Current translational potential and underlying molecular mechanisms of necroptosis

Tamás Molnár1,2, Anett Mázló1,2,3, Vera Tslaf1, Attila Gábor Szőlösi1, Gabriella Emri4 and Gábor Koncz1

Abstract

Cell death has a fundamental impact on the evolution of degenerative disorders, autoimmune processes, inflammatory diseases, tumor formation and immune surveillance. Over the past couple of decades extensive studies have uncovered novel cell death pathways, which are independent of apoptosis. Among these is necroptosis, a tightly regulated, inflammatory form of cell death. Necroptosis contribute to the pathogenesis of many diseases and in this review, we will focus exclusively on necroptosis in humans. Necroptosis is considered a backup mechanism of apoptosis, but the in vivo appearance of necroptosis indicates that both caspase-mediated and caspase-independent mechanisms control necroptosis. Necroptosis is regulated on multiple levels, from the transcription, to the stability and posttranslational modifications of the necrosome components, to the availability of molecular interaction partners and the localization of receptor-interacting serine/threonine-protein kinase 1 (RIPK1), receptor-interacting serine/threonine-protein kinase 3 (RIPK3) and mixed lineage kinase domain-like protein (MLKL). Accordingly, we classified the role of more than seventy molecules in necroptotic signaling based on consistent in vitro or in vivo evidence to understand the molecular background of necroptosis and to find opportunities where regulating the intensity and the modality of cell death could be exploited in clinical interventions. Necroptosis specific inhibitors are under development, but >20 drugs, already used in the treatment of various diseases, have the potential to regulate necroptosis. By listing necroptosis-modulated human diseases and cataloging the currently available drug-repertoire to modify necroptosis intensity, we hope to kick-start approaches with immediate translational potential. We also indicate where necroptosis regulating capacity should be considered in the current applications of these drugs.

Facts

- Necroptosis is closely associated with the pathogenesis of many human diseases.
- The in vivo appearance of necroptosis indicates that both caspase-independent and caspase-dependent mechanisms control this cell death pathway.
- More than 70 human molecules play a role in the regulation of necroptosis.
- More than 20 approved drugs have the potential to regulate necroptosis.

Open Questions

- How can we monitor and regulate necroptosis in human diseases?
- What are the main molecular targets in caspase independent regulatory mechanisms of necroptosis?
- How effective can the off-label use of already approved drugs in necroptosis-driven diseases be?

Introduction

The development and homeostasis of multicellular organisms depends on the balance between cell proliferation and cell death. In the past few years new regulated cell death pathways have been discovered and...
classified\(^1\). One of these tightly controlled inflammatory cell death pathways – necroptosis – has come to the center of attention because of its known contribution to the pathogenesis of many diseases\(^1,2\).

Many death-, pattern recognition-, DNA binding-, adhesion, and dependence-receptors, immune reactions, pathogens and various drugs have been identified as necroptosis triggers\(^1,3\). Necroptosis utilizes a signaling pathway requiring the involvement of receptor interacting protein kinase 3 (RIPK3)\(^4\), mixed lineage kinase domain-like protein (MLKL)\(^5\) and upon stimulation of death receptors (DR)\(^2\) RIPK1. RIPK3 oligomerization and its subsequent phosphorylation allows the RIPK3-MLKL interaction and the double phosphorylation of MLKL by RIPK3\(^6\). After this step, MLKL forms oligomers and translocates to the plasma membrane to execute necroptosis (Fig. 1). Generally, necroptosis requires inhibition of caspases\(^3,7\) or the absence of the pro-caspase-8-activating adaptor Fas-associated protein with death domain (FADD)\(^8\), demonstrating the crucial role of the apoptotic platform in the negative regulation of necroptosis. Active caspases block necroptosis\(^2\) preferentially through the cleavage of RIPK1\(^9\), RIPK3\(^3,10\), and cylindromatosis (CYLD) protein\(^11\) which acts as the de-ubiquitination enzyme of RIPK1. During DR-mediated signaling, inhibitors of apoptosis proteins (IAPs) initiate the ubiquitination of RIPK1 and this process favors cell survival\(^12\). Blockage of IAPs or the subsequent events of IAP-induced signaling strongly support necroptosis\(^13\). Various molecular pathways have been documented as regulators of downstream necrotic events beside MLKL-mediated membrane rupture, but the complexity of the signaling and regulation network of necroptosis are still not fully understood.

The immunological outcome of cell death can be classified as anti-inflammatory or pro-inflammatory and tolerogenic or immunogenic\(^1\). Dominance of apoptosis ensures the tolerogenic outcome of cell death under physiological conditions. When apoptosis signaling is blocked, necroptotic pathways are activated and the dying cells have the potential to initiate innate immune responses via production of damage associated molecules (DAMPs) resulting in an inflammatory response\(^14\). Signaling in necrotic cells also supports the cross priming capacity of dendritic cells (DCs)\(^15\).

In this review our goal was to understand the molecular background of necroptosis in humans and to find potential points of clinical intervention. We summarized the expression, posttranslational modification, and localization of necrotic molecules are regulated and what the interaction partners of the necrosome complex are. Finally, we provide an overview of drugs, which are already used in the clinic and have been shown to affect necroptosis.

**Necroptosis involved in human diseases**

Currently, necroptosis is mainly documented in various in vivo mice models\(^16,17\), but regulated necrosis contributes to the pathogenesis of many human diseases (Table 1). Both up and down-regulation of necroptosis and misregulation of the apoptosis-necroptosis transition which modifies the immunological outcome of cell death contribute to the evolution of degenerative disorders, autoimmune processes, inflammatory diseases or the immune surveillance of tumors.

Some physiological processes such as alteration of glucose level, oxygen deprivation or immune reactions resulted in elevated RIPK3 expression allowing in vivo emergence of necroptosis. Hyperglycemia (35–40 mM glucose) markedly enhanced the expression of RIPK3 in various cell lines and primed cells for necroptosis\(^18,19\). Similarly, upregulated expression of RIPK1, RIPK3 and MLKL, and increased RIPK1/3 complex formation have been observed in hypoxic cells\(^20–22\). At the same time caspase-8 mRNA, functioning as a negative regulator of necroptosis, was reported to be transiently decreased following the deprivation of oxygen and glucose (OGD)\(^23\). These processes are also involved in brain injury caused by hypoxia-ischemia and OGD-induced necroptosis\(^24,25\). Type I\(^26–28\) and type II\(^27,29\) interferons have been published to induce increased expression of RIPK3, while constitutive IFNβ signaling was demonstrated to increase the intracellular level of MLKL\(^28\). CD8+ T lymphocytes can trigger both apoptosis and necroptosis, which make these cells capable of killing tumor cells, even those that

![Fig. 1 Backbone of necroptosis signaling. Various extra- or intracellular signals activates the RIPK3 protein directly or through RIPK1. RIPK3-mediated phosphorylation induces MLKL membrane translocation and consequently, ion influx results in necroptosis\(^1\). Survival signals through upregulation of IAPs or activation of TAK1 kinase pathway blocks RIPK1-induced signaling and protects cells from unwanted necroptosis. Caspase-8-mediated cleavage of pro-necrotic RIPK1 and RIPK3 ensures the dominance of immunologically silent apoptosis to immune stimulant necroptosis](image-url)
| Disease                                     | Molecular changes in possible diagnosis                                                                 |
|--------------------------------------------|----------------------------------------------------------------------------------------------------------|
| Lipid storage disorders                    | Molecular changes in possible diagnosis                                                                 |
| Niemann–Pick disease                       | Increased expression of RIPK1 and RIPK3 in cerebellar tissue.                                            |
| Skin disorders                             | Molecular changes in possible diagnosis                                                                 |
| Toxic epidermal necrolysis                 | Upregulated RIPK3 expression and elevated MLKL phosphorylation in skin tissue sections                   |
| Cutaneous vasculitis                       | Strong phospho-MLKL signals in infiltrating tissue neutrophils in biopsy specimens                      |
| Psoriasis                                  | Molecular changes in possible diagnosis                                                                 |
| Lichen Planus                              | Detection of highly upregulated RIPK3 and increased phosphorylation of RIPK3 and MLKL                   |
| Systemic lupus erythematosus               | Molecular changes in possible diagnosis                                                                 |
| Cardiovascular diseases                    | Molecular changes in possible diagnosis                                                                 |
| Chronic Heart Failure                      | Elevated expression of RIPK1 and RIPK3, increased RIPK3 and MLKL phosphorylation, downregulation of active caspase-3 and 7 |
| Coronary artery disease                    | Patients with CAD plasma RIP3 levels were significantly higher than controls                              |
| Unstable atherosclerosis                   | High RIPK3 and MLKL expression. Increased phosphorylation of MLKL.                                      |
| Abdominal Aorta Aneurysm                   | Elevated levels of RIPK1 and RIPK3 in AAA tissue                                                       |
| Neurodegenerative disorders                | Molecular changes in possible diagnosis                                                                 |
| Multiple Sclerosis                         | High RIPK1 and RIPK3 expression. Increased phosphorylation of RIPK1 and RIPK3. Reduced expression of active Caspase-8. |
| Amyotrophic Lateral Sclerosis              | Elevated levels of RIPK1, RIPK3 and MLKL, increased RIPK1 and p-MLKL phosphorylation in both microglia and oligodendrocytes primarily localized in the white matter |
| Alzheimer’s disease                        | Detection of activated RIPK1                                                                           |
| Spinal cord injury                         | After SCI, strong RIP3-, phosphorylated-MLKL- (pMLKL) and HMGB1-immunoreactivities were detected.       |
| Gastrointestinal diseases                  | Molecular changes in possible diagnosis                                                                 |
| Alcoholic liver disease                    | Increased expression of RIPK3                                                                          |
| Non alcoholic fatty liver disease          | Increased RIPK3 and MLKL expression                                                                    |
| Drug-induced liver injury                  | Elevated phosphorylation of MLKL                                                                        |
| Crohn’s disease                            | Increased expression of RIPK3                                                                          |
| Primary biliary cholangitis                | Elevated expression of RIPK3, phosphorylation of MLKL, insoluble aggregates of RIPK1, RIPK3 and MLKL  |
| Ulcerative colitis                         | Strong phospho-MLKL signals in infiltrating tissue neutrophils in biopsy specimens                     |
| IBD in children                            | Increased expression of RIPK3 and MLKL and reduced caspase-8 in patient’s tissue                       |
| Autoimmune diseases, Immunodeficiency      | Loss-of-function mutations in RIPK1 detected with exome sequencing                                     |
| Immunodeficiency, arthritis and intestinal inflammation | Loss-of-function mutations in RIPK1 detected with exome sequencing                                     |
| Renal diseases                             | Molecular changes in possible diagnosis                                                                 |
| Acute kidney injury                        | Phosphorylation of RIPK3 and MLKL                                                                        |
| Autosomal dominant polycystic kidney disease | Phosphorylation of RIPK3 and MLKL                                                                        |
| Kidney ischemia-reperfusion injury         | Phosphorylation of MLKL                                                                                |
| Autoimmune vasculitis in the kidney        | Phosphorylation of MLKL                                                                                |

Table 1  Necroptosis related diseases in human

Molecular changes in possible diagnosis

Increased expression of RIPK1 and RIPK3 in cerebellar tissue.

Upregulated RIPK3 expression and elevated MLKL phosphorylation in skin tissue sections

Strong phospho-MLKL signals in infiltrating tissue neutrophils in biopsy specimens

Detection of highly upregulated RIPK3 and increased phosphorylation of RIPK3 and MLKL

Elevated expression of RIPK1 and RIPK3, increased RIPK3 and MLKL phosphorylation, downregulation of active caspase-3 and 7

Patients with CAD plasma RIP3 levels were significantly higher than controls

High RIPK3 and MLKL expression. Increased phosphorylation of MLKL.

Elevated levels of RIPK1 and RIPK3 in AAA tissue

High RIPK1 and RIPK3 expression. Increased phosphorylation of RIPK1 and RIPK3. Reduced expression of active Caspase-8.

Elevated levels of RIPK1, RIPK3 and MLKL, increased RIPK1 and p-MLKL phosphorylation in both microglia and oligodendrocytes primarily localized in the white matter.

Detection of activated RIPK1

After SCI, strong RIP3-, phosphorylated-MLKL- (pMLKL) and HMGB1-immunoreactivities were detected.

Increased expression of RIPK3

Increased RIPK3 and MLKL expression

Elevated phosphorylation of MLKL

Increased expression of RIPK3

Elevated expression of RIPK3, phosphorylation of MLKL, insoluble aggregates of RIPK1, RIPK3 and MLKL

Strong phospho-MLKL signals in infiltrating tissue neutrophils in biopsy specimens

Increased expression of RIPK3 and MLKL and reduced caspase-8 in patient’s tissue

Loss-of-function mutations in RIPK1 detected with exome sequencing

Phosphorylation of RIPK3 and MLKL

Phosphorylation of RIPK3 and MLKL

Phosphorylation of MLKL

Phosphorylation of MLKL in neutrophils
escaped apoptosis. T cell-mediated necroptotic cytolyis also plays a role in activation induced cell death, and can be critical in the development of autoimmune reactions.

