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Receptor Interacting Protein 3 Kinase Regulates Caspase-independent Programmed Necrosis

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ABSTRACT

Programmed necrosis is a specialized cell death that is distinct from apoptosis in morphology and mechanism. Unlike apoptosis, caspases are not required in programmed necrosis. In fact, programmed necrosis occurs optimally when caspases are inactivated, such as infections with viruses that encode caspase inhibitors. Thus, programmed necrosis may be an important anti-viral host defense strategy. The serine/threonine kinase receptor interacting protein1 (RIP1/RIPK1) plays an essential role in programmed necrosis and other caspase-independent cell death programs. However, the molecular basis by which RIP1 mediates caspase independent cell death is poorly understood. Here, we show that the kinase activity of RIP1 is induced in a cytoplasmic signaling complex termed Complex II during tumor necrosis factor alpha (TNFα)-induced programmed necrosis. Using a RNA interference (RNAi) screen, we identified RIP3 as a crucial downstream regulator for RIP1-mediated programmed necrosis. RIP3 binds to Complex II and undergoes phosphorylation in a RIP1-dependent manner. RIP3 triggers programmed necrosis by regulating downstream reactive oxygen species (ROS) production. Our results provide significant mechanistic insight on the molecular regulation of TNFα-induced programmed necrosis and reveal a physiological clue for RIP3-dependent programmed necrosis in a range of biological events.

Key words : Apoptosis, Programmed necrosis, Receptor interacting protein, Tumor necrosis factor alpha

Introduction

Cell death by programmed necrosis [1,2] is characterized by rapid loss of plasma membrane integrity prior to the presentation of phagocytic signals [3,4]. In fact, the apoptosis is recognized as one of programmed cell death (PCD) and requires caspases for executing cell death. Recently, a specialized and alternative cell death has been suggested under the specific conditions that is caspase-defective. This kind of caspase-independent programmed cell death is called programmed necrosis or necroptosis [5,6]. The release of endogenous "danger signals" from necrotic cells triggers inflammation and can activate immune responses, causing inflammatory diseases or cancer growth [7]. In addition, non-apoptotic or necrotic cell death has been shown to be closely associated with development of pathogenesis in animal models of hypoxic/ischemic injury [1], acute pancreatitis [8] and septic shock [9]. Consistent with these observations, blockade of necrosis was effective in slowing or reducing cell injury in models of cardiac infarct [10] and ischemic brain injury [1]. Recently, certain viral gene products were shown to potently inhibit programmed necrosis [11,12], suggesting that programmed necrosis is an important anti-viral host defense mechanism. In the presence of viral caspase inhibitors or the broad caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp (OMe)-fuoromethylketone (zVAD-fmk), tumor necrosis factor alpha (TNFα)-induced programmed necrosis in TNFα receptor-2+ (TNFR-2+) cells [11]. In addition to TNFR-2 signaling and caspase inhibition, the protein serine/threonine kinase receptor interacting protein 1 (RIP/RIP1/RIPK1) also plays an obligate role in programmed necrosis [11,13,14].

Recent evidence indicates that the transient TNFR-1 signal-

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ing complex on the cell surface (Complex I) is rapidly internalized and replaced by a more stable cytoplasmic signaling complex termed "Complex II". Interestingly, Complex II is devoid of TNFR-1, which may be a pre-requisite for the recruitment of proapoptotic factors including Fas-associated protein with death domain (FADD), caspase-8 and caspase-10 to Complex II [15]. Complex II critically regulates the balance between survival and apoptotic signals by modulating the recruitment of pro- and anti-apoptotic factors. Whether Complex II might play a similar regulatory role in programmed necrosis has not been investigated. The fact that TNFα can trigger distinct cell death programs raises questions about the physiological relevance of programmed necrosis. Because necrotic cells rapidly release "endogenous adjuvants", it is generally considered to be more inflammatory than apoptosis. Thus, programmed necrosis may facilitate innate and adaptive immunity by providing the critical pro-inflammatory signals [7]. In addition, programmed necrosis may serve as an alternative cell death pathway to circumvent viral inhibition of caspases and host cell apoptosis [16]. In support of this notion, programmed necrosis was essential for TNFα-induced cell death in cells infected with vaccinia virus (VV), which encodes a caspase and apoptosis inhibitor [11,17]. Hence, programmed necrosis may facilitate innate immune control of viral infections by promoting inflammation and by eliminating infected host cells.

