Seminars in Cell & Developmental Biology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Necroptosis: Pathway diversity and characteristics

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
Necroptosis
RIP1
RIP3
MLKL
TNF

ABSTRACT

Regulated cell death is a physiological process that controls organismal homeostasis. Deregulation of cell death can lead to the development of a number of human diseases and tissue damage. Apoptosis is a best-known model of caspase-dependent regulated cell death, but recently necroptosis has garnered a lot of attention as a form of regulated cell death not mediated by caspases. Different stimuli can trigger necroptosis, and all of them converge at the activation of the protein kinase RIP3 (receptor-interacting protein 3) and the pseudokinase MLKL (mixed lineage kinase domain-like). Necroptosis activation relies on the unique protein-interaction motif RHIM (RIP homology interaction motif). Different RHIM-containing proteins (RIP1, DAI and TRIF) transduce necroptotic signals from the cell death trigger to the cell death mediators RIP3-MLKL. RIP1 has a particularly important and complex role in necroptotic cell death regulation ranging from cell death activation to inhibition, often in a cell type and context dependent fashion.

Contents

Tissue homeostasis depends on the balance between cell death and cell survival that allows proliferation. There are several types of cell death (apoptosis, necroptosis, pyroptosis – inflammatory caspase-1 dependent cell death in response to pathogens, ferroptosis – iron-dependent form of nonapoptotic cell death that produces detrimental concentrations of ROS, etc.), reflecting the specificity and indispensable tight regulation of this process [1]. Apoptosis and necrosis are the best-studied cell death pathways and recent findings suggest they are further comprised of different subtypes of cell death that can be clearly defined. Indeed, necrotic cell death was associated with accidental damage or injury that leads to premature cell death. Nevertheless, necrosis encompasses distinct subclasses of cell death pathways, among them necroptosis, which is not a default death pathway but a highly regulated process. Necroptosis could be also considered a back-up mechanism for cells that are destined to die when apoptotic pathways are compromised.

Necrotic cell death has been observed in numerous pathologies such as ischemia/reperfusion injury (gangrene, heart attack, stroke, kidney failure), neurodegenerative pathologies, and retina detachment among others, thus making this form of cell death therapeutically attractive [2–4]. Regulation of necrosis in these diseases could minimize the resulting damage. In innate immunity cell death is critical for the control of pathogen infection by the host. Infected cells undergo apoptosis as the main mechanism of cell death [5,6]. However, if the apoptotic pathway is blocked, cells will engage necroptosis [7]. Thus, cell death could be beneficial in this situation. One of the complications of necrotic cell death is the associated inflammation caused by the early breakage of the plasma membrane and release of intracellular content. The intracellular space contains damage associated molecular patterns (DAMPs) and alarmins, which are recognized by receptors of the innate immune system [8]. This inflammation can be beneficial in pathogen defense; however, it can be detrimental in other situations such as ischemic traumas or neonatal development [9].

Necroptotic cell death can be differentiated form apoptotic cell death by different features [8] (Table 1):

- Induction of necroptosis

The best studied form of necroptotic cell death is initiated by TNFα (tumor necrosis factor), but necroptosis can also be induced

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http://dx.doi.org/10.1016/j.semcdb.2015.02.002
1084-9521/

Please cite this article in press as: de Almagro MC, Vucic D. Necroptosis: Pathway diversity and characteristics. Semin Cell Dev Biol (2015), http://dx.doi.org/10.1016/j.semcdb.2015.02.002

Table 1
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Apoptosis	Necroptosis
Cell shrinkage	Rapid cytoplasmic swelling
No swelling of organelles	Swelling of organelles
Plasma membrane blebbing and formation of apoptotic bodies	Early plasma membrane rupture and organelle breakdown
Chromatin condensation, and nuclear and DNA fragmentation	Not prominent
Caspase dependent	Caspase independent
RIP3 and MLKL are dispensable	RIP3 and MLKL are essential
Classically no inflammation. Phagocytes engulf apoptotic bodies with minimal disturbance of neighboring cells	Inflammation due to the release of intracellular content. Stimulation of the immune system in response to infection
Mitochondrial membrane permeability and release of intermembrane space proteins	Mitochondrial function not clear

by other members of the TNFα death ligand family (Fas and TRAIL/Apo2L), interferons, Toll-like receptors (TLRs) signaling and viral infection via the DNA sensor DAI (DNA-dependent activator of interferon regulatory factor) (Fig. 1) [1].