**Upregulation of necroptosis in human diseases**

Necroptosis takes part in the pathogenesis of human neurodegenerative disorders, such as Multiple Sclerosis (MS), Alzheimer’s disease (AD), and Amyotrophic Lateral Sclerosis (ALS). Defects in the activation of caspase-8 were demonstrated in the pathologic process of MS. Additionally, activated forms of RIPK1, RIPK3 and MLKL were detected in the cortical lesions of human MS samples. Activated RIPK1 as a marker of necroptosis was also observed in human AD brains correlating positively with Braak stage and negatively with brain mass and cognition. In ALS samples, multiple biochemical hallmarks of necroptosis including increased levels of RIPK1, RIPK3 and MLKL and elevated pRIPK1 and pMLKL were detected in both microglia and oligodendrocytes. Importantly, pMLKL was primarily localized in the white matter, where demyelination was found. In spinal cord injury strong RIPK3 expression and MLKL phosphorylation were detected.

In certain cardiovascular diseases, such as chronic heart failure (HF) cell loss and subsequent deterioration of contractile function is associated with elevated expression of RIPK1, RIPK3, and pRIPK3. On the other hand, the expression of caspase-8 was downregulated suggesting activation of necroptosis signaling. MLKL expression did not differ among the control and HF groups; however, pMLKL were present in all HF samples, which is in contrast to the controls where this was almost undetectable. A genetic variant in the RIP3 promoter region was associated with increased RIPK3 transcription, which contributed to the poor prognosis of HF patients.

In humans with unstable carotid atherosclerosis, expression of RIPK3 and MLKL was increased, while the phosphorylation of MLKL was detected in advanced atheromas. In patients with abdominal aorta aneurysm, the tissue showed elevated levels of RIPK1 and RIPK3 proteins. In coronary artery disease higher plasma RIPK3 levels were detected than in controls.

Regarding gastrointestinal diseases, increased RIPK3 expression was detected in liver biopsies from patients with alcoholic liver disease, while both RIPK3 and MLKL expression was increased in non-alcoholic fatty liver diseases, as well as elevated MLKL phosphorylation in drug-induced liver injury. High levels of RIPK3 and MLKL phosphorylation were also detected in the liver biopsies of patients with primary biliary cholangitis, in contrast with its low hepatic expression in healthy controls. Similarly, increased levels of RIPK3 were documented in the terminal ileum of patients with Crohn’s disease and elevated RIPK3 and MLKL levels were observed in inflamed tissues of inflammatory bowel disease (IBD) and allergic colitis patients, whereas the expression of caspase-8 in these tissues was reduced. The migration of human neutrophils to sites of inflammation was found to activate the RIPK3-MLKL pathway: a strong pMLKL signal was observed in infiltrating tissue neutrophils in samples collected from patients with cutaneous vasculitis, ulcerative colitis, and psoriasis.

Phosphorylation of MLKL molecules was also detected in human acute kidney injury biopsies, in biopsies taken immediately after excision for transplantation and in autosomal dominant polycystic kidney disease, representing involvement of necroptosis in renal disorders. Antineutrophil cytoplasmic antibody (ANCA) induces neutrophil extracellular traps via necroptosis and causes subsequent endothelial cell damage. ANCA-associated vasculitis exhibited a specific p-MLKL staining in glomerular neutrophils in human kidney biopsies.

Concerning skin diseases, human biopsy samples obtained from patients with Lichen Planus (LP) and Systemic lupus erythematosus (SLE) confirm the role of necroptosis in their development. RIPK3 and MLKL activation was demonstrated in podocytes in renal biopsies from patients with lupus nephritis. LP and SLE tissue sections showed enhanced epidermal expression of phosphorylated RIPK3. B cells from SLE patients also significantly displayed high expression levels of necroptosis-related genes. As we already mentioned, phosphorylation of MLKL in the infiltrated human neutrophils was also found in cutaneous vasculitis and psoriasis.
Upregulated RIPK3, and elevated MLKL phosphorylation were observed in the skin samples from patients with toxic epidermal necrolysis in correlation with unwanted necroptosis and subsequent inflammation 58.

Expression of RIPK3 and dynamin-related protein 1 (Drp1) was increased in lung tissue homogenates collected from patients suffering from chronic obstructive pulmonary disease, proving the role of necrotic cell death in pulmonary disease 59. In Kashin–Beck disease (KBD) necroptosis dominates as a cell death mechanism in the middle zone of cartilage from KBD children 60. Necrotic cell death is involved in the progression of chronic periodontitis, as gingival tissue in patients showed increased levels of RIPK1, RIPK3, and MLKL, as well as increased phosphorylation of MLKL 61.

Although RIPK1 is one of the key molecules required for execution of necroptosis, patients with its complete deficiency due to homozygous mutations suffered from recurrent infections, early-onset of IBD and progressive polyarthritis. In vitro, cells with RIPK1 deficiency showed impaired mitogen-activated protein kinase activation and cytokine secretion and were prone to necroptosis 62,63.

Role of necroptosis in cancers

An increasing number of studies have been published about the importance of necroptotic cell death in anticancer therapies, which have been extensively reviewed in recent papers 64,65.

Briefly, both pro- and anti-tumoral effects have been demonstrated following necroptosis in cancer development and progression. The anti-tumoral effect of necroptosis has been shown in many types of cancer in which the expression of RIPK3 56,67 or MLKL 68 was silenced or polymorphisms in their coding genes lead to modified expression of necrosomal components 66,69. In general, necroptosis resistance of cancer cells is a common process, and escape from necroptosis was suggested to be a potential hallmark of cancer, similar to the escape from apoptosis 64. Additionally, effective anti-cancer agents trigger immunogenic cell death, inducing the killing of the transformed cells and provoking the members of innate and adaptive immune system to attack. Beside the massive release of DAMPs, necrotic cells create a great possibility to trigger the activation of CD8+ T cells via cross presentation 75,76. The dual ability of necroptosis to activate innate and adaptive immunity simultaneously makes this cell death pathway a promising therapeutic target.

However, the tumor-promoting outcome of necroptosis has also been shown. RIPK3 and MLKL expression seems to vary among tissue samples from different subtypes and stages of cancer, and downregulation of necroptosis mediators has also been published in various cancers 71–73. Upregulated RIPK3 expression is a general phenomenon in tumor necrotic areas playing a critical role in tumor growth and metastasis 74. Necroptosis-induced inflammation contributes to tumorigenesis and necroptosis can also lead to an immunosuppressive tumor microenvironment 25. The immune-suppressing environment was associated with necroptosis-induced expression of the chemokine attractant CXCL1 71. It has also been shown that tumor cells induce necroptosis of endothelial cells, which promotes tumor cell extravasation and metastasis 76. Thus, we can conclude that necroptosis occurs in different phases during tumorigenesis and plays an ambivalent role in tumor formation.

Molecular mechanisms in the regulation of necroptosis

To understand the molecular background of necroptosis and to find potential points of clinical intervention we summarize below how the expression, the posttranslational modification, and the localization of key necrototic molecules (RIPK1, RIPK3 and MLKL) are regulated, while also highlighting the interaction partners of the necosome complex.

Regulation the expression level of necrototic proteins

RIPK3–RIPK3 homodimerization is sufficient to induce necroptosis; after which, its kinase domain stimulates the activation of RIPK3 through cis-autophosphorylation; a prerequisite step for the recruitment of MLKL 77–79. Thus, RIPK3 dimerization is probably the most critical point of necroptosis induction. Several lines of evidence support the idea that increased expression of RIPK3 can induce its oligomerization and can initiate necroptosis 42,80. RIPK1 dimerization, and accordingly upregulation of RIPK1, facilitates RIPK3 oligomerization, mainly upon death receptor stimuli.

All aspects of necrototic protein expression are intensely regulated, including their transcriptional activity, the stability of the expressed molecules and their degradation. Specificity protein 1 (Sp1), a zinc-finger transcription factor, directly regulates RIPK3 expression in cancer cells. Knockdown of endogenous Sp1 significantly decreases the transcription of RIPK3, while re-expression of Sp1 restores necroptotic response in vitro 81. Induction of necroptosis by interferon gamma (IFN-γ) resulted in elevated levels of RIPK3 27 and MLKL 28,29,82. This effect was found to depend on janus kinase 1 (JAK1) and its substrates; the signal transducer and activator of transcription 1 (STAT1) and interferon regulatory factor (IRF) transcription factors, pinpointing interferon-stimulated gene factor 3 (ISGF3) as a critical promoter 83. Bromodomain-containing protein 4 (BRD4), a member of the bromodomain and extraterminal domain (BET) family, has been shown to interact IRF1 and to upregulate MLKL transcription 84. Oncogenes such as BRAF and AXL have also been implicated in the regulation
of RIPK3 expression. The activity of RIPK3 promoter is tightly controlled by methylation (Fig. 2a). Ubiquitin-like PHD and RING finger domain-containing protein 1 (UHRF1) is essential for the maintenance of the hypermethylation of the RIPK3 promoter and thus contributes to the silencing of RIPK3 expression in quiescent cells.

Following transcriptional regulation multiple processes control the protein level of necrosome components. The heat shock protein 90 (HSP90) and CDC37 co-chaperone complex increases the stability of all RIPK1, RIPK3, and MLKL proteins. Consequently, inhibitors of HSP90 facilitated the degradation of these necrotic components and potently blocked necroptosis. Protein levels of RIPK1 and RIPK3 also decreased in FK506-binding protein 12 (FKBP12) knockdown cells.

On the contrary, cells treated with Hsp70 inhibitors underwent cell death, because Hsp70 enhances the stability of necroptosis antagonists, the RIPK1 regulators: cIAP1/2, x-linked inhibitor of apoptosis protein (XIAP), and the cellular FLICE-like inhibitor protein (cFLIP) (Fig. 2b).

The expression of necrotic molecules are down-regulated by cleavage and proteosomal degradation. The most well-known inhibitor of necroptosis, caspase-8 cleaves both RIPK1, RIPK3, and the necroptosis promoting deubiquitinase CYLD proteins. In macrophages, cathepsins were also reported to be capable of processing RIPK1, which resulted in significant decrease in necrotic cell death.

Several ubiquitin-ligases mediate K48-linked polyubiquitylation and the subsequent proteasomal degradation. The most well-known inhibitor of necroptosis, caspase-8 cleaves both RIPK1, RIPK3, and the necroptosis promoting deubiquitinase CYLD proteins. In macrophages, cathepsins were also reported to be capable of processing RIPK1, which resulted in significant decrease in necrotic cell death.

Posttranslational modifications in the regulation of necroptosis

Accumulating evidence suggests that cell death pathways are finely tuned by posttranslational modifications, such as ubiquitination and phosphorylation. Multiple excellent recent reviews go into extensive detail about the role of these processes in necroptosis, therefore we only provide a brief overview of these processes below. These pathways are mentioned in the tables and figures of this manuscript in the interest of providing a comprehensive visual guide to these processes as well (Fig. 2c).

The necrosome is formed due to the phosphorylation driven assembly of RIPK1, RIPK3, and MLKL. However several phosphorylation steps have been published to inhibit necroptosis, chief among them the transforming growth factor beta-activated kinase 1 (TAK1) complex, which is the most important hub for these necroptosis-damping signals. Various protein complexes are assembled along TNFR signaling; namely the survival (complex I), the apoptotic (complex IIa and IIb) and the necroptosis inducer (complex IIc) complexes. Upon activation TNFR recruits TRADD, RIPK1, TRAF2, TRAF5 proteins. The gathered E3 ubiquitin ligases, cIAP-1 and cIAP-2 molecules, and the linear ubiquitin chain assembly complex LUBAC (consisting of HOIP, HOIL-1L and Sharpin) poly-ubiquitinates RIPK1, and modified RIPK1 can now act as a scaffold for TAK1 and the IKK complex, which facilitates the degradation of these necroptotic components and potently blocked necroptosis.

Interaction partners of necrosome components

The activity of necrosome components are also mediated by molecular interactions (Fig. 2d). Three molecules, Aurora kinase A (AURKA), PPM1b, and HSP90 have been recently identified as binding partners of RIPK3 and/or RIPK1 in resting cells. AURKA and PPM1b act as local inhibitors against spontaneous necroptosis, since their silencing induces necroptosis. PPM1b as a phosphatase prevents RIPK3 autophosphorylation in resting cells together with its downstream target, Glycogen synthase kinase 3β (GSK3β) regulates the formation of RIPK1-RIPK3 and RIPK3-MLKL complexes. Silencing or blocking of AURKA, or inhibitors of GSK3β result in necroptosis without any other stimuli. Phosphorylation of GSK3β at Ser9 suppresses necroptosis through interfering with the formation of RIPK3-MLKL complex, however the direct targets of GSK3β still have not been identified. The third molecule which associates with RIPK3 in resting cells, HSP90, is required for proper activation of necroptosis. Formation of the HSP90–CDC37 complex is necessary for RIPK1–RIPK3 interaction, thus it mediates RIPK3 activation during necroptosis. Unsurprisingly HSP90 inhibitors can block TNF-induced systemic inflammatory response syndrome (SIRS) in rats. Additionally, membrane tethered mucins have been shown to interact with RIPK1 to block necroptosis in human bronchial epithelial cells in vitro.

The nuclear retinoic acid receptor gamma (RARγ) is released from the nucleus to initiate the formation of cell death signaling complexes by mediating RIPK1
dissociation from TNFR when cIAP activity is blocked. In vitro silencing of RARγ inhibited necroptosis and in vivo results also confirmed that RARγ was essential for TNF-induced RIPK1-initiated apoptosis and necroptosis (Table 2)115.

