Chapter 2
RIP1-Mediated Signaling Pathways in Cell Survival and Death Control

Yong Lin

2.1 Introduction

RIP1 was initially identified through a yeast two-hybrid screening as a Fas-interacting protein and an adaptor protein in the TNF receptor 1 (TNFR1) signaling complex (Stanger et al. 1995; Hsu et al. 1996). The human *rip1* gene is located on chromosome 6 and encodes a protein of 671 amino acids (aa) with a predicted molecular weight of 76 kDa (Hsu et al. 1996). In the 300 aa N-terminus resides a serine/threonine kinase domain (KD), while the C-terminal 112 aa contains a death domain (DD). The DD is homologous to the DD in the intracellular regions of Fas, TNFR1 TRAILR1 (DR4), and TRAILR2 (DR5). Because RIP1 can bind to these death receptors, it is thus called a death domain kinase. The DD can also bind TRADD and FADD in the TNFR1 signaling complex. Between the KD and DD is an intermediate domain (ID) that harbors a RIP homotypic interaction motif (RHIM) (Fig. 2.1). Since the discovery of RIP1, six other RIP-like proteins (RIP2-7) with serine/threonine kinase domain have been found which constitute the RIP family (Meylan and Tschopp 2005). It is noteworthy that other RIP family members cannot compensate RIP1deficiency in cells, indicating a unique cellular role for RIP1.

While RIP1 is a critical adaptor protein for TNFR1-mediated signaling to NF-κB activation, researches have determined RIP1 functions in diverse cell signaling pathways for either cell survival or death. These include death receptor (Fas, TNFR1, DR4, DR5, etc.)-mediated activation of MAPK (JNK, ERK, and p38) (Lin et al. 2000; Festjens et al. 2007), apoptosis and necrosis; Toll-like receptor (TLR)-3- and (TLR)-4-mediated activation of NF-κB and MAPK (Han et al. 2004; Meylan et al. 2004; Kaiser and Offermann 2005), apoptosis and necrosis; and genotoxic

Y. Lin, M.D., Ph.D. (✉)
Molecular Biology and Lung Cancer Program, Lovelace Respiratory Research Institute, 2425 Ridgecrest DR SE, Albuquerque, NM 87108, USA
e-mail: ylin@lrri.org

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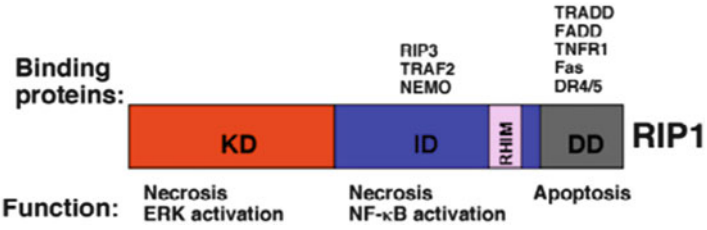

Fig. 2.1 Domain structure of RIP1. The kinase domain (KD), intermediate domain (ID), and the death domain (DD) are *highlighted*. RHIM, RIP homotypic interaction motif. The known interaction proteins and functions of each domain are *listed*

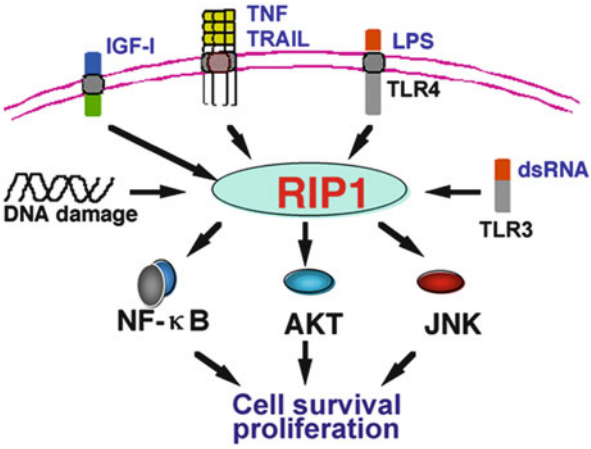

Fig. 2.2 RIP1-mediated cell survival pathways. Activation of receptors such as IGF-IR, TNFR1, DR4/5 (for TRAIL), TLR3 (for dsRNA), or TLR4 (for LPS) and cellular stresses such as DNA damage activate cell survival pathways (NF-κB, Akt, and JNK) depending on cellular context. See text for details

stress-induced activation of NF-κB, apoptosis and necrosis. Therefore, RIP1 is placed at a unique position to relay signals activated by diverse stimuli to different pathways (Fig. 2.2). It is apparent that RIP1 is a key player in regulating cells' fate, survival, or death, in response to different stimulations (Meylan and Tschopp 2005; Festjens et al. 2007; O'Donnell and Ting 2011; Zhang et al. 2011).

2.2 RIP in Cell Survival Signaling

2.2.1 RIP1 in Death Receptor-Mediated Survival Signaling

2.2.1.1 RIP1 in Death Receptor-Mediated NF-κB Activation

The most well-studied NF-κB activation pathway involving RIP1 is that mediated by TNFR1 (Karin and Gallagher 2009) (Fig. 2.3). Ligation of TNFα to its receptor results in the trimerization of TNFR1, which recruits TRADD to form a platform

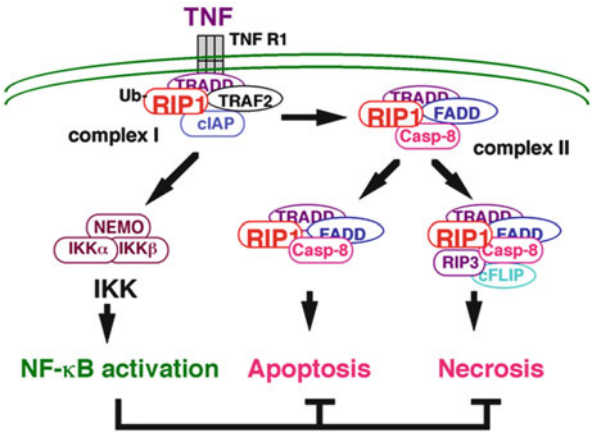

Fig. 2.3 RIP1 in TNF**α**-induced cell survival or death signaling. TNF**α** binding to TNFR1 induces formation of complex I containing TRADD, RIP1, TRAF2, and cIAP1/2. Ubiquitination of RIP1 by cIAP1/2 leads to IKK activation to turn on the NF-κB activation pathway. Internalization of complex I, deubiquitination of RIP1, and recruitment of FADD and caspas-8 result in formation of complex II. When caspase-8 is sufficiently activated, complex II mediates apoptosis. In contrast, if caspase-8 is suppressed by c-FLIP, complex II-mediates RIP3-dependent necrosis. See text for details

for assembling a so-called complex I that consists of RIP1 and TNF receptor-associated factor 2 (TRAF2) in the lipid rafts on the plasma membrane (Hsu et al. 1996; Micheau and Tschopp 2003). RIP1 is then Lys63 polyubiquitinated on its Lys377 within minutes (Ea et al. 2006; Skaug et al. 2009). The Lys63 polyubiquitin chain serves as a platform for binding of NEMO in the IκB kinase (IKK) complex (Wu et al. 2006a). Then IKK is activated by phosphorylation mediated by TAK1 or MEKK3 (Devin et al. 2000; Yang et al. 2001). The adaptor proteins, TAB2 and TAB3, bind to the Lys63 polyubiquitin chain to recruit TAK1 to the complex for IKK activation (Kanayama et al. 2004; Skaug et al. 2009). The activated IKK in turn phosphorylates the inhibitors of NF-κB (IκB), which retains NF-κB in the cytoplasm, to trigger their rapid polyubiquitination followed by degradation in the 26S proteasome. This process allows NF-κB to migrate to the nucleus and bind to the promoters of its target genes. Several of NF-κB's target genes such as c-IAP1, c-IAP2, XIAP, and c-FLIP are found to have anti-apoptotic properties (Karin et al. 2004). Induction of the antioxidant manganese superoxide dismutase (MnSOD) by NF-κB is also suggested to be anti-apoptotic (Kamata et al. 2005). Therefore, the TNFR1-mediated NF-κB activation is generally believed to be for survival through anti-apoptosis. Interestingly, the cIAPs are E3 ubiquitin ligases that execute RIP1 Lys63 polyubiquitination, which may be a positive feedback loop for NF-κB activation (Skaug et al. 2009; Xu et al. 2009). In contrast, A20 and CYLD, both NF-κB targets, remove the Lys63 polyubiquitin chains from RIP1 and promote binding of Lys48-linked ubiquitin chain to RIP1, resulting in proteasomal degradation of RIP1 (Shembade et al. 2008; Skaug et al. 2009). In addition, RIP1 is cleaved at Asp324

by caspase-8 at the early stage of TNFR1 signaling, which shuts off the NF-κB activation pathway and promotes apoptosis (Lin et al. 1999). Thus, RIP1 serves as an important checkpoint for TNFR1-mediated NF-κB activation, and regulation of RIP1 underlies one of the mechanisms for accurate induction of NF-κB activity in terms of extent and duration (Festjens et al. 2007; O'Donnell and Ting 2011).