In this report, we show that a kinase cascade involving RIP1 critically regulates TNFα-induced programmed necrosis. Using a targeted RNA interference screen, RIP3 has been identified as a second crucial kinase that regulates programmed necrosis executed by TNFα. Both RIP1 and RIP3 were recruited to Complex II during TNFα-induced programmed necrosis. We show that upon binding to Complex II, the RIP1 kinase activity was induced and was responsible for phosphorylating downstream effectors including RIP3. RIP3 regulates programmed necrosis in part by controlling reactive oxygen species (ROS) production. The physiological involvement of RIP3-dependent programmed necrosis against viral infection and for other biological processes could make it feasible to explore downstream signaling pathways of RIP1-RIP3 complex.

Materials and Methods

1. Reagents

Antibodies were purchased from the following vendors: RIP1, TRAF2, TRADD, CD3 and β-actin (BD Pharmingen and Santa Cruz); caspase-8 (MBL and Santa Cruz); mouse RIP3 (Axxora); β-actin, tubulin, vimentin (Sigma); GFP (Roche); FADD (Millipore and BD Pharmingen); TNFR-1 (R&D Systems). Human and mouse TNFα were purchased from Biosource.

2. Generation of rabbit polyclonal antibody against RIP3

Rabbit polyclonal antibody was generated against the human RIP3 peptides RIP3-N (19-EELENQELVGKGGFG-33) and RIP3-C (502-DPEAWSRPQGWYNHSGK-518). The polyclonal antiserum was purified on peptide conjugated affinity column. ELISA shows that the polyclonal antibody recognized the RIP3-N peptide with very little reactivity against the RIP3-C peptide.

3. IP kinase assays and Western blots

For immunoprecipitations, at least 5×10^7 cells were used per IP sample, except in RIP1-transfected cells, where 5×10^6 cells per sample were used. Cell lysates were prepared by lysis in 150 mM NaCl, 20 mM Tris-Cl [pH7.5], 0.2% NP-40, 1 mM EDTA, 3 mM NaF, 1 mM β-gylcerophosphate, 1 mM sodium orthovanadate and 10% glycerol. After pre-clearing with Sepharose 6B beads, TNFR-1, caspase-8, FADD or RIP1 were immunoprecipitated with specific antibodies and protein A agarose beads or Protein G Sepharose 4B beads for 4 hours to overnight at 4˚C. The resulting immune complexes were washed with lysis buffer and resolved on 4-12% NuPAGE gels (Invitrogen). For *in vitro* kinase assays, immune complexes were incubated in kinase reaction buffer (20 mM HEPES [pH 7.5], 2 mM DTT, 1 mM NaF, 1 mM Na_3VO_4, 20 mM β-glycerophosphate, 20 mM $MgCl_2$, 20 mM $MnCl_2$, 1 mM EDTA, 300 μM ATP) supplemented with 10 μCi [^{32}P] γ-ATP and 5 μg of MBP (Stressgen) for 30 minutes at 30˚C. Samples were resolved on 4-12% NuPAGE gels (Invitrogen) and exposed to autoradiographic films.

4. Phosphate labeling

Ten million FADD-deficient Jurkat cells were resuspended at 1×10^6 cells/mL of phosphate-free medium and incubated for 40 minutes at 37˚C. After that, cells were spun down and resuspended in phosphate-free medium containing 1 mCi of [^{32}P]-orthophosphate and incubated for 2 hours prior to stimu-

lation with TNFα for 2 hours. In some experiments, 30 μM of necrostatin-1 was added to the cells one hour prior to TNFα stimulation. After washes and cell lysis, the cell extracts were immunoprecipitated with RIP1-specific antibody.

5. Transfections of DNA plasmids

siRNAs were transfected into Jurkat cells, MEFs and L929 cells using HiPerfect (Qiagen) as per manufacturer's instructions. For plasmid transfections, Jurkat cells were transfected with 20 μg of expression vectors using the BTX 630 Electro Cell Manipulator (262V, 725 ohms, 1050 μFd). After 16-20 hours, cells were treated with the indicated stimuli and cell death was measured by flow cytometry using PI as an indicator of cell death. Cell death was determined in the GFP positive transfected populations. Percentage cell loss is calculated using the following formula: % cell loss=(1−(number of live cells in treated sample/number of live cells in untreated sample))×100. For 293T cells, plasmids were transfected using Fugene 6 as per manufacturer's protocol (Roche).

6. Induction of programmed necrosis

To induce programmed necrosis in Jurkat cells and L929 cells, we pretreated cells with 50 μM zVAD-fmk for one hour prior to stimulation with 100 ng/mL rhTNFα (rmTNFα for L929 cells). To induce programmed necrosis in caspase-8 and FADD deficient Jurkat cells, cells were treated with 100 ng/mL rhTNFα. Cell death was determined by propidium iodide staining and flow cytometry. In some experiments, we stained cells with Annexin V and FAM-FLICA to confirm that the cells were undergoing programmed necrosis, but not apoptosis. For the induction of autophagic cell death and oxidative stress, L929 cells were transfected with different siRNA. Twenty-four hours later, cells were treated with different doses of zVAD-fmk (Sigma) for 24-48 hours to induce autophagic cell death. Cell death was measured by MTS assay (Promega).