Although RIPK1 initiates RIPK3 activation during death receptor driven necroptosis, it plays an ambivalent role in the regulation of RIPK3 aggregation. Under special circumstances instead of activation, RIPK1 acts to suppress the spontaneous activation of RIPK3 by TIR-domain-containing adapter-inducing interferon-β (TRIF)116 or DNA-dependent activator of IFN-regulatory factors (DAI; also known as ZBP1)78,117. RIPK3 oligomerization is able to seed a RHIM dependent oligomer and this process is both sufficient and a necessary step in necroptosis. The stability of all RIPK1, RIPK3 and MLKL proteins are increased by HSP90 and CDC37 co-chaperone complex and by FKBP12. The level of both RIPK1 and RIPK3 are down-regulated by caspase-8-mediated cleavage. Cathepsins are also capable of processing RIPK1. A20, CHIP, Optn, PEL1 and Triad3a ubiquitin-ligases mediate K48-linked polyubiquitination and the subsequent proteasome dependent degradation of: RIPK1, RIPK3 and/or MLKL. Upon necroptosis human RIPK1 is autophosphorylated at ser14, ser15, ser161, ser166 and RIPK3 at ser199 and ser227 and ser277. The transient phosphorylation of RIPK1 at ser321 is phosphorylated transiently by TAK1 leading to RIPK1-independent apoptosis and the sustained phosphorylation of RIPK1 by TAK1 at ser321, ser332, ser334 and ser336 induces RIPK1 kinase activation106. IKKα/IKKβ also phosphorylate RIPK1 at ser25 and thereby block RIPK1 activity108,214,215. Mitogen-activated protein kinase-activated protein kinase 2 (MK2) mediates phosphorylation of RIPK1 at ser321 and ser336 and restrains integration of RIPK1 into the cytosolic death complex107. The phosphorylation at ser321 by a currently unknown kinase inhibits the RIPK1 kinase activity218. Ubiquitylation of RIPK1 at Lys115 by PEL1219 or Lys377 by cIAP1, cIAP2 and Parkin220 promotes necroptosis. LUBAC complex and the deubiquitinase CYLD regulates M1 ubiquitination of RIPK1220. Lys363 ubiquitylation of RIPK3 leads to its proteasomal degradation. RIPK3 is responsible for the phosphorylation of MLKL at thr357 and ser358. TAM (Tyro3, Axl, and Mer) family of receptor tyrosine kinases phosphorylate MLKL on Tyr376 to facilitate MLKL oligomerization145. MLKL is also phosphorylated on Ser441 by a still unidentified kinase222. Caspase-8 mediates the cleavage and inactivation of RIPK1 at asp324 and RIPK3 at asp328. O-GlcNAcylation of the RIPK3 at thr467 by OGT prevents necroptosis223. Red names indicate interaction partners of RIPK1, RIPK3, MLKL which activate necroptosis, blue marks necroptosis inhibitors.

Fig. 2 Direct interacting partners of main necroptotic signaling molecules. Sp1 transcription factor increases RIPK3 expression. INFγ-mediated up-regulation of RIPK3 and MLKL level depend on JAK1 kinase, and STAT1 and IRF transcription factors. BRD4 cooperating with IRF1 also increase MLKL transcription. Hypermethylation of the RIPK3 promoter by UHRF1 results in silenced RIPK3 expression. The stability of all RIPK1, RIPK3 and MLKL proteins are increased by HSP90 and CDC37 co-chaperone complex and by FKBP12. The level of both RIPK1 and RIPK3 are down-regulated by caspase-8-mediated cleavage. Cathepsins are also capable of processing RIPK1. A20, CHIP, Optn, PEL1 and Triad3a ubiquitin-ligases mediate K48-linked polyubiquitination and the subsequent proteasome dependent degradation of: RIPK1, RIPK3 and/or MLKL. Upon necroptosis human RIPK1 is autophosphorylated at ser14, ser15, ser161, ser166 and RIPK3 at ser199 and ser227 and ser277. The transient phosphorylation of RIPK1 at ser321 is phosphorylated transiently by TAK1 leading to RIPK1-independent apoptosis and the sustained phosphorylation of RIPK1 by TAK1 at ser321, ser332, ser334 and ser336 induces RIPK1 kinase activation106. IKKα/IKKβ also phosphorylate RIPK1 at ser25 and thereby block RIPK1 activity108,214,215. Mitogen-activated protein kinase-activated protein kinase 2 (MK2) mediates phosphorylation of RIPK1 at ser321 and ser336 and restrains integration of RIPK1 into the cytosolic death complex107. The phosphorylation at ser321 by a currently unknown kinase inhibits the RIPK1 kinase activity218. Ubiquitylation of RIPK1 at Lys115 by PEL1219 or Lys377 by cIAP1, cIAP2 and Parkin220 promotes necroptosis. LUBAC complex and the deubiquitinase CYLD regulates M1 ubiquitination of RIPK1220. Lys363 ubiquitylation of RIPK3 leads to its proteasomal degradation. RIPK3 is responsible for the phosphorylation of MLKL at thr357 and ser358. TAM (Tyro3, Axl, and Mer) family of receptor tyrosine kinases phosphorylate MLKL on Tyr376 to facilitate MLKL oligomerization145. MLKL is also phosphorylated on Ser441 by a still unidentified kinase222. Caspase-8 mediates the cleavage and inactivation of RIPK1 at asp324 and RIPK3 at asp328. O-GlcNAcylation of the RIPK3 at thr467 by OGT prevents necroptosis223. Red names indicate interaction partners of RIPK1, RIPK3, MLKL which activate necroptosis, blue marks necroptosis inhibitors.
Table 2 Molecules in necroptotic signaling

| Interaction partners | Outcome of silencing | Confirmed in KO mice | Interactions with... | Regulatory mechanism |
|----------------------|----------------------|----------------------|----------------------|----------------------|
|                      |                      |                      | RIPK1 | RIPK3 | MLKL |
| A20                  | ↑225,226             | The embryonic lethality of A20/−/− mice is inhibited by RIPK3 KO225,227. A20 protects T cells from necroptosis. | +225 | +225 |     |
| ABIN-1               | ↑67                  | The embryonic lethality of Abin-1/−/− mice is blocked by inhibition of RIPK1 or absence of RIPK3. |     | +150 |     |
| ADAM9 ADAM10         | ↓150                 |                      | +149 |     |     |
| ALIX and synctenin-1 | ↑149                 |                      |       |     |     |
| APC11                | ↓228                 |                      |        | +228 |     |
| Akt ½ mTOR           | ↓124,125             |                      | +124  |     |     |
| Atg5                 | ↓128                 |                      | +199  | +199 |     |
| AURKA                | ↑112                 | AURKA inhibitor stimulated MLKL phosphorylation and inhibited the growth of implanted tumors. AURKA and GSK3β Are Associated With Poor Prognosis in Human Pancreatic Cancer112. | +112 | +112 | +112 |
| Bax/Bak              | ↓229,230             |                      |       |     |     |
| BRD4                 | ↓84                  |                      |       |     |     |
| CAMKII               | ↓127                 | KO of CaMKII abrogated I/R-induced necrosis and blocked doxorubicin-induced contractile dysfunction, myocardial necrosis and mortality.127 | +127,231 |     |
| Caspase-2            | ↑232                 | Caspase-2 KO leads to embryonic lethality, but Casp8 KO mice fully viable when bred on RIPK3 KO234 or MLKL KO235. |       |       |     |
| Caspase-8            | ↑233                 | Caspase-8 KO leads to embryonic lethality, but Casp8 KO mice fully viable when bred on RIPK3 KO234 or MLKL KO235. | +4,115 | +4,115 |     |
| c-Cbl                | ↓228                 |                      | +228  |     |     |
| CDC37                | ↓91                  | CHIP/STUB1 KO mice showed postnatal lethality with intestinal defects, which is rescued by crossing with RIPK3 KO mice.97 | +91 | +91 |     |
| CypD                 | ↓10,127,173,196,237,238 | In vivo analysis in mice suggested the distinctness of CypD-mediated MPT from RIPK1/RIPK3-mediated necroptosis.133 |     |     |     |
| CYLD                 | ↓11,239–242          | Inhibition of CYLD catalytic activity in epidermal keratinocytes could delay the development of inflammatory skin lesions in FADD−/− mice.91 |     |     |     |
| Daxx                 | ↓244                 |                      | +244  | +244 |     |
| Drp1                 | ↓131,132 debated     | Drp1 KO mice showed postnatal lethality with intestinal defects, which is rescued by crossing with RIPK3 KO mice.97 | +131,132 | +131,132 |     |

RIPK3-KO elevates RIPK3 KS ubiquitination and RIPK1-RIPK3 complexes formation, but A20 replaces K63 polyubiquitin from RIPK1 with K48 polyubiquitin, leading to RIPK1 degradation.92

ABIN-1 is a ubiquitin-binding protein associated with TNFR and A20. Regulates the RIPK1 ubiquitylation/deubiquitylation mediated by LUBAC and pA20.92

MLKL binds with multiple ADAMs to mediate the shedding of cell-surface proteins.93

Phosphorylated MLKL was removed from membranes through ALIX–syntenin-1–mediated exocytosis.149

RIPK3-mediated activation of CaMKII, including direct phosphorylation and indirect ROS-mediated oxidation.127

Caspase-2 KO enhanced the phosphorylation of RIPK1 and MLKL.236

Caspase-8 cleaves RIPK1, RIPK3, and CYLD to block necroptosis.11

C-cbl promotes necroptosis induced by TNF/Sz/7Zvad, but upon TCZ, interaction with RIPK1 was detected upon RIPK1-dependent apoptosis.91

RIPK3 activation requires the activity of an HSP90 and CDC37/cochaperone complex.91

RIPK3 and RIPK1 expression level is negatively regulated by CHIP E3 ligase mediated ubiquitylation.97

Probably, cyclophilin-D (CypD) and RIPK3 mediate two independent form of programmed necrosis.103,196,237

CYLD deubiquitylates RIPK1 (both M1- and K63), facilitating the association of RIPK1 and RIPK3.17,239,283 CYLD promotes the dissociation of TRAF2 from MLKL.121

RIPK3 phosphorylated Daxx at Ser-668 triggering the nuclear export of Daxx.146

PGAM5S activates Drp1 by dephosphorylation, Drp1 facilitates...
**Table 2 continued**

