Novel, non-conventional pathways of necroptosis: molecular mechanisms and the inter-organelle interplay

Csaba Horvath[1], Izabela Jarabicova[1], Branislav Kura[2], Barbora Kalocayova[2], Eva Faurobert[3], Sean M. Davidson[4], Adriana Adameova[1,2]

1 Faculty of Pharmacy, Department of Pharmacology and Toxicology, Comenius University in Bratislava, Bratislava, Slovak Republic; adameova@fpharm.uniba.sk; horvath125@uniba.sk; jarabicova6@uniba.sk

2 Centre of Experimental Medicine, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic; adriana.duris.adameova@savba.sk; barbora.kalocayova@savba.sk; branislav.kura@savba.sk

3 French National Centre for Scientific Research, Institute for Advanced Biosciences; France; eva.faurobert@univ-grenoble-alpes.fr

4 The Hatter Cardiovascular Institute, University College London, United Kingdom; s.davidson@ucl.ac.uk

Correspondence: adameova@fpharm.uniba.sk

Abstract

Necroptosis, a cell death modality that is defined as a necrosis-like cell death depending on the receptor-interacting protein kinase 3 (RIP3) and mixed lineage kinase domain-like pseudokinase (MLKL), has been found to underlie the injury of various organs. Nevertheless, the molecular background of this cell loss seems to also involve, at least under certain circumstances, some novel axes, such as RIP3-PGAM5-Drp1 (mitochondrial protein phosphatase 5─dynamin-related protein), RIP3─CaMKII (Ca2+/calmodulin-dependent protein kinase II) and RIP3─JNK-BNIP3 (c-Jun N-terminal kinase─BCL2 Interacting Protein 3). In addition, endoplasmic reticulum stress and oxidative stress via the higher production of reactive oxygen species produced by the mitochondrial enzymes and the enzymes of the plasma membrane have been implicated in necroptosis, thereby depicting an inter-organelle interplay in the mechanisms of necroptosis. However, a role, as well as a relationship among the well-accepted canonical pathway and the novel, non-conventional signaling in terms of tissue- and/or disease-specific prioritisation, is completely unknown. In this review, we provide current knowledge on some other necroptotic pathways being not directly associated with RIP3─MLKL execution and report studies showing the role of respective circulating microRNAs as potential markers of necroptotic injury in the heart and in some other tissues having a high expression of the pro-necroptotic proteins.

Introduction

It has become evident that a programmed form of necrosis-like cell death called "necroptosis" is a crucial event underlying pathomechanisms of heart injury [1–5], neurodegeneration [6], cancer [7,8], as well as of some viral and microbial infections [9,10]. In addition, some lung [11–15], liver [16–22], intestinal [23–26], and renal diseases [27–31] have also been associated with active necroptosis. This wide and varied role for necrotic cell death is mainly mediated through the stimulation of death receptors such as Fas or TNFR [32], although genotoxic stress [33] has also been implicated. Execution of necroptosis involves the activation of receptor-interacting protein kinase 3 (RIP3, also called RIPK3) and its downstream substrate mixed lineage kinase domain-like pseudokinase (MLKL) [34–37]. Molecular signalling of necroptosis has previously also been associated with RIP1 (also called RIPK1), another member of the RIP family [38,39], indicating that the assembly of RIP1, RIP3 and MLKL - the so-called "necrosome" - is the main pro-necroptotic structure. In line with this, interventions targeting individual proteins (e.g: RIP1 inhibitors, RIP3 inhibitors and MLKL inhibitors), or dual-target inhibitors, (e.g: RIP1/RIP3 inhibitors), have been shown to elicit remarkable anti-cell death effects, and these are accompanied by the mitigation of organ dysfunction [39–45].

Although RIP1 is necessary for the promotion of necroptotic signalling under certain conditions, RIP3 activation can also occur without RIP1 kinase activity. For example, Toll-interleukin-1 receptor domain-containing adapter-inducing interferon (TRIF) can interact with RIP3 and induce its activation independently of RIP1 [46,47]. Likewise, necroptosis signalling triggered by cytomegalovirus infection can omit RIP1 by the direct interaction of RIP3 with the DNA-dependent activator of interferon regulatory factors/Z-DNA binding protein 1 (DAI/ZBP1) [48]. Since TRIF and DAI do not possess kinase activity, the activation of RIP3 under such conditions appears to be a result of conformational changes promoting the autophosphorylation of RIP3. Thus, the current understanding and definition of the signalling pathway of necroptosis execution emphasise a role for signalling via RIP3─MLKL and downplay the role of RIP1 [49,50]. Whether it is phosphorylated by RIP1 or by autophosphorylation, activated RIP3 then further phosphorylates and activates MLKL resulting in cell death. Some of the ways in which MLKL cause necroptosis include: i) alterations in ion homeostasis [51,52]; ii) shedding of various cell surface proteins including receptors, adhesion molecules, growth factors, and cytokines [53]; iii) and activation of the NACHT, LRR, and PYD domains containing protein 3 (NLRP3) inflammasome complex leading to the engagement of caspase-1 and subsequent interleukin-1β (IL-1β) production [54]. MLKL has also been suggested to cause cytotoxicity by permeabilizing the plasma membrane and inducing the loss of membrane integrity, as reviewed elsewhere [3,50].

The RIP1-RIP3–MLKL pathway promoting the disruption of the plasma membrane is referred to as the canonical pathway of necroptosis while the pathways dependent on the TRIF adaptor or DAI/ZBP1, with the unlikely function of RIP1, are considered to proceed with the RIP3-MLKL activation non-canonically [55]. It can be mentioned that the nomenclature referring to the canonical and non-canonical pathways of necroptosis seems to be used variously and inconsistently with the above-mentioned definition. In fact, some authors consider a pathway recognizing other non-RIP1, DAI/ZBP1 and TRIF activators or a pathway proceeding necroptosis via other non-MLKL adaptors of RIP3 also as the non-canonical signalling of necroptosis [47,48,55]. Irrespective of this various understanding of the definition of the non-canonical pathway, there is compelling evidence that RIP3 is the key common mediator of programmed necrosis. It can interact with other proteins, such as metabolic enzymes, including glycogen phosphorylase, glutamate-ammonia ligase, glutamate dehydrogenase 1, and pyruvate dehydrogenase to promote mitochondrial production of reactive oxygen species (ROS) [56,57]. In this regard, oxidative stress has been highlighted as a driving force in the activation of some pro-necroptotic proteins. For instance, cysteines of RIP1 have been found to be oxidized by mitochondrial ROS with resultant RIP1 autophosphorylation and subsequent formation of the necrosome [58,59]. However, since necroptosis in Jurkat cells and T-29 cells is reported to be ROS-independent [32,60], the role of ROS in the execution of necroptosis might be cell-specific, and this particular pathway linking ROS and RIP3 should be viewed with considerable caution. In addition, RIP3 activation by Ca2+/calmodulin-dependent protein kinase IIδ (CaMKIIδ) can promote mitochondrial permeability transition pore (mPTP) opening, thereby implicating mitochondria in necroptosis execution independently of mitochondrial ROS production [61]. Moreover, the activation of mitochondrial protein phosphatase PGAM5 by RIP3 leads to the dephosphorylation of the mitochondrial fission factor dynamin-related protein 1 (Drp1) at Ser637, and activation of its GTPase activity, which in turn can promote mitochondrial fragmentation, elevation in ROS production, and cell death [62]. Based on these findings indicating the involvement of mitochondria in the promotion of necroptotic cell death, it is evident that necroptosis can be executed by several additional signalling pathways apart from the constitution of membrane MLKL homo-oligomers or RIP3–MLKL hetero-oligomers. It should, however, be mentioned that the involvement of mitochondria in the process of necroptotic cell loss can be questioned, at least in some cells/tissue, since complete depletion of the mitochondria failed to alleviate the execution of necroptosis in NIH/3T3 fibroblasts [63]. Nevertheless, the above-indicated mitochondrial signalling pathways suggesting RIP3 as a multifunctional protein affecting mitochondrial function and dynamics can be viewed as other, alternative and non-conventional pathways of necroptosis. Furthermore, there are also some indications arguing for the

involvement of the endoplasmic reticulum (ER) in necroptosis, thereby extending the evidence on the non-plasma membrane-mediated signalling pathways of this cell death mode.

In this review, we examine the evidence for some novel, alternative pathways of necroptosis in the heart, in comparison to other relevant tissues, and propose a role for circulating microRNAs as potential markers of this type of cellular injury. It is, however, necessary to note that currently, the relationship between the canonical, well-accepted RIP3-MLKL executioner and the novel, alternative "non-canonical" necroptotic pathways and their relevance to specific tissue- and/or disease-specific is completely unknown.

Based on the data from the Human Protein Atlas (www.proteinatlas.org) it can be noted that the protein expression of some below-discussed alternative adapters of necroptosis (e.g., PGAM5 and Drp1) is comparable across several tissue types. On the other hand, although there is no comparative data on the protein expression of RIP3, its mRNA expression increases in order from the cerebral tissue through the liver, heart, lungs, and kidneys and reaches the highest levels in the intestines [64,65]. However, since both mRNA and protein expression can be highly altered by several factors important in disease processes, such as inflammation and oxidative stress [66], the abundance of pro-necroptotic proteins in healthy tissues may not directly correspond to the importance of the canonical or other, alternative necroptotic pathways in diseased tissue.

Accordingly, in this paper, we highlight the role of both the traditionally-viewed canonical or alternative, non-conventional executioners of necroptotic pathways and survey their roles in different tissues including the heart, intestines, kidneys, liver, central nervous system, lungs and blood vessels., We examine the role of potential inductors and associated pathological conditions along with tissue/cell specificity. Finally, we examine the role for miRNAs for regulating expression of the proteins involved, and thereby in the induction of necroptosis.

1. Evidence for additional, non-conventional pathways of necroptosis in the heart

Several major studies have demonstrated the importance of the necroptotic signalling pathways being not directly associated with the RIP1-RIP3-MLKL axis in the heart (reviewed in [3,67]). The RIP3—CaMKIIδ—mPTP pathway has been documented in the hearts subjected to 30 min ischemia (I) and 120 min reperfusion (R) [61]. Likewise, CaMKIIδ inhibition has been shown to be cardioprotective in acute models of myocardial I/R- and doxorubicin-induced cardiac damage by mitigating necrotic and necroptotic cell death [68,69]. Similarly, treatment of I/R hearts with KN-93, a CaMKII inhibitor, reduced the expression of RIP1 in the left ventricles of I/R hearts [69]. This association between RIP1 and mPTP is also supported by a

study using necrostatin-1 (Nec-1), a RIP1 inhibitor, which was able to retard both necroptotic cell death and mitochondria-related damage due to the reduction of the mPTP opening [70].

A role for the alternative RIP3─PGAM5─Drp1 pathway is indicated by a study in which mice with cardiac-specific PGAM5 knockout exhibited less MLKL phosphorylation, smaller infarct size and reduced cardiac inflammation and IL-6 expression [71]. In contrast, in a study examining the early phase of reperfusion (first 10 min) following ischaemia, no evidence of MLKL phosphorylation or alterations in other markers of necroptosis (either canonical or those referring to alternative ones) was observed, suggesting that necroptosis occurs later during reperfusion [45]. Interestingly, PGAM5 depletion has not altered the phosphorylation of Drp1 at Ser616[71], which is associated with the induction of mitochondrial fission [72], but preserved the phosphorylation at Ser637 [71]. Interestingly, with regards to RIP3, different studies have reported a RIP3-dependent increase in pSer616-Drp1 and a decrease in pSer637-Drp1 expression under in vivo myocardial I/R conditions [73] as well as in vitro hypoxia/reoxygenation (H/R) conditions [74]. Thus, since dephosphorylation of Drp1 at Ser637 has been associated with impaired cardiac function following I/R, direct inhibition of PGAM5, or by its upstream molecule – RIP3 may represent an important cardioprotective tool [67,75]. In support, a deficiency of RIP3 has been reported to improve ejection fraction and retard post-ischemic adverse remodelling during chronic left anterior descending coronary artery ligation [76].

Song et al. have described another mitochondria-associated necroptotic pathway [77]. They found that in cardiomyocytes under conditions of H/R, RIP3 deletion conferred protective effects on the mitochondria in terms of reducing mitochondrial oxidative stress and limiting mPTP opening as a result of reduced activation of c-Jun N-terminal kinase (JNK) and BCL2 Interacting Protein 3 (BNIP3) [77]. In a model of myocardial I/R, mPTP opening has also likely been preserved due to RIP3 inhibition however, such cardioprotection was not mediated by affecting the JNK─BNIP3 pathway [45]. Thus, such conflicting findings might suggest that the activation of the RIP3─JNK─BNIP3 pathway can be significantly affected by respective experimental conditions.

Of note, the promotion of necroptosis due to mPTP opening has been demonstrated to also involve the ER, indicating a novel inter-organelle molecular mechanism for necroptosis execution [78]. Indeed, under conditions of acute I/R, RIP3-evoked ER stress has led to the elevation in intracellular Ca2+ concentration in cardiomyocytes with subsequent upregulation in the expression of xanthine oxidase (XO). As a result, the elevated levels of XO caused ROS accumulation resulting in increased mPTP opening and finally cell death [78].

Although some studies employing cardiomyocytes have indicated that these other, additional pathways evidenced in the heart do have a place in these particular cardiac cells, it is unknown whether other types of cells in the heart (e.g., fibroblasts, vascular smooth muscle

cells, etc.) respond to the deleterious stimuli similarly. On the other hand, since cardiomyocytes have a high reliance on energy production by the mitochondria, these cardiac cells are likely more prone to mitochondria-related alternative necroptosis and the subsequent molecular events, including higher ROS production, can promote the signalling pathways in the neighbouring cells and thereby promote further damage e.g., fibrosis. From the above-mentioned discussion, it is, however, evident that the activation of either of these alternative necroptotic signalling pathways under conditions of myocardial damage might be associated with impaired mitochondrial function and dynamics via Drp1 allowing the division of one mitochondrion in two daughter mitochondria. It is also evident that RIP3 acts as a convergent point of multiple mitochondrial pathways apart from the plasma membrane RIP3–MLKL signalling. Therefore, it seems that the RIP3-targeting interventions may be a promising and powerful tool to elicit cardioprotection. In this regard, in our recent study employing an ex vivo model of a short period of I and R we have shown that RIP3 inhibition by two different agents is cardioprotective in terms of the prevention of membrane disruption and mitochondrial swelling. However, these pharmacological interventions targeting RIP3 did not affect the activation of known canonical (RIP1-RIP3-MLKL) and alternative pathways (RIP3-CaMKII, RIP3-PGAM5-Drp1, RIP3-JNK-BNIP3) of necroptosis [45]. Thus, such findings likely indicate that the role of RIP3 is rather more complex and more important in the fate of cells.