- Cell death ligands, TNFα

  TNFα induced necroptosis shares initial signaling steps with apoptosis and even with NF-κB signaling. TNFα binds to the TNFR1 (TNF receptor 1) prompting TNFR1 trimerization and formation of an intracellular complex, Complex-I. TRADD (TNF receptor associated death domain protein) binds to the intracellular death domain of TNFR1 and recruits the protein kinase RIP1 (receptor-interacting protein 1) through the death domain present in both proteins [10]. The adaptor protein TRAF2 (tumor necrosis factor receptor-associated factor 2) is also recruited to the TNFR1 complex, and it enlists the ubiquitin E3 ligases c-IAP1 and c-IAP2 (cellular inhibitor of apoptosis protein) [11].

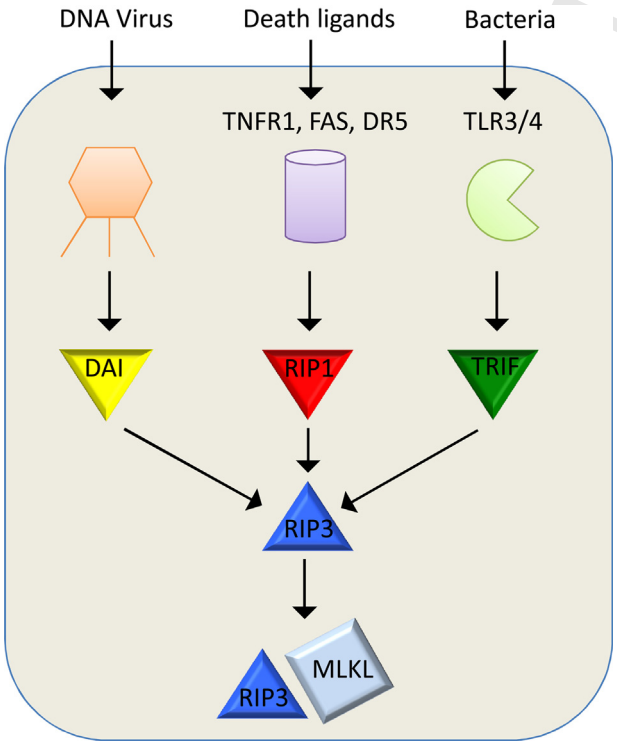

Fig. 1. Mediators of necroptotic pathways. DNA viruses, death ligands or bacterial products can activate necroptotic signaling by engaging adaptors DAI and TRIF and kinases RIP1 and RIP3 resulting in MLKL phosphorylation and cell death.

Proximity within TNFR1 complex permits c-IAP proteins dimerization, leading to the activation of their E3 ubiquitin ligase activity and ubiquitination of RIP1 with K63 and K11 polyubiquitin chains [12–14]. Ubiquitinated RIP1 is a platform for the recruitment of downstream components of the NF-κB and MAPK signaling pathways such as LUBAC (linear ubiquitin chain assembly complex), TAB2/3-TAK1, NEMO and IKK [10,15,16]. Activation of NF-κB and MAPK signaling lead to the translocation of transcription factors to the nucleus and expression of pro-survival and pro-inflammatory genes such as TNFα, cFLIP, and c-IAP2 [17].