NF-κB is also activated by other death receptors through a similar mechanism involving RIP1, although the extent is generally weak. Hsp90 binds and stabilizes RIP1, consequently facilitating TNF- or TRAIL-induced NF-κB activation (Lewis et al. 2000; Wang et al. 2006). While NF-κB activation protects cancer cells from TNF- or TRAIL-induced apoptosis, blocking NF-κB sensitizes TNF- or TRAIL-induced cytotoxicity in cancer cells (Wang et al. 2006; Ju et al. 2007; Bai et al. 2009, 2011; Lin et al. 2010).

2.2.1.2 RIP1 in Death Receptor-Mediated MAP Kinase Activation

Residing in complex I during TNFR1 signaling, RIP1 also contributes to activation of MAP kinases (JNK, ERK, and p38) (Devin et al. 2003). The activation of MAPKs also requires TRAF2 and involves sequential activation of the MAPKKK/MAPKK/MAPK cascade. The transient JNK activation appears to promote survival; however, sustained JNK activation leads to cell death (Lin and Dibling 2002; Ventura et al. 2004). Interestingly, NF-κB suppresses sustained JNK activation to maintain cell survival. How the signaling for NF-κB and JNK activation is balanced at RIP1 is still elusive. The role of ERK and p38 in TNFR1-induced cell death is not well understood. Because ERK activation requires the kinase activity of RIP1 and this activity is important for necrosis, it remains to be determined if ERK activation is involved in TNF-induced necrosis. Transient activation of JNK by TRAIL is partially dependent on RIP1 (Lin et al. 2000). In these settings, JNK functions as a cell survival signal to protect cells from death therefore could be a target for sensitizing anticancer chemotherapy (Lin et al. 2000; Wang et al. 2006; Bai et al. 2011).

2.2.2 RIP1 in Genotoxic Stress-Mediated NF-κB Activation

The involvement of RIP1 in DNA damage-induced NF-κB activation was first seen in RIP1−/− mouse embryonic fibroblasts (MEF). DNA topoisomerase inhibitors such as adriamycin and etoposide and ionizing radiation (IR), which cause double-strand DNA breaks (DSB), stimulated NF-κB in wild type but not RIP−/− MEF cells (Hur et al. 2003). Further studies revealed that when genomic DNA is insulted, distinct protein complexes are formed containing different isoforms of p53-induced protein with a death domain (PIDD) for mediating apoptosis, DNA repair, or NF-κB activation (Janssens and Tschopp 2006; Wu et al. 2006b). One complex, caspase-2 PIDDosome, consisting of PIDD, RIP-associated CH1/ECD3-homologous protein with death domain (RAIDD), and procaspase 2, initiates apoptosis in a mitochondria-dependent manner (Janssens et al. 2005; Janssens and Tschopp 2006). The complex

called NEMO PIDDosome, which contains PIDD, RIP1, and the NEMO/IKKγ subunit of IKK, is responsible for NF-κB activation. While PIDD and RIP1 interact directly through their DD, the interaction between NEMO and PIDD and activation of NEMO are mediated by RIP1, indicating the importance of RIP1 in genotoxic stress-induced NF-κB activation (Huang et al. 2003; Janssens et al. 2005; Wu et al. 2006b). Upon the induction of genotoxic stress, two parallel signaling pathways are independently activated for starting the NF-κB activation pathway. The first pathway promotes the nuclear translocation of PIDD followed by recruitment of RIP1 and NEMO to form a complex, where NEMO is rapidly sumoylated by protein inhibitor of activated STAT (PIAS) (Mabb et al. 2006). The second pathway activates the ATM kinase through phosphorylation. The two pathways merge at the point that the sumoylated NEMO and the active ATM kinase meet together. Then ATM phosphorylates NEMO to promote its ubiquitination. Activated NEMO is exported to the cytoplasm where it forms a complex with IKKα and IKKβ, resulting in an active IKK that phosphorylates IκB to trigger its degradation, thereby the downstream cascade for NF-κB activation is activated. The RIP1/NEMO and RAIDD/caspase-2 pathways are mutually exclusive, suggesting that the interaction between RIP1 and PIDD is solely for cell survival to counteract apoptosis mediated by the RAIDD/caspase-2 complex (Tinel et al. 2007; Janssens and Tinel 2012). Additionally, during DNA damage with DSB, RIP1 also interacts with arrest-defective 1 protein (ARD1). ARD1 migrates to the nucleus where the acetyltransferase activity of ARD1 is important for NF-κB activation (Park et al. 2012). It remains to be determined if RIP1 regulates ARD1 nuclear translocation and its acetyltransferase activity during genotoxic stress response.

Although NF-κB is well known as a transcriptional activator, it may function as a transcription repressor in certain circumstances as when cells are responding to DNA damage (Campbell et al. 2004). This may be through ARF-mediated and ATR- and CHK1-dependent phosphorylation of RelA at The505 or through deficiency in Ser536 phosphorylation and acetylation (Ho et al. 2005; Rocha et al. 2005). It is likely that the activation of NF-κB target genes and the cellular outcome in response to DNA damage-induced NF-κB activation are dependent on the cellular context (Wang et al. 2002; Janssens and Tschopp 2006). Accordingly, a pro-apoptosis role of NF-κB has been proposed (Campbell et al. 2004). This may partly be due to differences in cellular context, such as the genetic status of p53 and the current redox status, and the activity of other signaling pathways (Ganapathi et al. 2002; Wang et al. 2002; Lee et al. 2003; Janssens and Tschopp 2006; Chen et al. 2008). Thus, the role of RIP1-mediated NF-κB activation during DNA damage, particularly in cancer cells during chemo- or radiotherapy, requires careful evaluation.

2.2.3 RIP1 in TLR3- and TLR4-Mediated Cell Survival Signaling

TLR are pathogen-associated molecular pattern (PAMP) recognition receptors sensing a wide range of pathogens including bacteria, viruses, fungi, and protozoa.

TLRs are also involved in a wide range of pathophysiological responses such as that in immunity and cancer (Barton and Kagan 2009; Klein Klouwenberg et al. 2009). In the absence of RIP1, TLR3-mediated NF-κB activation, but not the JNK or interferon-β, was abolished. Therefore, TLR 3-induced NF-κB activation is dependent on RIP1 (Meylan et al. 2004). When the cognate ligands bind to TLR3 and TLR4, which are analogous to the dependence of TRADD in binding to the TNFR1 signaling complex, RIP1 is recruited to the receptor mediated by TIR-related adaptor protein inducing INF (TRIF) through the RIP RHIM motif. RIP1 is then phosphorylated followed by polyubiquitination. As an E3 ubiquitin ligase, TRAF6 is suggested to be responsible for RIP1 polyubiquitination (Festjens et al. 2007). Another E3 ubiquitin ligase, Peli1, which was found to bind to and ubiquitinate RIP1 for IKK activation induced by TLR3 and TLR4, suggesting that Peli1 is a ubiquitin ligase for RIP1 in transmission of TRIF-dependent TLR signals (Chang et al. 2009). The E3 ubiquitin ligase Triad3A is also suggested to ubiquitinate RIP1 for TLR signaling (Fearns et al. 2006). The modified RIP1 recruits IKK activating proteins to form a complex consisting of TRIF, TRAF6, RIP1, TAK1, TAB1, and TAB2, which mediates activation of IKKβ and eventually NF-κB (Cusson-Hermance et al. 2005; Festjens et al. 2007). The Bruton's tyrosine kinase (BTK) directly phosphorylates TLR3, leading to formation of the downstream TRIF/RIP1/TBK1 complex (Lee et al. 2012). Whether BTK also modifies TRIF and RIP1 for the signaling needs to be further determined. Interestingly, the RIP family member RIP2 is also involved in TLR3- and TLR4-mediated signaling (Kobayashi et al. 2002). Because TLR3 and TLR4 can induce both survival and death in cells, it remains to be determined if there is functional interaction between RIP1 and RIP2 in modulating cellular outcomes of signaling in these receptors. In addition, recent studies reveal that, despite TRIF, TRADD is also involved in TLR3-mediated RIP1 ubiquitination and NF-κB activation in bone marrow macrophages (Ermolaeva et al. 2008; Pobezinskaya et al. 2008). Thus, it is of interest to determine if TRIF and TRADD contribute to determination of cellular fate during TLR3 signaling.