2. Evidence of additional, non-conventional necroptosis in non-cardiac tissue types

2.1. Intestines

In a model of LPS-induced sepsis, necroptosis has been shown to be implicated in the loss of intestinal cells based on the upregulation of RIP1, RIP3 and MLKL, as well as PGAM5 and Drp1, indicating the involvement of both the canonical and alternative necroptosis [23]. Moreover, pre-treatment with a RIP1 inhibitor, Nec-1, was able to reverse the alterations in the expression of these proteins, mitigate jejunal morphological injury and improve digestive and barrier function [23]. Similarly, in a model of enterotoxigenic Escherichia coli-induced intestinal damage, treatment of intestinal porcine epithelial cells 1 (IPEC-1) with eicosapentaenoic or arachidonic acid reduced the protein levels of phosphorylated RIP3 and phosphorylated MLKL as well as the gene expression of PGAM5 and Drp1 [24]. The authors also found downregulated expression of NLRP3, caspase-1 and gasdermin D, indicating that the application of these polyunsaturated fatty acids resulted in the suppression of both necroptosis and pyroptosis and thus, cell inflammation [24].

p-JNK, a stress- and mitogen-activated protein kinase, has been found to serve as another potential mediator of alternative necroptosis in the intestines [25,26]. Treatment of

colon cancer cell lines with 2-methoxy-6-acetyl-7-methyljuglone, an inductor of necroptosis [79], promoted the formation of RIP1─RIP3 complex along with the sustained p-JNK activation and increased mitochondrial ROS production [26]. Similarly, Nec-1 as well as SP600125, a JNK inhibitor, were able to prevent these alterations, thereby confirming a mechanistic link between these molecular events. In support, in an in vitro model of inflammatory bowel disease, the silencing of a histone methyltransferase – enhancer of zeste homolog 2 (EZH2) increased the expression of p-JNK along with canonical proteins of necroptosis [25].

2.2. Kidneys

In subtotal nephrectomised rats, the remnant kidneys had elevated expression of RIP1, RIP3, MLKL and Drp1 while these changes were prevented by Nec-1 treatment. This anti-necroptotic agent also relieved kidney dysfunction and overall cell injury evidenced by transmission electron microscopy [27]. TNF-induced necroptosis of renal clear cell carcinoma cells has also been found to be mediated by Drp1, since the application of mitochondrial division inhibitor-1 (Mdivi-1), a Drp1 inhibitor, prevented the translocation of p-MLKL to the mitochondria, thereby suggesting a mechanistic link between Drp1 and MLKL that was RIP3-independent [28]. Interestingly, in addition to the indicated protective effects of Nec-1 on Drp1-mediated mitochondrial fission, the such pharmacological intervention was also able to decrease ROS production under these pathological conditions in the kidneys [27]. Likewise, in a mouse model of sepsis-induced acute kidney injury, activated RIP3 stimulated the pro-oxidant enzyme NADPH oxidase 4 (NOX4) independently of MLKL, and caused robust ROS production, induced mitochondrial dysfunction and thus, cell death [29]. Interestingly, the authors also detected higher urinary RIP3 levels and thus, identified a potential biomarker for such injury in the kidney [29]. In a model of renal I/R injury, RIP3 is also translocated to the mitochondria and promoted the release of mtDNA via degradation of the inner mitochondrial protein, Mitofilin, therefore exacerbating cell death [30]. Additionally, JNK has been associated with the process of necroptotic death under the conditions of renal I/R injury. However, it is assumed that JNK serves rather as an upstream activator of the canonical RIP3–MLKL axis, since the inhibition of JNK by CC-930 downregulated the core markers of necroptosis (p-RIP3 and p-MLKL) with concomitant mitigation of kidney dysfunction, tubular damage, and inflammation [31]. Furthermore, there are some indications that RIP3 independently of MLKL proceeds to renal fibrogenesis. It has been shown that both genetic deletion, as well as pharmacologic inhibition of RIP3, were protective against TGF-β1-mediated increased kidney fibrosis, while these results were not achieved by genetic depletion of MLKL [80].

2.3. Liver

In an early phase of acetaminophen (APAP)-induced liver injury, both RIP3 inhibition and genetic deletion attenuated necrosis-like cell death which was accompanied by the alleviation of mitochondrial dysfunction, Drp1 translocation and ROS production [16]. Furthermore, the application of Drp1 inhibitor to hepatocytes isolated from mice exposed to APAP resulted in reduced necrosis compared to non-treated cells [16], supporting the relevance of the RIP3—Drp1-mediated necroptosis under such liver damage. Similar protection has been found in another study using the same model of APAP-induced hepatotoxicity in which RIP1 inhibition decreased the expression of RIP3 and prevented necroptosis. In addition, the such pharmacological intervention has been linked to the attenuation of mitochondrial JNK activation and apoptosis-inducing factor (AIF) nuclear translocation, depicting a novel RIP1—RIP3—AIF-mediated necroptosis signalling [17]. A signalling pathway linking RIP3-mediated necroptosis with oxidative stress has also been delineated in a model of hepatotoxicity induced by chronic ethanol intake [18]. Depletion of hepatic RIP3 prevented hepatocytes injury, oxidative stress and JNK activation. Because the upregulation of hepatic RIP3 in mice fed with ethanol required ROS produced by ethanol metabolic transformation such a complex interplay between RIP3 and oxidative stress has supported the previous findings on the role of ROS in the alternative pathway of necroptosis in the liver.

In addition to mitochondrial dysfunction and overproduction of ROS, ER stress has also been implicated in necroptosis signalling under the conditions of hepatic injury. In a tunicamycin- or d-galactosamine-induced acute liver damage, both necroptotic cell loss and ER stress have been identified, while pharmacological inhibition of ER stress displayed a protective effect against hepatocyte necroptosis [19].

As previously mentioned, RIP3 is currently considered a core component of necroptosis signalling, however, in the liver several RIP3-independent molecular axes have been identified. In fact, Günther et al. [20] have reported that in a model of inflammation-dependent hepatitis, MLKL played an essential role in mediating programmed necrosis, which, indeed, occurred independently of RIP3 activation and rather required RIP1 kinase activity. Furthermore, MLKL, but not RIP3 upregulation was induced in the interferon-gamma (IFN-γ)—signal transducer and activator of transcription 1 (STAT1)-dependent manner. In support, alternative necroptosis mediated only by MLKL has also been described in a model of non-alcoholic steatohepatitis [21] and hepatocellular carcinoma cells treated with a monoclonal antibody against CD147 [22]. Although the underlying molecular mechanisms, including some other MLKL-activating kinases, have not been identified yet it is worth mentioning that these findings might indicate a novel view on necroptosis signalling in the liver. Likewise, it should also be noted that a simple comparison of the findings of these studies investigating additional

pro-necroptotic pathways in the liver would not be accurate as different methodical approaches have been adopted. Therefore, it is intended just to indicate an observation that in drug-induced liver damage, RIP3 mediates mitochondria and/or ER-related necroptosis, while inflammation-associated damage of hepatocytes is rather characterized by MLKL activation only.

2.4. Central nervous system

Several alternative pathways of necroptosis have recently been implicated in the pathophysiology of central nervous system (CNS) diseases. Indeed, in both in vitro and in vivo models of hypoxic/ischemic brain damage, the knockdown of RIP3 attenuated necroptotic cell loss via decreasing the phosphorylation of CaMKIIδ, one of the first identified mediators of alternative necroptosis [81]. Moreover, the such intervention also prevented mitochondrial membrane potential dissipation and MLKL plasma membrane translocation [82]. On the other hand, despite the indication of CaMKIIδ being downstream of RIP3 [81], others have shown that in an early stage of cerebral I/R injury, CaMKIIα, another isoform of CaMKII being highly expressed in the brain, acted rather as an upstream activator of the RIP1—RIP3 complex, since pretreatment of rats with CaMKII inhibitor, KN-93, markedly reduced such interaction as well as the activation of Drp1 [83].

In addition to CaMKII and Drp1, several other proteins related to mitochondrial damage have been proposed to mediate necroptosis execution under the conditions of cerebral I/R. It has been shown that AIF, an inter-membrane mitochondrial protein inducing caspase-independent apoptosis [84], co-localized with RIP3 in the cytoplasm while this complex subsequently translocated into the nucleus and promoted DNA degradation and necroptosis of neurons damaged by I/R [85,86]. Interestingly, it is worth mentioning that such RIP3—AIF-mediated necroptosis was suppressed by treatment with both RIP1 and RIP3 inhibitors [85,86], as well as with an inhibitor of JNK, SP600125 [85], thereby pointing out novel crosstalk between the RIP3—AIF axis and the JNK inflammatory pathway in necroptosis execution. Such interplay was further supported by an observation that pharmacologic inhibition of RIP3 by GSK’872 decreased the levels of both p-JNK and IL-6 [85]. These signalling pathways involving RIP3, AIF and JNK are also likely involved in the pathophysiology of intracerebral haemorrhage (ICH). Indeed, RIP3 has been found to bind with both CaMKII and AIF under such pathological conditions of the brain [87]. The formation of these complexes was inhibited by RIP3 knockdown and treatment with the combination of KN-93, a CaMKII inhibitor, and cyclosporine A, an inhibitor of mPTP opening, while all these interventions resulted in a significant improvement in neurological functions [87]. Investigating the same model, Huang et al. [88] have found that treatment with GSK’872 reversed an ICH-induced increase in the expression of RIP3, p-MLKL, p-JNK, IL-6 and monocyte chemoattractant protein-1 (MCP-1),

a prominent proinflammatory protein [89], as well as disturbed the interactions between RIP3 and MCP-1. These findings have suggested a comprehensive role of RIP3 in mediating both the canonical necroptosis and the MCP-1—JNK—IL-6 proinflammatory signalling in brain injury due to intracerebral haemorrhage. Besides the mentioned RIP3 downstream effectors, death-associated protein (DAXX) has recently been identified as a novel substrate of RIP3. Administration of Nec-1 before inducing cerebral I/R in rats suppressed RIP3 phosphorylation and both RIP1—RIP3 and RIP3—DAXX interactions. Furthermore, DAXX activation was able to improve cognitive functions and increase neuronal survival [90].

The mitochondria-associated necroptosis pathway involving RIP3, PGAM5 and Drp1 and/or overproduction of ROS has also been found to promote CNS injury. In an in vivo model of multiple sclerosis, the expression of the canonical necrosome components along with the levels of PGAM5 and Drp1 were elevated in the spinal cord [91]. PGAM5 seems to play a key role under these conditions since its silencing ameliorated the disease via suppressing Drp1 activation, necroptosis of microglia, inflammation and ROS production [91]. An interplay between necroptosis, inflammatory response and oxidative stress has also been described in a model of traumatic brain injury. Under such pathological conditions, RIP3 deficiency improved cognitive functions and alleviated brain damage while the underlying mechanisms included the mitigation of the NLRP3-inflammatory pathway, apoptosis, oxidative stress and canonical necroptosis [92]. Thus, it is plausible that both the conventional and novel, alternative signalling of necroptosis including mitochondria/oxidative stress-related pathways as well as the other RIP3-mediated deleterious events might act in cooperation to promote brain damage.

The knowledge of non-conventional necroptosis in CNS has been extended by another study implicating NLRP3 inflammasome in necroptotic cell loss in a cerebral I/R model. In fact, the treatment of rats with a novel pan-caspase inhibitor, Q-VD-OPH, which was used to induce necroptosis, resulted in both the canonical necrosome and the NLRP3 inflammasome activation in the ischemic neurons while the treatment with NLRP3 inhibitor significantly reduced the infarct volume [93]. However, such interconnection depicted in this study warrants further investigation since the precise mechanism underlying NLRP3 activation in cells undergoing necroptotic death remains unknown.

Another described mechanism of alternative necroptosis in CNS has been shown to involve the stress of ER. In a model of spinal cord injury, both MLKL and RIP3 translocated into the ER of microglia/macrophages, which were also positive for increased levels of ER stress sensor, GRP78 [94]. The participation of ER stress in necroptosis execution has been further confirmed in an in vitro model of ischemia, in which both ER stress and necroptosis co-existed while necroptotic cell loss was suppressed by the treatment with an ER stress inhibitor

[94]. Such proposed interplay was also strongly supported by evidence on both p-MLKL and GRP78 upregulation in microglia/macrophages isolated from human injured spinal cord [94].

2.5. Lungs

Current studies on various models of pulmonary diseases strongly support a concept of additional pathways of necroptosis which is mediated, at least partly, via alterations in oxidative stress and/or mitochondrial activity. In A549 human lung adenocarcinoma cells, tanshinol A, a cytotoxic compound, induced MLKL-mediated necroptosis which was completely independent of both RIP1 and RIP3. Furthermore, the authors of this study have shown that MLKL activation was a downstream event of ROS generation since treatment with antioxidants prevented its phosphorylation, membrane translocation and cell death [95]. Such findings agree with several other studies showing that necroptosis was almost completely abolished by treatment with ROS scavengers [11,96,97]. Likewise, studies reporting that Nec-1 suppressed ROS production and increased the expression of antioxidant proteins, such as heme oxygenase 1 (HO-1), nuclear factor erythroid 2-related factor 2 (NRF2) and superoxide dismutase 2 (SOD2) [11,98] support a link between oxidative stress and necroptosis. Further evidence of such phenomenon comes from a study investigating a model of Streptococcus pneumoniae infection, where RIP3 formed a complex with RIP1, MLKL and the mitochondrial calcium uniporter (MCU) with resultant necroptosis of macrophages via mitochondrial ROS-induced mPTP opening [15]. Interestingly, besides mediating necroptosis, RIP3 also promoted the activation of the NLRP3 inflammasome through the mitochondrial ROS–AKT pathway [15]. A link between necroptosis and mitochondrial dysfunction was also indicated in a model of cigarette smoke-induced chronic obstructive pulmonary disease (COPD), in which both Drp1 inhibition by Mdivi-1 and knockdown of a mitophagy regulator, PTEN-induced kinase 1 (PINK1), resulted in reduced phosphorylation of MLKL [12], suggesting a crosstalk between necroptosis and autophagy. In addition, BNIP3, a pro-death protein inducing mitochondrial dysfunction [99], has also been suggested as a mediator of alternative necroptosis in the lungs. In A549 cancer cells, treatment with TNF resulted in necroptotic cell loss which was executed via ROS-dependent mitochondrial insertion of BNIP3, while this effect was suppressed by the administration of both Nec-1 and N-acetylcysteine [100].