If TNFR1 activation coincides with c-IAPs absence (IAP antagonist treatment, which will prevent RIP1 ubiquitination), translation inhibition (cyclohexamide treatment), or deubiquitination of RIP1 by the deubiquitinating enzyme (DUB) CYLD, the translocation of RIP1 to a secondary cytoplasmatic complex, Complex-II, will occur [18–20]. Cyclohexamide is thought to inhibit the translation of pro-survival genes after TNFα stimulation, among them c-IAP2 and cFLIP, which eventually allows RIP1 translocation to Complex-II [10,21]. Complex-II is formed by the death domain containing protein FADD (Fas-associated Protein), caspase-8 and cFLIP. Once complex-II is formed it will trigger caspase-8 activation, resulting in apoptotic cell death initiation [21]. However, if caspases are not fully activated or their activity is blocked, for example by viral inhibitors, the protein kinase RIP3 would get recruited to the complex, forming a necrosome, which will lead to necroptotic cell death [22,23]. The obligatory components of necrosome are RIP1 and RIP3 but necrosome complex frequently also contains caspase-8, FADD and cFLIP. The relevance of caspase activity blockade in necroptotic cell death is highlighted in caspase-8 and FADD knockout mice models. Both knockout mice strains die at the same embryonic stage and with a similar phenotype. However, Casp8$^{-/-}$RIP3$^{-/-}$ and FADD$^{-/-}$RIP3$^{-/-}$ mice survive, suggesting the potential involvement of necroptotic cell death [24–26]. This positions caspase-8 as a necroptosis inhibitor, in addition to its traditional role as a cell death trigger [27]. The necroptosis protective role of caspase-8 may be explained in part by its cleavage of RIP1, RIP3, and CYLD [28–31]. CYLD, as well as A20, remove ubiquitin chains from RIP1 during TNFR1-mediated signaling enabling RIP1 translocation to death platforms [18,32,33].

Once the necrosome is formed, RIP1 and RIP3 engage in a series of auto and cross phosphorylation events that are essential for necroptotic cell death. Necroptosis can be completely blocked either by the kinase inactivating mutation in any of the two kinases, or chemically by RIP1 kinase inhibitors (necrostatins), or RIP3 kinase inhibitors [34–36]. Although RIP1 phosphorylation is associated with activation of its kinase activity, phosphorylation at a particular residue on RIP1, Ser89, was reported to have the opposite effect: it decreases RIP1 kinase activity without affecting RIP3 binding [37]. Phosphorylation of RIP3 allows the binding and phosphorylation of pseudokinase MLKL (mixed lineage kinase domain-like), a key component of necroptotic cell death [38,39].

One interesting feature of the necrosome complex is the formation of amyloid structures [40]. RIP1 and RIP3 interact through the RHIM motif forming fibril structures and puncta that can be clearly observed by immunohistochemistry. The importance of these fibrils was demonstrated by the addition of reagents that block amyloid oligomerization [40]. Prevention of RIP1 and RIP3 oligomerization blocks necroptotic cell death. Interestingly, RIP3 oligomerization is sufficient for necroptosis induction. Nonetheless, even after induced dimerization of RIP3 by artificial dimerizers, RIP3 RHIM motif is required for cell death [41]. This finding supports the idea that necroptosis

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requires not just amyloid structures, but higher oligomerization structures mediated by the RHIM motif [41]. In general, the RHIM motif appears to be important for RIP3 and RIP1 kinase activity, and thus cell death [40]. However, it is not clear if RIPs oligomerization through the RHIM motif is crucial for its activity, or if mutations in the RHIM domain just disrupt RIPs structure, since the kinase domain constructs of RIP proteins are active kinases without the need of RHIM-oligomerization [42].

- Toll-like receptor (TLR)

Cell death is not always a detrimental process; it can be protective against pathogen infection. TLRs are a family of receptors that sense pathogen-associated molecular patterns (PAMPs). TLR2 senses peptidoglycan, TLR3 dsRNA, TLR4 lipopolysaccharide (LPS), TLR5 flagellin and TLR9 unmethylated CpG DNA motifs [43]. Once TLR receptors get activated they recruit Toll/IL-1R (TIR) domain-containing adapters and initiate NF-κB and IRF3/IRF7 signaling pathways, which lead to the expression of cytokines, chemokines and interferons [36]. TLRs engage the adaptor proteins MyD88 (myeloid differentiation primary response gene), while TLR3 and TLR4 also recruit TRIF (Toll/IL-1 receptor domain-containing adaptor inducing IFN-β), which is a RHIM-containing protein [44,45].