| Interaction partners | Outcome of silencing | Confirmed in KO mice | Interactions with... | Regulatory mechanism |
|----------------------|----------------------|-----------------------|----------------------|---------------------|
| ESCRT-III components | ↑51,245              |                       | RIPC3, MLKL          | mitochondrial fragmentation, ESCRT-III machinery, CHMP2A, CHMP4B, VPS4B, IST1 controls the duration of plasma membrane integrity, when MLKL activation is limited or reversed (192,245)  |
| ESCRT-I components  | ↑283                 |                       | RIPC3, MLKL          | FADD functions together with caspase-8 in the repression of necroptotic signaling. |
| FADD                 | ↑92                  | Fadd KO mice are fully viable when bred RIPK3 KO (162,270) or Mli KO backgrounds (93,248) | +4,101,240,250, +4,101,216, +253 | Protein levels of RIPK1 and RIPK3 decreased significantly in FKBP12 knockout cells  |
| FKBP12               | ↑2,226 ↓252          | FKBP12 is essential for TNFα-induced systemic inflammatory response syndrome. | +253 | c-FLIPS/L, procaspase-8 heterodimers inhibit RIPK1 and RIPK3 (193,254)  |
| cFLIP                |                       |                       | cFLIP KO (as well as caspase-8 KO or FADD KO) results in embryonic lethality, FLIP KO, FADD KO, RIPK3 KO mice are viable (142) | cFLIP, and cFLIPL simple block procaspase-8 activation (22) |
| Flotillin1-2         | ↑149                 | Flotillin-null mice were highly sensitive to TZ-induced SIRS (146) | +149 | Phosphorylated MLKL was removed from membranes through flotillin-mediated endocytosis (140)  |
| Gy10                 | ↓157                 |                       |                       | In complex with GB2 and Src regulates intracellular trafficking of necrosomes (157)  |
| GSK3b                | ↑132                 | AURKA and GSK3β are associated with poor prognosis in human pancreatic cancer (117). | +77 | Phosphorylation of GSK3β at Ser9 by AURKA suppresses the formation of the RIPK3-MLKL complex.  |
| GLUD1                | ↓77                  |                       |                       | Targets of RIPK3, contributing to TNF-β-induced ROS. GLUL and GLUD1 play a role in using glutamine as a supplementary substrate for the TCA cycle.  |
| GLUL                 | ↓77                  |                       |                       | HACE1 is required for RIPK1-dependent apoptosis via TRAF2 ubiquitination. HACE1 KO leads to necroptosis dominance to apoptosis (78).  |
| HACE1                | 0                    | Increased susceptibility of hace–1 KO mice to DSS-induced colitis depends on RIPK3 (135) | +149 | Hsp70 is sustaining the stability of necroptosis inhibitors, cIAP1/2, XIAP, and cFLIPs (93)  |
| HSP70                | ↑99                  |                       |                       | Hsp70 regulates the stability of RIPK1, RIPK3, and MLKL (160,174) and blocks the membrane translocation of MLKL (236).  |
| HSP90                | ↓90,91,256           | HSP 90 inhibitor delayed death in TNF-α-induced SIRS in rats, but not in mice (93) | +91 | HtrA2 promoted RIPK1 degradation during necroptosis (125) and induced monoubiquitination of its substrate UCH-L1 during TNF-induced necroptosis (252)  |
| HtrA2/Omi            | ↓257                 | Inhibitor of HtrA2, significantly alleviated DSS-induced colitis (138) | +258 | cIAP1 and cIAP2 mediates RIPK1 ubiquitination, allowing the recruitment of LUBAC (262–264).  |
| cIAP1/cIAP2          | ↑240,259             | RIPK1 +/− allowed XIAP and cIAP1 double KO to survive past birth, and prolonged cIAP2 and cIAP1 double KO survival (2,259). | +240,261 | Loss of XIAP results in aberrantly elevated ubiquituation of RIPK1 outside of TNFR complex (256).  |
| XIAP                 | ↑2,544,265           | RIPK1 +/− allowed XIAP and cIAP1 double KO to survive past birth (259). | +261 | XIKA and IKKβ, in addition to their known function in NF-κB activation directly phosphorylate RIPK1 (148,214)  |
| IKKα                 | ↑108                 | The lethality induced by TNF + TPCA-1 results from both RIPK1 kinase-dependent apoptosis and necroptosis (193). RIPK3 is activated in Ikka/β-deficient livers, but does not control cholestasis (14). | +266 | NEMO inhibits necroptosis by binding to ubiquitinated RIPK1 (117), blocks the RIPK1-caspase-8 interaction, activates NF-κB (256).  |
| IKKβ                 | ↑2,564,267           | JEC-specific FADD KO combined with RIPK3 KO prevented colitis development in NEMO IEC-KO mice (198,209) | +266 | Phosphorylated inositol products dissociate the auto-inhibitory region from MLKL. IP kinases needs to MLKL oligomerization and membrane localization (117).  |
| IKK/NEMO            | ↑2,564,267           |                       |                       | IFNAR1-deficient macrophages displayed greatly reduced IFNγ transcript levels (149).  |
| IPMK                 | ↑142,143             |                       |                       | IRF1 contributes to IFNγ-dependent and also IFNγ-independent necroptosis (120).  |
| ITPK                 | ↑270                 |                       |                       |  |
| IPPK                 | ↑83                  |                       |                       |  |
| IFNAR1               | ↑83                  | IFNAR1-deficiency protects against LPS/2/2ad induced septic shock (149). |  |  |
| Interaction partners | Outcome of silencing | Confirmed in KO mice | Interactions with... | Regulatory mechanism |
|----------------------|----------------------|----------------------|----------------------|----------------------|
|                      |                      |                      | RIPK1 | RIPK3 | MLKL |
| JAK1                 | ↓ 27,271             |                      |        | + 228 |       |
| Stat1                |                      |                      |        |       |       |
| LRRK2                | ↓ 228                |                      |        |       |       |
| CIR1                 | ↑ 104,236,245        |                      | + 265  | + 221 |       |
|                     |                      | Lubac complex        |        |       |       |
| (HOIP, HOIL1, Sharpin)|                    |                      |        |       |       |
| MKRN1               | ↑ 277                |                      |        |       |       |
| MK2                 | ↑ 107,216            |                      | + 216,217 | + 217 |    |
| MUC1                | ↑ 114                |                      |        | + 114 |       |
| OGT                 | ↑ 223                |                      |        | + 223 |       |
| OPTN                | ↑ 35                 |                      |        | + 35  |       |
| Otulin              | ↑ 278                |                      |        |       |       |
| Parkin              | ↓ 279                |                      |        | + 220 |       |
| Parp1               | ↓ 130                | debated              |        | + 130 |       |
|                     |                      | in Ref.281           |        |       |       |
| PDC                 | ↓ 134                |                      |        | + 134 |       |
| PEL1                | ↓ 99                |                      | + 99,219 | + 99  |       |
|                     |                      | In toxic epidermal   |        |       |       |
|                     |                      | necrosis the        |        |       |       |
|                     |                      | expression level of  |        |       |       |
|                     |                      | PEL1 decreases      |        |       |       |
| PGAM5               | ↓ 131                | debated              |        | + 131 | + 131 |
|                     |                      | in Ref.132,135       |        |       |       |
| PiPs                | ↓ 138                |                      |        |       |       |
| PITPa               | ↓ 144                |                      |        | + 144 |       |
| PKR                 | ↓ 27,286             |                      |        | + 27  |       |
| PPM1b               | ↑ 113                |                      |        | + 113 |       |
|                      |                      | Ppm1b protects mice  |        |       |       |
|                      |                      | from TNF-induced     |        |       |       |
|                      |                      | SIRS through        |        |       |       |
|                      |                      | dephosphorylating    |        |       |       |
|                      |                      | RIPK3               |        |       |       |
| PYGL                | ↓ 77                |                      |        | + 77  |       |
|                     |                      | Target of RIPK3      |        |       |       |
|                     |                      | contributing to     |        |       |       |
|                     |                      | TNF-induced ROS     |        |       |       |
|                     |                      | PYGL regulates pyruvate production. | | | |
Table 2

| Interaction partners | Outcome of silencing | Confirmed in KO mice | Interactions with... | Regulatory mechanism |
|----------------------|----------------------|-----------------------|----------------------|---------------------|
|                      |                      |                       | RIPK1 | RIPK3 | MLKL |
| RARγ                 | $\downarrow^{115}$   | RARγ KO mice are protected from TNF + Z-vad induced death$^{115}$. | + $115$ |                      | RARγ facilitates RIPK1 dissociation from TNF receptor and the formation of death signaling complexes$^{115}$. |
| RelA                 | $\uparrow^{282}$    | Embryonic lethality of RelA KO mice is partially prevented by the KO of RIPK3 or MLKL, and it is fully rescued by the combined ablation of Fadd and RIPK3 or MLKL or RIPK1$^{114,282}$. |                      | RelA KO leads to TNF-induced activation of FADD-dependent apoptosis and RIPK3-dependent necroptosis. |
| RGMb                 | $\uparrow^{122}$    | Renal tubule-specific RGMb knockout mice exhibited severe tubular injury, after renal ischemia/reperfusion$^{122}$. |                      | RGMb inhibits MLKL membrane translocation or membrane binding$^{122}$. |
| RIPK1                | $\downarrow^{78,118}$ | Caspase-8/RIPK1 double-knockout animals die shortly after birth, ablation of RIPK3 to triple knockouts, rescues the viability of these animals. Deficiency in either RIPK3 or MLKL prevented the development of skin lesions in RIPK1E-KO mice$^{117-120}$. | + $4$ | $+^{283}$ | In a kinase-independent function of RIPK1 the RHIM domains of RIPK1 competes with RHIM domain of TRIF or DAI to RHIM-mediated RIPK3 aggregation, but RIPK1 oligomerization is initiative of death domain driven necroptosis$^{78}$. |
| Sp1                  | $\downarrow^{81}$   | In contrary to the in vitro data Spata2 deficiency sensitizes mice to SIRS induced by TNFα$^{284,285}$. |                      | Sp1 specifically binds to RIPK3 promoter and regulates transcription$^{284,285}$. |
| STAT1                | $\downarrow^{17,81,118}$ | IFN-γ failed to induce Mlkl transcription in Stat1$^{−/−}$ mice$^{19}$. |                      | SPATA2 binds CYLD into the TNF-RSC and to HOIP. SPATA2 KO reduces phosphorylation of RIPK1 and MLKL in TNF-α-induced necroptosis$^{284,285}$. |
| TAB1/2               | $\uparrow^{286}$    | Various tissue injuries have been published in the absence of Tak1. These symptoms are associated primarily with apoptosis and were not rescued by RIPK3 deletion$^{98}$. | + $259$ |                      | Interacting with Gγ10-Gβ2 complex regulates intracellular trafficking of necrosomes$^{97}$. |
| TAK1                 | $\uparrow^{102,103}$ | TAK1 inhibition triggered the degradation of cIAP2, FLIP, and NFκB-p65. TAK1 blocks RIPK1-RIPK3-FADD complex formation$^{102,111}$. Intermediate domain of RIPK1 is phosphorylated transiently by TAK1$^{106,289}$. Downstream targets of TAK1 phosphorylates RIPK1 (see, MK2, IKK, RelA) TAK1 inhibition regulates transcription of SIRS$^{106,289}$. | + $102,103,259$ |                      | TAK1 inhibition regulated the degradation of cIAP2, FLIP, and NFκB-p65. TAK1 blocks RIPK1-RIPK3-FADD complex formation$^{102,111}$. Intermediate domain of RIPK1 is phosphorylated transiently by TAK1$^{106,289}$. Downstream targets of TAK1 phosphorylates RIPK1 (see, MK2, IKK, RelA) TAK1 inhibition regulated the degradation of cIAP2, FLIP, and NFκB-p65. TAK1 blocks RIPK1-RIPK3-FADD complex formation$^{102,111}$. Intermediate domain of RIPK1 is phosphorylated transiently by TAK1$^{106,289}$. Downstream targets of TAK1 phosphorylates RIPK1 (see, MK2, IKK, RelA) |
| TAM kinases          | $\downarrow^{145}$  | Tyro3AxlMertk triple KO mice were completely resistant to the SIRS$^{48}$. | $+^{145}$ |                      | TAM (Tyro3, Axl, and Mer) receptor tyrosine kinases phosphorylate MLKL to promote MLKL oligomerization and necroptosis$^{95}$. |
| TRAF2                | $\uparrow^{121,290}$ | TRAF2 deletion causes morbidity, RIPK3 KO delays TRAF2 KO mortality$^{112,260}$ and suppressing TRAF2 augments ischemic brain damage through necroptosis mechanism$^{292}$. | $+^{121}$ |                      | TRAF2-MLKL association suppresses the interaction of MLKL with RIPK3$^{27,121}$. |
| Triad3a              | $\uparrow^{98}$     |                               |                      |                      | Triad3a induces K48 ubiquitination and the degradation of RIPK1, FADD and Caspase-8$^{98}$. |
| TRIF                 | $\downarrow^{83,116}$ | Mice without functional TRIF did not show macrophage loss and elevation of inflammatory cytokines upon LPS/Z-vad$^{293}$. | $+^{294}$ | $+^{116,294}$ | Activates necroptosis through RHIM dependent association of TRIF with RIPK3 kinase$^{10}$. |
| TRPM7                | $\downarrow^{146}$  |                               | $+^{146}$ |                      | TRPM7 is a target of MLKL for the induction of Ca$\left(2+\right)$ influx$^{146}$. |
| TRX1                 | $\uparrow^{123}$    |                               | $+^{123}$ |                      | TRX1 blocks necroptosis by maintaining MLKL in a reduced inactive state$^{123}$. |
| UCH-L1               | $\downarrow^{128,257}$ |                               |                      |                      | Htra2/Omi induces monoubiquitination of UCH-L1$^{257}$. |
| UHRF1                | $\uparrow^{81}$     |                               |                      |                      | UHRF1 silences RIPK3 expression via promoter hypermethylation. Sp1 initiates RIPK3 transcription in the absence of UHRF1$^{131}$. |

MLKL association with RIPK3 is also suppressed by a constitutive interaction of MLKL with a competitive inhibitor, TRAF2, in resting cells. TRAF2 deubiquitination by CYLD promotes the dissociation of TRAF2 from MLKL and allows necroptosis$^{121}$. Two other molecules inhibit cell death by blocking MLKL association with pro-necroptotic components: Repulsive guidance molecule b (RGMb) inhibits MLKL membrane translocation or
membrane binding and Redox regulator thioredoxin-1 (TRX1) blocks MLKL disulfide bond formation, and through it the critical polymerization of MLKL.

Various molecules have been published to act as downstream targets of RIPK3 and others to regulate MLKL localization and/or activation. RIPK3 constitutes an important upstream kinase of death associated protein (Daxx), triggering its nuclear export. The Akt/mTOR pathway, and Ca$^{2+}$/calmodulin-dependent protein kinase II (CaMKII) are also active effectors of downstream necroptotic signaling. Accordingly, several models suggest that effects on these signaling routes modify necroptotic intensity. Poly [ADP-ribose] polymerase 1 (PARP-1) (debated in ref. 129,130) and phosphoglycerate mutase family member 5 (PGAM5) (debated in ref. 132) have been documented as cell type specific regulators of downstream necroptotic events (Table 2).

Glucose metabolism and ROS production in necroptosis

Reactive oxygen species (ROS) have long been considered to contribute to necroptosis. Oxidation of specific cysteine residues in RIPK1 by ROS activates RIPK1 autophosphorylation. A positive feedback loop is generated because silencing of RIPK1 or RIPK3 reduces ROS production. RIPK1 autophosphorylation is also promoted by mitochondrial ROS and is essential for RIPK3 recruitment into the necrosome. However, necroptosis could occur without ROS induction in some cell lines.

Metabolic enzymes — human liver glycogen phosphorylase (PYGL), glutamate-ammonia ligase (GLUL), glutamate dehydrogenase 1 (GLUD1) — increase pyruvate production from glycogen or play a role in glutamine catabolism. These enzymes are activated by RIPK3, resulting in enhancement of aerobic respiration and thus likely contribute to TNF-induced ROS production. Pyruvate dehydrogenase complex (PDC) converts pyruvate to acetyl-CoA, and triggers the entrance of metabolic flux into the tricarboxylic acid cycle. Activated RIPK3 in the necrosome enhances PDC activity by phosphorylating the PDC E3 at T135 and plays a major role in increasing aerobic respiration. Based on in vitro studies, activation of these enzymes has additive effects to aerobic respiration and ROS production (Table 2).