Like in other tissues reported in this paper, the role of ER stress in the novel, additional pathways of necroptosis has also been indicated as a pathomechanism of lung damage. In an in vitro model of lung I/R injury, the canonical necrosome formation proceeded into the stress of ER with subsequent mitochondrial calcium overload, overproduction of ROS and necrotic cell loss [13]. Moreover, this study indicated that the formation of such a pro-necroptotic complex was dependent on calpain-induced STAT3 phosphorylation [13], thereby

indicating that necroptosis might also be related to disrupted calcium homeostasis under the conditions of I/R.

The involvement of mitogen-activated protein kinases (MAPKs) in necroptosis has also been discussed under the conditions of lung injury. In an in vitro model of heat stress-induced damage to lungs, pharmacological inhibition of either extracellular signal-regulated kinase (ERK) or nuclear factor-kappa B (NF-κB) or c-Jun suppressed necroptotic cell loss along with preventing cytosolic translocation of high mobility group box 1 protein (HMGB1) [101]. Moreover, RIP1/RIP3 siRNA transfection negatively regulated the activation of c-Jun and ERK, while Nec-1 decreased NF-κB p65 nuclear translocation [101], indicating the involvement of such non-conventional mediators downstream of the RIP1─RIP3 necrosome. JNK, another member of the MAPK family, has also been implemented in necroptosis in the lungs. It was shown that treatment of A549 lung cancer cells with an anti-cancer agent, 2-methoxy-6-acetyl-7-methyljuglone, induced necroptosis which was reversed by both JNK silencing as well as pharmacologic inhibition by SP600125. In fact, the activation of JNK was ROS-dependent and resulted in increased nitrosative stress with the subsequent promotion of necroptosis via mitochondrial alterations and downregulation of pro-survival genes [14].

2.6. Blood vessels

Mitochondrial damage due to excessive mPTP opening seems to play a pivotal role in necroptosis that can occur in cells of the vasculature. It has been found that pharmacological inhibition of mPTP, as well as deficiency of a key mPTP regulator, cyclophilin D (cypD), in microvascular endothelial cells exposed to necroptosis-inducing agents, suppressed MLKL phosphorylation and necroptotic cell death with the resultant decrease in HMGB1 release [102]. Similarly, in an in vitro model of hypoxia/reoxygenation, both cypD inhibition and deletion inhibited necroptosis of endothelial cells while the underlying mechanism included the prevention of AIF translocation into the nucleus [103]. In support, in a mouse model of cardiac transplantation, cypD deficiency in donor's hearts showed protective effects in terms of reducing endothelial damage, graft rejection and mortality possibly due to suppressing necroptosis mediated by the mitochondrial release of AIF and its subsequent nuclear translocation [102,103]. Furthermore, in the context of cardiac microvascular I/R injury, the mitochondrial phosphatase PGAM5 activated by RIP3 has been found to directly colocalize with cypD while promoting its phosphorylation which in turn led to the opening of mPTP and endothelial necroptosis [104]. Another mPTP-related mechanism of I/R-induced microvascular endothelial necroptosis has been found to include intracellular calcium overload and MCU upregulation subsequently leading to excessive mPTP opening [105]. Interestingly, overexpression of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a) prevented the calcium─MCU─mPTP necroptosis pathway and also normalized the I/R-

induced upregulation of both RIP3 and PGAM5 with resultant improvements in endothelial function [105].

Other mechanisms of necroptosis promoted via CaMKII signalling have also been shown to significantly contribute to the pathogenesis of vessel injury. In a model of coronary vascular endothelial damage induced by bisphenol A, CaMKIIα has been suggested to stimulate necroptosis downstream of RIP3, since RIP3 silencing prevented both p-CaMKIIα upregulation and necroptotic cell loss. On the other hand, CaMKII inhibition by KN-93 did not affect RIP3 expression but also suppressed necroptosis, thereby supporting a role for RIP3 signalling to CaMKIIα rather than CaMKIIα to RIP3 [106]. The concept of CaMKII acting as a downstream molecule of the "classical" necrosome has been further supported by a study on a model of abdominal aortic aneurysm showing that RIP3 deficiency, as well as MLKL knockdown, diminished CaMKIIδ phosphorylation, but knockdown of CaMKIIδ failed to affect the expression of such proteins [107]. This study also indicated that MLKL rather than RIP3 might serve as an upstream activator of CaMKIIδ since co-immunoprecipitation studies revealed no direct interactions between RIP3 and CaMKIIδ [107]. Therefore, it is clear that CaMKII is closely associated with the mediators of the canonical pathway of necroptosis but the precise relationship between RIP3–MLKL and CaMKII might vary depending on the CaMKII isoform, the specific pathological conditions and the particular type of injured cells.

p38 MAPK has been proposed to mediate necroptosis via RIP3 in a model of high glucose (HG)-induced damage of human umbilical vein endothelial cells. It has been reported that treatment of cells with a p38 inhibitor, SB203580, suppressed RIP3 upregulation and both Nec-1 administration and RIP3 silencing reduced the phosphorylation of p38 while all these interventions reversed HG-induced cytotoxicity [108]. Thus, it is likely that a positive feedback loop between p38 and necroptosis might exist. Furthermore, both RIP3 silencing and SB203580 treatment attenuated ROS production and prevented the loss of mitochondrial membrane potential [108] indicating that such events might, at least partially, contribute to the necroptotic loss of endothelial cells induced by HG. Consistent with these reports, necroptosis induced by tert-butyl hydroperoxide in the endothelial cells has been shown to be mediated by both p38 MAPK and mitochondrial alterations. In fact, inhibition of p38, as well as mitochondrial ROS production, prevented necroptotic cell loss while silencing of the canonical necrosome components suppressed p38 activation, generation of mitochondrial ROS and loss of mitochondrial membrane potential [109]. Based on these data, it is plausible that both p38 signalling and oxidative stress generated by mitochondria cooperate to execute necroptosis in endothelial cells.

Finally, receptor for advanced glycation end products (RAGE) has been identified as another non-conventional mediator of necroptosis in a murine model of red blood cell transfusion. RAGE knockout in mice has been found to attenuate necrosis-like cell death as

evidenced by decreased plasma levels of RIP3 and HMGB1 [110]. Further in vitro, investigations have revealed that knockdown of RAGE suppressed necroptosis of human lung endothelial cells while RAGE was found to directly interact with RIP3 in both the nucleus and cytosol [110] supporting the relevance of this novel identified mediator of necroptosis signalling.

3. MicroRNAs in necroptosis regulation

As indicated above, necroptosis is a tightly regulated process. One mechanism controlling necroptosis has been identified as microRNAs (miRNAs) [111]. miRNA regulation of necroptotic pathways has mostly been studied in models of cardiovascular diseases. The specific miRNAs regulating the canonical pathway include miRNA-181b-1, -19, -874, -512-3p, -128a, -155, -21, -223-5p/3p, -24-3p, -325-3p, -103 as reviewed elsewhere [111,112]. Regarding the alternative pathways delineated in this review, increased expression of miRNA-105 and miRNA-873 has been shown to prevent necroptosis through the direct downregulation of RIP proteins in cardiomyocytes [113,114]. In adult mouse cardiomyocytes, miRNA-874 has been reported to promote necroptosis by suppressing the expression of caspase-8 [115]. Nevertheless, in a study using a transgenic mouse model with heart-specific overexpression of miR-223, the death receptors such as tumour necrosis factor receptor 1 (TNFR1) and death receptor 6 (DR6), known to initiate necroptosis signalling, have been identified as targets of miRNA-223-5p. On the other hand, miRNA-223-3p has been found to directly suppress the expression of NLRP3 and inhibitory kappa B kinase alpha (IKKα) [116]. Interestingly, increased expression of miRNA-103/107 targeting the Fas-associated death domain (FADD), a negative regulator of necroptosis, has been associated with the progression of myocardial infarction [117]. However, in TNF–induced necroptosis, the participation of such miRNA-103/107─FADD pathway has not been confirmed [117]. miRNA-2861 has been reported to regulate H_2O_2-induced cardiomyocyte necroptosis by targeting adenine nucleotide translocator 1 (ANT1), a protein which facilitates the exchange of cytosolic ADP and mitochondrial ATP across the mitochondrial inner membrane [118]. Another study demonstrated that delivery of exosomal miRNA-17-3p to the heart alleviated the programmed necrosis associated with cardiac I/R injury by regulating tissue inhibitor of metalloproteinases 3 (TIMP3) expression [119]. miRNA-214 induced by ischemic injury has been shown to protect cardiomyocytes from damage through the suppression of CaMKII and cypD [120]. Interestingly, oxidative stress and the inflammatory response as well as MAPK signalling have been suggested to be involved in miRNA-200a-5p-induced additional pathways of necroptosis in cardiomyocytes [121].

In the liver, miRNAs have been reported to regulate necroptotic cell death via the inhibition of caspase-8. Visalli et al. [122] suggested three miRNAs including miRNA-371-5p, miRNA-373 and miRNA-543 induce necroptosis in hepatocellular carcinoma by directly targeting the caspase-8 gene. Comparably to cardiac tissue, the downregulation of FADD by miRNA-675 has been found to promote liver necroptosis in response to inflammatory signals [123]. Gu et al. [124] suggest that miRNA-425-5p negatively controls the RIP1-mediated necroptotic signalling cascades in a mouse model of sepsis-related liver damage. In addition, miRNA-21 has been shown to modulate necroptosis in primary mouse hepatocytes by targeting the cell cycle regulator cyclin-dependent kinase 2-associated protein 1 (CDK2AP1), which is a known modulator of RIP3 [125]. In the liver damage induced by LPS, miRNA-155 has been suggested to regulate necroptosis by targeting TNF receptor-associated factor 3 (TRAF3), thereby activating the JNK pathway, thus promoting the respective alternative non-conventional pathway of this cell death [126].

In a model of LPS-induced lung damage, necroptosis execution has been associated with increased expression of miRNA-16-5p targeting phosphatidylinositol 3-kinase (PI3K) with the resultant inhibition of the PI3K/AKT pathway [127]. LPS stimulation has also been found to inhibit miRNA-15a, and simultaneously increase the expression of JNK, NLRP3, caspase-1, RIP1, RIP3, and MLKL, thereby collectively promoting pulmonary necroptosis in chicken lungs. Furthermore, in LPS-induced lung injury in four-month-old chickens, miRNA-15a/JNK has been suggested to serve as a modulator of both necroptosis and oxidative stress [128]. Nevertheless, a different study by Carnino et al. [129] has depicted that miRNA-185-5p promoted necroptosis in alveolar type II cells via modulating the FADD/caspase-8 pathway.

So far, there is only a limited number of studies investigating the regulation of necroptosis by miRNAs in renal diseases. Zhao et al. [130] have found that overexpression of miRNA-381-3p blocks TNF-induced necroptosis in renal cancer cells by inhibiting the activation of RIP3 and MLKL. Likewise, miRNA-500a-3p has been shown to alleviate kidney injury by targeting MLKL-mediated necroptosis in renal epithelial cells [131]. Shen et al. [132] have identified a necroptotic pathway linking hypoxia-inducible factor 1-α (HIF-1α), miRNA-26a, transient receptor potential channel 6 (TRPC6), and poly(ADP-ribose) polymerase 1 (PARP1) in ischemic acute kidney injury model. Mechanistically, HIF-1α has been suggested to bind to the promoter region of miRNA-26a which in turn affect TRPC6.

Comparably to the kidneys, the available literature offers only scattered studies dealing with the regulation of necroptosis by miRNAs in the CNS and intestines. miRNA-425 has been shown to target RIP1 and promote the phosphorylation of MLKL and necroptosis in the brains of patients with Parkinson`s disease [133]. Cui et al. [134] have shown that selenium deficiency induced necroptosis through the regulation of TNFR1 by miRNA-29a-3p in the pig brain. In another study, selenium deficiency induced the deregulation of the miR-130–CYLD

axis and caused RIP3-dependent necroptosis in the pig cerebellum [135]. In a model of enterocolitis, miR-141-3p has been found to protect intestinal epithelial cells from LPS damage by suppressing RIP1-mediated necroptosis [136].

Concluding remarks

From the aforementioned discussion as well as from Table 1, summarising evidence of some of the additional. non-conventional pathways of necroptosis, it is evident that the scientific view on necroptosis and its regulation has advanced since several mitochondrial and ER-related proteins have been suggested, at least under some circumstances, to promote necroptotic cell loss. Thus, it is clear that the conventional concept of necroptosis signalling involving the RIP3–MLKL axis as the executioner might be challenged and other, additional adapters of necroptotic signalling might be involved in the final proceeding of necroptotic cell death (Figure 1). Although many issues need to be clarified, it is clear that some of the additional pathways, being not directly and not exclusively associated with the traditionally accepted terminal pro-necroptotic protein, have been identified in specific pathological conditions of some organs only while under other conditions, or in other organs, the authors have suggested a role of different ones. They are schematically depicted in Figure 2. These observations support very complex and possibly organ and injury-type specific events. However, it is also possible that a lack of studies in this field explains this level of knowledge. Irrespective of these facts, we propose that a simple approach considering only the genetically determined expression levels of pro-necroptotic proteins in different healthy tissues is unlikely to provide sufficient information on the regulation and preferential promotion of either of the alternative signalling pathways since the gene and protein expression among various cells does not significantly differ under basal conditions. On the other hand, it should be mentioned that although the basal mRNA levels of RIP3 are lower in the heart compared to the lungs, this protein kinase seems to be crucially involved in all of the proposed cardiac alternative molecular pathways of necroptosis. In contrast, the cells in the lungs seem to undergo necroptosis independently of RIP3, and rather in an MLKL-dependent manner. It is also worth pointing out that some of the proposed molecular targets of the novel, additional pathways are also known to be involved in the regulation of other types of programmed cell death. For instance, AIF is implemented in apoptosis activation while at the same time, it has also been found to promote necroptosis via nuclear translocation of RIP3–AIF complex in some organs. Collectively, the additional pathways shown to mediate cell loss via necroptosis have partially challenged the conventional definition of necroptotic death referred to as “a modality of regulated cell death triggered by perturbations of extracellular or intracellular homeostasis that critically depends on MLKL, RIP3, and (at least in some settings) on the kinase activity of

RIP1" [49]. Thus, further intensive experimental work in this area of biology as well as reconsideration of the definition of this cell death mode might be expected in near future. Accordingly, the design of novel anti-necroptotic approaches mitigating necroptotic injury due to targeting either of the other, alternative axes along with the canonical pathway of necroptosis might be significantly affected.