In addition to NF-κB, MAPK and IRF3/IRF7 signaling, TLR activation can stimulate cell death as well. If caspases get activated, apoptosis is the preferential cell death mechanism [46]. But, if caspase activity is compromised, necroptotic cell death can ensue [44]. Necroptotic cell death initiated by the adaptor protein MyD88 is RIP1 and TNFα dependent. This is consequence of an indirect cell death mechanism, since the activation of the majority of TLRs causes TNFα production, which engages TNFR1 necroptotic cell death [36]. On the other hand, TLR3/4 necroptotic cell death is TNFα independent [36,44]. In contrast to other TLRs, TLR3/4 necroptotic cell death is a direct consequence of the RHIM-containing protein TRIF binding to RIP3 to form a necrosome [36,44]. RIP1 seems to have a cell lineage specific role in TLR3/4 cell death. In TLR3 signaling, RIP1 is needed for NF-κB signaling, however, NF-κB and IRF3 activation do not affect the necroptotic outcome [36,44,45]. Fibroblasts and endothelial cells undergo TLR3-dependent necroptosis independently of RIP1, nevertheless, macrophages require of RIP1 to commit to TLR3/4 mediated necroptotic cell death [36].

- DNA-dependent activator of interferon regulatory factors (DAI)

DNA viruses can trigger a different innate immune pathway than TLRs, the DAI pathway. DAI can provoke cell death to protect cells from viral propagation. DAI is an intracellular DNA sensor that activates the IRF3 and NF-κB pathways to promote the synthesis of interferons and cytokines [47].

DAI also contains a RHIM-motif that allows it to bind to RIP1 and RIP3 [47–49]. DAI can bind RIP1 to stimulate NF-κB activation, however, DAI-dependent necroptosis does not rely on RIP1 [47]. DAI-dependent necroptosis requires RIP3 binding through RHIM-motif interactions [49].

- Virus inhibition of apoptosis and necroptosis

TLRs and DAI-cell death is intended to protect a host from pathogen infection as cell death prevents viral replication and propagation. Nevertheless, there is a group of viruses that have adapted to this host defense mechanism by blocking apoptotic or necroptotic cell death in the infected cell. Some viruses express caspase inhibitors to inhibit apoptotic cell death. Among them are: vaccinia virus with B13R/Spi2, cowpox virus through CrmA, and MCMV with M36-encoded viral inhibitor of caspase-8 activation (vICA) [24,44]. These viral proteins can inhibit caspase-8 and elude apoptosis, however, that permits the infected cell to switch to necroptotic cell death and counteract viral cell death evasion [23].

Still, some viruses have evolved to block necroptosis as well. This phenomenon can be appreciated in MCMV, which also encodes a RHIM-motif protein, the M45 or viral inhibitor of RIP activation (vIRA) [50]. The vIRA inhibits RIP1-RIP3 interaction as well as other RIP3-RHIM dependent necroptotic pathways, like TRIF and DAI [48,50]. vIRA competes for the binding of these RHIM-containing proteins, blocking necroptosis and thus allowing viral replication in the infected host.

- MLKL

For a long time, RIP3 downstream effectors were an unsolved mystery, but in 2012 two groups discovered MLKL as RIP3 substrate [38,39]. One group discovered MLKL by screening a kinase/phosphatase shRNA library in the human cell line HT29 upon necroptotic stimulus [38]. The other group found MLKL through a screen of chemical compounds that can inhibit necroptosis also in HT29 cells. The drug necrosulfonamide ((E)-N-(4-(N-(3-methoxypyrazin-2-yl)sulfamoyl) phenyl)-3-(5-nitrothiophene-2-yl)acrylamide) was found to target MLKL and inhibit necroptosis. Necrosulfonamide is specific for human MLKL since the cysteine 86 of human MLKL is not present in mouse MLKL protein [38,39].

