negative breast cancer (TNBC) (100). Overexpression of miR-182 inhibits the CYLD action on the ubiquitin chains on RIP1, leading to caspase-8-dependent apoptosis in TNF-α-treated TNBC cells (99). Additionally, miR-145, which is downregulated in TNBC, targeted cIAP1 and reduced the formation of the RIP1/FADD-caspase-8 complex (100). Therefore, miRNAs can be perceived as a novel approach for RIP1 regulation. However, their potential effects in disease intervention requires further research (Table II).

Table I. Direct and indirect inhibitors of RIP1/RIP3.

Compound	Targeted proteins	Applications	(Refs.)
Nec-1	RIP1	Nec-1 is the most active, compared with Nec-3, Nec-4 and Nec-5	(2)
GSK2982772 (compound 5)	RIP1	Phase 2a clinical studies for rheumatoid arthritis and ulcerative colitis	(88)
7-oxo-2,4,5,7-tetrahydro-6H-pyrazolo[3,4-c]pyridine (compound 22)	RIP1	Oral administration (10 mg/kg, twice a day) attenuated disease progression in a mouse experimental autoimmune encephalomyelitis model of multiple sclerosis	(27)
RIPA-56 (compound 56)	RIP1	For the treatment of systemic inflammatory response syndrome	(89)
Tozasertib (also called as VX-680 and MK-0457)	pan-Aurora kinase, RIP1	Protects against TNF-induced necroptosis	(90)
Ponatinib	RIP1, RIP3	Ponatinib can directly bind RIP1 and block RIP3 autophosphorylation	(91)
Pazopanib	Preferentially targets RIP1	Similar to Nec-1, RIP1 is the main target of pazopanib	(91)
Dabrafenib	RIP3, B-Raf (V600E)	Alleviates acetaminophen-induced liver injury	(93)
Murine cytomegalovirus M45	RIP3	Binds to RIP3 through RHIM domain	(95)
Kongensin A	HSP90 (indirectly inhibits RIP3)	Kongensin A covalently binds to HSP90 and inhibits the stability of RIP3/MLKL	(102)
Compound 17AAG	HSP90 (indirectly inhibits RIP3)	Promotes degradation of MLKL and RIP3 through the proteasome pathway	(104)
Alvespimycin (17-DMAG)	HSP90 (indirectly inhibits RIP1/RIP3)	Destabilizes the necroptosis proteins RIP1 and RIP3	(105)
Dyngo 4a	TLR4 (indirectly inhibits RIP3)	Blocks the internalization of TLR4 and prevents RIP3-induced necroptosis of macrophages	(108)

RIP, receptor-interacting serine/threonine kinase; Nec, necrostatin; TNF, tumor necrosis factor; RHIM, RIP homotypic interaction motif; HSP90, heat shock protein 90; MLKL, mixed lineage kinase domain like protein; TLR4, Toll-like receptor 4.

Table II. Applications of targeting miRNAs in necroptosis.

miRNA	Targeted mRNAs	Sequence (5'-3')	(Refs.)
miR-155	RIP1	UUAAUGCUAAUUGUGAUAGGGG	(96)
miR-103	FADD	AGCAGCAUUGUACAGGGCUAUGA	(97,98)
miR-107	FADD	AGCAGCAUUGUACAGGGCUAUCA	(98)
miR-182	CYLD	UUUGGCAAUGGUAGAACUCACACU	(99)
miR-145	cIAP1	GUCCAGUUUUCCCAGGAAUCCCUGGAUUCCUGGGAAAA CUGGACUU	(100)

miRNA, microRNA; RIP, receptor-interacting serine/threonine kinase; FADD, Fas-associated protein with death domain; CYLD, CYLD lysine 63 deubiquitinase; cIAP1, cellular inhibitor of apoptosis 1.