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References

[1] A. Adameova, E. Goncalvesova, A. Szobi, N.S. Dhalla, Necroptotic cell death in failing heart: relevance and proposed mechanisms, Heart Fail Rev. 21 (2016) 213–221. https://doi.org/10.1007/s10741-016-9537-8.

[2] A. Adameova, J. Hrdlicka, A. Szobi, V. Farkasova, M. Murarikova, J. Neckar, F. Kolar, T. Ravingerova, Evidence of necroptosis in hearts subjected to various forms of ischemic insults, (2017) 1–29. https://doi.org/10.1139/cjpp-2016-0609.

[3] A. Adameova, C. Horvath, S. Abdul-Ghani, Z. v. Varga, M.S. Suleiman, N.S. Dhalla, Interplay of Oxidative Stress and Necrosis-like Cell Death in Cardiac Ischemia/Reperfusion Injury: A Focus on Necroptosis, Biomedicines. 10 (2022). https://doi.org/10.3390/BIOMEDICINES10010127.

[4] M. Lichý, A. Szobi, J. Hrdlička, C. Horváth, V. Kormanová, T. Rajtík, J. Neckář, F. Kolář, A. Adameová, Different signalling in infarcted and non-infarcted areas of rat failing hearts: A role of necroptosis and inflammation, J Cell Mol Med. 23 (2019). https://doi.org/10.1111/jcmm.14536.

[5] A. Szobi, E. Gonçalvesová, Z. v. Varga, P. Leszek, M. Kuśmierczyk, M. Hulman, J. Kyselovič, P. Ferdinandy, A. Adameová, Analysis of necroptotic proteins in failing human hearts, J Transl Med. 15 (2017) 86. https://doi.org/10.1186/s12967-017-1189-5.

[6] S. Zhang, M.B. Tang, H.Y. Luo, C.H. Shi, Y.M. Xu, Necroptosis in neurodegenerative diseases: a potential therapeutic target, Cell Death Dis. 8 (2017). https://doi.org/10.1038/CDDIS.2017.286.

[7] L.E. Colbert, S.B. Fisher, C.W. Hardy, W.A. Hall, B. Saka, J.W. Shelton, A. v Petrova, M.D. Warren, B.G. Pantazides, K. Gandhi, J. Kowalski, D.A. Kooby, B.F. El-Rayes, C.A. Staley, N. Volkan Adsay, W.J. Curran, J.C. Landry, S.K. Maithel, D.S. Yu, Pronecrotic Mixed Lineage Kinase Domain-Like Protein Expression Is a Prognostic Biomarker in Patients With Early-Stage Resected Pancreatic Adenocarcinoma, Cancer. 119 (2013) 3148–3155. https://doi.org/10.1002/cncr.28144.

[8] M. bin Meng, H.H. Wang, Y.L. Cui, Z.Q. Wu, Y.Y. Shi, N.G. Zaorsky, L. Deng, Z.Y. Yuan, Y. Lu, P. Wang, Necroptosis in tumorigenesis, activation of anti-tumor immunity, and cancer therapy, Oncotarget. 7 (2016) 57391–57413. https://doi.org/10.18632/ONCOTARGET.10548.

[9] H. Guo, S. Omoto, P.A. Harris, J.N. Finger, J. Bertin, P.J. Gough, W.J. Kaiser, E.S. Mocarski, Herpes Simplex Virus Suppresses Necroptosis in Human Cells, Cell Host Microbe. 17 (2015) 243–251. https://doi.org/10.1016/j.chom.2015.01.003.

[10] K. Kitur, D. Parker, P. Nieto, D.S. Ahn, T.S. Cohen, S. Chung, S. Wachtel, S. Bueno, A. Prince, Toxin-induced necroptosis is a major mechanism of Staphylococcus aureus lung damage, PLoS Pathog. 11 (2015). https://doi.org/10.1371/JOURNAL.PPAT.1004820.

[11] M. Hussain, V. Zimmermann, S.J.L. van Wijk, S. Fulda, Mouse lung fibroblasts are highly susceptible to necroptosis in a reactive oxygen species-dependent manner, Biochem Pharmacol. 153 (2018) 242–247. https://doi.org/10.1016/j.bcp.2018.01.025.

[12] K. Mizumura, S.M. Cloonan, K. Nakahira, A.R. Bhashyam, M. Cervo, T. Kitada, K. Glass, C.A. Owen, A. Mahmood, G.R. Washko, S. Hashimoto, S.W. Ryter, A.M.K. Choi, Mitophagy-dependent necroptosis contributes to the pathogenesis of COPD, Journal of Clinical Investigation. 124 (2014) 3987–4003. https://doi.org/10.1172/JCI74985.

[13] H. Kim, R. Zamel, X.-H. Bai, C. Lu, S. Keshavjee, S. Keshavjee, M. Liu, Ischemia-reperfusion induces death receptor-independent necroptosis via calpain-STAT3 activation in a lung transplant setting, Am J Physiol Lung Cell Mol Physiol. 315 (2018) 595–608. https://doi.org/10.1152/ajplung.00069.2018.-Ischemia.

[14] W. Sun, J. Bao, W. Lin, H. Gao, W. Zhao, Q. Zhang, C.H. Leung, D.L. Ma, J. Lu, X. Chen, 2-Methoxy-6-acetyl-7-methyljuglone (MAM), a natural naphthoquinone, induces NO-dependent apoptosis and necroptosis by H2O2-dependent JNK activation in cancer cells, Free Radic Biol Med. 92 (2016) 61–77. https://doi.org/10.1016/j.freeradbiomed.2016.01.014.

[15] H.R. Huang, S.J. Cho, R.M. Harris, J. Yang, S. Bermejo, L. Sharma, C.S. dela Cruz, J.F. Xu, H.W. Stout-Delgado, RIPK3 activates MLKL-mediated necroptosis and inflammasome signaling during streptococcus infection, Am J Respir Cell Mol Biol. 64 (2021) 579–591. https://doi.org/10.1165/rcmb.2020-0312OC.

[16] A. Ramachandran, M.R. Mcgill, Y. Xie, H.M. Ni, W.X. Ding, H. Jaeschke, Receptor interacting protein kinase 3 is a critical early mediator of acetaminophen-induced hepatocyte necrosis in mice, Hepatology. 58 (2013) 2099–2108. https://doi.org/10.1002/hep.26547.

[17] Y.F. Zhang, W. He, C. Zhang, X.J. Liu, Y. Lu, H. Wang, Z.H. Zhang, X. Chen, D.X. Xu, Role of receptor interacting protein (RIP)1 on apoptosis-inducing factor-mediated necroptosis during acetaminophen-evoked acute liver failure in mice, Toxicol Lett. 225 (2014) 445–453. https://doi.org/10.1016/j.toxlet.2014.01.005.

[18] S. Roychowdhury, M.R. Mcmullen, S.G. Pisano, X. Liu, L.E. Nagy, Absence of receptor interacting protein kinase 3 prevents ethanol-induced liver injury, Hepatology. 57 (2013) 1773–1783. https://doi.org/10.1002/hep.26200.

[19] R.D. Tian, Y.Q. Chen, Y.H. He, Y.J. Tang, G.M. Chen, F.W. Yang, Y. Li, W.G. Huang, H. Chen, X. Liu, S. de Lin, Phosphorylation of eIF2α mitigates endoplasmic reticulum stress and hepatocyte necroptosis in acute liver injury, Ann Hepatol. 19 (2020) 79–87. https://doi.org/10.1016/j.aohep.2019.05.008.

[20] C. Günther, G.W. He, A.E. Kremer, J.M. Murphy, E.J. Petrie, K. Amann, P. Vandenabeele, A. Linkermann, C. Poremba, U. Schleicher, C. Dewitz, S. Krautwald, M.F. Neurath, C. Becker, S. Wirtz, The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 during hepatitis, J Clin Invest. 126 (2016) 4346–4360. https://doi.org/10.1172/JCI87545.

[21] X. Wu, K.L. Poulsen, C. Sanz-Garcia, E. Huang, M.R. McMullen, S. Roychowdhury, S. Dasarathy, L.E. Nagy, MLKL-dependent signaling regulates autophagic flux in a murine model of non-alcohol-associated fatty liver and steatohepatitis, J Hepatol. 73 (2020) 616–627. https://doi.org/10.1016/j.jhep.2020.03.023.

[22] K. Pomlok, S. Pata, M. Kulaphisit, R. Pangnuchar, J. Wipasa, D.R. Smith, W. Kasinrerk, P. Lithanatudom, An IgM monoclonal antibody against domain 1 of CD147 induces non-canonical RIPK-independent necroptosis in a cell type specific manner in hepatocellular carcinoma cells, Biochim Biophys Acta Mol Cell Res. 1869 (2022). https://doi.org/10.1016/j.bbamcr.2022.119295.

[23] Y. Liu, Q. Xu, Y. Wang, T. Liang, X. Li, D. Wang, X. Wang, H. Zhu, K. Xiao, Necroptosis is active and contributes to intestinal injury in a piglet model with lipopolysaccharide challenge, Cell Death Dis. 12 (2021). https://doi.org/10.1038/S41419-020-03365-1.

[24] K. Xiao, Y. Yang, Y. Zhang, Q. Lv, F. Huang, D. Wang, J. Zhao, Y. Liu, Long-chain PUFA ameliorate enterotoxigenic Escherichia coli-induced intestinal inflammation and cell

injury by modulating pyroptosis and necroptosis signaling pathways in porcine intestinal epithelial cells, Br J Nutr. (2021). https://doi.org/10.1017/S0007114521005092.
[25] X. Lou, H. Zhu, L. Ning, C. Li, S. Li, H. Du, X. Zhou, G. Xu, EZH2 Regulates Intestinal Inflammation and Necroptosis Through the JNK Signaling Pathway in Intestinal Epithelial Cells, Dig Dis Sci. 64 (2019) 3518–3527. https://doi.org/10.1007/S10620-019-05705-4.
[26] W. Sun, X. Wu, H. Gao, J. Yu, W. Zhao, J.J. Lu, J. Wang, G. Du, X. Chen, Cytosolic calcium mediates RIP1/RIP3 complex-dependent necroptosis through JNK activation and mitochondrial ROS production in human colon cancer cells, Free Radic Biol Med. 108 (2017) 433–444. https://doi.org/10.1016/J.FREERADBIOMED.2017.04.010.
[27] Y. Zhu, H. Cui, H. Gan, Y. Xia, L. Wang, Y. Wang, Y. Sun, Necroptosis mediated by receptor interaction protein kinase 1 and 3 aggravates chronic kidney injury of subtotal nephrectomised rats, Biochem Biophys Res Commun. 461 (2015) 575–581. https://doi.org/10.1016/J.BBRC.2015.03.164.
[28] R.S. Al-Lamki, W. Lu, P. Manalo, J. Wang, A.Y. Warren, A.M. Tolkovsky, J.S. Pober, J.R. Bradley, Tubular epithelial cells in renal clear cell carcinoma express high RIPK1/3 and show increased susceptibility to TNF receptor 1-induced necroptosis, Cell Death Dis. 7 (2016) e2287. https://doi.org/10.1038/CDDIS.2016.184.
[29] A. Sureshbabu, E. Patino, K.C. Ma, K. Laursen, E.J. Finkelsztein, O. Akchurin, T. Muthukumar, S.W. Ryter, L. Gudas, A.M.K. Choi, M.E. Choi, RIPK3 promotes sepsis-induced acute kidney injury via mitochondrial dysfunction, JCI Insight. 3 (2018). https://doi.org/10.1172/JCI.INSIGHT.98411.
[30] Y. Feng, A. Imam Aliagan, N. Tombo, D. Draeger, J.C. Bopassa, RIP3 Translocation into Mitochondria Promotes Mitofilin Degradation to Increase Inflammation and Kidney Injury after Renal Ischemia-Reperfusion, Cells. 11 (2022) 1894. https://doi.org/10.3390/CELLS11121894.
[31] K. Grynberg, E. Ozols, W.R. Mulley, R.J. Davis, R.A. Flavell, D.J. Nikolic-Paterson, F.Y. Ma, JUN Amino-Terminal Kinase 1 Signaling in the Proximal Tubule Causes Cell Death and Acute Renal Failure in Rat and Mouse Models of Renal Ischemia/Reperfusion Injury, Am J Pathol. 191 (2021) 817–828. https://doi.org/10.1016/J.AJPATH.2021.02.004.
[32] D. Moquin, F.K.-M. Chan, The molecular regulation of programmed necrotic cell injury, Trends Biochem Sci. 35 (2010) 434–441. https://doi.org/https://doi.org/10.1016/j.tibs.2010.03.001.
[33] A. Oberst, C.P. Dillon, R. Weinlich, L.L. McCormick, P. Fitzgerald, C. Pop, R. Hakem, G.S. Salvesen, D.R. Green, Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis, Nature. 471 (2011) 363–367. https://doi.org/10.1038/nature09852.
[34] S. Grootjans, T. vanden Berghe, P. Vandenabeele, Initiation and execution mechanisms of necroptosis: an overview, Cell Death Differ. 24 (2017) 1184–1195. https://doi.org/10.1038/cdd.2017.65.
[35] M. Feoktistova, M. Leverkus, Programmed necrosis and necroptosis signalling, FEBS J. 282 (2014) 19–31. https://doi.org/10.1111/febs.13120.
[36] D. Huang, X. Zheng, Z. Wang, X. Chen, W. He, Y. Zhang, J.-G. Xu, H. Zhao, W. Shi, X. Wang, Y. Zhu, J. Han, The MLKL Channel in Necroptosis Is an Octamer Formed by Tetramers in a Dyadic Process, 2017. https://doi.org/10.1128/MCB.00497-16.