MLKL is a pseudokinase, which lacks a phosphate-binding glycine-rich P loop and the key aspartate 349 residue that coordinates magnesium [39]. MLKL can bind ATP, but it is catalytically inactive [51]. An N-terminus coiled-coil domain region and a C-terminal kinase-like domain characterize MLKL. MLKL binds the kinase domain of RIP3 through its C-terminal kinase-like domain [39]. Necrosulfonamide does not inhibit RIP3-MLKL binding as it binds to the N-terminal coiled-coil domain region of MLKL [39]. The binding of RIP3 to MLKL is dependent on RIP3 kinase activity and RIP3 needs to be phosphorylated at S227 (T231 and S232 in mouse) to bind MLKL [39]. On the other hand, MLKL is phosphorylated by RIP3 at T357 and S358 in human and S345, S347, and T349 residues in mouse [39,51]. These phosphorylation sites are necessary for necroptosis since mutation of both sites inhibits necroptotic cell death [39,51]. Structural studies of MLKL allowed the identification of functionally important residues in MLKL. K219M and Q343A mutations (residues involved in ATP binding) in mouse MLKL protein result in a constitutively active MLKL [51]. The same effect was observed for a phospho-mimic of one of the sites targeted by RIP3, S345D. These mutants not only cause necroptosis in unstimulated cells, but also in the absence of RIP3, suggesting that the crucial role of RIP3 in necroptosis is MLKL phosphorylation.

MLKL knockout mice show no abnormalities under non-stress conditions: they are viable, fertile and look healthy [51,52]. MLKL deficient cells can undergo apoptosis but they are resistant to TNFα induced necroptotic cell death, as well as to LPS, oxLDL and partially cyclohexamide necroptosis, indicating that MLKL is a common downstream effector of different necroptotic death pathways [52]. The physiological importance of MLKL and thus necrosis can be appreciated in the resistance of MLKL knockout mice to cerulean-induced acute pancreatitis, a disease that involves necrosis [52].

Recently, several studies have deciphered the role of MLKL in necroptosis. MLKL oligomerizes through its N-terminal four-helix bundle, and that triggers its translocation to the plasma membrane [53–56]. Oligomerization of MLKL is induced by RIP3 mediated phosphorylation at the kinase-like domain of MLKL. Nevertheless, artificial oligomerization of the N-terminal region of MLKL is sufficient to trigger necroptotic cell death. It is interesting that the two different coiled-coil (CC) domains of MLKL are essential for necroptotic cell death although for different functions. CC2 is responsible for MLKL oligomerization, while the CC1 is involved in the recruitment to membranes [53]. The role of MLKL in membranes is not completely clear. One study states that

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MLKL in the plasma membrane binds to the ion channel TRMP7 (transient receptor potential melastatin related 7), which leads to the influx of Ca^{2+} ions and the subsequent cell death [53]. Another study claims that the importance of MLKL in membranes stems from its regulation of Na^{+} channels that triggers Na^{+} entrance, increase in the osmotic pressure and cause plasma membrane rupture [55]. However, there are other studies that show that oligomerized MLKL binds membrane lipids through the positively charged amino acids in its N-terminus, and that MLKL may be directly responsible for pore formation and membrane disruption [54,56].

At the same time with MLKL, PGAM5 (Phosphoglycerate mutase family member 5) was identified to be another target of RIP3 by one of the groups [57]. According to this report, RIP3 can phosphorylate PGAM5, causing PGAM5 to de-phosphorylate Drp1, a protein responsible for mitochondrial fission [57]. However, the role of PGAM5 in necroptosis does not appear to be that crucial [51,58]. Moreover, the role of mitochondria in necroptosis is not very clear either, not only because of the uncertain involvement of PGAM5-Drp1, but also because of ROS contribution. Upon mitochondria removal, cells become resistant to the mitochondrial apoptotic pathway but not to TNF-induced necroptosis, questioning the importance of mitochondria and mitochondrial proteins in necroptotic cell death [59].

- The controversy: RIP1 as a cell death activator and inhibitor

The real function of RIP1 in cell death remains elusive and contradictory. The cell death observed in RIP1 knockout models draws RIP1 as a cell death inhibitor; however, necroptosis resistance detected in studies where just the kinase activity of RIP1 is abolished suggests that RIP1 is a necroptotic activator [9,35,60].