The evidence showing RIP1 is involved in TLR4-mediated signaling to phosphatidylinositol 3 kinase (PI3K)/Akt activation was from RIP1 (–/–) mouse splenocytes that failed to proliferate and undergo isotype switching in response to LPS. These cells had impaired Akt phosphorylation and increased apoptosis, suggesting that RIP1 is essential for cell survival after TLR4 signaling through mediating the PI3K/Akt pathway (Vivarelli et al. 2004). How RIP1 mediates LPS-/TLR4-induced Akt activation remains to be elucidated.

2.2.4 RIP1 in Other Cell Survival Pathways

RIP1 is also reported to contribute to other cell survival/proliferation pathways. For example, RIP1 was found in the signaling complex of insulin-like growth factor 1 receptor (IGF-1R) for JNK activation, which contributes to cell proliferation (Lin et al. 2006). Also, it was suggested that RIP1 is involved in epidermal growth

factor receptor (EGFR)-mediated signaling (Habib et al. 2001). In addition, RIP1 is overexpressed in glioblastoma. In glioblastoma cells, RIP1 activates PI3K-Akt through dual mechanisms: activates PI3K-Akt by interrupting the mTOR negative feedback loop through negatively regulating mTOR transcription via a NF-κB-dependent pathway and downregulates cellular PTEN levels independent of NF-κB activation. Furthermore, RIP1 suppresses p27 (Kip1) expression to facilitate cell proliferation through the PI3K-/Akt-forkhead pathway (Park et al. 2008, 2009). All these pathways need more attention to their roles in death/survival regulation in different cell types.

2.3 RIP1 in Cell Death Signaling

Although RIP1 possesses a DD and artificial overexpression of RIP1 causes apoptotic cell death that can be rescued by co-expression of the viral caspase-8 inhibiting protein CrmA, in early researches RIP1 was found not to be required for death receptor-mediated cell death under the conditions of transcriptional or translational inhibition (Ting et al. 1996; Kelliher et al. 1998; Lin et al. 1999, 2000; Festjens et al. 2007). Thus, for a long time, RIP1 was not thought to be a death mediator. However, later studies clearly demonstrate that RIP1 actively contributes to cell death, in both apoptosis and necroptosis.

2.3.1 RIP1 in Mediating Apoptosis

2.3.1.1 RIP1 in Death Receptor-Mediated Apoptosis

During TNFα-induced signaling, the TNFR1 complex I that contains TRADD, TRAF2, RIP1, cIAP1, and cIAP2 is internalized and converted into complex II with recruitment of FADD and caspase-8. Complex II mediates signaling to caspase-8 activation and subsequent activation of executor caspases to initiate apoptosis (Fig. 2.3). It has been puzzling that although RIP1 resides in complex II, it appeared not to be involved in apoptosis signaling (Ting et al. 1996; Kelliher et al. 1998). These findings were made in experiments that used RIP1 knockout mouse embryonic fibroblasts (MEF) or RIP1 mutated leukemia cell line Jurkat with addition of TNF or TRAIL in combination with transcription inhibitor or translation inhibitor to block gene expression (Ting et al. 1996; Kelliher et al. 1998; Lin et al. 1999, 2000; Festjens et al. 2007). A later research using stable short-hairpin RNA (shRNA) knockdown (KD) in human tumor cells and immunoprecipitation demonstrated competitive binding of RIP1and TRADD to TNFR1. While FADD is necessary for FasL- or TRAIL- but not TNF-induced apoptosis, RIP1 is required for TNF-induced apoptosis. Furthermore, RIP1 KD abrogated complex II formation after TNF exposure. These observations, although adding more complexity to the roles of death

receptor signaling, suggest that RIP1 contributes to apoptosis induced by TNFR1 in certain tumor cells (Jin and El-Deiry 2006).

Compelling evidence showing RIP1 is involved in TNFR1-mediated apoptosis is that different subtypes of apoptosis are induced by TNFα. With a comparison of apoptosis induced with TNFα combined with cycloheximide that inhibits protein synthesis or second mitochondria-derived activator of caspases (Smac) mimic that targets cIAP1 and cIAP2 for degradation, two distinct caspase-8 activation-mediated apoptosis pathways were identified (Wang et al. 2008). The first well-studied pathway is negatively regulated by the endogenous caspase-8 inhibitor c-FLIP. Cycloheximide eliminates c-FLIP rapidly to promote caspase-8 activation. The second pathway is uncovered with Smac mimetic, which triggers autodegradation of cIAP1 and cIAP2, resulting in the release of RIP1 from complex I to form a caspase-8-activating complex consisting of RIP1, FADD, and caspase-8. While Lys63 polyubiquitination of RIP1 is critical for NF-κB activation, deubiquitination of RIP1 by CYLD is crucial for RIP1/FADD/caspase-8 complex formation and caspase-8 activation (Wang et al. 2008). Thus, it is clear that RIP1 contributes to TNFR1-mediated apoptosis under the condition of cIAP1/2 suppression or CYLD activation. The recently identified CLIP-170-related 59 kDa protein (CLIPR-59) is involved in the formation of complex II and downregulation of TNFα-induced ubiquitination of RIP1 through binding to CYLD, resulting in the formation of complex II and thus promoting caspase-8 activation and apoptosis (Fujikura et al. 2012).

In addition to TNFR1 signaling to apoptosis, other non-death receptor members of the TNFR superfamily also utilize RIP1 for apoptosis. CD40, a cytokine with a prominent role in antitumor immune response, induces apoptosis in cancer cells when its survival signals are blocked. Apoptosis is initiated within a cytosolic death-inducing signaling complex containing RIP1, which is required for CD40 ligand-induced caspase-8 activation and tumor cell killing. Degradation of cIAP1/2 amplifies, whereas inhibition of CYLD reduces the CD40-mediated cytotoxic effect through impacting the ubiquitination on RIP1 (Knox et al. 2011). TNF-like weak inducer of apoptosis (TWEAK, TNFSF12, CD255) induces apoptosis in certain cancer cells via autocrine TNFα. During TWEAK-induced apoptosis, a RIP1-FADD-caspse-8 complex is assembled. Knockdown of RIP1 by siRNA prevented TWEAK-induced association of FADD and caspase-8, suggesting a crucial role of RIP1 in the proapoptotic activity of TWEAK in cancer cells (Ikner and Ashkenazi 2011). A synergy in inducing apoptosis in pediatric acute lymphoblastic leukemia (ALL) occurs with combination of inhibitors of IAPs and various anticancer drugs such as AraC, gemcitabine, doxorubicin, etoposide, vincristine, and Taxol that depends on the formation of a RIP1/FADD/caspase-8 complex via an autocrine/paracrine loop of TNFα. RIP1 is essential for the formation of this complex and subsequent activation of caspase-8 and caspase-3, mitochondrial perturbations, and apoptosis. These findings substantiate the role of RIP1 in cancer therapy that involves death receptor-mediated apoptosis activation with IAP inhibitors and conventional chemotherapy (Loder et al. 2012). Similarly, the Smac mimetic BV6 sensitizes the first-line chemotherapeutic agent in the treatment of glioblastoma temozolomide (TMZ) through apoptosis activation mediated by a RIP1/caspase-8/

FADD complex. Knockdown of RIP1 significantly reduces BV6- and TMZ-induced caspase-8 activation and apoptosis, substantiating that RIP1 is necessary for apoptosis induction and antitumor activity of this therapy regimen (Wagner et al. 2012).

2.3.1.2 RIP1 in DNA Damage-Induced Apoptosis

When DNA damage occurs, PIDDosome is formed for either NF-κB activation-mediated cell survival or caspase-2 activation-mediated apoptosis. While the RIP1 and NEMO containing PIDDosome negatively regulates DNA damage-induced apoptosis through NF-κB activation (Tinel et al. 2007), recent reports show that RIP1 plays a role in facilitating apoptosis when cells acquire DNA damage. Upon excessive DNA damage, ATM is activated to stimulate cytokine secretion, which alerts neighbor cells and induces apoptosis to eliminate the afflicted cell. Extensive DNA lesions stimulate two sequential NF-κB activation phases that induce TNFα-TNFR1 feedforward signaling and drive RIP1 phosphorylation-mediated JNK3 activation, resulting in FADD-mediated pro-apoptotic caspase-8 activation. Thus, in the context of excessive DNA damage, RIP1 kinase participates in TNFα autocrine-mediated apoptosis (Biton and Ashkenazi 2011). Additionally, RIP1-mediated JNK activation has been suggested to be one of the critical components involved in mediating DNA damage-induced and p53-independent cell death (Hur et al. 2006).