7. Detection of intracellular ROS generated by TNFα stimulation

L929 cells were exposed to both TNFα and zVAD at the indicated concentrations for 4 h. For the detection of ROS, DCFDA (1 μM) was added to cells, which were further kept in an incubator for 30 min so as to emit fluorescence derived from oxidized DCFDA. Intracellular ROS production was observed under a fluorescence microscope. Additionally, to examine membrane integrity of the cells, an indicator of necrotic cell death, cells were stained with PI, which permeates damaged cells, and were microscopically imaged.

Results

RIP1 is a pleiotropic adaptor that mediates TNFα-induced nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation and programmed necrosis via separate domains [11,13,14,18]. The kinase function of RIP1 is not required for NF-κB activation, but is essential for programmed necrosis [11,13]. We hypothesized that the kinase activity of

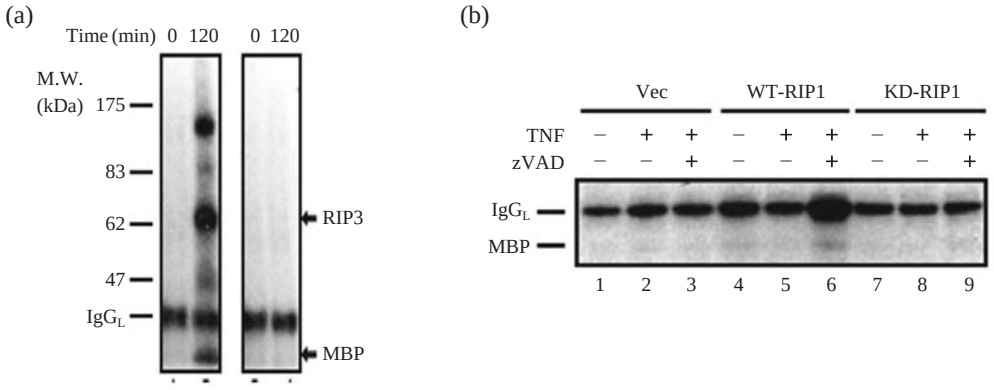

Fig. 1. RIP1 is required for the induction of Complex II kinase activity during programmed necrosis. (a) Ligand-dependent activation of Complex II kinase activity requires RIP1. FADD complexes from caspase-8 deficient (lanes 1-2) or RIP1-deficient (lanes 3-4) Jurkat cells were subjected to *in vitro* kinase assay using MBP as substrate. The arrows indicate the phosphorylated MBP and RIP3 components. (b) An intact RIP1 kinase is required for the induction of FADD associated kinase activity. RIP1-deficient Jurkat cells were transfected with GFP vector alone (lanes 1-3), RIP1-GFP (lanes 4-6) or KD-RIP1-GFP (lanes 7-9) expression vectors. Cells were stimulated for 2 hours with TNFα, TNFα/zVAD or left untreated as indicated. *In vitro* kinase assay was performed with the FADD complexes using MBP as substrate.