In the absence of RIP1 certain cells become more sensitive to TNFα-dependent cell death, making RIP1 a cell death inhibitor [27,60]. One explanation for this phenomenon could be the role of RIP1 in mediating NF-κB signaling. NF-κB blockade, such as by the IκBα super-repressor, sensitizes cells to apoptosis upon TNFα stimulation [61]. RIP1 activation of NF-κB signaling could enable the expression of pro-survival genes such as cFLIP [62]. cFLIP is a natural inhibitor of caspase-8, and absence of cFLIP provokes apoptotic cell death upon TNFα stimulation [63,64]. Nevertheless, in the absence of RIP1 the NF-κB pathway can still be activated, although the signaling is weaker [65]. In RIP1 knockout mice, elevated levels of cFLIP can be observed in the skin [9]. Moreover, RIP1 knockout mice die perinatally, while cFLIP knockouts die at E10.5, thus RIP1 might not be responsible for cFLIP expression during embryonic development [9]. These observations suggest that deficient expression of NF-κB dependent pro-survival genes may not be the main cause of increased cell death sensitivity in the absence of RIP1.

Another possibility is that the presence of ubiquitinated RIP1 in the TNFR1 signaling complex inhibits the formation of the death platform complex-II independently of NF-κB activation [58]. After TNFα stimulation, RIP1 depleted L929 cells undergo apoptotic cell death [27]. Combined knockdown of RIP1 and TRADD or RIP1 and caspase-8 blocks apoptosis, however it can lead to RIP3 dependent necroptosis [27]. A similar situation can be observed in the intestines of RIP1 knockout neonate mice; the intestinal defects can be rescued by deleting caspase-8 or TNFR1 [9]. Moreover, $RIP1^{-/-}$ progenitor hematopoietic cells cannot self-renew causing their death; however if TNFα signaling is blocked cells survive [9]. This phenomenon could be a result of the binding of TRADD instead of RIP1 to TNFR1 leading to the formation of complex IIa [66,67]. In addition, mutation of the RIP1 ubiquitination site K377 renders cells more sensitive to apoptotic cell death by allowing disassociation from the TNFR1 associated complex [18]. RIP1 ubiquitination within the TNFR1 complex preferentially leads to NF-κB signaling while at the same time it prevents the formation of cell death inducing complex II [61].

However, the perinatal lethality of RIP1 knockout mice, which cannot be rescued by TNFR1 or caspase-8 deletion in C57BL/6 mice, suggests that RIP1 could have a different cell death inhibitory role independent of TNFα signaling and caspase-8, through RIP3 regulation [9]. Examination of RIP1 knockout mice revealed apoptotic regions but also intense necrotic regions in the liver, thymus, lung and intestine as well as systemic inflammation [9]. Interestingly, in RIP1 deficient mice, deletion of RIP3 or MLKL remarkably reduces cell death and the consequent systemic inflammation result of the release of DAMPs, such as IL-1α and IL-33 from necrotic cells. $RIP1^{-/-}$ $Myd88^{-/-}$ double gene ablation relieves the inflammation but it allows the mice to live for just a couple of days. However, in all those crosses the mice still die due to $RIP1^{-/-}$ intestinal defects. $RIP1^{-/-}RIP3^{-/-}Casp8^{-/-}$ mice are viable and fertile; the intestinal defects are eliminated with the lack of caspase-8, and the necrotic cell death and inflammation with RIP3 absence [9,68,69]. These genetic models clearly indicate tissue-specific functional importance of RIP1.

In contrast to RIP1 knockout mice, RIP1 kinase dead knockins are viable, indicating that RIP1 possesses an adaptor role that inhibits perinatal cell death [35,60]. However, these studies with RIP1 kinase-dead knockin animals, as well as with the drug necrostatin, clearly indicate that the kinase activity of RIP1 is required for TNF-, TRIF-, and viral-initiated necroptosis [34,35,60]. This is particularly true for caspase-8 knockout mice, which die at E10.5; nevertheless, $caspase8^{-/-}$ $RIP1^{-/-}$ survive until birth (as well as $caspase8^{-/-}$ $RIP3^{-/-}$), when $RIP1^{-/-}$ phenotype leads to death, suggesting that RIP1 may be required to undergo necroptosis during embryogenesis [9]. Even though RIP1 knockout mice die in part due to necroptosis, there are a number of RIP1 deficient cells and cell lines that are also resistant to necroptosis [60].