A more recent study shed lights on the mechanism of RIP1 in DNA damage-induced apoptotic cell death. Upon genotoxic stress, a large protein complex about 2 MDa called the Ripoptosome is formed to serve as a cell death-inducing platform that can stimulate caspase-8-mediated apoptosis as well as caspase-independent necrosis. Containing RIP1, FADD, and caspase-8, this complex is assembled in response to genotoxic stress-induced depletion of the IAPs (XIAP, cIAP1, and cIAP2). Ripoptosome formation is independent of either death receptors or mitochondria but requires RIP1's kinase activity. The formation and activity of the Ripoptosome are negatively regulated by IAPs. Mechanistically, IAPs serve as a brake for Ripoptosome through mediating RIP1 ubiquitination to keep caspase-8 inactive. These observations shed light on fundamental mechanisms by which RIP1 contributes to chemotherapeutic-induced apoptosis in cancer cells (Tenev et al. 2011). c-$FLIP_L$ prevents, while c-$FLIP_S$ promotes Ripoptosome formation. When cIAPs are absent, caspase activity controlled by c-FLIP isoforms in the Ripoptosome functions as determinants for a cell's fate: RIP3-dependent necroptosis or caspase-dependent apoptosis. While RIP1 is the core component of the complex and the Ripoptosome critically influences the outcome of genotoxic stress, the differential quality of cell death mediated by the Ripoptosome may cause important pathophysiological consequences (Feoktistova et al. 2011).

It should be noted that although RIP1 mediates a cell death pathway in response to DNA damage, it also transduces cell survival signals such as NF-κB. When RIP1 expression is suppressed by gene knockout in MEFs or knockdown in cancer cells, DNA damage-induced cytotoxicity is significantly increased (Yang et al. 2011;

Wang et al. 2014), suggesting that cell survival signaling is predominant in RIP1-mediated genotoxic stress signaling and other cell death pathways independent of RIP1 are sufficient to kill the cells.

2.3.1.3 RIP1 in TLR3- and TLR4-Mediated Apoptotic Death

TLR activation by viral infection can result in apoptosis that is dependent on RIP1, FADD, and caspase. Interestingly, contrasted to TNFR1 signaling, RIP1 functions upstream of FADD in TLR3- and TLR4-induced apoptosis (Ruckdeschel et al. 2004). TRIF physically interacts with the RHIM motif in RIP1. RIP1 recruits FADD and caspase-8 that are essential for apoptosis (Kaiser and Offermann 2005). Engagement of TLR3 by dsRNA in lung cancer cells induces the formation of an atypical caspase-8-containing complex that is devoid of death receptors of the TNFR superfamily. The recruitment of caspase-8 to TLR3 is dependent on RIP1-mediated recruitment of FADD. The TLR3/RIP1/caspase-8 complex is negatively modulated by RIP1 ubiquitination by a ubiquitin ligase complex containing cIAP2-TRAF2-TRADD. These observations uncover the molecular mechanisms underlying TLR3-induced apoptosis (Estornes et al. 2012). Viruses encode proteins suppressing TLR-mediated apoptosis. The murine cytomegalovirus M45 protein directly interacts with RIP 1 and RIP3 via RHIM to suppress cell death. The interaction between M45 and RIP1 underlies the cell tropism role of M45 in preventing premature death of endothelial cells during murine cytomegalovirus infection. Thus, suppressing RIP1 provides a direct cell type-dependent replication benefit to the virus (Mack et al. 2008; Upton et al. 2008). Ribonucleotide reductase R1 subunits of herpes simplex virus type 1 protect cells against TLR3-induced apoptosis by interacting with RIP1 and caspase-8. Collectively, RIP1 is the molecular target for certain viruses to impair the host defense apoptotic mechanism prompted by dsRNA (Dufour et al. 2011).

2.3.2 RIP1 in Programmed Necrosis (Necroptosis)

Necrosis has long been regarded as an uncontrolled, passive, and accidental process where cells experience extreme physicochemical stress conditions. Only in the last decade is it becoming clear that necrotic cell death is an active programmed cellular event and the term necroptosis was coined. Necrotic cell death may be an important cell death mode that is both pathologically and physiologically relevant (Festjens et al. 2006; Vandenabeele et al. 2010). It appears that necrotic cell death is not simply a result of one well-described signaling cascade but is the consequence of extensive cross talk between several biochemical and molecular events at different cellular levels (Festjens et al. 2006; Vandenabeele et al. 2010). Necrotic cell death initiates proinflammatory signaling by actively releasing inflammatory cytokines and releasing cellular contents that can stimulate inflammatory responses.

Necrosis is capable of killing tumor cells that have developed strategies to evade apoptosis. Thus, detailed knowledge of necrosis may be exploited in cancer therapeutic strategies (Festjens et al. 2006).

2.3.2.1 RIP1 in TNFα-Induced Necrosis

Although it was noticed that TNFα induces necrotic cell death long ago, uncovering the first piece of the puzzle of the underlying mechanism was not made until the discovery that RIP1 plays a key role in this pathway. In primary T cells, TNFα-, TRAIL-, or FasL-induced caspase-independent necrotic death is absent when FADD or RIP1 is deficient. In contrast to RIP1's role in NF-κB activation, RIP1 kinase activity is required for necrotic death signaling (Holler et al. 2000). With use of RIP1 knockout MEF cells, it was determined that RIP1-mediated cellular ROS, mainly superoxide, accumulation is crucial for TNF-induced nonapoptotic cell death (Lin et al. 2004). RIP1 is essential for Nox1 recruitment to form a signaling complex for activation of Nox1 which plays a key role in TNF-induced necrotic cell death (Kim et al. 2007).

A genome-wide siRNA screen revealed that another member of the RIP kinase family, RIP3, is required for mediating RIP1-dependent necrosis. Upon induction of necrosis, RIP3 is recruited to RIP1 to form a necrosis-inducing complex and the kinase activity of RIP3 is essential for necrosis execution (He et al. 2009). RIP3 regulates necrosis-specific RIP1 phosphorylation. The phosphorylation of RIP1 and RIP3 stabilizes their association within the pronecrotic complex, activates pronecrotic kinase activity, and triggers downstream ROS production. Furthermore, the pronecrotic RIP1–RIP3 complex is induced during vaccinia virus infection, resulting in tissue necrosis, inflammation, and viral replication suppression (Cho et al. 2009). By activating key enzymes of metabolic pathways, RIP3 regulates TNF-induced ROS production and necrosis, substantiating that modulation of energy metabolism in response to death stimuli has an important role in the choice between apoptosis and necrosis (Zhang et al. 2009). With the RIP3 kinase inhibitor necrosulfonamide, the mixed lineage kinase domain-like protein (MLKL) was identified as the RIP3 interacting target. RIP3 phosphorylates MLKL at the threonine 357 and serine 358 residues for executing necrosis, implicating MLKL as a key mediator of necrosis signaling downstream of the kinase RIP3 (Sun et al. 2012). An independent study with screening a kinase/phosphatase shRNA library also identified MLKL as a key RIP3 downstream component of TNFα-induced necrosis. MLKL functions downstream of RIP1 and RIP3 and is recruited to the necrosome through its interaction with RIP3 for the generation of ROS and the late-phase activation of JNK during TNF-induced necrosis (Zhao et al. 2012). In the RIP1- and RIP3-containing protein complexes resides the mitochondrial protein phosphatase PGAM5. Both two splice variants, PGAM5L (long form) and PGAM5S (short form), are involved in necrosis signaling through ROS production in mitochondria. Upon necrosis induction, PGAM5S binds to the mitochondrial fission factor Drp1 to activate its GTPase activity through dephosphorylation of Drp1, resulting in mitochondrial

fragmentation, an early and obligatory step for necrosis execution (Wang et al. 2012). These observations establish a pathway consisting of RIP1, RIP3, MLKL, and PGAM5 for mitochondria-mediated necrosis in response to TNFα and other stimulations.

Necroptosis could be a target for overcoming cancer's chemoresistance (Kreuzaler and Watson 2012; He et al. 2013). For example, in addition to sensitizing apoptosis-proficient cells to TNFα-mediated and caspase-dependent apoptosis, Smac mimetic primes apoptosis-resistant cells lacking FADD or caspase-8 to TNFα-induced, RIP1-dependent, and caspase-independent necroptosis, highlighting the importance of therapeutic exploitation of necroptosis as an alternative cell death program to overcome chemoresistance (Laukens et al. 2011). Through suppressing RIP1 kinase activity, cIAP1 protects cells from TNFα-induced necrosis by preventing RIP1-/RIP3-dependent ROS production, indicating that cIAPs are key in regulating necrosis and thus appear to be a main target for sensitizing cancer cells to necrosis (Vanlangenakker et al. 2011b). By inhibiting RIP1 recruitment to the death receptor signaling complex, PKC activation suppresses the death receptor-mediated necrotic cell death pathway (Byun et al. 2006).