[37] H. Wang, L. Sun, L. Su, J. Rizo, L. Liu, L.F. Wang, F.S. Wang, X. Wang, Mixed Lineage Kinase Domain-like Protein MLKL Causes Necrotic Membrane Disruption upon Phosphorylation by RIP3, Mol Cell. 54 (2014) 133–146. https://doi.org/10.1016/j.molcel.2014.03.003.
[38] M. Feoktistova, P. Geserick, D. Panayotova-Dimitrova, M. Leverkus, Pick your poison: The Ripoptosome, a cell death platform regulating apoptosis and necroptosis, Cell Cycle. 11 (2012) 460–467. https://doi.org/10.4161/cc.11.3.19060.
[39] M.I.F.J. Oerlemans, J. Liu, F. Arslan, K. den Ouden, B.J. van Middelaar, P.A. Doevendans, J.P.G. Sluijter, Inhibition of RIP1-dependent necrosis prevents adverse cardiac remodeling after myocardial ischemia–reperfusion in vivo, Basic Res Cardiol. 107 (2012) 270. https://doi.org/10.1007/s00395-012-0270-8.
[40] A. Iannielli, S. Bido, L. Folladori, A. Segnali, C. Cancellieri, A. Maresca, L. Massimino, A. Rubio, G. Morabito, L. Caporali, F. Tagliavini, O. Musumeci, G. Gregato, E. Bezard, V. Carelli, V. Tiranti, V. Broccoli, Pharmacological Inhibition of Necroptosis Protects from Dopaminergic Neuronal Cell Death in Parkinson's Disease Models, Cell Rep. 22 (2018) 2066–2079. https://doi.org/10.1016/j.celrep.2018.01.089.
[41] S. Cruz, Z. Qin, AlexandreF.R. Stewart, H.-H. Chen, Dabrafenib, an inhibitor of RIP3 kinase-dependent necroptosis, reduces ischemic brain injury, Neural Regen Res. 13 (2018) 252. https://doi.org/10.4103/1673-5374.226394.
[42] S. Koshinuma, M. Miyamae, K. Kaneda, J. Kotani, V.M. Figueredo, Combination of necroptosis and apoptosis inhibition enhances cardioprotection against myocardial ischemia-reperfusion injury, J Anesth. 28 (2014) 235–241. https://doi.org/10.1007/s00540-013-1716-3.
[43] Y. v. Dmitriev, S.M. Minasian, E.A. Demchenko, M.M. Galagudza, Study of cardioprotective effects of necroptosis inhibitors on isolated rat heart subjected to global ischemia-reperfusion, Bull Exp Biol Med. 155 (2013) 245–248. https://doi.org/10.1007/s10517-013-2124-2.
[44] B. Yan, L. Liu, S. Huang, Y. Ren, H. Wang, Z. Yao, L. Li, S. Chen, X. Wang, Z. Zhang, Discovery of a new class of highly potent necroptosis inhibitors targeting the mixed lineage kinase domain-like protein†, 2017. https://doi.org/10.1039/c7cc00667e.
[45] C. Horvath, M. Young, I. Jarabicova, L. Kindernay, K. Ferenczyova, T. Ravingerova, M. Lewis, M.S. Suleiman, A. Adameova, Inhibition of Cardiac RIP3 Mitigates Early Reperfusion Injury and Calcium-Induced Mitochondrial Swelling without Altering Necroptotic Signalling, International Journal of Molecular Sciences 2021, Vol. 22, Page 7983. 22 (2021) 7983. https://doi.org/10.3390/IJMS22157983.
[46] S. He, Y. Liang, F. Shao, X. Wang, Toll-like receptors activate programmed necrosis in macrophages through a receptor-interacting kinase-3-mediated pathway, Proc Natl Acad Sci U S A. 108 (2011) 20054–20059. https://doi.org/10.1073/PNAS.1116302108.
[47] W.J. Kaiser, H. Sridharan, C. Huang, P. Mandal, J.W. Upton, P.J. Gough, C.A. Sehon, R.W. Marquis, J. Bertin, E.S. Mocarski, Toll-like Receptor 3-mediated Necrosis via TRIF, RIP3, and MLKL, J Biol Chem. 288 (2013) 31268. https://doi.org/10.1074/JBC.M113.462341.
[48] J.W. Upton, W.J. Kaiser, E.S. Mocarski, DAI/ZBP1/DLM-1 complexes with RIP3 to mediate virus-induced programmed necrosis that is targeted by murine cytomegalovirus vIRA, Cell Host Microbe. 11 (2012) 290–297. https://doi.org/10.1016/J.CHOM.2012.01.016.

[49] L. Galluzzi, I. Vitale, S.A. Aaronson, J.M. Abrams, D. Adam, P. Agostinis, E.S. Alnemri, L. Altucci, I. Amelio, D.W. Andrews, M. Annicchiarico-Petruzzelli, A. v. Antonov, E. Arama, E.H. Baehrecke, N.A. Barlev, N.G. Bazan, F. Bernassola, M.J.M. Bertrand, K. Bianchi, M. v. Blagosklonny, K. Blomgren, C. Borner, P. Boya, C. Brenner, M. Campanella, E. Candi, D. Carmona-Gutierrez, F. Cecconi, F.K.M. Chan, N.S. Chandel, E.H. Cheng, J.E. Chipuk, J.A. Cidlowski, A. Ciechanover, G.M. Cohen, M. Conrad, J.R. Cubillos-Ruiz, P.E. Czabotar, V. D'Angiolella, T.M. Dawson, V.L. Dawson, V. de Laurenzi, R. de Maria, K.M. Debatin, R.J. Deberardinis, M. Deshmukh, N. di Daniele, F. di Virgilio, V.M. Dixit, S.J. Dixon, C.S. Duckett, B.D. Dynlacht, W.S. El-Deiry, J.W. Elrod, G.M. Fimia, S. Fulda, A.J. García-Sáez, A.D. Garg, C. Garrido, E. Gavathiotis, P. Golstein, E. Gottlieb, D.R. Green, L.A. Greene, H. Gronemeyer, A. Gross, G. Hajnoczky, J.M. Hardwick, I.S. Harris, M.O. Hengartner, C. Hetz, H. Ichijo, M. Jäättelä, B. Joseph, P.J. Jost, P.P. Juin, W.J. Kaiser, M. Karin, T. Kaufmann, O. Kepp, A. Kimchi, R.N. Kitsis, D.J. Klionsky, R.A. Knight, S. Kumar, S.W. Lee, J.J. Lemasters, B. Levine, A. Linkermann, S.A. Lipton, R.A. Lockshin, C. López-Otín, S.W. Lowe, T. Luedde, E. Lugli, M. MacFarlane, F. Madeo, M. Malewicz, W. Malorni, G. Manic, J.C. Marine, S.J. Martin, J.C. Martinou, J.P. Medema, P. Mehlen, P. Meier, S. Melino, E.A. Miao, J.D. Molkentin, U.M. Moll, C. Muñoz-Pinedo, S. Nagata, G. Nuñez, A. Oberst, M. Oren, M. Overholtzer, M. Pagano, T. Panaretakis, M. Pasparakis, J.M. Penninger, D.M. Pereira, S. Pervaiz, M.E. Peter, M. Piacentini, P. Pinton, J.H.M. Prehn, H. Puthalakath, G.A. Rabinovich, M. Rehm, R. Rizzuto, C.M.P. Rodrigues, D.C. Rubinsztein, T. Rudel, K.M. Ryan, E. Sayan, L. Scorrano, F. Shao, Y. Shi, J. Silke, H.U. Simon, A. Sistigu, B.R. Stockwell, A. Strasser, G. Szabadkai, S.W.G. Tait, D. Tang, N. Tavernarakis, A. Thorburn, Y. Tsujimoto, B. Turk, T. vanden Berghe, P. Vandenabeele, M.G. vander Heiden, A. Villunger, H.W. Virgin, K.H. Vousden, D. Vucic, E.F. Wagner, H. Walczak, D. Wallach, Y. Wang, J.A. Wells, W. Wood, J. Yuan, Z. Zakeri, B. Zhivotovsky, L. Zitvogel, G. Melino, G. Kroemer, Molecular mechanisms of cell death: Recommendations of the Nomenclature Committee on Cell Death 2018, Cell Death Differ. 25 (2018) 486–541. https://doi.org/10.1038/s41418-017-0012-4.

[50] P.K. Mishra, A. Adameova, J.A. Hill, C.P. Baines, P.M. Kang, J.M. Downey, J. Narula, M. Takahashi, A. Abbate, H.C. Piristine, S. Kar, S. Su, J.K. Higa, N.K. Kawasaki, T. Matsui, Guidelines for evaluating myocardial cell death, (2019). https://doi.org/10.1152/ajpheart.00259.2019.

[51] Z. Cai, S. Jitkaew, J. Zhao, H.C. Chiang, S. Choksi, J. Liu, Y. Ward, L.G. Wu, Z.G. Liu, Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis, Nat Cell Biol. 16 (2014) 55–65. https://doi.org/10.1038/ncb2883.

[52] X. Chen, W.W. Li, J. Ren, D. Huang, W. He, Y. Song, C. Yang, W.W. Li, X. Zheng, P. Chen, J. Han, Translocation of mixed lineage kinase domain-like protein to plasma membrane leads to necrotic cell death, Cell Res. 24 (2014) 105–121. https://doi.org/10.1038/cr.2013.171.

[53] Z. Cai, A. Zhang, S. Choksi, W. Li, T. Li, X.M. Zhang, Z.G. Liu, Activation of cell-surface proteases promotes necroptosis, inflammation and cell migration, Cell Res. 26 (2016) 886–900. https://doi.org/10.1038/cr.2016.87.

[54] S.A. Conos, K.W. Chen, D. de Nardo, H. Hara, L. Whitehead, G. Núñez, S.L. Masters, J.M. Murphy, K. Schroder, D.L. Vaux, K.E. Lawlor, L.M. Lindqvist, J.E. Vince, Active

MLKL triggers the NLRP3 inflammasome in a cell-intrinsic manner, Proc Natl Acad Sci U S A. 114 (2017) E961–E969. https://doi.org/10.1073/pnas.1613305114.
[55] S. Grootjans, T. vanden Berghe, P. Vandenabeele, Initiation and execution mechanisms of necroptosis: an overview, Cell Death & Differentiation 2017 24:7. 24 (2017) 1184–1195. https://doi.org/10.1038/cdd.2017.65.
[56] D.E. Christofferson, J. Yuan, Necroptosis as an alternative form of programmed cell death, Curr Opin Cell Biol. 22 (2010) 263–268. https://doi.org/https://doi.org/10.1016/j.ceb.2009.12.003.
[57] Z. Yang, Y. Wang, Y. Zhang, X. He, C.Q. Zhong, H. Ni, X. Chen, Y. Liang, J. Wu, S. Zhao, D. Zhou, J. Han, RIP3 targets pyruvate dehydrogenase complex to increase aerobic respiration in TNF-induced necroptosis, Nat Cell Biol. 20 (2018) 186–197. https://doi.org/10.1038/S41556-017-0022-Y.
[58] B. Schenk, S. Fulda, Reactive oxygen species regulate Smac mimetic/TNFα-induced necroptotic signaling and cell death, Oncogene. 34 (2015) 5796–5806. https://doi.org/10.1038/ONC.2015.35.
[59] Y. Zhang, S.S. Su, S. Zhao, Z. Yang, C.Q. Zhong, X. Chen, Q. Cai, Z.H. Yang, D. Huang, R. Wu, J. Han, RIP1 autophosphorylation is promoted by mitochondrial ROS and is essential for RIP3 recruitment into necrosome, Nat Commun. 8 (2017). https://doi.org/10.1038/NCOMMS14329.
[60] X. Guo, Y. Chen, Q. Liu, Necroptosis in heart disease: Molecular mechanisms and therapeutic implications, J Mol Cell Cardiol. 169 (2022) 74. https://doi.org/10.1016/J.YJMCC.2022.05.006.
[61] T. Zhang, Y. Zhang, M. Cui, L. Jin, Y. Wang, F. Lv, Y. Liu, W. Zheng, H. Shang, J. Zhang, M. Zhang, H. Wu, J. Guo, X. Zhang, X. Hu, C. Cao, R. Xiao, CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress – induced myocardial necroptosis, Nat Med. (2016). https://doi.org/10.1038/nm.4017.
[62] Z. Wang, H. Jiang, S. Chen, F. Du, X. Wang, The Mitochondrial Phosphatase PGAM5 Functions at the Convergence Point of Multiple Necrotic Death Pathways, Cell. 148 (2012) 228–243. https://doi.org/https://doi.org/10.1016/j.cell.2011.11.030.
[63] S.W.G. Tait, A. Oberst, G. Quarato, S. Milasta, M. Haller, R. Wang, M. Karvela, G. Ichim, N. Yatim, M.L. Albert, G. Kidd, R. Wakefield, S. Frase, S. Krautwald, A. Linkermann, D.R. Green, Widespread mitochondrial depletion via mitophagy does not compromise necroptosis, Cell Rep. 5 (2013) 878–885. https://doi.org/10.1016/J.CELREP.2013.10.034.
[64] M. Uhlén, L. Fagerberg, B.M. Hallström, C. Lindskog, P. Oksvold, A. Mardinoglu, Å. Sivertsson, C. Kampf, E. Sjöstedt, A. Asplund, I.M. Olsson, K. Edlund, E. Lundberg, S. Navani, C.A.K. Szigyarto, J. Odeberg, D. Djureinovic, J.O. Takanen, S. Hober, T. Alm, P.H. Edqvist, H. Berling, H. Tegel, J. Mulder, J. Rockberg, P. Nilsson, J.M. Schwenk, M. Hamsten, K. von Feilitzen, M. Forsberg, L. Persson, F. Johansson, M. Zwahlen, G. von Heijne, J. Nielsen, F. Pontén, Tissue-based map of the human proteome, Science (1979). 347 (2015). https://doi.org/10.1126/SCIENCE.1260419/SUPPL_FILE/1260419_UHLEN.SM.PDF.
[65] Y. Yang, W. Hu, S. Feng, J. Ma, M. Wu, RIP3 beta and RIP3 gamma, two novel splice variants of receptor-interacting protein 3 (RIP3), downregulate RIP3-induced apoptosis, Biochem Biophys Res Commun. 332 (2005) 181–187. https://doi.org/10.1016/J.BBRC.2005.04.114.