The role of RIP1 in TLR3/4-dependent necroptosis is also tissue dependent. The kinase activity of RIP1 contributes to necroptosis triggered by poly(I:C) or LPS in macrophages [36,60]. However, in other cell lineages such as fibroblasts and endothelial cells this necroptotic cell death is RIP1 independent [36].

The dichotomy surrounding the role of RIP1 in necroptosis was also assessed by using artificial dimerization of RIP3. Chemical dimerization of RIP3 or stabilization of RIP3 protein levels showed the difference between the absence of RIP1 and the inhibition of its kinase activity [41]. Nec-1 decreases RIP3 oligomerization and necroptosis, while the absence of RIP1 induces more necroptotic cell death in those conditions. The authors theorized that in basal conditions RIP3 dimerizes and RIP1 binding inhibits further oligomerization. However, upon TNFα stimulation RIP1 also binds RIP3, but in this case RIP1 phosphorylates RIP3 and triggers RIP3 oligomerization [41]. This hypothesis correlates with the reports of necroptosis in the absence of RIP1 due to high levels of RIP3 or another RHIM-containing protein such as DAI or TRIF, as well as the necroptosis observed in skin tissues deficient for RIP1, where higher levels of RIP3 and MLKL can be observed [9,19,48,70].

Taken together, these results indicate that RIP1 can have a cell death activator or inhibitor role depending not only on the tissue and cell type, but also the developmental stage. RIP1 is shown here as a pleiotropic molecule, that can regulate the same pathway in completely opposite directions depending on the circumstances (Fig. 2). RIP1 is an example of the special attention that needs to be given to genetic models, since the cytotoxic results of the complete deletion of RIP1 convert the protein in a death inhibitor, while genetic models based on the necroptosis resistance of the kinase dead version of RIP1 point to RIP1 as a pro-death protein. These results remind us that even though the enzymatic activity of a protein may seem its most important

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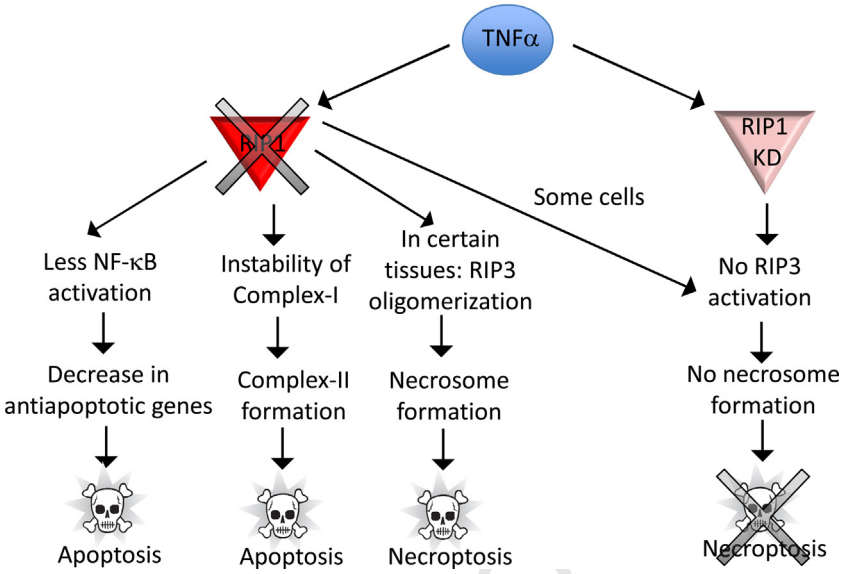

Fig. 2. Pro- and anti-cell death functions of RIP1. The absence of RIP1 can trigger cell death by decreasing the expression of anti-apoptotic genes or by enhancing Complex-II or necrosome formation in certain tissues. On the other hand, RIP1 absence or inactivation of its kinase activity prevents necroptotic cell death. Thus, depending on its expression in various tissues and its kinase activity RIP1 can be a cell death activator or inhibitor.

feature, one should not forget about its non-enzymatic roles such as adaptor that allows the formation of signaling complexes.