2.3.2.2 RIP1 in ROS- and PARP-Mediated Necrotic Cell Death

ROS are the main players for propagation and execution of necrotic cell death through directly or indirectly provoking protein, lipid, and DNA damages, culminating in disruption of organelle and cell integrity (Festjens et al. 2006). Oxidative stress and ROS elicit and modulate necrotic cell death. RIP1−/− MEF cells are resistant to ROS-induced cell death. Upon H_2O_2 exposure, RIP1 and TRAF2 form a complex in lipid rafts, which is independent of TNFR1. RIP1 and TRAF2 mediate ROS-induced cell death through JNK activation (Shen et al. 2004). JNK1 subsequently phosphorylates the key DNA repair protein poly(ADP-ribose) polymerase-1 (PARP-1), resulting in sustained activation of PARP-1 (Zhang et al. 2007). Activated PARP1 catalyzes NAD+ into nicotinamide and poly-ADP ribose, resulting in depletion of NAD+ and cellular energy failure that leads to necrotic cell death (Festjens et al. 2006). However, with using the DNA alkylating agent *N*-methyl-*N*′-nitro-N-nitrosoguanidine, a potent PARP-1 activator, JNK was shown to be required for PARP-1-induced mitochondrial dysfunction and subsequent cell death. In this necrosis model, RIP1 is upstream of JNK but downstream of PARP-1 (Xu et al. 2006a). Thus, although RIP1 is clearly involved in ROS- and PARP1-mediated necrosis, the defined mechanisms need further study.

2.3.2.3 RIP1 in Necrotic Cell Death Induced by Other Stimulations

RIP1 is involved in necrosis induced by other cellular stresses. For example, following TLR4 ligation by LPS, when NF-κB and caspase-8 are suppressed, cells undergo necrosis depending on RIP1 (Ma et al. 2005). The interaction between viral proteins and RIP1 prevents necrotic cell death during infection (Mack et al. 2008;

Upton et al. 2008). Thus, RIP1 is the molecular target for certain viruses to modulate necrotic cell death (Dufour et al. 2011).

5-Aminolevulinic acid (5-ALA) for glioblastoma therapy mainly activates a necrotic type of cell death depending on a pronecrotic complex containing RIP3 and RIP1 that mediates singlet oxygen production. Interestingly, the pronecrotic complex is devoid of caspase-8 and FADD, two proteins usually part of the necrosome or Ripoptosome, suggesting different complexes consisting of RIP1 or RIP3 are formed for necrosis under different conditions (Coupienne et al. 2011). Heme leaked from hemolysis or myonecrosis has proinflammatory and cytotoxic effects partly through TLR4-dependent production of TNFα and subsequent necrosis that requires RIP1- and RIP3-mediated ROS production (Fortes et al. 2012). Thus, RIP1 functions as a central player in programmed necrosis. However, TCR-induced necroptosis does not require RIP3 (Osborn et al. 2010). In contrast, RIP1-independent but RIP3-mediated necroptosis in the context of TNFα signaling in particular conditions was also reported (Vanlangenakker et al. 2011a). Therefore, necrosis signaling may be more complex and the role of RIP1 in this context needs further study.

2.3.3 RIP1 in Autophagic Cell Death

Autophagy is a cellular process for degradation and recycling of long-lived proteins and organelles, which is important for cell survival under nutrient starvation conditions and for housekeeping through removal of exhausted, redundant, and unwanted cellular components. However, in certain circumstances autophagy leads to cell death (Todde et al. 2009; Mizushima and Komatsu 2011). LPS induces autophagy in macrophages through a pathway regulated by TRIF-dependent and MyD88-independent TLR4 signaling. RIP1 is downstream of TRIF and MyD88 for inducing autophagy, which contributes to caspase-independent macrophage necrotic cell death (Xu et al. 2006b, 2008). In TRAIL-induced cytoprotective autophagy, RIP1 and TRAF2 mediate JNK activation to blunt apoptosis in cancer cells. Thus, suppression of this RIP1-involved pathway could be utilized for sensitizing cancer cells to therapy with TRAIL (He et al. 2012). On the other hand, in acute lymphoblastic leukemia (ALL), RIP1 is not involved in induction of autophagy but is required for autophagy-mediated necroptosis (Bonapace et al. 2010). These studies reveal a role for RIP1 in autophagic cell death.

2.4 Convergence and Interplay Between RIP1-Mediated Cell Survival and Death Pathways

While RIP1 is involved in both cell survival and death signaling, strict and accurate regulations must be installed to maintain tissue homeostasis and for response to physiological and pathological stimuli. For example, in TNFα-induced signaling to

NF-κB, apoptosis, and necroptosis, multiple shared proteins residing in the TNFR1 complex I and II are involved (Vanlangenakker et al. 2011a). There are two levels of regulation during TNFR1 signaling. The first one is the decision to proceed to survival or death, mainly through regulation of NF-κB. Two cell death checkpoints following TNF stimulation may be involved: an early transcription-independent checkpoint where NEMO restrains RIP1 from activating the caspase cascade, followed by a later checkpoint dependent on NF-κB-mediated transcription of pro-survival genes (Legarda-Addison et al. 2009). Rapid activating expression of NF-κB target anti-apoptosis factors cIAPs that activate RIP1 through ubiquitination and c-FLIP that suppresses caspase-8 is critical for cell survival (Bertrand et al. 2008). In contrast, shutting off cell survival signaling shifts cells' fate to death. In this regard, cleavage of RIP1 by caspase-8 at early time points plays an important role, which blocks NF-κB and enhances apoptosis (Lin et al. 1999). Also, deubiquitination of RIP1 through suppressing cIAPs and activating CYLD shifts RIP1-mediated signaling to death. The second-level regulation is for the modes of cell death. The suppression of caspase-8 by c-FLIP plays a pivotal role for ensuring RIP1-mediated necrosis (Arslan and Scheidereit 2011). Cleavage of RIP1 may also help to suppress necrosis to ensure apoptosis (Sato et al. 2008). In addition, competitive binding of RIP1 and TRADD to TNFR1 may also play a role in determining cells' fate by modulating NF-κB, apoptosis, and nonapoptotic death signals (Zheng et al. 2006).

Analogous to TNFR1 complex II, the main determinant in the Ripoptosome for the mode of cell death is likely the activity of caspase-8. In this regard, c-FLIP plays an important role (Feoktistova et al. 2011). Other mechanisms such as modulation of FADD may exist. In response to Taxol, the mitotic kinase Aurora A and the polo-like kinase Plk1 cooperatively phosphorylate FADD to enhance recruitment of caspase-8 for apoptosis, while dissociation of RIP1 from FADD for necrotic cell death (Jang et al. 2011). Certainly, more defined mechanisms for cell death control need further studies.

2.5 Summary and Perspective

Current research places RIP1 at an important position in mediating cell signaling to cell survival or death. Because cell survival and death control is vital for a variety of cellular functions as well as in disease pathophysiology, further research on RIP1 biology will undoubtedly contribute to elucidation of the mechanisms of pathogenesis in important diseases such as cancer. RIP1 is overexpressed in a portion of human caners without induction of apoptosis as seen in in vitro RIP1 overexpression experiments. Understanding the mechanisms by which the RIP1-mediated apoptosis pathway is attenuated in cancer cells would help to elucidate the role of RIP1 in carcinogenesis and develop new anticancer therapy. Realizing the complexity of RIP1 signaling, one should keep in mind that the role of RIP1 in cell survival and death regulation might be cell context- and stimulus-specific (Wang et al. 2002; Janssens and Tschopp 2006). For example, in some circumstances, RIP1 is

dispensable for TNFR1-mediated NF-κB activation (Wong et al. 2010). With tremendous efforts devoted to researches on cell survival and death signaling involving RIP1, it would be expected that approaches targeting RIP1-mediated pathways will be developed and applied clinically in the near future.

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References

Arslan SC, Scheidereit C (2011) The prevalence of TNFalpha-induced necrosis over apoptosis is determined by TAK1-RIP1 interplay. PLoS One 6:e26069

Bai L, Chen W, Wang X, Ju W, Xu S, Lin Y (2009) Attenuating Smac mimetic compound 3-induced NF-kappaB activation by luteolin leads to synergistic cytotoxicity in cancer cells. J Cell Biochem 108:1125–1131

Bai L, Xu S, Chen W, Li Z, Wang X, Tang H, Lin Y (2011) Blocking NF-kappaB and Akt by Hsp90 inhibition sensitizes Smac mimetic compound 3-induced extrinsic apoptosis pathway and results in synergistic cancer cell death. Apoptosis 16:45–54

Barton GM, Kagan JC (2009) A cell biological view of Toll-like receptor function: regulation through compartmentalization. Nat Rev Immunol 9:535–542

Bertrand MJ, Milutinovic S, Dickson KM, Ho WC, Boudreault A, Durkin J, Gillard JW, Jaquith JB, Morris SJ, Barker PA (2008) cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. Mol Cell 30:689–700

Biton S, Ashkenazi A (2011) NEMO and RIP1 control cell fate in response to extensive DNA damage via TNF-alpha feedforward signaling. Cell 145:92–103

Bonapace L, Bornhauser BC, Schmitz M, Cario G, Ziegler U, Niggli FK, Schafer BW, Schrappe M, Stanulla M, Bourquin JP (2010) Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance. J Clin Invest 120:1310–1323