Indirect inhibition of RIP1/RIP3. Previously, Li *et al* (101) revealed that the cochaperone complex of HSP90 and CDC37 regulated the stability and function of RIP3 and MLKL, and participated in the RIP3 activation process during necroptosis. The HSP90 inhibitor kongensin A (KA) disrupted the association of HSP90 and CDC37, leading to the inhibition of RIP3-dependent necroptosis (101,102). These results suggested that KA was an effective HSP90 inhibitor with a potential anti-RIP3 effect in both RIP3-dependent necroptosis and inflammation. In addition, the disruption of HSP90 function

prevented necrosome formation, reduced MLKL phosphorylation and inhibited TNF-induced necroptosis (103). The compound 17AAG destabilized the interaction of MLKL and RIP3 by inhibiting HSP90, resulting in degradation of MLKL and RIP3 via the proteasome pathway (104). Alvespimycin (17-DMAG), an inhibitor of HSP90, facilitated the degradation of RIP3 following HSP90 inactivation (105). Therefore, pharmacological modulation of RIP3-induced necroptotic cell death through HSP90 could be a promising strategy for therapy in several settings (Table I).

In TNFα-induced necroptosis, as RIP1 and RIP3 form a protein complex through their common RHIM domain, phosphorylation and activation of RIP3 and downstream MLKL occur (106). In addition, RHIM-containing proteins, such as TLR, and interferon regulatory factors (Z-DNA binding protein 1, also known as DAI or DLM1) are known to activate RIP3 and further transduce necrosis signals to MLKL (107). TLR3 or TLR4 directly activate necroptosis through the RHIM-dependent association of TRIF with RIP3. This pathway proceeds independently of RIP1, but remains dependent on MLKL downstream of RIP3 kinase (46). Dyngo 4a blocks the internalization of TLR4 and prevents RIP3-induced necroptosis of macrophages (108). A previous study unveiled DAI as the RIP3 partner to interact with RIP3, mediating virus-induced necrosis analogous to the RIP1/RIP3 complex controlling TNF-induced necroptosis (109). Table I summarizes the direct and indirect inhibitors of RIP3.

7. Application potential of RIP1/RIP3 inhibition in disease therapy

RIP1/RIP3-dependent necroptosis in cardiovascular disease. Necroptosis participates in the development of several diseases, such as atherosclerosis cardiovascular disease, a leading cause of mortality worldwide (110). Overexpression of RIP3 during necroptosis of primary macrophages induced by oxidized LDL (ox-LDL) facilitates the development of the disease (111). Furthermore, monoclonal antibodies can be detected in the core of atherosclerotic plaques, specifically recognizing the phosphorylation form of RIP3 at Ser232 (112). Notably, the mortality of apolipoprotein E/RIP3 double-knockout mice was delayed dramatically (112). These findings indicated that RIP3-mediated necroptosis in atherosclerotic plaques may release pro-inflammatory cytokines that exacerbate atherosclerosis. Of note, PS-341, a potent and specific proteasome inhibitor, was demonstrated to impair macrophage necroptosis through stabilization of cIAPs and disruption of the formation of the RIP1/RIP3 complex (113).

RIP1 inhibition leads to a reduction of infarct size, implying a functional importance of necroptosis in myocardial ischemia (MI) (114). Luedde *et al* (20) analyzed RIP3 expression in murine hearts and highlighted the potential functional significance of RIP3-dependent necroptosis in the modulation of post-ischemic adverse remodeling in MI. A previous study demonstrated that RIP3 was upregulated in murine hearts subjected to ischemia-reperfusion (IR) injury, as well as in cardiomyocytes treated with LPS and hydrogen peroxide (115). This study further illustrated that upregulated RIP3 evoked endoplasmic reticulum (ER) stress, ultimately resulting in cardiomyocyte necroptosis in the setting of cardiac IR injury (115). In addition, in a mouse cardiac hypertrophy model established by transverse abdominal aortic constriction, both mRNA and protein expression levels of RIP1 and RIP3 were increased significantly. Losartan downregulated the expression of RIP1/RIP3, resulting in the inhibition of necroptosis and to the alleviation of cardiac hypertrophy (116). RIP1/RIP3 may thus be an attractive target for future therapies that aim to limit the adverse consequences of cardiac disease.