[66] K.A. Walker, N. Basisty, D.M. Wilson, L. Ferrucci, Connecting aging biology and inflammation in the omics era, J Clin Invest. 132 (2022). https://doi.org/10.1172/JCI158448.
[67] S.M. Davidson, A. Adameová, L. Barile, H.A. Cabrera-fuentes, A. Lazou, Mitochondrial and mitochondrial-independent pathways of myocardial cell death during ischaemia and reperfusion injury, (2020) 1–12. https://doi.org/10.1111/jcmm.15127.
[68] M. Vila-Petroff, M.A. Salas, M. Said, C.A. Valverde, L. Sapia, E. Portiansky, R.J. Hajjar, E.G. Kranias, C. Mundiña-Weilenmann, A. Mattiazzi, CaMKII inhibition protects against necrosis and apoptosis in irreversible ischemia-reperfusion injury, Cardiovasc Res. 73 (2007) 689–698. https://doi.org/10.1016/J.CARDIORES.2006.12.003.
[69] A. Szobi, T. Rajtik, S. Carnicka, T. Ravingerova, A. Adameova, Mitigation of postischemic cardiac contractile dysfunction by CaMKII inhibition: Effects on programmed necrotic and apoptotic cell death, Mol Cell Biochem. 388 (2014) 269–276. https://doi.org/10.1007/s11010-013-1918-x.
[70] S.Y. Lim, S.M. Davidson, M.M. Mocanu, D.M. Yellon, C.C.T. Smith, The cardioprotective effect of necrostatin requires the cyclophilin-D component of the mitochondrial permeability transition pore, Cardiovascular Drugs and Therapy / Sponsored by the International Society of Cardiovascular Pharmacotherapy. 21 (2007) 467. https://doi.org/10.1007/S10557-007-6067-6.
[71] H. Zhu, Y. Tan, W. Du, Y. Li, S. Toan, D. Mui, F. Tian, H. Zhou, Phosphoglycerate mutase 5 exacerbates cardiac ischemia-reperfusion injury through disrupting mitochondrial quality control, Redox Biol. 38 (2021) 101777. https://doi.org/10.1016/J.REDOX.2020.101777.
[72] M. Tong, D. Zablocki, J. Sadoshima, The role of Drp1 in mitophagy and cell death in the heart, J Mol Cell Cardiol. 142 (2020) 138–145. https://doi.org/10.1016/J.YJMCC.2020.04.015.
[73] L. She, H. Tu, Y.-Z. Zhang, L. Tang, N. Li, Q.-L. Ma, B. Liu, Q. Li, X.-J. Luo, J. Peng, Inhibition of Phosphoglycerate Mutase 5 Reduces Necroptosis in Rat Hearts Following Ischemia/Reperfusion Through Suppression of Dynamin-Related Protein 1, Cardiovasc Drugs Ther. 33 (2019) 13–23. https://doi.org/10.1007/s10557-018-06848-8.
[74] H. Hou, Y. Wang, Q. Li, Z. Li, Y. Teng, J. Li, X. Wang, J. Chen, N. Huang, The role of RIP3 in cardiomyocyte necrosis induced by mitochondrial damage of myocardial ischemia–reperfusion, Acta Biochim Biophys Sin (Shanghai). 50 (2018) 1131–1140. https://doi.org/10.1093/abbs/gmy108.
[75] W.W. Sharp, Y.H. Fang, M. Han, H.J. Zhang, Z. Hong, A. Banathy, E. Morrow, J.J. Ryan, S.L. Archer, Dynamin-related protein 1 (Drp1)-mediated diastolic dysfunction in myocardial ischemia-reperfusion injury: Therapeutic benefits of Drp1 inhibition to reduce mitochondrial fission, FASEB Journal. 28 (2014) 316–326. https://doi.org/10.1096/FJ.12-226225.
[76] M. Luedde, M. Lutz, N. Carter, J. Sosna, C. Jacoby, M. Vucur, J. Gautheron, C. Roderburg, N. Borg, F. Reisinger, H.J. Hippe, A. Linkermann, M.J. Wolf, S. Rose-John, R. Lüllmann-Rauch, D. Adam, U. Flögel, M. Heikenwalder, T. Luedde, N. Frey, RIP3, a kinase promoting necroptotic cell death, mediates adverse remodelling aftermyocardial infarction, Cardiovasc Res. 103 (2014) 206–216. https://doi.org/10.1093/cvr/cvu146.
[77] X. Song, T. Li, Ripk3 mediates cardiomyocyte necrosis through targeting mitochondria and the JNK-Bnip3 pathway under hypoxia-reoxygenation injury, Journal of Receptors

and Signal Transduction. 39 (2019) 331–340. https://doi.org/10.1080/10799893.2019.1676259.
[78] P. Zhu, S. Hu, Q. Jin, D. Li, F. Tian, S. Toan, Y. Li, H. Zhou, Y. Chen, Ripk3 promotes ER stress-induced necroptosis in cardiac IR injury: A mechanism involving calcium overload/XO/ROS/mPTP pathway, Redox Biol. 16 (2018) 157–168. https://doi.org/10.1016/j.redox.2018.02.019.
[79] W. Sun, J. Bao, W. Lin, H. Gao, W. Zhao, Q. Zhang, C.H. Leung, D.L. Ma, J. Lu, X. Chen, 2-Methoxy-6-acetyl-7-methyljuglone (MAM), a natural naphthoquinone, induces NO-dependent apoptosis and necroptosis by H2O2-dependent JNK activation in cancer cells, Free Radic Biol Med. 92 (2016) 61–77. https://doi.org/10.1016/J.FREERADBIOMED.2016.01.014.
[80] M. Imamura, J.S. Moon, K.P. Chung, K. Nakahira, T. Muthukumar, R. Shingarev, S.W. Ryter, A.M. Choi, M.E. Choi, RIPK3 promotes kidney fibrosis via AKT-dependent ATP citrate lyase, JCI Insight. 3 (2018). https://doi.org/10.1172/JCI.INSIGHT.94979.
[81] T. Zhang, Y. Zhang, M. Cui, L. Jin, Y. Wang, F. Lv, Y. Liu, W. Zheng, H. Shang, J. Zhang, M. Zhang, H. Wu, J. Guo, X. Zhang, X. Hu, C.M. Cao, R.P. Xiao, CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress-induced myocardial necroptosis, Nat Med. 22 (2016) 175–182. https://doi.org/10.1038/NM.4017.
[82] Y. Qu, J. Tang, H. Wang, S. Li, F. Zhao, L. Zhang, Q.R. Lu, D. Mu, RIPK3 interactions with MLKL and CaMKII mediate oligodendrocytes death in the developing brain, Cell Death Dis. 8 (2017). https://doi.org/10.1038/cddis.2017.54.
[83] L. Zhan, Z. Lu, X. Zhu, W. Xu, L. Li, X. Li, S. Chen, W. Sun, E. Xu, Hypoxic preconditioning attenuates necroptotic neuronal death induced by global cerebral ischemia via Drp1-dependent signaling pathway mediated by CaMKIIα inactivation in adult rats, FASEB J. 33 (2019) 1313–1329. https://doi.org/10.1096/FJ.201800111RR.
[84] S.P. Cregan, V.L. Dawson, R.S. Slack, Role of AIF in caspase-dependent and caspase-independent cell death, Oncogene. 23 (2004) 2785–2796. https://doi.org/10.1038/sj.onc.1207517.
[85] W. Hu, X. Wu, D. Yu, L. Zhao, X. Zhu, X. Li, T. Huang, Z. Chu, Y. Xu, Regulation of JNK signaling pathway and RIPK3/AIF in necroptosis-mediated global cerebral ischemia/reperfusion injury in rats, Exp Neurol. 331 (2020). https://doi.org/10.1016/j.expneurol.2020.113374.
[86] Y. Xu, J. Wang, X. Song, L. Qu, R. Wei, F. He, K. Wang, B. Luo, RIP3 induces ischemic neuronal DNA degradation and programmed necrosis in rat via AIF, Sci Rep. 6 (2016). https://doi.org/10.1038/srep29362.
[87] Y. Xu, X. Wu, W. Hu, D. Yu, Z. Shao, W. Li, T. Huang, J. Zhang, X. Zhu, X. Li, H. Yang, Z. Chu, K. Lv, RIP3 facilitates necroptosis through CaMKII and AIF after intracerebral hemorrhage in mice, Neurosci Lett. 749 (2021). https://doi.org/10.1016/j.neulet.2021.135699.
[88] S. Huang, W. Hu, D. Rao, X. Wu, Q. Bai, J. Wang, Z. Chu, Y. Xu, RIPK3-Dependent Necroptosis Activates MCP-1-Mediated Inflammation in Mice after Intracerebral Hemorrhage: RIPK3 activates MCP-1, Journal of Stroke and Cerebrovascular Diseases. 31 (2022). https://doi.org/10.1016/j.jstrokecerebrovasdis.2021.106213.
[89] B.J. Rollins, Monocyte chemoattractant protein 1: a potential regulator of monocyte recruitment in inflammatory disease, Mol Med Today. 2 (1996) 198–204. https://doi.org/10.1016/1357-4310(96)88772-7.

[90] R. Yang, K. Hu, J. Chen, S. Zhu, L. Li, H. Lu, P. Li, R. Dong, Necrostatin-1 protects hippocampal neurons against ischemia/reperfusion injury via the RIP3/DAXX signaling pathway in rats, Neurosci Lett. 651 (2017) 207–215. https://doi.org/10.1016/j.neulet.2017.05.016.
[91] Y. Wang, Y. Bi, Z. Xia, W. Shi, B. Li, B. Li, L. Chen, L. Guo, Butylphthalide ameliorates experimental autoimmune encephalomyelitis by suppressing PGAM5-induced necroptosis and inflammation in microglia, Biochem Biophys Res Commun. 497 (2018) 80–86. https://doi.org/10.1016/j.bbrc.2018.02.024.
[92] Z.M. Liu, Q.X. Chen, Z.B. Chen, D.F. Tian, M.C. Li, J.M. Wang, L. Wang, B.H. Liu, S.Q. Zhang, F. Li, H. Ye, L. Zhou, RIP3 deficiency protects against traumatic brain injury (TBI) through suppressing oxidative stress, inflammation and apoptosis: Dependent on AMPK pathway, Biochem Biophys Res Commun. 499 (2018) 112–119. https://doi.org/10.1016/j.bbrc.2018.02.150.
[93] X. Teng, W. Chen, Z. Liu, T. Feng, H. Li, S. Ding, Y. Chen, Y. Zhang, X. Tang, D. Geng, NLRP3 inflammasome is involved in Q-VD-OPH induced necroptosis following cerebral ischemia-reperfusion injury, Neurochem Res. 43 (2018) 1200–1209. https://doi.org/10.1007/s11064-018-2537-4.
[94] H. Fan, H.B. Tang, J. Kang, L. Shan, H. Song, K. Zhu, J. Wang, G. Ju, Y.Z. Wang, Involvement of endoplasmic reticulum stress in the necroptosis of microglia/macrophages after spinal cord injury, Neuroscience. 311 (2015) 362–373. https://doi.org/10.1016/j.neuroscience.2015.10.049.
[95] X. Liu, Y. Zhang, H. Gao, Y. Hou, J. jian Lu, Y. Feng, Q. Xu, B. Liu, X. Chen, Induction of an MLKL mediated non-canonical necroptosis through reactive oxygen species by tanshinol A in lung cancer cells, Biochem Pharmacol. 171 (2020). https://doi.org/10.1016/j.bcp.2019.113684.
[96] Y. Jiang, S. Shan, L. Chi, G. Zhang, X. Gao, H. Li, X. Zhu, J. Yang, Methyl methanesulfonate induces necroptosis in human lung adenoma A549 cells through the PIG-3-reactive oxygen species pathway, Tumor Biology. 37 (2016) 3785–3795. https://doi.org/10.1007/s13277-015-3531-y.
[97] Y.M. Ma, Y.M. Peng, Q.H. Zhu, A.H. Gao, B. Chao, Q.J. He, J. Li, Y.H. Hu, Y.B. Zhou, Novel CHOP activator LGH00168 induces necroptosis in A549 human lung cancer cells via ROS-mediated ER stress and NF-κB inhibition, Acta Pharmacol Sin. 37 (2016) 1381–1390. https://doi.org/10.1038/aps.2016.61.
[98] B. Lin, Z. Jin, X. Chen, L. Zhao, C. Weng, B. Chen, Y. Tang, L. Lin, Necrostatin-1 protects mice from acute lung injury by suppressing necroptosis and reactive oxygen species, Mol Med Rep. 21 (2020) 2171–2181. https://doi.org/10.3892/mmr.2020.11010.
[99] J. Zhang, P.A. Ney, Role of BNIP3 and NIX in cell death, autophagy, and mitophagy, Cell Death & Differentiation 2009 16:7. 16 (2009) 939–946. https://doi.org/10.1038/cdd.2009.16.
[100] J.Y. Kim, Y.J. Kim, S. Lee, J.H. Park, BNip3 is a mediator of TNF-induced necrotic cell death, Apoptosis. 16 (2011) 114–126. https://doi.org/10.1007/s10495-010-0550-4.
[101] W. Huang, W. Xie, J. Gong, W. Wang, S. Cai, Q. Huang, Z. Chen, Y. Liu, Heat stress induces RIP1/RIP3-dependent necroptosis through the MAPK, NF-κB, and c-Jun signaling pathways in pulmonary vascular endothelial cells, Biochem Biophys Res Commun. 528 (2020) 206–212. https://doi.org/10.1016/j.bbrc.2020.04.150.
[102] I. Gan, J. Jiang, D. Lian, X. Huang, B. Fuhrmann, W. Liu, A. Haig, A.M. Jevnikar, Z.X. Zhang, Mitochondrial permeability regulates cardiac endothelial cell necroptosis and