- The unexpected role of RIP3 as an apoptotic inhibitor

One of the most surprising findings about RIP3 has been its ability to inhibit apoptosis. RIP3 has been described as a death-inducing protein but emerging evidence suggests that RIP3 kinase activity is important for apoptosis inhibition [35]. How this process occurs is still not well understood, but genetic (kinase-dead knockin mice) or chemical (RIP3 kinase inhibitors) approaches have reached the same conclusion [35,36,71]. The phenotypic differences between the deficiency of RIP3 kinase activity and RIP3 absence are intriguing. Both RIP3 alterations lead to resistance to necroptotic cell death, however, RIP3 knockout mice are viable while RIP3-KD knockin mice die due to apoptotic cell death [35] (Fig. 3). The apoptotic cell death triggered by kinase dead RIP3 depends on RIP1 and caspase-8, pointing to a reverse pathway regulation in comparison to necroptosis.

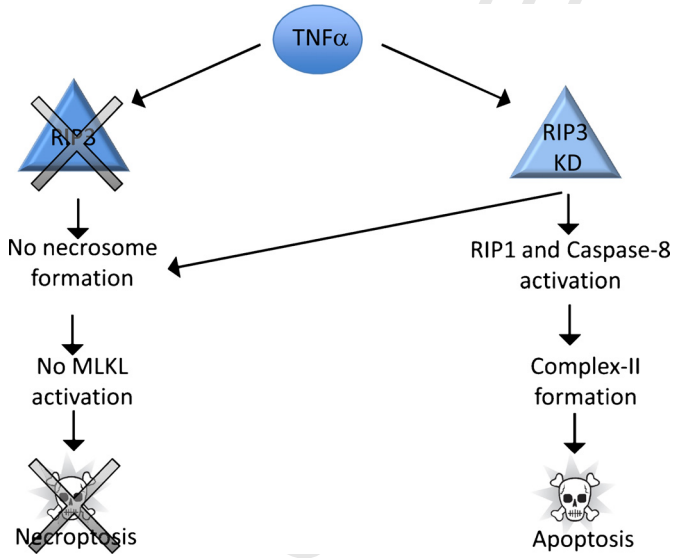

Fig. 3. Pro- and anti-cell death functions of RIP3. Genetic ablation or catalytic inactivation of RIP3 blocks necroptotic cell death. However, catalytically dead RIP3 can trigger apoptotic cell death thus giving RIP3 dual ability to positively and negatively regulate cell death.

Another interesting study claims that in the murine cell line L929, absence of RIP3 or MLKL prevents necroptotic cell death initially, but latter a switch to apoptotic cell death occurs [58]. This apoptotic cell death would be dependent on RIP1 kinase activity, since the drug Nec-1 prevents it. In this study, in contrast to the mouse models, apoptosis is induced in the absence of RIP3, not just the deficiency of its kinase activity. For that reason the authors do not discount the possibility that in L929 cells, in absence of the necroptotic mediators, TNFα can induce apoptotic cell death as the default cell death pathway [58]. It is worth noting that in L929 just TNFα alone, without the need for caspase inhibitors, induces necroptosis – thus, a switch to apoptosis if the necroptotic pathway is blocked is a reasonable possibility.

In conclusion, cell death pathways show high degree of diversity and flexibility: from the initiation by different stimuli to the convergence at the same downstream "core" players, such as RIP3 kinase. At the same time, RIP1 and RIP3 proteins can have multiple and opposing functions depending on the cell type and cell conditions. Cell death is a tightly regulated process, which is beneficial in some cases (development, tissue homeostasis and immunity) or detrimental in the others (tissue injury or neurodegeneration). Harnessing cell death pathways for therapeutic benefit will require further advancement of our understanding of guiding principles that control mediation and execution of these processes.

Acknowledgments

The authors thank colleagues at Genentech for insightful comments and suggestions.

Both authors are employees of Genentech.

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Please cite this article in press as: de Almagro MC, Vucic D. Necroptosis: Pathway diversity and characteristics. Semin Cell Dev Biol (2015), http://dx.doi.org/10.1016/j.semcdb.2015.02.002

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