Byun HS, Park KA, Won M, Yang KJ, Shin S, Piao L, Kwak JY, Lee ZW, Park J, Seok JH, Liu ZG, Hur GM (2006) Phorbol 12-myristate 13-acetate protects against tumor necrosis factor (TNF)-induced necrotic cell death by modulating the recruitment of TNF receptor 1-associated death domain and receptor-interacting protein into the TNF receptor 1 signaling complex: Implication for the regulatory role of protein kinase C. Mol Pharmacol 70:1099–1108

Campbell KJ, Rocha S, Perkins ND (2004) Active repression of antiapoptotic gene expression by RelA(p65) NF-kappa B. Mol Cell 13:853–865

Chang M, Jin W, Sun SC (2009) Peli1 facilitates TRIF-dependent Toll-like receptor signaling and proinflammatory cytokine production. Nat Immunol 10:1089–1095

Chen W, Wang X, Bai L, Liang X, Zhuang J, Lin Y (2008) Blockage of NF-kappaB by IKKbeta- or RelA-siRNA rather than the NF-kappaB super-suppressor IkappaBalpha mutant potentiates adriamycin-induced cytotoxicity in lung cancer cells. J Cell Biochem 105:554–561

Cho YS, Challa S, Moquin D, Genga R, Ray TD, Guildford M, Chan FK (2009) Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 137:1112–1123

Coupienne I, Fettweis G, Rubio N, Agostinis P, Piette J (2011) 5-ALA-PDT induces RIP3-dependent necrosis in glioblastoma. Photochem Photobiol Sci 10:1868–1878

Cusson-Hermance N, Khurana S, Lee TH, Fitzgerald KA, Kelliher MA (2005) Rip1 mediates the Trif-dependent toll-like receptor 3- and 4-induced NF-{kappa}B activation but does not contribute to interferon regulatory factor 3 activation. J Biol Chem 280:36560–36566

Devin A, Cook A, Lin Y, Rodriguez Y, Kelliher M, Liu Z (2000) The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. Immunity 12:419–429

Devin A, Lin Y, Liu ZG (2003) The role of the death-domain kinase RIP in tumour-necrosis-factor-induced activation of mitogen-activated protein kinases. EMBO Rep 4:623–627

Dufour F, Bertrand L, Pearson A, Grandvaux N, Langelier Y (2011) The ribonucleotide reductase R1 subunits of herpes simplex virus 1 and 2 protect cells against poly(I. C)-induced apoptosis. J Virol 85:8689–8701

Ea CK, Deng L, Xia ZP, Pineda G, Chen ZJ (2006) Activation of IKK by TNFalpha requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. Mol Cell 22:245–257

Ermolaeva MA, Michallet MC, Papadopoulou N, Utermohlen O, Kranidioti K, Kollias G, Tschopp J, Pasparakis M (2008) Function of TRADD in tumor necrosis factor receptor 1 signaling and in TRIF-dependent inflammatory responses. Nat Immunol 9:1037–1046

Estornes Y, Toscano F, Virard F, Jacquemin G, Pierrot A, Vanbervliet B, Bonnin M, Lalaoui N, Mercier-Gouy P, Pacheco Y, Salaun B, Renno T, Micheau O, Lebecque S (2012) dsRNA induces apoptosis through an atypical death complex associating TLR3 to caspase-8. Cell Death Differ 19(9):1482–1494

Fearns C, Pan Q, Mathison JC, Chuang TH (2006) Triad3A regulates ubiquitination and proteasomal degradation of RIP1 following disruption of Hsp90 binding. J Biol Chem 281: 34592–34600

Feoktistova M, Geserick P, Kellert B, Dimitrova DP, Langlais C, Hupe M, Cain K, MacFarlane M, Hacker G, Leverkus M (2011) cIAPs block Ripoptosome formation, a RIP1/caspase-8 containing intracellular cell death complex differentially regulated by cFLIP isoforms. Mol Cell 43:449–463

Festjens N, Vanden Berghe T, Cornelis S, Vandenabeele P (2007) RIP1, a kinase on the crossroads of a cell's decision to live or die. Cell Death Differ 14:400–410

Festjens N, Vanden Berghe T, Vandenabeele P (2006) Necrosis, a well-orchestrated form of cell demise: signalling cascades, important mediators and concomitant immune response. Biochim Biophys Acta 1757:1371–1387

Fortes GB, Alves LS, de Oliveira R, Dutra FF, Rodrigues D, Fernandez PL, Souto-Padron T, De Rosa MJ, Kelliher M, Golenbock D, Chan FK, Bozza MT (2012) Heme induces programmed necrosis on macrophages through autocrine TNF and ROS production. Blood 119:2368–2375

Fujikura D, Ito M, Chiba S, Harada T, Perez F, Reed JC, Uede T, Miyazaki T (2012) CLIPR-59 regulates TNF-alpha-induced apoptosis by controlling ubiquitination of RIP1. Cell Death Dis 3:e264

Ganapathi R, Vaziri SA, Tabata M, Takigawa N, Grabowski DR, Bukowski RM, Ganapathi MK (2002) Inhibition of NF-kappaB and proteasome activity in tumors: can we improve the therapeutic potential of topoisomerase I and topoisomerase II poisons. Curr Pharm Des 8:1945–1958

Habib AA, Chatterjee S, Park SK, Ratan RR, Lefebvre S, Vartanian T (2001) The epidermal growth factor receptor engages receptor interacting protein and nuclear factor-kappa B (NF-kappa B)-inducing kinase to activate NF-kappa B. Identification of a novel receptor-tyrosine kinase signalosome. J Biol Chem 276:8865–8874

Han KJ, Su X, Xu LG, Bin LH, Zhang J, Shu HB (2004) Mechanisms of the TRIF-induced interferon-stimulated response element and NF-kappaB activation and apoptosis pathways. J Biol Chem 279:15652–15661

He W, Wang Q, Xu J, Xu X, Padilla MT, Ren G, Gou X, Lin Y (2012) Attenuation of TNFSF10/TRAIL-induced apoptosis by an autophagic survival pathway involving TRAF2- and RIPK1/RIP1-mediated MAPK8/JNK activation. Autophagy 8:1811–1821

He S, Wang L, Miao L, Wang T, Du F, Zhao L, Wang X (2009) Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell 137:1100–1111

He W, Wang Q, Srinivasan B, Xu J, Padilla MT, Li Z, Wang X, Liu Y, Gou X, Shen HM, Xing C, Lin Y (2013) A JNK-mediated autophagy pathway that triggers c-IAP degradation and necroptosis for anticancer chemotherapy. Oncogene. Jul 13. [Epub ahead of print]

Ho WC, Dickson KM, Barker PA (2005) Nuclear factor-kappaB induced by doxorubicin is deficient in phosphorylation and acetylation and represses nuclear factor-kappaB-dependent transcription in cancer cells. Cancer Res 65:4273–4281

Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, Bodmer JL, Schneider P, Seed B, Tschopp J (2000) Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 1:489–495

Hsu H, Huang J, Shu HB, Baichwal V, Goeddel DV (1996) TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. Immunity 4:387–396

Huang TT, Wuerzberger-Davis SM, Wu ZH, Miyamoto S (2003) Sequential modification of NEMO/IKKgamma by SUMO-1 and ubiquitin mediates NF-kappaB activation by genotoxic stress. Cell 115:565–576

Hur GM, Kim YS, Won M, Choksi S, Liu ZG (2006) The death domain kinase RIP has an important role in DNA damage-induced, p53-independent cell death. J Biol Chem 281:25011–25017

Hur GM, Lewis J, Yang Q, Lin Y, Nakano H, Nedospasov S, Liu ZG (2003) The death domain kinase RIP has an essential role in DNA damage-induced NF-kappa B activation. Genes Dev 17:873–882

Ikner A, Ashkenazi A (2011) TWEAK induces apoptosis through a death-signaling complex comprising receptor-interacting protein 1 (RIP1), Fas-associated death domain (FADD), and caspase-8. J Biol Chem 286:21546–21554

Jang MS, Lee SJ, Kang NS, Kim E (2011) Cooperative phosphorylation of FADD by Aur-A and Plk1 in response to taxol triggers both apoptotic and necrotic cell death. Cancer Res 71:7207–7215

Janssens S, Tinel A (2012) The PIDDosome, DNA-damage-induced apoptosis and beyond. Cell Death Differ 19:13–20

Janssens S, Tinel A, Lippens S, Tschopp J (2005) PIDD mediates NF-kappaB activation in response to DNA damage. Cell 123:1079–1092

Janssens S, Tschopp J (2006) Signals from within: the DNA-damage-induced NF-kappaB response. Cell Death Differ 13:773–784

Jin Z, El-Deiry WS (2006) Distinct signaling pathways in TRAIL-versus tumor necrosis factor-induced apoptosis. Mol Cell Biol 26:8136–8148