Intracellular localization of necrosome components

The intracellular localization of necrosome components seems to be crucial in the regulation of necroptosis. The RHIM domain of RIPK1 and RIPK3 mediates the assembly of heterodimeric filamentous structures, and the amyloid-like aggregation of RIPK1/RIPK3 complexes. Compromised cluster formation correlated with decreased programmed necrosis. MLKL has also been reported to form SDS-resistant, disulfide bond-dependent polymers during necroptosis and it has been shown that these MLKL polymers were independent of RIPK1/RIPK3 fibers.

MLKL translocation to the cell membrane is an obligatory step in necroptotic signaling. Phosphatidyl-inositol phosphates (PIPs) as critical binders of MLKL are required for plasma membrane targeting of MLKL and subsequent membrane permeabilization in necroptosis. Highly phosphorylated inositol products, but not weakly phosphorylated precursors are able to displace the MLKL auto-inhibitory brace region, which is a necessary event for late plasma membrane breakdown and cell death. Accordingly, necroptosis requires inositol polyphosphate-specific kinase activity and in cells containing mutant IP kinases, MLKL failed to oligomerize and localize to membranes despite proper RIPK3-dependent phosphorylation. Deletion of inositol polyphosphate multikinase (IPMK), inositol-tetrakisphosphate 1-kinase (ITPK1) or inositol pentakisphosphate 2-kinase (IPPK) inhibited necroptosis. Connected to this, phosphatidylinositol transfer protein alpha (PITPa) interacts with MLKL which facilitates MLKL oligomerization and plasma membrane translocation. Following membrane localization TAM (Tyro3, Axl, and Mer) family of receptor tyrosine kinases phosphorylate MLKL to promote MLKL oligomerization and necroptosis. Beside their direct pore forming ability, membrane-localized MLKL regulates transient receptor potential cation channel, subfamily M, member 7 (TRPM7), a non-voltage-sensitive ion channel, for the mediation of Ca$^{2+}$ influx.

Once MLKL is membrane associated, all the endosomal sorting complexes required for transport III machinery (ESCRT-III), flotillin-mediated endocytosis and ALIX-syntenin-1-mediated exocytosis act to sustain survival of the cell. The ESCRT-III-driven plasma membrane repair machinery limits the duration of the loss of plasma membrane integrity upon MLKL activation, while endo- and exocytosis removes phospho-MLKL from the plasma membrane. MLKL also forms a complex with multiple membrane metalloproteases upon necroptotic stimulus. A disintegrin and metalloprotease (ADAM)-enzymes are activated to mediate the shedding of cell-surface proteins in response to necroptotic stimuli and through this process also play a key role in promoting necroptosis, but only in adherent cells.

RIPK1, RIPK3, and MLKL have all been reported to localize to the nucleus and these localizations preceded necroptotic death. RIPK3 and MLKL have been shown to became activated in the nucleus, and after their cooperative nuclear export, they contribute to cytosolic necroosome formation. Following the interaction of RIPK3 and MLKL, the translocation of this complex to mitochondria-associated membranes has also been.
demonstrated and this relocation was found to be essential for necroptosis signaling. The intracellular trafficking of necrosomes is regulated by the TNF-induced guanine nucleotide-binding protein y 10 (Gy10) – Src signaling pathway, however, RIPK1/RIPK3 kinase activity has no direct interaction with Gy10 or on Src kinase.

**Drugs to regulate necroptosis intensity**

In vitro studies prefer to use caspase inhibitors to activate necroptosis, however we still do not fully understand how necroptosis is activated under physiological conditions. The in vivo appearance of necroptosis indicates that in addition to caspase-mediated processes various caspase independent regulatory mechanisms control necroptosis. Drugs affecting either the expression or the activity of necroptosis mediators, or that modify the indirect regulators of necroptosis may have therapeutic potential (Tables 3 and 4).

**Regulation the expression level of necrosome components**

Drugs that control the promoters of RIPK3 or MLKL or modify the stability and degradation of these molecules can regulate necroptosis sensitivity. Interferons, hypomethylating agents such as decitabine (5-aza-2’-deoxycytidine) and 5-azacytidine (used in Myelodysplastic syndromes and AML), histone deacetylase inhibitor valproic acid (VPA), anti-fungal miconazole, traditional Chinese medicine drugs (shikonin, resibufogenin, bufalin, youdujing, emodin), and components found in different plants (matrine, genipine, lycorine, quercetin, curcumin, Bulnesia sarmientoi) were all found to upregulate the expression of RIPK1 or RIPK3.

On the other hand, various inhibitors of the HSP90 have been documented to downregulate necroptosis (Kongensin A, G-TPP, geldanamycin, gamitrinib, DHQ3 and 17-demethoxy-reblastatin). Cyclosporine, Cyclosporine′ hypomethylating agents such as decitabine (5-aza-2’-deoxycytidine) and 5-azacytidine (used in Myelodysplastic syndromes and AML), histone deacetylase inhibitor valproic acid (VPA), anti-fungal miconazole, traditional Chinese medicine drugs (shikonin, resibufogenin, bufalin, youdujing, emodin), and components found in different plants (matrine, genipine, lycorine, quercetin, curcumin, Bulnesia sarmientoi) were all found to upregulate the expression of RIPK1 or RIPK3.

On the other hand, various inhibitors of the HSP90 have been documented to downregulate necroptosis (Kongensin A, G-TPP, geldanamycin, gamitrinib, DHQ3 and 17-demethoxy-reblastatin). Cyclosporine, Cyclosporine′ hypomethylating agents such as decitabine (5-aza-2’-deoxycytidine) and 5-azacytidine (used in Myelodysplastic syndromes and AML), histone deacetylase inhibitor valproic acid (VPA), anti-fungal miconazole, traditional Chinese medicine drugs (shikonin, resibufogenin, bufalin, youdujing, emodin), and components found in different plants (matrine, genipine, lycorine, quercetin, curcumin, Bulnesia sarmientoi) were all found to upregulate the expression of RIPK1 or RIPK3.

**Regulation the activity of necrosome components**

Beside the expression of necrosome components, the activity of these enzymes is also modified by various drugs. Promising specific inhibitors are currently being developed for the central molecules of necroptosis. RIPK1, RIPK3, and MLKL (reviewed in refs. 181,182) which may interfere with unwanted cell death and subsequent inflammation. Multiple second mitochondria-derived activator of caspase (SMAC) mimetics and TAK-1 (reviewed in refs. 183,184) inhibitors are being tested in clinical trials to activate necroptosis for therapeutic intervention, by restoring the sensitivity of apoptosis-resistant tumors to cell death. Since these drugs are reviewed elsewhere, we focus on currently available necroptosis regulators.

Drugs currently used for the treatment of different forms of tumors display anti-necrototic activity (Dabrafenib, Sorafenib, Pazopanib, Ponatinib, and Carfilzomib) as does the anti-epilepsy drug Phenytoin (a clinically used anti-convulsant) or herbal components such as uogonin and aucubin inhibit RIPK1 activity. All these drugs provide immediate translational potential to dampen necroptosis-driven tissue degradation. Presumably, these drugs will be additive to the above-mentioned necroptosis inhibitors which downregulate the expression of necrosome components.

On the other hand, radiation, or chemotherapeutic agents such as anthracyclines and oxaliplatin, cisplatin, 5-fluorouracil or the pan-BCL-2 inhibitor Obatoclax (several phase two trials have been completed), traditional Chinese medicines such as resibufogenin (also tested in phase II of a clinical trial on pancreatic cancer), aucubin, tanshinone or neoaconulon have been documented to upregulate necroptosis. Based on current results, these drugs regulate the activity, and not the expression of necrototic component. As a mono-therapy these group of necroptosis regulators could be ineffective in tumors that down-regulate the level of RIPK3 or MLKL, but these medicines may increase the effect of the above listed mediators in combination therapy following the restoration of RIPK1 or RIPK3 expression in cancer cells.

**Regulation the signaling of necrototic pathway**

Some drugs regulate necroptosis by modulating the level or activity of partner molecules of the necosome. For example, VPA induces the release of SMAC from mitochondria thereby upregulating necroptosis similarly to the widely tested SMAC mimetics. Dimethyl fumarate (DMF, which is currently used in relapsing-remitting multiple sclerosis) induces necroptosis via downregulation of the negative regulators of necroptosis such as IAPs and cFLIPs. Aurora kinase inhibitors have been shown to directly induce necroptosis and stimulated intra-tumoral phosphorylation of MLKL. Drugs antagonizing Trx1function as necroptosis inducers. PX-12 (completed phase I of a clinical trial on advanced metastatic cancer) and DMF target TRX1 and have been shown to sensitize tumor cells to necroptosis.

Various drugs activate necroptosis via regulation of downstream components of necroptosis. Adiponectin
## Table 3 Available drugs to modify necroptosis intensity

| Drug/Agent | Effect on necroptosis | Mechanism | Cells tested in necroptosis | Application/clinical trial in general |
|------------|-----------------------|-----------|-----------------------------|-------------------------------------|
| Anthracine mitoxantrone | ↑ | Induces MLKL phosphorylation | Inhibits TC1 and EL4 cell lines-induced tumor growth in vivo in mice | Used in chemotherapy for various cancers |
| Bonezomib/PS-341 | ↓ | Disrupts the formation of RIPK1-RIPK3 complex through stabilizing of cIAPs | In vitro studies on primary bone marrow-derived macrophages | Bonezomib (PS-341) is used in Multiple Myeloma treatment |
| Carftizomib | ↓ | Inhibits induction of pRIPK3 and pMLKL | HT-29 cells | Approved on Multiple Myeloma |
| Cisplatin | ↑ | Induces necroosome formation | In vitro in various cell lines
and in vivo in rats | Widely used immunosuppressive drug |
| Cyclosporine A | ↓ | Reduction in necroptosis markers RIPK1 and RIPK3 | In vivo in rat cerebral ischemia-reperfusion injury | |
| Dabrafenib | ↓ | RIPK3 inhibition by competing with ATP binding | In vitro in normal human hepatocytes and in vivo in mouse models of ischemic injury. | Approved in BRAF-mutant melanoma |
| Dasatinib | ↑ | Plays a role in HMGB1-induced necroptosis. | CCC-HEH-2 human embryonic cardiac tissue-derived cell lines | Used as an anticancer drug in CML patients |
| Dexmedetomidine | ↓ | Inhibition of HMGB1 expression | H9C2 embryonic rat heart-derived cells | Used in the intensive care setting for light to moderate sedation |
| Diacerein | ↓ | Decreased renal expression of RIPK3 and MLKL | Prevents necroptosis in acute kidney injury in rats | Registered in some European Union and Asian countries to treat joint diseases |
| Dimethyl Fumarate | ↑ | Depletion of GSH, increases MAPK and ROS activation, inhibits the Tnx1/Nrf2 axis | Gastrointestinal CT26 and lymphoid cancer cell lines Se-Ax, HH and CEM cells | Used in relapsing-remitting Multiple Sclerosis |
| Fluorouracil | ↑ | Reduces cIAP1 protein level, stabilizes binding between RIP1 and RIP3 | In vivo xenograft experiments with HT29 cells blocked tumor growth | Used in chemotherapy for various cancers |
| Hypomethylating agents (decitabine, 5-azacytidine and RG108) | ↑ | Restores RIPK3 in cancer cells where RIPK3 had previously been silenced. | Human breast tumor and AML samples | Decitabine and 5-Acetyldcytidine are used in Myelodysplastic syndrome and AML |
| Interferons, Type I- II | ↑ | Increases expression of RIPK3 and/ or MLKL | In vitro mose studies in septic model | Used in different diseases |
| Lithium | ↑ | Induces AKT- and mTOR-mediated necroptosis | In vitro RT4 cells and human primary schwannoma cells | Lithium is used as the first line treatment in bipolar disorders |
| Melatonin | ↓ | Represses the RIPK3-PGAMS-CypD-mPTP pathway | In vivo mose studies in cardiac ischemia-reperfusion | Used for Jet Lag sleep disorder |
| Miconazole | ↑ | Upregulates RIPK3 and MLKL | MDA-MB-231 cells | Anti-fungal medication |
| Oxaliplatin | ↑ | Induces ATP release in RIPK3 and MLKL expressing cells | Inhibits TC1 and EL4 cell lines-induced tumor growth in vivo in mice | Used in colorectal cancer |
| Phenhydantin | ↓ | Suppresses phosphorylation and activation of RIPK1, RIPK3, and MLKL | In vitro MEFs, L929, NIH3T3, HT-29, U937, and Jurkat mouse and human cell lines | Used as an anti-convulsive drug |
| Phenytoin | ↓ | Partial inhibition of RIPK1 | HT29 cells and RAW 264, human colon cancer cell lines | Used as anti-arrhythmic class IIb and as anti-convulsant |
| Pazopanib | ↓ | Inhibits RIPK1 | FADD-deficient Jurkat cells | Approved for renal cell carcinoma and soft tissue sarcoma |
| Ponatinib | ↓ | Inhibits both RIPK1 and RIPK3 | FADD-deficient Jurkat cells | Approved in some chronic myeloid leukemia and some acute lymphoblastic leukemia |
| Rapamycin | ↓ | Inhibits RIP-1 expression | Experimental retinal detachment in rats | Approved for Prevention of transplant rejection in Lymphangioleiomyomatosis, and to prevent restenosis in coronary arteries following balloon angioplasty |
| SAHA/Vorinostat | ↓ | HDAC inhibitor, activates NFkB and p38 MAPK, inactivates JNK and Akt kinase; enhances cFLIPL expression | In vitro L929 cells and human neuroblastoma SH-SY5Y cells | Approved for the treatment of Cutaneous T cell lymphoma |
| Sorafenib | ↓ | Reduces interaction of RIPK1 with RIPK3, inhibits kinase activity of RIPK1 and RIPK3 | In vitro various cells and in vivo protects against TNF-induced SIRS and renal ischemia-reperfusion injury | Approved for advanced thyroid and renal cell cancer, hepatocellular carcinoma |
| Valproic acid | ↑ | Histone deacetylase inhibitor, induces JNK1 activation and RIPK1 expression | In vitro rat PC12 cells | Used in epilepsy and mood disorders |

Official journal of the Cell Death Differentiation Association
receptor agonists (tested in various clinical trials), DMI, neoablonol induce ROS production. Lithium, clinically used for treating bipolar disorders, facilitates AKT-mTOR-mediated necroptosis, while dasatinib (used drug in CML) induces HMGB1-mediated necroptosis.