The quaternary nitrogen herbicide paraquat is a highly toxic pro-oxidant that triggers oxidative stress and multi-organ failure, including that of the heart. Recently, Zhang *et al* (117) revealed that Nec-1 pretreatment prevented cardiac contractile dysfunction, reduced RIP1/RIP3 interaction, downregulated the RIP1/RIP3/MLKL signaling pathway, and dramatically inhibited the production of ROS in paraquat-challenged mice. Thus, the RIP1/RIP3/MLKL signaling cascade may represent an innovative therapeutic direction for paraquat poisoning-induced cardiac contractile dysfunctions (Table III).

RIP1/RIP3-dependent necroptosis in cancer therapy. Abundant research on RIP1/RIP3 has highlighted its role in cancer, which is due to its necroptosis-inducing function (118). Chen *et al* (119) have noted that necroptosis is a critical cell-killing mechanism in response to severe stress and blocked apoptosis, and have proposed that it can serve as an alternative cell death program to prevent cancer. Previous studies have indicated that increased RIP3 expression was correlated with cancer development, including colon and lung cancers, nasopharyngeal carcinoma and non-Hodgkin lymphoma (120-122). The topoisomerase inhibitor SN38, an active metabolite of irinotecan, was demonstrated to mediate cytotoxicity through the TNF/TNFR signaling pathway in a panel of colon cancer cells (123). SN38 also promoted the progression of necroptosis, inhibited cell proliferation and induced DNA damage accumulation (123). This suggested that the SN38-induced activation of RIP1 and subsequent necroptosis may exert the therapeutic efficacy on colorectal carcinoma (123). Furthermore, Xin *et al* (124) reported that degradation of suppressor of cytokine signaling 1, a key negative regulator of IFN-γ signaling, was prevented by TNF through RIP1/RIP3 signaling. The authors suggested that necroptotic inhibition might be a novel strategy for the treatment of acute myeloid leukemia through the combination of RIP1/RIP3 inhibitor with IFN-γ. Recently, bufalin was demonstrated to increase the expression of necroptosis mediators RIP1/RIP3 and ROS, leading to poly(ADP-ribose) polymerase (PARP)-dependent tumor cell death and tumor growth inhibition in MCF-7 and MDA-MB-231 human breast cancer cells (125). The promising role of RIP1/RIP3-dependent necroptosis in cancer therapy warrants attention in future studies (Table III).

RIP1/RIP3-dependent necroptosis in metabolic diseases. Glucose and its metabolism have a crucial role in driving necroptosis4 (1). Larocca *et al* (126) found that hyperglycemia upregulated necroptosis and shifted from apoptosis to necroptosis, associated with increased expression of RIP1, RIP3 and MLKL proteins. Sequentially, levels of RIP1 and MLKL increased in cerebral tissue from hyperglycemic neonatal mice that underwent hypoxia-ischemia brain injury (127). Current studies have documented that

Table III. Potential of RIP1/RIP3-regulated necroptosis in diseases therapy.