Ju W, Wang X, Shi H, Chen W, Belinsky SA, Lin Y (2007) A critical role of luteolin-induced reactive oxygen species in blockage of tumor necrosis factor-activated nuclear factor-kappaB pathway and sensitization of apoptosis in lung cancer cells. Mol Pharmacol 71:1381–1388

Kaiser WJ, Offermann MK (2005) Apoptosis induced by the toll-like receptor adaptor TRIF is dependent on its receptor interacting protein homotypic interaction motif. J Immunol 174:4942–4952

Kamata H, Honda S, Maeda S, Chang L, Hirata H, Karin M (2005) Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell 120:649–661

Kanayama A, Seth RB, Sun L, Ea CK, Hong M, Shaito A, Chiu YH, Deng L, Chen ZJ (2004) TAB2 and TAB3 activate the NF-kappaB pathway through binding to polyubiquitin chains. Mol Cell 15:535–548

Karin M, Gallagher E (2009) TNFR signaling: ubiquitin-conjugated TRAFfic signals control stop-and-go for MAPK signaling complexes. Immunol Rev 228:225–240

Karin M, Yamamoto Y, Wang QM (2004) The IKK NF-kappa B system: a treasure trove for drug development. Nat Rev Drug Discov 3:17–26

Kelliher MA, Grimm S, Ishida Y, Kuo F, Stanger BZ, Leder P (1998) The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. Immunity 8:297–303

Kim YS, Morgan MJ, Choksi S, Liu ZG (2007) TNF-induced activation of the Nox1 NADPH oxidase and its role in the induction of necrotic cell death. Mol Cell 26:675–687

Klein Klouwenberg P, Tan L, Werkman W, van Bleek GM, Coenjaerts F (2009) The role of Toll-like receptors in regulating the immune response against respiratory syncytial virus. Crit Rev Immunol 29:531–550

Knox PG, Davies CC, Ioannou M, Eliopoulos AG (2011) The death domain kinase RIP1 links the immunoregulatory CD40 receptor to apoptotic signaling in carcinomas. J Cell Biol 192:391–399

Kobayashi K, Inohara N, Hernandez LD, Galan JE, Nunez G, Janeway CA, Medzhitov R, Flavell RA (2002) RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. Nature 416:194–199

Kreuzaler P, Watson CJ (2012) Killing a cancer: what are the alternatives? Nat Rev Cancer 12(6):411–424

Laukens B, Jennewein C, Schenk B, Vanlangenakker N, Schier A, Cristofanon S, Zobel K, Deshayes K, Vucic D, Jeremias I, Bertrand MJ, Vandenabeele P, Fulda S (2011) Smac mimetic bypasses apoptosis resistance in FADD- or caspase-8-deficient cells by priming for tumor necrosis factor alpha-induced necroptosis. Neoplasia 13:971–979

Lee CT, Seol JY, Lee SY, Park KH, Han SJ, Yoo CG, Han SK, Shim YS, Kim YW (2003) The effect of adenovirus-IkappaBalpha transduction on the chemosensitivity of lung cancer cell line with resistance to cis-diammine dichloroplatinum(II) (cisplatin) and doxorubicin (adriamycin). Lung Cancer 41:199–206

Lee KG, Xu S, Kang ZH, Huo J, Huang M, Liu D, Takeuchi O, Akira S, Lam KP (2012) Bruton's tyrosine kinase phosphorylates Toll-like receptor 3 to initiate antiviral response. Proc Natl Acad Sci U S A 109:5791–5796

Legarda-Addison D, Hase H, O'Donnell MA, Ting AT (2009) NEMO/IKKgamma regulates an early NF-kappaB-independent cell-death checkpoint during TNF signaling. Cell Death Differ 16:1279–1288

Lewis J, Devin A, Miller A, Lin Y, Rodriguez Y, Neckers L, Liu ZG (2000) Disruption of hsp90 function results in degradation of the death domain kinase, receptor-interacting protein (RIP), and blockage of tumor necrosis factor-induced nuclear factor-kappaB activation. J Biol Chem 275:10519–10526

Lin A, Dibling B (2002) The true face of JNK activation in apoptosis. Aging Cell 1:112–116

Lin Y, Bai L, Chen W, Xu S (2010) The NF-kappaB activation pathways, emerging molecular targets for cancer prevention and therapy. Expert Opin Ther Targets 14:45–55

Lin Y, Choksi S, Shen HM, Yang QF, Hur GM, Kim YS, Tran JH, Nedospasov SA, Liu ZG (2004) Tumor necrosis factor-induced nonapoptotic cell death requires receptor-interacting protein-mediated cellular reactive oxygen species accumulation. J Biol Chem 279:10822–10828

Lin Y, Devin A, Cook A, Keane MM, Kelliher M, Lipkowitz S, Liu ZG (2000) The death domain kinase RIP is essential for TRAIL (Apo2L)-induced activation of IkappaB kinase and c-Jun N-terminal kinase. Mol Cell Biol 20:6638–6645

Lin Y, Devin A, Rodriguez Y, Liu ZG (1999) Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. Genes Dev 13:2514–2526

Lin Y, Yang Q, Wang X, Liu ZG (2006) The essential role of the death domain kinase receptor-interacting protein in insulin growth factor-I-induced c-Jun N-terminal kinase activation. J Biol Chem 281:23525–23532

Loder S, Fakler M, Schoeneberger H, Cristofanon S, Leibacher J, Vanlangenakker N, Bertrand MJ, Vandenabeele P, Jeremias I, Debatin KM, Fulda S (2012) RIP1 is required for IAP inhibitor-mediated sensitization of childhood acute leukemia cells to chemotherapy-induced apoptosis. Leukemia 26:1020–1029

Ma Y, Temkin V, Liu H, Pope RM (2005) NF-kappaB protects macrophages from lipopolysaccharide-induced cell death: the role of caspase 8 and receptor-interacting protein. J Biol Chem 280:41827–41834

Mabb AM, Wuerzberger-Davis SM, Miyamoto S (2006) PIASy mediates NEMO sumoylation and NF-kappaB activation in response to genotoxic stress. Nat Cell Biol 8:986–993

Mack C, Sickmann A, Lembo D, Brune W (2008) Inhibition of proinflammatory and innate immune signaling pathways by a cytomegalovirus RIP1-interacting protein. Proc Natl Acad Sci U S A 105:3094–3099

Meylan E, Burns K, Hofmann K, Blancheteau V, Martinon F, Kelliher M, Tschopp J (2004) RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kappa B activation. Nat Immunol 5:503–507

Meylan E, Tschopp J (2005) The RIP kinases: crucial integrators of cellular stress. Trends Biochem Sci 30:151–159

Micheau O, Tschopp J (2003) Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 114:181–190

Mizushima N, Komatsu M (2011) Autophagy: renovation of cells and tissues. Cell 147:728–741

O'Donnell MA, Ting AT (2011) RIP1 comes back to life as a cell death regulator in TNFR1 signaling. FEBS J 278:877–887

Osborn SL, Diehl G, Han SJ, Xue L, Kurd N, Hsieh K, Cado D, Robey EA, Winoto A (2010) Fas-associated death domain (FADD) is a negative regulator of T-cell receptor-mediated necroptosis. Proc Natl Acad Sci U S A 107:13034–13039

Park J, Kanayama A, Yamamoto K, Miyamoto Y (2012) ARD1 binding to RIP1 mediates doxorubicin-induced NF-kappaB activation. Biochem Biophys Res Commun 422(2): 291–297

Park S, Ramnarain DB, Hatanpaa KJ, Mickey BE, Saha D, Paulmurugan R, Madden CJ, Wright PS, Bhai S, Ali MA, Puttaparthi K, Hu W, Elliott JL, Stuve O, Habib AA (2008) The death domain-containing kinase RIP1 regulates p27(Kip1) levels through the PI3K-Akt-forkhead pathway. EMBO Rep 9:766–773

Park S, Zhao D, Hatanpaa KJ, Mickey BE, Saha D, Boothman DA, Story MD, Wong ET, Burma S, Georgescu MM, Rangnekar VM, Chauncey SS, Habib AA (2009) RIP1 activates PI3K-Akt via a dual mechanism involving NF-kappaB-mediated inhibition of the mTOR-S6K-IRS1 negative feedback loop and down-regulation of PTEN. Cancer Res 69:4107–4111

Pobezinskaya YL, Kim YS, Choksi S, Morgan MJ, Li T, Liu C, Liu Z (2008) The function of TRADD in signaling through tumor necrosis factor receptor 1 and TRIF-dependent Toll-like receptors. Nat Immunol 9:1047–1054

Rocha S, Garrett MD, Campbell KJ, Schumm K, Perkins ND (2005) Regulation of NF-kappaB and p53 through activation of ATR and Chk1 by the ARF tumour suppressor. EMBO J 24:1157–1169