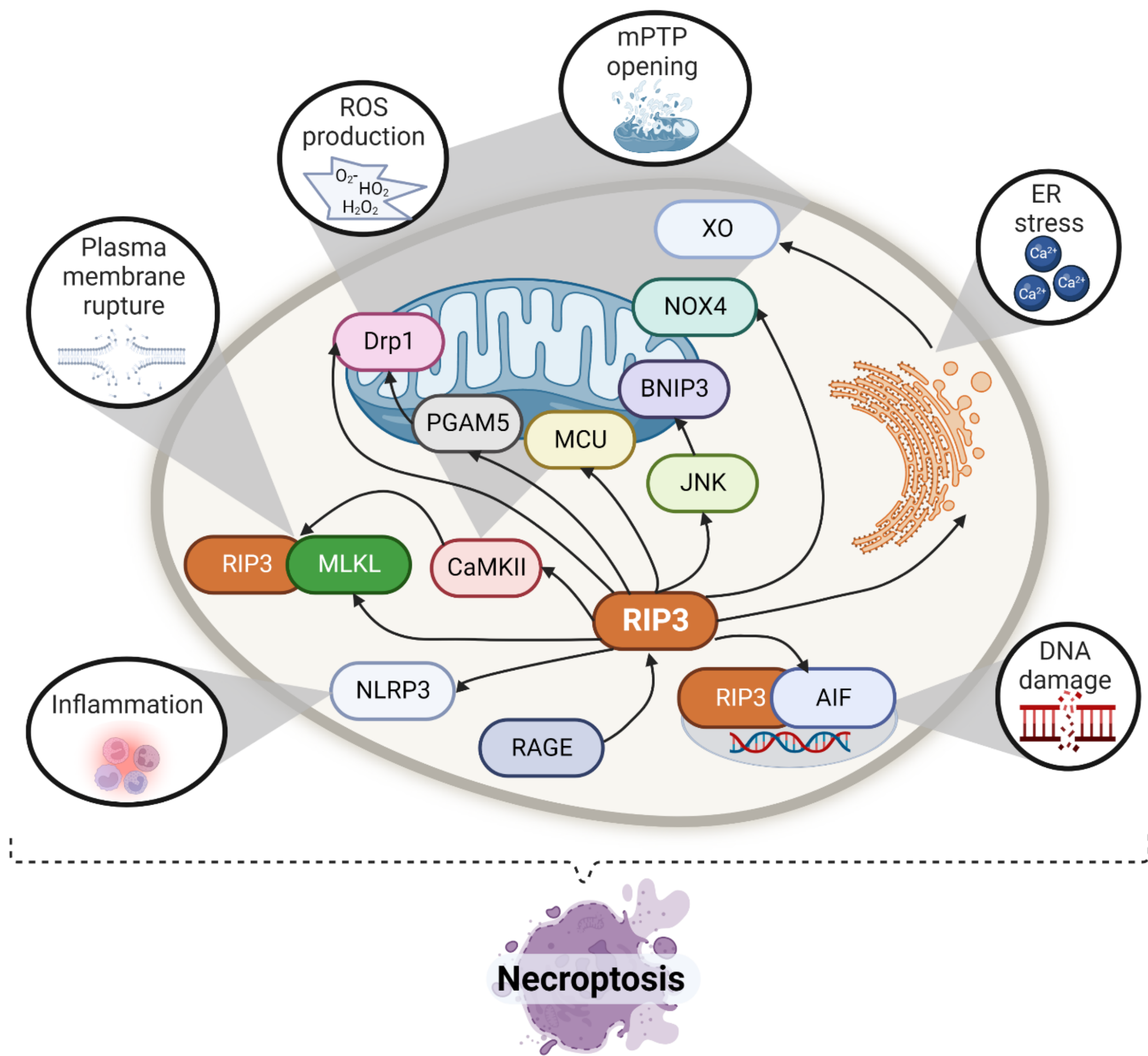
cardiac allograft rejection, Am J Transplant. 19 (2019) 686–698. https://doi.org/10.1111/AJT.15112.
[103] A. Qamar, J. Zhao, L. Xu, P. McLeod, X. Huang, J. Jiang, W. Liu, A. Haig, Z.X. Zhang, Cyclophilin d regulates the nuclear translocation of aif, cardiac endothelial cell necroptosis and murine cardiac transplant injury, Int J Mol Sci. 22 (2021). https://doi.org/10.3390/ijms222011038.
[104] H. Zhou, D. Li, P. Zhu, Q. Ma, S. Toan, J. Wang, S. Hu, Y. Chen, Y. Zhang, Inhibitory effect of melatonin on necroptosis via repressing the Ripk3-PGAM5-CypD-mPTP pathway attenuates cardiac microvascular ischemia–reperfusion injury, J Pineal Res. 65 (2018). https://doi.org/10.1111/jpi.12503.
[105] C. Li, Q. Ma, S. Toan, J. Wang, H. Zhou, J. Liang, SERCA overexpression reduces reperfusion-mediated cardiac microvascular damage through inhibition of the calcium/MCU/mPTP/necroptosis signaling pathways, Redox Biol. 36 (2020). https://doi.org/10.1016/j.redox.2020.101659.
[106] P. Reventun, S. Sanchez-Esteban, A. Cook, I. Cuadrado, C. Roza, R. Moreno-Gomez-Toledano, C. Muñoz, C. Zaragoza, R.J. Bosch, M. Saura, Bisphenol A induces coronary endothelial cell necroptosis by activating RIP3/CamKII dependent pathway, Sci Rep. 10 (2020). https://doi.org/10.1038/s41598-020-61014-1.
[107] T. Zhou, E. Deroo, H. Yang, A. Stranz, Q. Wang, R. Ginnan, H.A. Singer, B. Liu, Mlkl and camkii are involved in ripk3-mediated smooth muscle cell necroptosis, Cells. 10 (2021). https://doi.org/10.3390/cells10092397.
[108] J. Lin, X. Li, Y. Lin, Z. Huang, W. Wu, Exogenous sodium hydrosulfide protects against high glucose-induced injury and inflammation in human umbilical vein endothelial cells by inhibiting necroptosis via the p38 MAPK signaling pathway, Mol Med Rep. 23 (2021). https://doi.org/10.3892/mmr.2020.11706.
[109] W. Zhao, H. Feng, W. Sun, K. Liu, J.J. Lu, X. Chen, Tert-butyl hydroperoxide (t-BHP) induced apoptosis and necroptosis in endothelial cells: Roles of NOX4 and mitochondrion, Redox Biol. 11 (2017) 524–534. https://doi.org/10.1016/j.redox.2016.12.036.
[110] H. Faust, L.M. Lam, M.J. Hotz, D. Qing, N.S. Mangalmurti, RAGE interacts with the necroptotic protein RIPK3 and mediates transfusion-induced danger signal release, Vox Sang. 115 (2020) 729–734. https://doi.org/10.1111/vox.12946.
[111] J.H. Jang, T.-J. Lee, The role of microRNAs in cell death pathways, Yeungnam Univ J Med. 38 (2021) 107–117. https://doi.org/10.12701/yujm.2020.00836.
[112] Y. Cai, Y. Zhou, Z. Li, P. Xia, X. ChenFu, A. Shi, J. Zhang, P. Yu, Non-coding RNAs in necroptosis, pyroptosis, and ferroptosis in cardiovascular diseases, Front Cardiovasc Med. 9 (2022). https://doi.org/10.3389/fcvm.2022.909716.
[113] S. Shin, J.-W. Choi, H. Moon, C.Y. Lee, J.-H. Park, J. Lee, H.-H. Seo, G. Han, S. Lim, S. Lee, S.W. Kim, K.-C. Hwang, Simultaneous Suppression of Multiple Programmed Cell Death Pathways by miRNA-105 in Cardiac Ischemic Injury, Mol Ther Nucleic Acids. 14 (2019) 438–449. https://doi.org/10.1016/j.omtn.2018.12.015.
[114] K. Wang, F. Liu, C.-Y. Liu, T. An, J. Zhang, L.-Y. Zhou, M. Wang, Y.-H. Dong, N. Li, J.-N. Gao, Y.-F. Zhao, P.-F. Li, The long noncoding RNA NRF regulates programmed necrosis and myocardial injury during ischemia and reperfusion by targeting miR-873, Cell Death Differ. 23 (2016) 1394–1405. https://doi.org/10.1038/cdd.2016.28.

[115] K. Wang, F. Liu, L.-Y. Zhou, S.-L. Ding, B. Long, C.-Y. Liu, T. Sun, Y.-Y. Fan, L. Sun, P.-F. Li, miR-874 regulates myocardial necrosis by targeting caspase-8, Cell Death Dis. 4 (2013) e709–e709. https://doi.org/10.1038/cddis.2013.233.
[116] D. Qin, X. Wang, Y. Li, L. Yang, R. Wang, J. Peng, K. Essandoh, X. Mu, T. Peng, Q. Han, K.-J. Yu, G.-C. Fan, MicroRNA-223-5p and -3p Cooperatively Suppress Necroptosis in Ischemic/Reperfused Hearts, Journal of Biological Chemistry. 291 (2016) 20247–20259. https://doi.org/10.1074/jbc.M116.732735.
[117] J.-X. Wang, X.-J. Zhang, Q. Li, K. Wang, Y. Wang, J.-Q. Jiao, C. Feng, S. Teng, L.-Y. Zhou, Y. Gong, Z.-X. Zhou, J. Liu, J.-L. Wang, P. Li, MicroRNA-103/107 Regulate Programmed Necrosis and Myocardial Ischemia/Reperfusion Injury Through Targeting FADD, Circ Res. 117 (2015) 352–363. https://doi.org/10.1161/CIRCRESAHA.117.305781.
[118] K. Wang, B. Long, N. Li, L. Li, C.-Y. Liu, Y.-H. Dong, J.-N. Gao, L.-Y. Zhou, C.-Q. Wang, P.-F. Li, MicroRNA-2861 regulates programmed necrosis in cardiomyocyte by impairing adenine nucleotide translocase 1 expression, Free Radic Biol Med. 91 (2016) 58–67. https://doi.org/10.1016/j.freeradbiomed.2015.11.031.
[119] Z. Liu, D. Zhu, F. Yu, M. Yang, D. Huang, Z. Ji, W. Lu, G. Ma, Exosomal miR-17-3p Alleviates Programmed Necrosis in Cardiac Ischemia/Reperfusion Injury by Regulating TIMP3 Expression, Oxid Med Cell Longev. 2022 (2022) 1–13. https://doi.org/10.1155/2022/2785113.
[120] A.B. Aurora, A.I. Mahmoud, X. Luo, B.A. Johnson, E. van Rooij, S. Matsuzaki, K.M. Humphries, J.A. Hill, R. Bassel-Duby, H.A. Sadek, E.N. Olson, MicroRNA-214 protects the mouse heart from ischemic injury by controlling Ca2+ overload and cell death, Journal of Clinical Investigation. 122 (2012) 1222–1232. https://doi.org/10.1172/JCI59327.
[121] T. Yang, C. Cao, J. Yang, T. Liu, X.G. Lei, Z. Zhang, S. Xu, miR-200a-5p regulates myocardial necroptosis induced by Se deficiency via targeting RNF11, Redox Biol. 15 (2018) 159–169. https://doi.org/10.1016/j.redox.2017.11.025.
[122] M. Visalli, M. Bartolotta, F. Polito, R. Oteri, A. Barbera, R. Arrigo, R. Di Giorgio, G. Navarra, M. Aguennouz, miRNA expression profiling regulates necroptotic cell death in hepatocellular carcinoma, Int J Oncol. (2018). https://doi.org/10.3892/ijo.2018.4410.
[123] R. Harari-Steinfeld, M. Gefen, A. Simerzin, E. Zorde-Khvalevsky, M. Rivkin, E. Ella, T. Friehmann, M. Gerlic, J. Zucman-Rossi, S. Caruso, M. Leveille, J.L. Estall, D.S. Goldenberg, H. Giladi, E. Galun, Z. Bromberg, The lncRNA H19-Derived MicroRNA-675 Promotes Liver Necroptosis by Targeting FADD, Cancers (Basel). 13 (2021) 411. https://doi.org/10.3390/cancers13030411.
[124] C. Gu, C. Hou, S. Zhang, miR-425-5p improves inflammation and septic liver damage through negatively regulating the RIP1-mediated necroptosis, Inflammation Research. 69 (2020) 299–308. https://doi.org/10.1007/s00011-020-01321-5.
[125] M.B. Afonso, P.M. Rodrigues, A.L. Simão, M.M. Gaspar, T. Carvalho, P. Borralho, J.M. Bañales, R.E. Castro, C.M.P. Rodrigues, miRNA-21 ablation protects against liver injury and necroptosis in cholestasis, Cell Death Differ. 25 (2018) 857–872. https://doi.org/10.1038/s41418-017-0019-x.
[126] Z. Zhirong, Z. Qiaojian, X. Chunjing, W. Shengchen, L. Jiahe, L. Zhaoyi, L. Shu, Methionine selenium antagonizes LPS-induced necroptosis in the chicken liver via the miR-155/TRAF3/MAPK axis, J Cell Physiol. 236 (2021) 4024–4035. https://doi.org/10.1002/jcp.30145.

[127] L. Wang, X. Shi, S. Zheng, S. Xu, Selenium deficiency exacerbates LPS-induced necroptosis by regulating miR-16-5p targeting PI3K in chicken tracheal tissue, Metallomics. 12 (2020) 562–571. https://doi.org/10.1039/c9mt00302a.
[128] B. Wang, Y. Cui, Q. Zhang, S. Wang, S. Xu, Selenomethionine alleviates LPS-induced JNK/NLRP3 inflammasome-dependent necroptosis by modulating miR-15a and oxidative stress in chicken lungs, Metallomics. 13 (2021). https://doi.org/10.1093/mtomcs/mfab048.
[129] J.M. Carnino, H. Lee, X. He, M. Groot, Y. Jin, Extracellular vesicle-cargo miR-185-5p reflects type II alveolar cell death after oxidative stress, Cell Death Discov. 6 (2020) 82. https://doi.org/10.1038/s41420-020-00317-8.
[130] C. Zhao, Y. Zhou, Q. Ran, Y. Yao, H. Zhang, J. Ju, T. Yang, W. Zhang, X. Yu, S. He, MicroRNA-381-3p Functions as a Dual Suppressor of Apoptosis and Necroptosis and Promotes Proliferation of Renal Cancer Cells, Front Cell Dev Biol. 28 (2020) 290. https://doi.org/10.3389/fcell.2020.00290.
[131] L. Jiang, X.-Q. Liu, Q. Ma, Q. Yang, L. Gao, H.-D. Li, J.-N. Wang, B. Wei, J. Wen, J. Li, Y.-G. Wu, X.-M. Meng, hsa-miR-500a-3P alleviates kidney injury by targeting MLKL-mediated necroptosis in renal epithelial cells, The FASEB Journal. 33 (2019) 3523–3535. https://doi.org/10.1096/fj.201801711R.
[132] B. Shen, M. Mei, Y. Pu, H. Zhang, H. Liu, M. Tang, Q. Pan, Y. He, X. Wu, H. Zhao, Necrostatin-1 Attenuates Renal Ischemia and Reperfusion Injury via Meditation of HIF-1α/mir-26a/TRPC6/PARP1 Signaling, Mol Ther Nucleic Acids. 17 (2019) 701–713. https://doi.org/10.1016/j.omtn.2019.06.025.
[133] Y.-B. Hu, Y.-F. Zhang, H. Wang, R.-J. Ren, H.-L. Cui, W.-Y. Huang, Q. Cheng, H.-Z. Chen, G. Wang, miR-425 deficiency promotes necroptosis and dopaminergic neurodegeneration in Parkinson’s disease, Cell Death Dis. 10 (2019) 589. https://doi.org/10.1038/s41419-019-1809-5.
[134] J. Cui, H. Liu, S. Xu, Selenium-deficient diet induces necroptosis in the pig brain by activating TNFR1 via mir-29a-3p, Metallomics. 12 (2020) 1290–1301. https://doi.org/10.1039/d0mt00032a.
[135] L. Jiao, Z. He, S. Wang, C. Sun, S. Xu, miR-130-CYLD Axis Is Involved in the Necroptosis and Inflammation Induced by Selenium Deficiency in Pig Cerebellum, Biol Trace Elem Res. 199 (2021) 4604–4613. https://doi.org/10.1007/s12011-021-02612-6.
[136] X. Li, Y. Wang, Y. Wang, X. He, MiR-141-3p ameliorates RIPK1-mediated necroptosis of intestinal epithelial cells in necrotizing enterocolitis, Aging. 12 (2020) 18073–18083. https://doi.org/10.18632/aging.103608.