Necroptosis can be inactivated via the regulation of interacting partners of the necrosome or by downstream

| Drug/Agent | Effect on necroptosis | Mechanism | Cells tested in necroptosis | Application/ clinical trial in general |
|------------|-----------------------|-----------|-----------------------------|----------------------------------------|
| Aucubin | ↓ | Inhibits MLKL and RIPK1 activation | Lithium-pilocarpine induced epilepsy rat model in vivo | Component of Eucommia ulmoides Oliv., a traditional Chinese medicine |
| Bufalin | ↑ | Increases the expression of RIPK1 and RIPK3 | MCF-7 and MDA-MB-231 human breast cancer cells and in a mouse xenograft model of human breast cancer | Bufalin is a component of Chinese medicine. Completed phase II of a clinical trial on pancreatic cancer. |
| Bulnesia sarmientoi | ↑ | Induction of RIPK1 | Human lung carcinoma cell lines A-S49, and H661, normal human lung fibroblast MRC-5 | Analgesic, wound-healing and anti-inflammatory medicinal plant |
| Curcumin | ↑ | Upregulates the expression of RIPK1 and RIPK3 | Human HSC-LX2 cells | Extracted from the roots of the herb *Rhizoma Curcumae* |
| Emodin | ↑ | Emodin upregulated the levels of TNF-α, RIP1, RIPK3 and MLKL | Inhibits U-251 glioblastoma cell line proliferation | Compound extracted from traditional Chinese medicines |
| Genipin | ↓ | Attenuation of increased levels of RIPK3, RIPK1/RIPK3 complexes and p-MLKL | In vivo acute liver failure model in mice | Major active compound of the gardenia fruit |
| Gomisin J | ↑ | Mechanism is not described | Human breast cancer cell lines (MCF7 and MDA-MB-231) | A component of Schisandra chinensis fruit *A Chinese herbal medicine* |
| Lycarine | ↑ | Upregulates RIPK1 and RIPK3 expression | Multiple myeloma cell line ARH-77 | Chinese medicinal herb |
| Matrine alkaloid | ↑ | Increases RIPK3 expression; increases ROS production | In vitro in CCA QBC939 and Mz-Cha-1 cell lines | Component of the traditional Chinese medical herb *Sophora flavescens* Ait. |
| Neoablonol | ↑ | Increase of RIPK1/RIPK3 colocalization, down-regulates cIAP1/2 and TNF receptor-associated factors TRAFs | Nasopharyngeal carcinoma cell line C666-1 | Compound isolated from the fungus, *Albatrellus confluens* |
| Patchouli alcohol | ↓ | Down-regulates RIPK3 and MLKL proteins. | DSS (dextran sulfate sodium)-induced mouse colitis in vivo | *Pogostemon* (patchouli) leaves used in traditional medicine |
| Resibufogenin | ↑ | Upregulation of RIPK3 and phosphorylation of MLKL | In vitro MEF cells, Human CRC cell lines (SW480, HCT-116) and SW480 cells xenografted to BALB/c-nu mice | Used as traditional Chinese medicine component. Completed phase II of a clinical trial on pancreatic cancer |
| Shikonin | ↑ | RIPK1 and RIPK3- dependent necroptosis | Various human cell lines | Used in traditional Chinese medicine as a wound healing ointment |
| Tanshinone IIA | ↑ | Especially in the presence of caspase inhibitors forms RIPK1/RIPK3 complex | In human hepatocellular carcinoma HepG2 cells | Constituent of the traditional medicinal plant *Salvia miltiorrhiza* |
| Youdujing | ↑ | Increases RIP1 expression | In ectocervical Ect1/E6E7 cell line | Traditional Chinese herbal formula |
| Wogonin | ↓ | Inhibited RIPK1 by occupying the ATP-binding pocket | Inhibits necroptosis in cisplatin-induced AKI mouse model | Herbal compound, was found in *Scutellaria baicalensis*, ingredient of a Japanese herbal supplement |
Necroptosis has fundamental roles in various human diseases, as well. The proteasome inhibitor Bortezomib\(^\text{207}\) (used in Multiple Myeloma treatment) and a HDAC inhibitor Vorinostat\(^\text{208}\) (approved for the treatment of Cutaneous T cell lymphoma) have been demonstrated to inhibit necroptosis through the upregulation of necroptosis inhibitors, sequentially stabilizing IAPs or increasing FLIP expression.

Various ROS scavengers have been implicated in the modulation of necroptosis\(^\text{209,210}\). Dexmedetomidine (used for jetlag sleep disorder) blocks melatonin\(^\text{212}\) (used for jetlag sleep disorder) inhibits HMGB1 production\(^\text{211}\).

While there are no drugs on the market directly approved to regulate necroptosis, various medicines have the potential to both up and downregulate necroptosis, for example, the HDAC inhibitor Vorinostat\(^\text{208}\) (approved for the treatment of Cutaneous T cell lymphoma) and the proteasome inhibitor Bortezomib\(^\text{207}\) (used in Multiple Myeloma treatment) and mib\(^\text{207}\) (used in Multiple Myeloma treatment) and a variety of other drugs.

References

1. Galluzzi, L. et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. 25, 486–541 (2018).
2. Holger, N. et al. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat. Immunol. 1, 489–495 (2000).
3. Vandenhove, T., Kaiser, W. J., Bertrand, M. J. & Vandemaele, P. Molecular crosstalk between apoptosis, necroptosis, and survival signaling. Mol. Cell. Oncol. 2, e975093 (2015).
4. He, S. et al. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell 137, 1100–1111 (2009).
5. Zhao, J. et al. Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. Proc. Natl Acad. Sci. USA 109, 5322–5327 (2012).
6. Sun, L. et al. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. Cell 148, 213–227 (2012).
7. Oberst, A. et al. Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. Nature 471, 363–367 (2011).
8. Zhang, H. et al. Functional complementation between FADD and RIP1 in embryos and lymphocytes. Nature 471, 373–376 (2012).
9. Lin, Y., Devin, A., Rodriguez, Y. & Liu, Z. G. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. Genev. Des. 13, 2514–2526 (1999).
10. Chen, L., Tsau, J. S., Molkentin, J. D., Komatsu, M. & Hedrick, S. M. Mechanisms of necroptosis in T cells. J. Exp. Med. 208, 633–641 (2011).
11. O’Donnell, M. A. et al. Caspase 8 inhibits programmed necrosis by processing CYLD. Nat. Cell Biol. 13, 1437–1442 (2011).
12. Park, S. M., Yoon, J. B. & Lee, H. Receptor interacting protein is ubiquitinated by cellular inhibitor of apoptosis proteins (c-IAP1 and c-IAP2) in vitro. FEBS Lett. 566, 151–156 (2004).
13. Moulin, M. et al. IAPs limit activation of RIP kinases by TNF receptor 1 during development. EMBO J. 31, 1679–1691 (2012).
14. Kaczmarek, A., Vandemaele, P. & Kryska, D. V. Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. Immunity 38, 209–223 (2013).
15. Yamada, T. et al. RIPK1 and NF-kappaB signaling in dying cells determines cross-priming of CD8(+) T cells. Science 350, 328–334 (2015).
16. Wallace, J., Tang, T. B., Yang, S. H. & Kovalenko, A. The in vivo significance of necroptosis: lessons from exploration of caspase-8 function. Cytokine Growth Factor Rev. 25, 157–165 (2014).
17. Josan-Lanhout, S. et al. Necroptosis, in vivo detection in experimental disease models. Semin Cell Dev. Biol. 35, 2–13 (2014).
18. Lin, J. et al. Exogenous hydrogen sulfide protects human umbilical vein endothelial cells against high glucose-induced injury by inhibiting the necroptosis pathway. Int. J. Mol. Med. 41, 1477–1486 (2018).
19. Liang, W. et al. A novel damage mechanism: Contribution of the interaction between necroptosis and ROS to high glucose-induced injury and inflammation in H9c2 cardiac cells. Int. J. Mol. Med. 40, 201–208 (2017).
20. Huang, C. Y., Kuo, W. T., Huang, Y. C., Lee, T. C. & Yu, L. C. Resistance to hypoxia-induced necroptosis is conferred by glycolytic pyruvate scavenging of mitochondrial superoxide in colorectal cancer cells. Cell Death Dis. 4, e622 (2013).
21. Yang, X. S. et al. Hypoxia-inducible factor-1 alpha is involved in RIP-induced necroptosis caused by in vitro and in vivo ischemic brain injury. Sci. Rep. 7, 5818 (2017).
22. Zhou, Y. et al. The degradation of mixed lineage kinase domain-like protein promotes neuroprotection after ischemic brain injury. Oncotarget 8, 66395–66401 (2017).
23. Vieira, M. et al. Ischemic insults induce necroptotic cell death in hippocampal neurons through the up-regulation of endogenous RIP3. Neurobiol. Dis. 68, 26–36 (2014).
24. McCaig, W. D. et al. Hyperglycemia potentiates a shift from apoptosis to RIP1-dependent necroptosis. Cell Death Discos. 5, 58 (2015).
25. LaRocca, T. J., Sonoun, S. A., Shakerley, N. L., Ten, V. S. & Ratner, A. J. Hyperglycemic conditions prime cells for RIP1-dependent necroptosis. J. Biol. Chem. 291, 13753–13761 (2016).
26. Li, Y. et al. Type I IFN operates pyroptosis and necroptosis during multidrug-resistant A. baumannii infection. Cell Death Differ. 25, 1304–1318 (2018).
27. Thapa, R. J. et al. Interferon-induced RIP1/RIP3-mediated necrosis requires PI3K and is licensed by FADD and caspases. Proc. Natl Acad. Sci. USA 110, E3109–E3118 (2013).
28. Sarhan, J. et al. Constitutive interferon signaling maintains critical threshold of Mx1 expression to license necroptosis. Cell Death Differ. https://doi.org/10.1038/s41418-018-0122-7 (2018).
29. Gunther, C. et al. The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIP3s during hepatitis. J. Clin. Invest. 126, 4346–4350 (2016).
30. Ivanova, O. K. et al. CD3(+) CD8(+) NKGD(+) T lymphocytes induce apoptosis and necroptosis in HLA-negative cells via FasL-Fas interaction. J. Cell Biochem. 118, 3359–3366 (2017).
Of Molnár et al. 39. Hu, D. et al. A common variant of RIP3 promoter region is associated with
38. Szobi, A. et al. Analysis of necroptotic proteins in failing human hearts.
37. Fan, H. et al. Reactive astrocytes undergo M1 microglia/macrophage-
36. Xu, D. et al. TBK1 Suppresses RIPK1-Driven Apoptosis and In
35. Ito, Y. et al. RIPK1 mediates axonal degeneration by promoting inflammation
and necroptosis in ALS. Science 353, 603–608 (2016).
34. Xu, D. et al. TBK1 Suppresses RIPK1-Driven Apoptosis and Inflammation during Development and in Aging Cell 174, 1477–1491 e1419 (2018).
33. Fan, H. et al. Reactive astrocytes undergo M1 microglia/macrophages-
32. Ofengeim, D. et al. Activation of necroptosis in multiple sclerosis.
31. Kesarwani, P. et al. Blocking TCR restimulation induced necroptosis in
30. Afonso, M. B. et al. Activation of necroptosis in human and experimental
29. Gong, Y. N. et al. ESCRT-III acts downstream of MLKL to regulate necroptotic death and necroptosis in the progress of chronic periodontitis. Science 364, 352–356 (2019).
28. McComb, S. et al. Type-I interferon signaling through ISGF3 complex is
27. Knuth, A. K. et al. Interferons Transcriptionally Up-Regulate MLKL Expression
26. Strilic, B. et al. Tumour-cell-induced endothelial cell necroptosis via death
25. Ertao, Z. et al. Prognostic value of mixed lineage kinase domain-like protein
24. Li, J. et al. The R1P/RIP3 necrosome forms a functional armoylated signalling complex required for programmed necrosis. Cell 150, 213–226 (2012).
23. Zhang, D. W. et al. RIP3, an energy metabolism regulator that switches TNF-
22. Xiong, Y. et al. The bromodomain protein BRD4 positively regulates
21. Lu, J. et al. The R1P/RIP3 necrosome forms a functional armoylated signalling complex required for programmed necrosis. Cell 150, 213–226 (2012).
20. Yang, Z. et al. 2-HG Inhibits Necroptosis by Stimulating DNMT1-Dependent
19. Kesarwani, P. et al. Blocking TCR restimulation induced necroptosis in
18. Jiao, D. et al. Necroptosis of tumor cells leads to tumor necrosis and pro-
17. Seifert, L. et al. The necrosome promotes pancreatic oncogenesis via CXCL1 and Mincle-induced immune suppression. Nature 532, 245–249 (2016).
16. Snyder, A. G. et al. Intratumoral activation of the necroptotic pathway
15. Van, X. N. et al. Distinct roles of RIP1-RIP3 hetero- and RIP3-RIP3 homo-
14. He, L. et al. Mixed lineage kinase domain-like protein MLKL modulates tumor metastasis.
13. Verrier, M. et al. Mixed lineage kinase domain-like protein MLKL modulates tumor metastasis.
12. Kesarwani, P. et al. Blocking TCR restimulation induced necroptosis in
11. Min, K. S. et al. Upregulated RIP3 Expression Potentiates MLKL Phosphorylation-Mediated Programmed Necrosis in Tumor Pathogenesis. Cell Death Differ 135, 2021–2030 (2015).
10. Li, Y. et al. Human RIPK1 deficiency causes combined immunodeficiency and
9. Qin, X., Ma, D., Tan, Y. K., Wang, H. Y. & Cai, Z. The role of necroptosis in cancer. A double-edged sword? Biochim. Biophys. Acta. Rev. Cancer 1871, 259–266 (2019).
8. Messmer, M. N., Snyder, A. G. & Oberst, A. Comparing the effects of different cell death programs in tumor progression and immunotherapy. Cell Death Differ. 26, 115–129 (2019).
7. Coney, N. V. et al. RIP3 expression as a potential predictive and prognostic marker in early breast cancer. Clin. Cancer Res. 142, E31–E8 (2018).
6. Dzedzic, S. A. et al. ABIN-1 regulates RIPK1 activation by linking Met1 ubi-
5. Wang, X., Youse, S. & Ofengeim, D. Necroptosis and RIPK1-mediated neu-
4. Jiao, D. et al. Necroptosis of tumor cells leads to tumor necrosis and pro-
3. Yang, B. et al. Interleukin-1 receptor activation aggravates autosomal domi-
2. Ito, Y. et al. RIPK1 mediates axonal degeneration by promoting inflammation
1. Hu, D. et al. A common variant of RIP3 promoter region is associated with poor prognosis in heart failure patients by in...
tumor necrosis factor-induced nuclear factor-kappaB activation. J. Biol. Chem. 275, 10519–10526 (2000).