Ruckdeschel K, Pfaffinger G, Haase R, Sing A, Weighardt H, Hacker G, Holzmann B, Heesemann J (2004) Signaling of apoptosis through TLRs critically involves toll/IL-1 receptor domain-containing adapter inducing IFN-beta, but not MyD88, in bacteria-infected murine macrophages. J Immunol 173:3320–3328

Sato T, Machida T, Takahashi S, Murase K, Kawano Y, Hayashi T, Iyama S, Takada K, Kuribayashi K, Sato Y, Kobune M, Takimoto R, Matsunaga T, Kato J, Niitsu Y (2008) Apoptosis supercedes necrosis in mitochondrial DNA-depleted Jurkat cells by cleavage of receptor-interacting protein and inhibition of lysosomal cathepsin. J Immunol 181:197–207

Shembade N, Harhaj NS, Parvatiyar K, Copeland NG, Jenkins NA, Matesic LE, Harhaj EW (2008) The E3 ligase Itch negatively regulates inflammatory signaling pathways by controlling the function of the ubiquitin-editing enzyme A20. Nat Immunol 9:254–262

Shen HM, Lin Y, Choksi S, Tran J, Jin T, Chang L, Karin M, Zhang J, Liu ZG (2004) Essential roles of receptor-interacting protein and TRAF2 in oxidative stress-induced cell death. Mol Cell Biol 24:5914–5922

Skaug B, Jiang X, Chen ZJ (2009) The role of ubiquitin in NF-kappaB regulatory pathways. Annu Rev Biochem 78:769–796

Stanger BZ, Leder P, Lee TH, Kim E, Seed B (1995) RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. Cell 81: 513–523

Sun L, Wang H, Wang Z, He S, Chen S, Liao D, Wang L, Yan J, Liu W, Lei X, Wang X (2012) Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. Cell 148:213–227

Tenev T, Bianchi K, Darding M, Broemer M, Langlais C, Wallberg F, Zachariou A, Lopez J, MacFarlane M, Cain K, Meier P (2011) The Ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. Mol Cell 43:432–448

Tinel A, Janssens S, Lippens S, Cuenin S, Logette E, Jaccard B, Quadroni M, Tschopp J (2007) Autoproteolysis of PIDD marks the bifurcation between pro-death caspase-2 and pro-survival NF-kappaB pathway. EMBO J 26:197–208

Ting AT, Pimentel-Muinos FX, Seed B (1996) RIP mediates tumor necrosis factor receptor 1 activation of NF-kappaB but not Fas/APO-1-initiated apoptosis. EMBO J 15:6189–6196

Todde V, Veenhuis M, van der Klei IJ (2009) Autophagy: principles and significance in health and disease. Biochim Biophys Acta 1792:3–13

Upton JW, Kaiser WJ, Mocarski ES (2008) Cytomegalovirus M45 cell death suppression requires receptor-interacting protein (RIP) homotypic interaction motif (RHIM)-dependent interaction with RIP1. J Biol Chem 283:16966–16970

Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G (2010) Molecular mechanisms of necroptosis: an ordered cellular explosion. Nat Rev Mol Cell Biol 11:700–714

Vanlangenakker N, Bertrand MJ, Bogaert P, Vandenabeele P, Vanden Berghe T (2011a) TNF-induced necroptosis in L929 cells is tightly regulated by multiple TNFR1 complex I and II members. Cell Death Dis 2:e230

Vanlangenakker N, Vanden Berghe T, Bogaert P, Laukens B, Zobel K, Deshayes K, Vucic D, Fulda S, Vandenabeele P, Bertrand MJ (2011b) cIAP1 and TAK1 protect cells from TNF-induced necrosis by preventing RIP1/RIP3-dependent reactive oxygen species production. Cell Death Differ 18:656–665

Ventura JJ, Cogswell P, Flavell RA, Baldwin AS Jr, Davis RJ (2004) JNK potentiates TNF-stimulated necrosis by increasing the production of cytotoxic reactive oxygen species. Genes Dev 18:2905–2915

Vivarelli MS, McDonald D, Miller M, Cusson N, Kelliher M, Geha RS (2004) RIP links TLR4 to Akt and is essential for cell survival in response to LPS stimulation. J Exp Med 200(3): 399–404

Wagner L, Marschall V, Karl S, Cristofanon S, Zobel K, Deshayes K, Vucic D, Debatin KM, Fulda S (2012) Smac mimetic sensitizes glioblastoma cells to Temozolomide-induced apoptosis in a RIP1- and NF-kappaB-dependent manner. Oncogene 32:988–997

Wang L, Du F, Wang X (2008) TNF-alpha induces two distinct caspase-8 activation pathways. Cell 133:693–703

Wang Q, Chen W, Bai L, Chen W, Padilla MT, AS Lin, Shi S, Wang X. Lin Y (2014) Receptor-interacting protein 1 increases chemoresistance by maintaining inhibitor of apoptosis protein levels and reducing reactive oxygen species through a microRNA-146a-mediated catalase pathway. J Biol Chem. Jan 14 [Epub ahead of print]

Wang S, Kotamraju S, Konorev E, Kalivendi S, Joseph J, Kalyanaraman B (2002) Activation of nuclear factor-kappaB during doxorubicin-induced apoptosis in endothelial cells and myocytes is pro-apoptotic: the role of hydrogen peroxide. Biochem J 367:729–740

Wang X, Ju W, Renouard J, Aden J, Belinsky SA, Lin Y (2006) 17-allylamino-17-demethoxygeldanamycin synergistically potentiates tumor necrosis factor-induced lung cancer cell death by blocking the nuclear factor-kappaB pathway. Cancer Res 66:1089–1095

Wang Z, Jiang H, Chen S, Du F, Wang X (2012) The mitochondrial phosphatase PGAM5 functions at the convergence point of multiple necrotic death pathways. Cell 148:228–243

Wong WW, Gentle IE, Nachbur U, Anderton H, Vaux DL, Silke J (2010) RIPK1 is not essential for TNFR1-induced activation of NF-kappaB. Cell Death Differ 17:482–487

Wu CJ, Conze DB, Li T, Srinivasula SM, Ashwell JD (2006a) Sensing of Lys 63-linked polyubiquitination by NEMO is a key event in NF-kappaB activation [corrected]. Nat Cell Biol 8:398–406

Wu ZH, Shi Y, Tibbetts RS, Miyamoto S (2006b) Molecular linkage between the kinase ATM and NF-kappaB signaling in response to genotoxic stimuli. Science 311:1141–1146

Xu M, Skaug B, Zeng W, Chen ZJ (2009) A ubiquitin replacement strategy in human cells reveals distinct mechanisms of IKK activation by TNFalpha and IL-1beta. Mol Cell 36:302–314

Xu Y, Huang S, Liu ZG, Han J (2006a) Poly(ADP-ribose) polymerase-1 signaling to mitochondria in necrotic cell death requires RIP1/TRAF2-mediated JNK1 activation. J Biol Chem 281: 8788–8795

Xu Y, Kim SO, Li Y, Han J (2006b) Autophagy contributes to caspase-independent macrophage cell death. J Biol Chem 281:19179–19187

Xu Y, Liu XD, Gong X, Eissa NT (2008) Signaling pathway of autophagy associated with innate immunity. Autophagy 4:110–112

Yang J, Lin Y, Guo Z, Cheng J, Huang J, Deng L, Liao W, Chen Z, Liu Z, Su B (2001) The essential role of MEKK3 in TNF-induced NF-kappaB activation. Nat Immunol 2:620–624

Yang Y, Xia F, Hermance N, Mabb A, Simonson S, Morrissey S, Gandhi P, Munson M, Miyamoto S, Kelliher MA (2011) A cytosolic ATM/NEMO/RIP1 complex recruits TAK1 to mediate the NF-kappaB and p38 mitogen-activated protein kinase (MAPK)/MAPK-activated protein 2 responses to DNA damage. Mol Cell Biol 31:2774–2786

Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, Dong MQ, Han J (2009) RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science 325:332–336

Zhang J, Zhang H, Li J, Rosenberg S, Zhang EC, Zhou X, Qin F, Farabaugh M (2011) RIP1-mediated regulation of lymphocyte survival and death responses. Immunol Res 51:227–236

Zhang S, Lin Y, Kim YS, Hande MP, Liu ZG, Shen HM (2007) c-Jun N-terminal kinase mediates hydrogen peroxide-induced cell death via sustained poly(ADP-ribose) polymerase-1 activation. Cell Death Differ 14:1001–1010

Zhao J, Jitkaew S, Cai Z, Choksi S, Li Q, Luo J, Liu ZG (2012) Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. Proc Natl Acad Sci U S A 109:5322–5327

Zheng L, Bidere N, Staudt D, Cubre A, Orenstein J, Chan FK, Lenardo M (2006) Competitive control of independent programs of tumor necrosis factor receptor-induced cell death by TRADD and RIP1. Mol Cell Biol 26:3505–3513