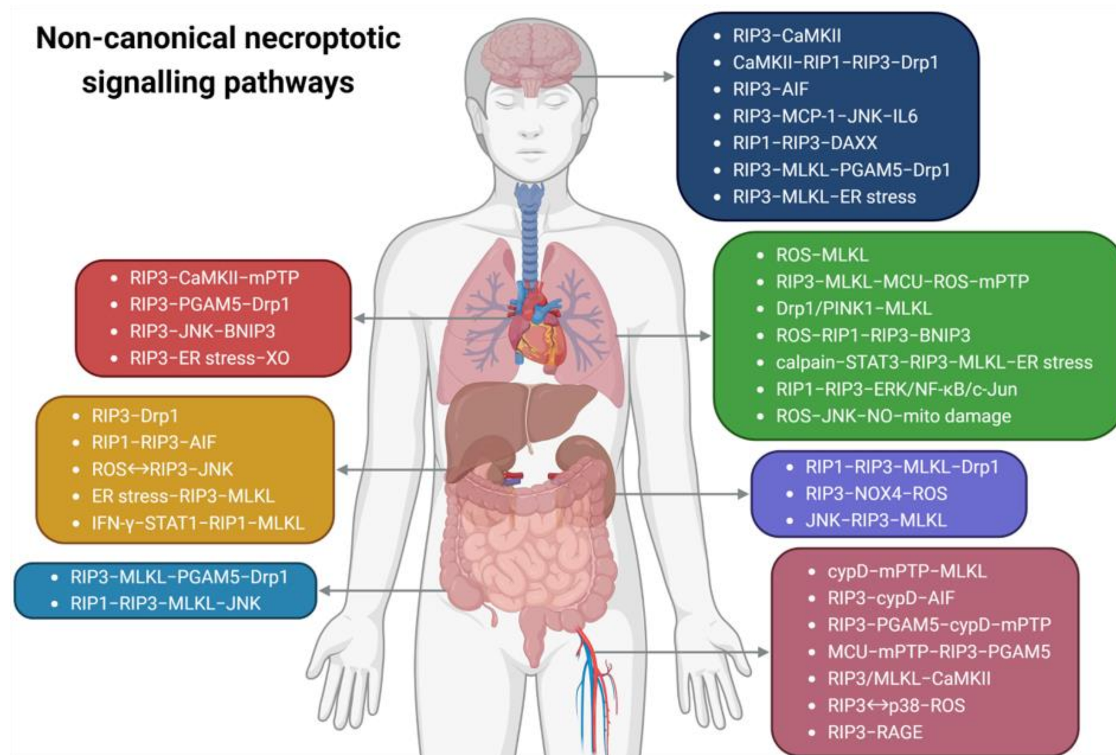
Figure legend

Figure 1: A schematic illustration of the canonical RIP1-RIP3-MLKL pathway of necroptosis and some of additional, non-conventional axes found to promote necroptosis via inducing inflammation, oxidative stress, stress of the endoplasmic reticulum, mPTP opening and DNA damage apart from the rupture of the plasma membrane.



Abbreviations: AIF – apoptosis-inducing factor; BNIP3 – BCL2 Interacting Protein 3; CaMKII – Ca2+/calmodulin-dependent protein kinase II; Drp1 – dynamin-related protein 1; ER – endoplasmic reticulum; JNK – c-Jun N-terminal kinase; MCU – mitochondrial calcium uniporter; MLKL – mixed lineage kinase domain-like pseudokinase; mPTP – mitochondrial permeability transition pore; NOX4 – NADPH oxidase 4; PGAM5 – mitochondrial serine/threonine protein phosphatase; XO – xanthin oxidase; RAGE – receptor for advanced glycation end products; RIP1 – receptor-interacting protein kinase 1; RIP3 – receptor-interacting protein kinase 3; ROS – reactive oxygen species;

Figure 2: Summary of the studied additional, non-conventional pathways of necroptosis in various tissues including the central nervous system, heart, intestines, kidneys, liver, lungs and vessels.



Abbreviations: AIF – apoptosis-inducing factor; BNIP3 – BCL2 Interacting Protein 3; CaMKII – Ca2+/calmodulin-dependent protein kinase II; cypD – cyclophilin D; DAXX – death-associated protein; Drp1 – dynamin-related protein 1; ER – endoplasmic reticulum; ERK – extracellular signal-regulated kinase; IFN-γ – interferon-gamma; IL-6 – interleukin 6; JNK – c-Jun N-terminal kinase; MCP-1 – monocyte chemoattractant protein-1; MCU – mitochondrial calcium uniporter; MLKL – mixed lineage kinase domain-like pseudokinase; mPTP – mitochondrial permeability transition pore; NF-κB – nuclear factor-kappa B; NO – nitric oxide; NOX4 – NADPH oxidase 4; PGAM5 – mitochondrial serine/threonine protein phosphatase; XO – xanthin oxidase; PINK1 – PTEN-induced kinase 1; RAGE – receptor for advanced glycation end products; RIP1 – receptor-interacting protein kinase 1; RIP3 – receptor-interacting protein kinase 3; ROS – reactive oxygen species; SERCA2a – sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 2a; STAT1 – signal transducer and activator of transcription 1; STAT3 – signal transducer and activator of transcription 3. Symbols: “—” – signalling order; ”↔” – possibility of mutual activation.

Table 1. A list of some of alternative pathways of necroptosis in various tissues and main findings supporting their relevance apart from the canonical pathway.

Tissue	Model	Findings	Ref.
Heart	H/R injury	- increased expression of JNK, BNIP3 - gene knockout of RIP3 decreased JNK and BNIP3 and improved cell viability	[77]
	I/R injury	- increased expression of CaMKII, PGAM5, pSer616-Drp1 and XO; decreased expression of pSer637-Drp1 - inhibition of CaMKII, gene knockout of PGAM5 reduced mPTP opening, mitigated cardiac I/R injury and inflammation	[61,69,73,74,78]
Intestines	LPS-induced injury	- increased expression of PGAM5 and Drp1 - necroptosis inhibition decreased PGAM5, Drp1, and improved digestive function	[23]
	Escherichia coli-induced damage	- increased expression of PGAM5 and Drp1 - PUFAs reduce necroptotic and pyroptotic cell death and inflammation	[24]
	Inflammatory bowel disease	- increased expression of JNK - necroptosis and JNK inhibition alleviated cell death, ROS accumulation and intestinal inflammation	[25,26]
Kidneys	Chronic kidney disease due to partial nephrectomy	- increased expression of Drp1 - necroptosis inhibition improved renal function and renal pathologic changes	[27]
	TNF-induced damage	- increased expression of Drp1 - necroptosis and Drp1 inhibition reduced mitochondrial fission and ROS production and improved cell viability	[28]
	Sepsis-induced acute kidney injury	- RIP3 mediates mitochondrial damage via NOX4 and causes increased ROS production	[29]
	I/R injury	- increased expression of p-JNK and decreased expression of Mitofilin - RIP3 translocates to the mitochondria and degrades Mitofilin, leading to mitochondrial dysfunction and ROS elevation - gene knockout of RIP3 and inhibition of JNK mitigate renal I/R injury and inflammation	[30,31]
Liver	APAP-induced damage	- RIP3 inhibition or deletion reduced mitochondrial damage, Drp1 translocation and ROS production, Drp1 inhibition improved cell viability - RIP1 inhibition decreased JNK activation and AIF nuclear translocation	[16,17]
	Ethanol-induced hepatotoxicity	- depletion of RIP3 reduced JNK activation and oxidative stress	[18]
	Tunicamycin/d-galactosamine-induced damage	- ER stress inhibition prevented hepatocytes necroptosis	[19]
	Inflammation-dependent hepatitis	- necroptosis was mediated by RIP1 and MLKL independently of RIP3 - IFN-γ–STAT1 pathway activation induced MLKL upregulation	[20]
	Non-alcoholic steatohepatitis	- necroptosis was mediated by MLKL independently of RIP3	[21]

	Hepatocellular carcinoma cells	- treatment with anti-CD147 monoclonal antibody-induced RIP3-independent necroptosis mediated by MLKL	[22]
Central nervous system	I/R injury	- knockdown of RIP3 decreased CaMKII phosphorylation and prevented mitochondrial membrane potential dissipation - CaMKII inhibition reduced RIP1–RIP3 complex formation and Drp1 activation - necroptosis mediated by nuclear translocation of RIP3–AIF complex was suppressed by inhibition of RIP1, RIP3 and JNK - RIP3 inhibition decreased p-JNK and IL-6 expression - RIP1 inhibition prevented RIP3–DAXX-induced necroptosis - necroptosis inductor, pan-caspase inhibitor, activated NLRP3 inflammasome, NLRP3 inhibitor reduced infarct volume	[82,83,85,86,90,93]
	Intracerebral haemorrhage	- necroptosis mediated by both RIP3–AIF and RIP3–CaMKII complexes was supressed by RIP3 knockdown or the combination of CaMKII and mPTP inhibitor - RIP3 inhibition decreased the expression of p-JNK, IL-6 and MCP-1 and prevented RIP3 and MCP-1 interactions	[87,88]
	Multiple sclerosis	- increased expression of PGAM5 and Drp1 - PGAM5 silencing suppressed Drp1 activation, necroptosis, inflammation and ROS production	[91]
	Traumatic brain injury	- RIP3 deficiency suppressed necroptosis along with apoptosis, NLRP3 inflammatory pathway and oxidative stress	[92]
	Spinal cord injury	- both RIP3 and MLKL translocated into the ER, ER stress inhibitor suppressed necroptosis, p-MLKL and ER stress marker were upregulated also in human tissue samples	[94]
Lungs	Tashinol A-induced damage	- inhibition of ROS production prevented MLKL activation and necroptosis	[95]
	TNF- and LPS-induced damage	- necroptosis inhibition suppressed ROS production and increased the expression of antioxidant enzymes HO-1, NRF2 and SOD2	[11,98]
	Streptococcus pneumoniae infection	- RIP3 formed a complex with MLKL and MCU, induced AKT-dependent activation of the NLRP3 inflammasome, aggravated mPTP opening and necroptotic death	[15]
	COPD	- Drp1 inhibition and PINK1 knockdown reduced the phosphorylation of MLKL and necroptosis	[12]
	TNF-induced damage	- necroptosis inhibition and administration of N-acetylcysteine prevented ROS-dependent insertion of BNIP3 into the mitochondria	[100]
	I/R injury	- formation of the necrosome was p-STAT3 dependent and evoked ER stress, Ca2+ overload, ROS production and cell death	[13]
	Heat stress-induced damage	- inhibition of ERK or NF-κB or c-Jun suppressed necroptosis and decreased the release of HMGB1 - RIP1/RIP3 silencing negatively regulated the activation of c-Jun and ERK - necroptosis inhibition decreased NF-κB p65 nuclear translocation	[101]
	2-methoxy-6-acetyl-7-methyljuglone treatment	- silencing and pharmacologic inhibition of JNK suppressed ROS generation, nitrosative stress and necroptosis and preserved the activity of pro-survival genes	[14]

Vessels	TNF-induced damage	- increased cypD activity - inhibition of mPTP opening suppressed necroptotic death and the release of HMGB1	[102]
	H/R; cardiac transplantation	- increased cypD activity and AIF translocation to the nucleus - cypD inhibition and deletion inhibited necroptosis and ameliorated endothelial dysfunction, graft rejection and reduced mortality	[103]
	Cardiac microvascular I/R injury	- RIP3-induced PGAM5 co-localization with cypD, Ca2+ overload and MCU upregulation led to aggravated mPTP opening and endothelial necroptosis	[100, 101]
	Bisphenol A-induced damage	- increased phosphorylation of CaMKII - RIP3 silencing prevented p-CaMKII upregulation and necroptotic cell loss	[106]
	Abdominal aortic aneurysm	- increased expression and phosphorylation of CaMKII - RIP3 deficiency and MLKL knockdown diminished CaMKII phosphorylation and mitigated endothelial damage	[107]
	High glucose-induced damage	- increased phosphorylation of p38 - Inhibition of p38 suppressed RIP3 upregulation, attenuated ROS production and reversed high glucose-induced cytotoxicity	[108]
	Tert-butyl hydroperoxide-induced damage	- inhibition of p38 and mitochondrial ROS production, prevented necroptotic cell loss - silencing of RIP3 and MLKL suppressed p38 activation, generation of mitochondrial ROS and loss of mitochondrial membrane potential	[109]
	Red blood cell transfusion	- RAGE knockout attenuated necroptotic death and decreased the plasma levels of RIP3 and HMGB1	[110]

Abbreviations: AIF – apoptosis-inducing factor; APAP – acetaminophen; BNIP3 – BCL2 Interacting Protein 3; CaMKII – Ca2+/calmodulin-dependent protein kinase II; COPD – chronic obstructive pulmonary disease; cypD – cyclophilin D; DAXX – death-associated protein; Drp1 – dynamin-related protein 1; ER – endoplasmic reticulum; ERK – extracellular signal-regulated kinase; HMGB1 – high mobility group box 1 protein; HO-1 – heme oxygenase-1; H/R - hypoxia/reoxygenation; IFN-γ – interferon-gamma; IL-6 – interleukin 6; I/R - ischemia/reperfusion; JNK – c-Jun N-terminal kinase; LPS – lipopolysaccharide; MCP-1 – monocyte chemoattractant protein-1; MCU – mitochondrial calcium uniporter; MLKL – mixed lineage kinase domain-like pseudokinase; mPTP – mitochondrial permeability transition pore; NF-κB – nuclear factor-kappa B; NLRP3 – NACHT, LRR, and PYD domains containing protein 3; NOX4 – NADPH oxidase 4; NRF2 – nuclear factor erythroid 2-related factor 2; PGAM5 – mitochondrial serine/threonine protein phosphatase; XO – xanthin oxidase; PINK1 – PTEN-induced kinase 1; PUFA – polyunsaturated fatty acid; RAGE – receptor for advanced glycation end products; RIP1 – receptor-interacting protein kinase 1; RIP3 – receptor-interacting protein kinase 3; ROS – reactive oxygen species; SOD2 – superoxide dismutase 2; STAT1 – signal transducer and activator of transcription 1; STAT3 – signal transducer and activator of transcription 3; TNF – tumour necrosis factor.