89. Wang, Z. et al. Inhibition of HSP90alpha protects cultured neurons from oxygen-glucose deprivation induced necroptosis by decreasing RIP3 expression. J. Cell Physiol. 233, 4864–4874 (2018).

90. Zhao, X. M. et al. Hsp90 modulates the stability of MLKL and is required for TNF-induced necroptosis. Cell Death Dis. 7, e2089 (2016).

91. Li, D. et al. A cytosolic heat shock protein 90 and cochaperone CDC37 complex is required for RIP3 activation during necroptosis. Proc. Natl Acad. Sci. USA 112, 5017–5022 (2015).

92. Wang, Z., Feng, J., Yu, J. & Chen, G. FKBP12 mediates necroptosis by initiating RIPK1/RIPK3/MLKL signal transduction in response to TNF receptor 1 ligation. J. Cell Sci. https://doi.org/10.1242/jcs.227777 (2019).

93. Sinnvasa, S. R. et al. Heat Shock Protein 70 (Hsp70) suppresses RIP1-Dependent Apoptotic and Necroptotic Cascades. Mol. Cancer Res. 16, 58–68 (2018).

94. Feng, S. et al. Cleavage of RIP3 inactivates its caspase-independent apoptosis pathway by removal of kinase domain. Cell Signal. 19, 2056–2067 (2007).

95. Mortaza N. S. carcinoma cells act via IRE1α, XBP1, and necroptosis through cleavage of RIP1 kinase. J. Immunol. 192, 5671–5678 (2014).

96. Wertz, I. E. et al. De-ubiquitination and ubiquitin ligase domains of A20 limit RipK1-dependent cytokine production and necroptosis. J. Immunol. 189, 5497–5507 (2012).

97. Alturk, N. A. et al. TriDelta induces the degradation of early necroptosis to limit Ripk1-dependent cytokine production and necroptosis. Cell Death Dis. 9, 592 (2018).

98. Choi, S. W. et al. PELI1 Selectively Targets Kinase-Active RIP3 for Ubiquitylation-Dependent Proteasomal Degradation. Mol. Cell 70, 920–935 e927 (2018).

99. Molnár et al. Dondelinger, Y. et al. RIPK3 contributes to TNFR1-mediated RIPK1 kinase-dependent apoptosis in epidermal growth factor receptor-activating kinase 3 (ERK5)-dependent lineage kinase-like (MLKL) signaling pathway, which is counterregulated by autophagy. J. Allergy Clin. Immunol. 140, 1632–1642 (2017).

100. Wang, Y., Zhang, Q., Wang, B., Li, P. & Liu, P. LCL1 treatment induces programmed cell death of Schwannoma cells through AKT- and MTOR-mediated necroptosis. Neurochem. Res. 42, 2363–2371 (2017).

101. Zhang, T. et al. CaMINI is a RIP3 substrate mediating ischemia- and oxidative stress-induced myocardial necroptosis. Nat. Med. 22, 175–182 (2016).

102. Sohma, J. et al. Differences and similarities in TRAIL- and tumor necrosis factor-mediated necroptotic signaling in cancer cells. Mol. Cell. Biol. 36, 2626–2644 (2016).

103. Sohma, J. et al. TNF-induced necroptosis and PARP-1-mediated necrosis represent distinct routes to programmed necrotic cell death. Cell Mol. Life Sci. 71, 331–348 (2014).

104. Jiang, K. H., Jiang, T., Son, E., Choi, S. & Kim, E. Kinase-independent role of nuclear RIPK1 in regulating parthanatos through physical interaction with PARP1 upon oxidative stress. Biochim. Biophys. Acta Mol. Cell Res. 1865, 132–141 (2018).

105. Zhang, W., Jiang, H., Chen, S., Du, F. & Wang, X. The mitochondrial phosphatase PAG5 functions at the convergence point of multiple necrotic death pathways. Cell 148, 228–243 (2012).

106. Remijn, Q. et al. Depletion of RIPK3 or MLKL blocks TNF-driven necroptosis and switches towards a delayed RIPK1 kinase-dependent apoptosis. Cell Death Dis. 5, e1004 (2014).

107. Chen, X. et al. Translocation of mixed lineage kinase domain-like protein to plasma membrane leads to necrotic cell death. Cell Res. 24, 105–121 (2014).

108. Yang, Z. et al. RIP3 targets pyruvate dehydrogenase complex to increase aerobic respiration in TNF-induced necroptosis. Nat. Cell Biol. 20, 186–197 (2018).

109. Schenk, B. & Fulda, S. Reactive oxygen species regulate Smac mimetic/ TNFα-induced necroptotic signaling and cell death. Oncogene 34, 5796–5806 (2015).

110. Tai, S. W. et al. Widespread mitochondrial depletion via mitophagy does not confer necromembrane necroptosis. J. Biol. Chem. 289, 17101–17124 (2017).

111. Liu, S. et al. MLKL forms disulfide bond-dependent amyloid-like polymers to induce necroptosis. Proc. Natl Acad. Sci. USA 114, E7450–E7459 (2017).

112. McIlhanna, D. E., Quarto, G., Guy, C. S., Green, D. R. & Molgoaveanu, T. Characterization of MLKL-mediated plasma membrane rupture in necroptosis. J. Vis. Exp. https://doi.org/10.3791/58088 (2018).

113. Quarto, G. et al. Sequential engagement of distinct MLKL phosphatidylinositol-binding sites executes necroptosis. Mol. Cell 61, 589–601 (2016).

114. Ross, U. et al. Necroptosis execution is mediated by plasma membrane nanopores independent of calcium. Cell Rep. 19, 175–187 (2017).

115. Dondelinger, Y. et al. MLKL compromises plasma membrane integrity by binding to phosphatidylinositol phosphates. Cell Rep. 7, 971–981 (2016).

116. Doye, C. M. et al. MLKL requires the nositol phosphate code to execute necroptosis. Mol. Cell 70, 936–948 e937 (2018).
McNamara, D. E. et al. Direct activation of human MLKL by a select repertoire of inositol phosphate metabolites. Cell Chem. Biol., https://doi.org/10.1016/j.chembiol.201903010 (2019).

Jing, L. et al. MLKL-PITPalpha signaling-mediated necroptosis contributes to cisplatin-triggered cell death in lung cancer A549 cells. Cancer Lett. 414, 136–146 (2018).

Najafov, A. et al. TAM kinases promote necroptosis by regulating oligomerization of MLKL. Mol. Cell, https://doi.org/10.1016/j.molcel.201905022 (2019).

Cai, Z. et al. Plasma membrane translocation of trimethylated MLKL protein is required for TNF-induced necrosis. Nat. Cell Biol. 16, 55–65 (2014).

Xia, B. et al. MLKL forms cation channels. Cell Res. 26, 517–528 (2016).

Tornus, W., Gernhardt, F., Hugo, C. & Linkermann, A. Die later with ESCRTI Oncotarget 8, 41790–41791 (2017).

Fan, W. et al. Follitin-mediated endocytosis and ALIX-synteny-1-mediated exocytosis protect the cell membrane from damage caused by necroptosis. Sci. Signal. 12, https://doi.org/10.1126/scisignal.aaw3423 (2019).

Cai, Z. et al. Activation of cell-surface proteases promotes necroptosis, inflammation and cell migration. Cell Res. 26, 886–900 (2016).

Janssens, S., Xu, L., Liang, X. & Jorgensen, P. DED mediates NF-kappaB activation in response to DNA damage. Cell 123, 1097–1092 (2005).

Li, M., Feng, S. & Wu, M. Multiple roles for nuclear localization signal (NLS) aa 442-472 of receptor interacting protein 3 (RIP3). Biochem. Biophys. Res. Commun. 372, 850–855 (2008).

Yang, Y., Ma, J., Chen, Y. & Wu, M. Nuclear-cytoplasmic shuttling of receptor-interacting protein 3 (RIP3) identification of novel nuclear export and import signals in RIP3. J. Biol. Chem. 279, 38820–38829 (2004).

Yoon, S., BogdanoV, K., Kovalenko, A. & Wallach, D. Necroptosis is preceded by nuclear translocation of the signaling proteins that induce it. Cell Death Differ. 23, 253–260 (2016).

Weber, K., Roelant, R., Bruggeman, I., Estomes, Y. & Vandendaele, P. Nuclear RIPK3 and MLKL contribute to cytosolic necrosonic formation and necroptosis. Commun. Biol. 1, 6 (2018).

Chen, W. et al. Diverse sequence determinants control human and mouse necroptosis. Cell Res. 26, 886–900 (2016).

Fakharnia, F., Khodagholi, F., Dargahi, L. & Ahmadiani, A. Prevention of cyclophilin D-mediated mPTP opening using cyclosporine-A alleviates the elevation of necroptosis, autophagy and apoptosis-related markers following global cerebral ischemia-reperfusion. J. Mol. Neurosci. 61, 52–60 (2017).

Abd-Elatif, R. N. et al. Diacerein protects against glycolipid-induced acute kidney injury: modulating oxidative stress, inflammation, apoptosis and necroptosis. Chem. Biol. Interact. 306, 47–53 (2019).

Ding, J. et al. Reparmin inhibits photocytotoxic Necroptosis and Protected the Retina by Activation of Autophagy in Experimental Retinal Detachment. Curr. Eye Res., 1–7, https://doi.org/10.1007/s12623-019-15883-1 (2019).

Qu, C. et al. Patchouli alcohol ameliorates dextran sodium sulfate-induced experimental colitis and suppresses tryptophan catabolism. Pharm. Res. 121, 70–82 (2017).

Nikseresht, S., Khodagholi, F. & Ahmadiani, A. Protective effects of ex-527 on cerebral ischemia-reperfusion injury through necroptosis signaling pathway attenuation. J. Cell Physiol. 234, 1816–1826 (2019).

Chen, X. et al. Identification of TAK-632 and its analogs as potent inhibitors of necroptosis by targeting RIP1 and RIP3. Br. J. Pharmacol, https://doi.org/10.1111/bph.14653 (2019).

Degterev, A. & Linkermann, A. Generation of small molecules to interfere with regulated necrosis. Cell Mol. Life Sci. 73, 2251–2267 (2016).

Fulda, S. Smac mimetics to therapeutically target IAP proteins in cancer. Int. Rev. Cell Mol. Biol. 330, 157–169 (2017).

Kilty, I. & Jones, L. H. TAK1 selective inhibition: state of the art and future opportunities. Future Med. Chem. 7, 23–33 (2015).

Li, J.X et al. The B-Raf/VEGFR inhibitor dabrafenib selectively inhibits RIP3 and alleviates acetylcholinesterase-induced liver injury. Cell Death Dis. 5, e1278 (2014).