Disease	Compound or treatment	Pathological mechanism	(Refs.)
Atherosclerosis	PS-341	Impairing macrophage necroptosis and inflammation	(111-113)
Myocardial ischemia	Nec-1	Reducing the infarct size induced by necroptosis	(114)
Cardiac hypertrophy	Losartan	Inhibiting the RIP1/RIP3-induced necroptosis of cardiac cell injury	(116)
Hyperglycemia	Nec-1s	High glucose promotes necroptosis and the expression of RIP1, RIP3, and MLKL proteins	(126-128)
Acetaminophen-mediated liver injury	Nec-1	Inhibiting acetaminophen-induced hepatic JNK phosphorylation and mitochondrial Bax translocation	(131)
Concanavalin A-induced autoimmune hepatitis	Nec-1 or RIP3$^{-/-}$	Inhibiting the necroptosis of hepatocytes	(130)
Alcoholic fatty liver disease	RIP3$^{-/-}$	Mice lacking RIP3 were protected from ethanol-induced steatosis, hepatocyte injury, and expression of proinflammatory cytokines	(134)
Nonalcoholic fatty liver disease	RIP3$^{-/-}$	Attenuating choline-deficient diet-induced liver injury, steatosis, inflammation, fibrosis and oxidative stress	(135)
CCl4-induced liver fibrosis	Melatonin	Preventing liver fibrosis by inhibiting necroptosis-associated inflammatory signaling	(136)
Renal ischemia-reperfusion injury	Nec-1, RIP3$^{-/-}$	Inhibiting the necroptosis of organ damage, independent of the immune system	(138)
Kidney inflammation, interstitial fibrosis	Nec-1	Inhibiting the necroptosis of kidney injury induced by unilateral ureteral obstruction	(140)
Colorectal carcinoma	Topoisomerase inhibitor SN38 (active metabolite of irinotecan)	Promoting RIP1-depended necroptosis of HT29 and HCT116 cell lines	(123)
Acute myeloid leukemia	Nec-1 combined with interferon-γ	Combination treatment inactivates RIP1/RIP3-mediated necroptotic signaling	(124)
Breast cancer	Bufalin	Increasing the necroptosis mediators RIP1/RIP3 and reactive oxygen species in MCF-7 and MDA-MB-231 human breast cancer cells	(125)
Retinal detachment	Nec-1, RIP3$^{-/-}$	Preventing necroptosis and reducing oxidative stress	(142)
Retinitis pigmentosa	RIP3$^{-/-}$	Necroptosis promotes cone photoreceptor degeneration	(143)
Amyotrophic lateral sclerosis (induced by optineurin deficiency)	Nec-1s	Necroptosis and inflammation exacerbate disease progression	(147)
Hyperuricemia	RIP3$^{-/-}$	Reducing pro-inflammatory cytokines and necroptosis of kidney cells	(128)
Donor organ injury	Nec-1, RIP3$^{-/-}$	Improving renal function	(149)
Paraquat-induced cardiac contractile dysfunction	Nec-1	Downregulating the RIP1/RIP3/MLKL signaling pathway and preventing cardiac contractile dysfunction	(117)
Toxic epidermal necrolysis	Dabrafenib	Preventing RIP3-mediated necroptosis	(150)

RIP, receptor-interacting serine/threonine kinase; Nec, necrostatin-1; Nec-1s, Nec-1 stable.

the intensity of necroptosis is closely associated with high glucose levels.

Hyperuricemia (HU) is closely related to metabolic syndrome. Wang *et al* (128) demonstrated that RIP3 was strongly expressed in mice with HU, whereas RIP3 deficiency attenuated HU symptoms. Using RIP3-knockout mice, various effects were observed following HU compared with RIP wild-type mice, namely downregulation of circulating and kidney pro-inflammatory cytokines (IL-1β, TNF-α and IL-6), a decrease of FADD, cleaved caspase-8/-3 and PARP expression levels, and a decrease in TUNEL apoptotic staining in renal samples (128). These results suggested that RIP3 may have a crucial role in HU, and it may serve as a novel target for future therapeutic strategies (Table III).

RIP1/RIP3-dependent necroptosis in liver injury. The death of hepatocytes initiates and aggravates chronic inflammation and fibrosis during liver injury, ultimately leading to liver cirrhosis and hepatocellular carcinoma. Increasing evidence indicates that necroptosis has a key role in acute liver injury and chronic liver injury (129). Deutsch *et al* (130) indicated that RIP1 and RIP3 have different roles in drug-induced or immunological acute liver injuries. In Concanavalin A (ConA)-induced autoimmune hepatitis, RIP3 deletion delayed hepatic injury, while RIP1 inhibition markedly exacerbated ConA-induced hepatitis (130). Conversely, in acetaminophen (APAP)-mediated liver injury, blockade of RIP1 or RIP3 ameliorated APAP toxicity (130). Zhang *et al* (131) demonstrated that RIP1 was strongly expressed and promoted acute liver failure in mice treated with APAP (300 mg/kg, intraperitoneally injected). Further analysis demonstrated that Nec-1, the inhibitor of RIP1, significantly inhibited APAP-induced hepatic JNK phosphorylation and mitochondrial Bax translocation (131). Using the APAP model in RIP3$^{-/-}$ mice, Ramachandran *et al* (132) found that RIP3 knockout significantly reduced hepatotoxicity after 6 h of APAP treatment (200-300 mg/kg), while the protective effect of RIP3 knockout on the liver was not obvious after 24 h of APAP treatment.