Cruz, S. A., Qin, Z., Stewart, A. F. & Chen, H. H. Dabrafenib, an inhibitor of RIP3 kinase-dependent necroptosis, reduces ischemic brain injury. Neuronal Regen. Res. 13, 252–256 (2018).

Martens, S. et al. Sorafenib tosylate inhibits directly necrosonic complex formation and protects in mouse models of inflammation and tissue injury. Cell Death Dis. 8, e2904 (2017).

Feldmann, F., Schenk, B., Martens, S., Vandendaele, P. & Fulda, S. Sorafenib inhibits therapeutic induction of necroptosis in acute leukemia cells. OncoResearch 8, 68208–68220 (2017).

Fauster, A. et al. A cellular screen identifies FBXO32 as a novel regulator of cell death. Cell Death Dis. 5, e1376 (2015).

Ali, M. & Mocarski, E. S. Proteasome inhibition blocks necroptosis by attenuating cell death. Cell Death Dis. 8, 346 (2017).

Wang, H. et al. Contribution of RIP3 and MLKL to immunogenic cell death signaling in cancer chemotherapy. Oncoimmunology 5, e1149673 (2016).

Xu, Y. et al. Cisplatin-induced necroptosis in TNFalpha dependent and independent pathways. Cell Signal. 31, 112–123 (2017).
Wang, Y. et al. PELI1 functions as a dual modulator of necroptosis and apoptosis. *Cell Death Differ.* 24, 1605 (2016).

Deng, Q. et al. Neealbacconol induces energy depletion and multiple cell death in cancer cells by targeting PDK1-PI3K-Akt signaling pathway. *Cell Death Dis.* 4, e8004 (2013).

Xie, X. et al. Dimethyl fumarate induces necroptosis in colon cancer cells through GSH depletion/ROS increase/MAPKs activation pathway. *Biochim. Biophys. Acta BMB Rep.* 1859, 730–736 (2016).

Yu, X. et al. Neealbacconol induces cell death through necroptosis by regulating RIPK-dependent autocrine TNFalpha and ROS production. *Onco Targets + Therapy* 6, 1995–2008 (2015).

Zhang, Y. et al. Proteasome inhibitor PS-341 limits macrophage necroptosis by promoting cIAPs-mediated inhibition of RIP1 and RIP3 activation. *Biochim. Biophys. Acta Mol. Cell Mol. Biol.* 1841, 1202–1218 (2014).

Wang, L. et al. TNF-alpha induced stress activates RIPK1 kinase by promoting cIAPs-mediated inhibition of RIP1 and RIP3 activation. *Biochim. Biophys. Acta Mol. Cell Mol. Biol.* 1841, 1202–1218 (2014).

Zhang, H. et al. Functional complement between FADD and RIP1 in regulating RIPK-dependent autocrine TNFalpha and ROS production. *Cell Death Dis.* 10, e230 (2019).

Newton, K. et al. RIPK3 deficiency or catalytically inactive RIPK1 provides greater benefit than MLKL deficiency in mouse models of inflammation and tissue injury. *Cell Death Differ.* 23, 1565–1576 (2016).

Amin, P. et al. Regulation of a distinct activated RIPK1 intermediate bridging complex I and complex II in TNFalpha-mediated apoptosis. *Proc. Natl Acad. Sci. USA* 115, E9534–E9538 (2018).

Karch, J. et al. Necroinflammatory cytokines with MOMP and the MPTP in mediating cell death. *Plos ONE* 10, e0130205 (2015).

Tischner, D., Woess, C., Ortme, E. & Villunger, A. Bcl-2-regulated cell death signalling in the prevention of autoimmunity. *Cell Death Dis.* 1, e403 (2014).

Qu, Y. et al. RIPK3 interactions with MLKL and CalpK mediate oligodenronocyte cell death in the developing brain. *Cell Death Dis.* 8, e3329 (2017).

Zamaraeva, A. V., Kopena, G. S., Buchbinder, J. H., Zhivotovskiy, B. & Lavrik, I. N. Caspase-2 is a negative regulator of necroptosis. *Int. J. Biochem. Cell Biol.* 102, 101–108 (2018).

Chen, Z. F. & Ramaekers, L. TNF alpha mediates resistance and susceptibility to mycobacteria via mitochondrial reactive oxygen species. *Cell 153*, 521–534 (2013).

Kaiser, W. J. et al. RIP3 mediates the embryonic lethality of caspase-8-deficient mice. *Nature 471*, 368–372 (2011).

Alvarez-Diaz, S. et al. The Pseudokinase MLKL and the kinase RIPK3 have distinct roles in autoimmunity caused by loss of death-receptor-induced apoptosis. *Immunity 45*, 513–526 (2016).

Lu, J. V. et al. Complementary roles of Fas-associated death domain (FADD) and receptor interacting protein kinase 3 (RIPK3) in T-cell homeostasis and autoimmune inflammation. *Proc. Natl Acad. Sci. USA* 108, 15312–15317 (2011).

Linkermann, A. et al. Two independent pathways of regulated necrosis mediate ischemia-reperfusion injury. *Proc. Natl Acad. Sci. USA* 110, 12024–12029 (2013).

Roja, F. et al. Ramakrishnan, L. TNF alpha mediates resistance and susceptibility to mycobacteria via mitochondrial reactive oxygen species. *Cell 153*, 521–534 (2013).

Hitomi, J. et al. Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. *Cell 135*, 1311–1323 (2008).

Bonnert, M. C. et al. The adaptor protein FADD protects epidermal keratinocytes from necroptosis in vivo and prevents skin inflammation. *Immunity 35*, 572–582 (2011).

Moquin, D. M., McQuade, T. & Chan, F. K. CYLD deubiquitinates RIP1 in the TNFalpha-induced necrosome to facilitate kinase activation and programmed necrosis. *PLoS ONE* 8, e76841 (2013).

Dobrski, P. et al. LUBAC regulates gene activation and cell death by exerting opposing effects on linear ubiquitin in signaling complexes. *Cell Rep.* 13, 2258–2272 (2015).

Lee, Y. S. et al. Daxx is a key downstream component of receptor interacting protein kinase 3 mediating retinal ischemic cell death. *FEBS Lett.* 587, 266–271 (2013).

Yoon, S., Kovalenko, A., Bogdanov, K. & Wallach, D. MLKL, the protein that mediates necroptosis, also regulates endosomal trafficking and extracellular vesicle generation. *Immunity 47*, 51–65 e57 (2017).

Zhang, H. et al. Functional complementation between FADD and RIP1 in embryos and lymphocytes. *Nature 471*, 373–376 (2011).

Dillon, C. P. et al. Survival function of the FADD-CASPASE-8-cFLIP(L) complex. *Cell Rep.* 1, 401–407 (2012).

Zhang, X. et al. MLKL and FADD are critical for suppressing progressive lymphoproliferative disease and activating the NLRP3 inflammasome. *Cell Rep.* 16, 3247–3259 (2016).
Zhao, Q. et al. RIPK3 mediates necroptosis during embryonic development and perinatal inflammation in fadd-deficient mice. Cell Rep. 19, 798–808 (2017).

Petersen, S. L. et al. Autocrine TNFalpha signaling renders human cancer cells susceptible to Smac-mimetic-induced apoptosis. Cancer Cell 12, 445–456 (2007).

Lafont, E. et al. The linear ubiquitin chain assembly complex regulates TRAIL-induced cell death and activation of cFLIP. EMBO J. 36, 1147–1166 (2017).

Feoktistova, M. et al. cIAPs block Ripoptosome formation, a RIP1/caspase-8 containing intracellular cell death complex differentially regulated by cFLIP isoforms. Mol. Cell 43, 449–463 (2011).

Hughes, M. A. et al. Co-operative and hierarchical binding of c-FLIP and Caspase-8 is used in intracellular cell death complex differentially controlled by cFLIP isoforms. Mol. Cell 43, 449–463 (2011).

Toritaka, L. et al. The tumor suppressor Hace1 is a critical regulator of TNFR1-mediated cell death. Cell Rep. 15, 1481–1492 (2016).

Jacobson, A. V. et al. HSP90 activity is required for MLK oligomerization and membrane translocation and the induction of necrotic cell death. Cell Death Dis. 7, e2051 (2016).

Sosna, J. et al. The proteases HtrA2/Omi and UCH-L1 regulate TNF-induced necroptosis. Cell 173, 1360–1371 (2018).

Ozkaya, M. et al. Autocrine TNFalpha signaling renders human cancer cells susceptible to Smac-mimetic-induced apoptosis. Cancer Cell 12, 445–456 (2007).

Petersen, S. L. et al. Autocrine TNFalpha signaling renders human cancer cells susceptible to Smac-mimetic-induced apoptosis. Cancer Cell 12, 445–456 (2007).

Rickard, J. A. et al. TNFR1-dependent cell death drives inflammation in Shp2-deficient mice. Elife 3, https://doi.org/10.7554/elife.03464 (2014).

Haas, T. L. et al. Recruitment of the linear ubiquitin chain assembly complex stabilizes the TNF-R1 signaling complex and is required for TNF-mediated gene induction. Mol. Cell 36, 831–944 (2009).

Lee, E. W. et al. Ubiquitination and degradation of the FADD adaptor protein regulate death receptor-mediated apoptosis and necroptosis. Nat. Commun. 3, 978 (2012).

Heger, K. et al. OTULIN limits cell death induction by deubiquitinating LUBAC. Nature 559, 120–124 (2018).

Dionisio, P. E. A., Oliveira, S. R., Amaral, J. & Rodrigues, C. M. P. Loss of microglial parkin inhibits necroptosis and contributes to neuroinflammation. Mol. Neurobiol., https://doi.org/10.1007/s12035-018-1264-9 (2018).

Xu, X. et al. The role of PARP activation in glutamate-induced necroptosis in HT-22 cells. Brain Res. 1343, 206–212 (2010).

Jouan-Lanhouet, S. et al. TRAIL induces necroptosis involving RIPK1/RIPK3-dependent PARP-1 activation. Cell Death Differ. 19, 2003–2014 (2012).

Xu, C. et al. Embryonic lethality and host immunity of RELA-deficient mice are mediated by both apoptosis and necroptosis. J. Immunol. 200, 271–285 (2018).

Zhao, J. et al. Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necroptosis. Proc. Natl Acad. Sci. USA 109, 5322–5327 (2012).

Wagner, S. A., Satpathy, S., Beli, P. & Choudhary, C. SPATA2 links CnLD to the TNF-alpha receptor signaling complex and modulates the receptor signaling outcomes. EMBO J. 35, 1868–1884 (2016).

Kupka, S. et al. SPATA2-mediated binding of CYLD to HOIP enables CYLD recruitment to signaling complexes. Cell Rep. 16, 2271–2280 (2016).

Mihaly, S. R., Monioka, S., Ninomiya-Tsuji, J. & Takeo, G. Activated macrophage survival is coordinated by TAK1 binding proteins. PLoS ONE 9, e94982 (2014).

Broglie, P., Matsumoto, K., Akira, S., Brautigan, D. L. & Ninomiya-Tsuji, J. Transforming growth factor beta-activated kinase 1 (TAK1) kinase adaptor, TAK1-binding protein 2, plays dual roles in TAK1 signaling by recruiting both an activator and an inhibitor of TAK1 kinase in tumor necrosis factor signaling pathway. J. Biol. Chem. 285, 2333–2339 (2010).

Mihaly, S. R., Ninomiya-Tsuji, J. & Takeo, G. Activated macrophage survival is coordinated by TAK1 binding proteins. PLoS ONE 9, e94982 (2014).

Guo, X. et al. Cardioprotective role of tumor necrosis factor receptor-associated factor 2 by suppressing apoptosis and necroptosis. Circulation 136, 729–742 (2017).

Li, J. et al. TRAF2 protects against cerebral ischemia-induced brain injury by suppressing necroptosis. Cell Death Dis. 10, 328 (2019).

He, S., Liang, Y., Shao, F. & Wang, X. Toll-like receptors activate programmed necrosis in macrophages through a receptor-interacting kinase-3-mediated pathway. Proc. Natl Acad. Sci. USA 108, 20054–20059 (2011).

Meylan, E. et al. RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kappa B activation. Nat. Immunol. 5, 503–507 (2004).

Dasari, S. & Tchouwou, P. B. Cisplatin in cancer therapy: molecular mechanisms of action. Eur. J. Pharmac. 740, 364–378 (2014).

Sun, Y. et al. Down-regulation of RIP3 potentiates cisplatin chemosensitivity by triggering HSP90-BR Kinase pathway mediated DNA repair in esophageal squamous cell carcinoma. Cancer Lett. 418, 97–108 (2018).

Xu, Z. et al. High-mobility group box 1 protein-mediated necroptosis contributes to dasatinib-induced cardiotoxicity. Toxicol. Lett. 296, 39–47 (2018).

Stephen, L. J. Drug treatment of epilepsy in elderly people: focus on valproic Acid. Drugs Aging 20, 141–152 (2003).

Jung, S. et al. Anticancer activity of gomisin J from Schisandra chinensis fruit. Oncol. Rep. 41, 711–717 (2019).

Chen, C. et al. Shikonin induces apoptosis and necroptosis in pancreatic cancer via regulating the expression of RIP1/RIP3 and synergizes the activity of gemcitabine. Am. J. Transl. Res. 9, 5507–5517 (2017).