Chronic liver injury usually includes alcoholic fatty liver disease (AELD), nonalcoholic fatty liver disease (NAFLD), liver fibrosis and cirrhosis. It has been reported that the expression of RIP3 in the liver tissue of patients with AFLD is abnormally elevated, and a series of pathological changes, such as hepatocyte lipid accumulation and elevated transaminase, are observed in the liver (133,134). Additionally, Afonso *et al* (135) reported that the expression of RIP3 and phosphorylated MLKL was increased in the liver tissue of patients with NAFLD, while RIP3 knockout significantly reduced the steatosis and inflammatory response in mice with methionine-choline diet. Choi *et al* (136) found that melatonin reduced the expression of RIP1, RIP3 and MLKL in rat fibrotic liver induced by CCl_4, and inhibited the expression of high-mobility group box 1 (HMGB1) and IL-1α. This suggested that melatonin can alleviate liver fibrosis by inhibiting the inflammatory signaling associated with programmed necrosis (136). Further studies are required to fully elucidate the role of RIP1/RIP3-dependent necroptosis in liver injury (Table III).

RIP1/RIP3-dependent necroptosis in kidney injury. Increasing evidence has demonstrated that necroptosis has an important role in the pathogenesis of multiple types of kidney injury. Linkermann *et al* (137) first determined the presence of necroptosis in a murine model of renal ischemia-reperfusion injury (IRI). The detection of RIP1 and RIP3 in whole-kidney lysates and freshly isolated murine proximal tubules revealed the contribution of necroptosis in renal injury (137). A subsequent study demonstrated that, in a mouse model of IRI, necroptosis lead to primary organ damage, and RIP3-knockout mice were protected from IRI (138). Another study used MLKL-knockout mice to investigate the role of necroptosis in acute kidney injury (139). Their results revealed the indispensable role of MLKL in the necroptotic pathway (139). Additionally, Xiao *et al* (140) reported that the effect of necroptosis and the RIP1/RIP3/MLKL signaling pathway in renal inflammation and interstitial fibrosis was associated with primitive tubulointerstitial injury. Inhibition of necroptosis reduced the inflammatory response and interstitial fibrosis in renal tissues (140). Therefore, the signaling pathways and the main regulators of necroptosis may serve as potential candidates for therapeutic strategies in kidney injury (Table III).

RIP1/RIP3-dependent necroptosis in ocular disease. Apoptosis was previously demonstrated to be a significant form of cell loss in photoreceptor death, but RIP-mediated necrosis was recently discovered to be a crucial mode of photoreceptor cell loss in an experimental model of retinal detachment (141). Expression of RIP3 undergoes a 10-fold increase after retinal detachment (142). Nevertheless, Nec-1 or RIP3 deficiency substantially prevent necroptosis and reduce oxidative stress of apoptosis-inducing factor (142). Additionally, cone photoreceptor death in retinitis pigmentosa (RP) is widely considered as a necroptotic mechanism, while rod photoreceptor death is characterized by apoptotic features (143). Murakami *et al* (144) reported that RIP3 expression was elevated in rd10 mouse retinas in the cone phase, but not in rod degeneration, and thus suggested that RIP3 may be a potential target in protecting cone photoreceptors. On the other hand, Sato *et al* (145) found that RIP1/RIP3 accelerated both cone and rod photoreceptor degeneration in interphotoreceptor retinoid-binding protein (Irbp)$^{-/-}$ mice. It is worth noting that Irbp deficiency displays severe early and progressive photoreceptor degeneration. Based on these studies, RIP1/RIP3 may be regarded as a potential therapeutic target to prevent or delay photoreceptor degeneration in patients with RP.

In an animal model of retinal detachment (RD), cleaved IL-1β was reported in infiltrated macrophages undergoing RIP3-dependent necroptosis, rather than dying photoreceptors (146). In addition, Ito *et al* (147) suggested a direct connection between amyotrophic lateral sclerosis induced by optineurin deficiency and RIP1-regulated necroptosis and inflammation. Because it promotes both inflammation and cell death, RIP1/RIP3 may be a common mediator of human degenerative diseases characterized by axonal degeneration (Table III).

Other properties of RIP1/RIP3-dependent necroptosis. Donor organ injury is invariably mentioned in transplantation, due to immune responses related to IRI and alloimmune rejection (148). Lau *et al* (149) reported that in caspase-inhibited tubular epithelial cells (TECs), necroptosis was triggered *in vitro*, following inhibition by Nec-1 or RIP3$^{-/-}$ TEC. Following transplantation, recipients receiving RIP3$^{-/-}$ kidneys had longer survival and improved renal function (149). These results suggested that inhibition of RIP3-dependent necroptosis and inflammatory injury in donor organs may provide clinical benefit. Toxic epidermal necrolysis (TEN) is a severe adverse drug reaction with a high mortality rate (150). Kim *et al* (151) detected upregulated expression of RIP3 and elevated phosphorylation of MLKL in skin sections from patients with TEN. Dabrafenib notably prevented RIP3-mediated MLKL phosphorylation and decreased necroptosis, by inhibiting RIP3 in a TEN model (151). Based on these findings, it can be speculated that RIP may represent a potential target for treatment of TEN. Table III presents a list of existing studies on the role of RIP1/RIP3-mediated necroptosis in disease.

8. Conclusion and perspectives

It is well-known that necroptosis, a physiologically relevant form of cell death, is involved in pathological cell death resulting from IRI. RIP1/RIP3 is a key cascade involved in necroptosis, which is regulated by caspase activation and ubiquitination. RIP1 regulates cell death and survival, while RIP3 mediates apoptosis and necroptosis. Both TLR and virus-mediated activation are recognized or identified by specific receptors or sensors placed either inside or on the surface of cells. RIP1/RIP3 mediates the initiation of the necroptotic response to different stimuli. Following ligand binding and receptor activation, RIP3 enters complex II via interaction with RIP1, which then activates MLKL participation in inflammation, immune response, embryonic development and metabolic abnormality.

In conclusion, an improved understanding of the molecular events regulating necroptotic cell death would be extremely beneficial for disease therapy, as these mechanisms are implicated in a variety of human illnesses. Further research in this area can be expected to provide promising opportunities regarding therapeutic exploitation of cell death programs. Due to its crucial roles in organ growth and tumor cell proliferation, RIP1/RIP3 may be a key potential therapeutic strategy in multiple severe diseases. Therefore, by using small molecules that specifically target necroptosis, it may be possible to alleviate symptoms and prolong the lives of patients. In coming years, further research on this alternative cell death pathway may include identification of additional RIP1 and RIP3 substrates. RIP3 appears to be the foremost promoter of necroptosis. In the future, studies exploiting RIP3 inhibitors may provide crucial insight into the diagnosis and treatment of necroptosis-associated diseases.

Acknowledgements

Not applicable.

Funding

This study was sponsored by the National Science Foundation of China (grant no 81802504), the Sichuan National Science Research Funding (grant no. 2018JY0645), Sichuan Health and Family Planning Commission Funding (grant no. 16ZD0253), Chengdu National Science Research Funding (grant no. 2018-YFYF-00146-SN), and funding from the Sichuan Scientific Research Grant for Returned Overseas Chinese Scholars for Dr Yi Wang. This study was also supported by the National Key Research and Development Plan of China (grant no. 2017YFC0113901), the Key Research and Development Plan of Sichuan Science and Technology Bureau (grant no. 2019YFS0278), the Health Care for government officials of Sichuan Province and the Sichuan Health and Family Planning Commission Funding (grant no. 120094) for Dr Yuping Liu.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YW, RT and YL conceived and designed the review. DZ, TLiu, TLei, SD and LG wrote the manuscript. TLei, DQ and CL prepared the figures. YW, RT and YL reviewed and edited the manuscript. All authors read and approved the final manuscript, and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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