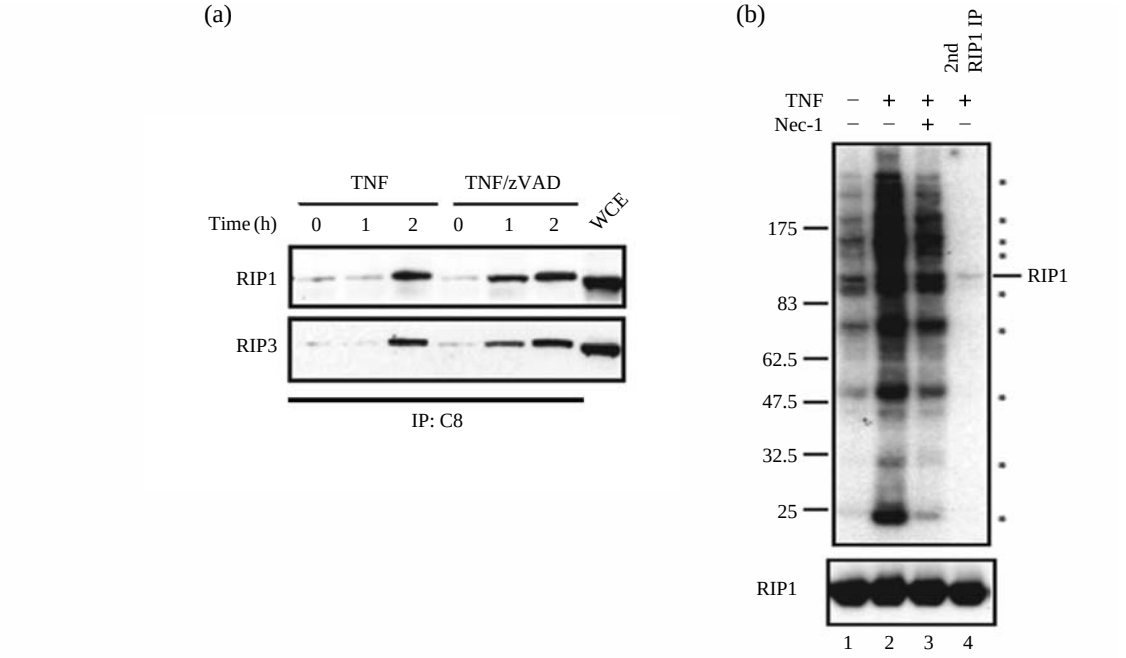

Fig. 2. RIP1 and RIP3 were recruited to Complex II and differentially phosphorylated during programmed necrosis. (a) RIP3 is recruited to Complex II. Complex II was isolated using caspase-8 specific antibody from wild type Jurkat cells stimulated with TNFα ± zVAD-fmk and the recruitment of RIP1 and RIP3 was determined by Western blot. WCE: whole cell extract, C8: caspase-8 (b) RIP1 was differentially phosphorylated upon TNFα stimulation in cells undergoing programmed necrosis. Caspase-8 deficient Jurkat cells (clone J3.2) were labeled with [32P]-orthophosphate. After TNFα stimulation for 2 hours, RIP1 was immunoprecipitated and resolved on SDS-PAGE. In lane 3, cells were treated with 30 μM necrostatin-1 before TNFα stimulation. In lane 4, the RIP1 immune complex was boiled in 2% SDS, diluted in lysis buffer, and subjected to a second immunoprecipitation with RIP1-specific antibody. The phospho-RIP1 band and other TNFα-induced phospho-proteins were indicated.

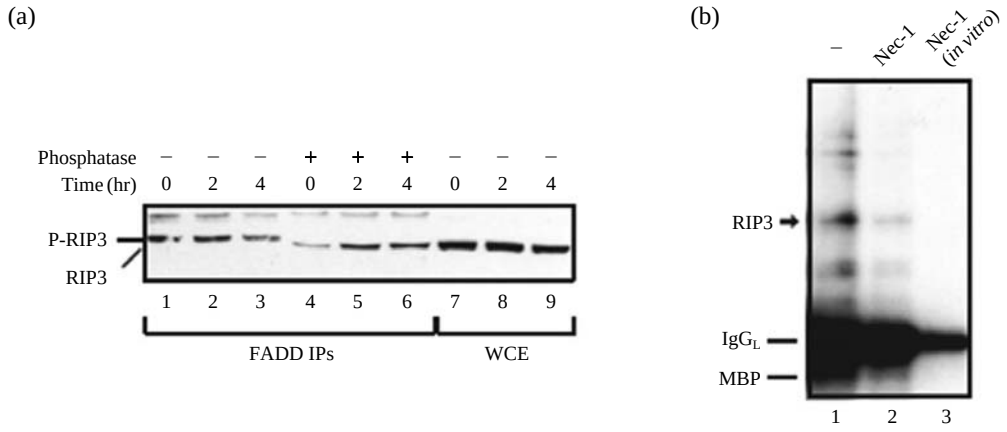

Fig. 3. RIP3 phosphorylation was stimulated by RIP1 in Complex II. (a) RIP3 was phosphorylated in Complex II. FADD associated complexes from TNFα-stimulated caspase-8 deficient Jurkat cells were treated with calf alkaline phosphatase and compared to samples without phosphatase treatment. Note the mobility shift in the phosphatase treated samples. Total cellular extracts were shown in lanes 7-9 for comparison. (b) RIP1 is the apical kinase that regulates Complex II kinase activity. *In vitro* kinase assays were performed as described above on Complex II isolated from TNFα-treated caspase-8 deficient Jurkat cells. Necrostatin-1 (30 μM) was either added to the cells prior to TNFα stimulation (lane 2) or added to the purified Complex II during the *in vitro* kinase assay (lane 3).

RIP1 is activated upon binding to either TNFR-1 associated Complex I or Complex II. Since no significant kinase activity was observed with Complex I (data not shown), we examined the kinase activities of Complex II in caspase-8-deficient Jurkat cells, which undergo programmed necrosis exclusively in response to TNFα [11]. For comparison, we carried out similar

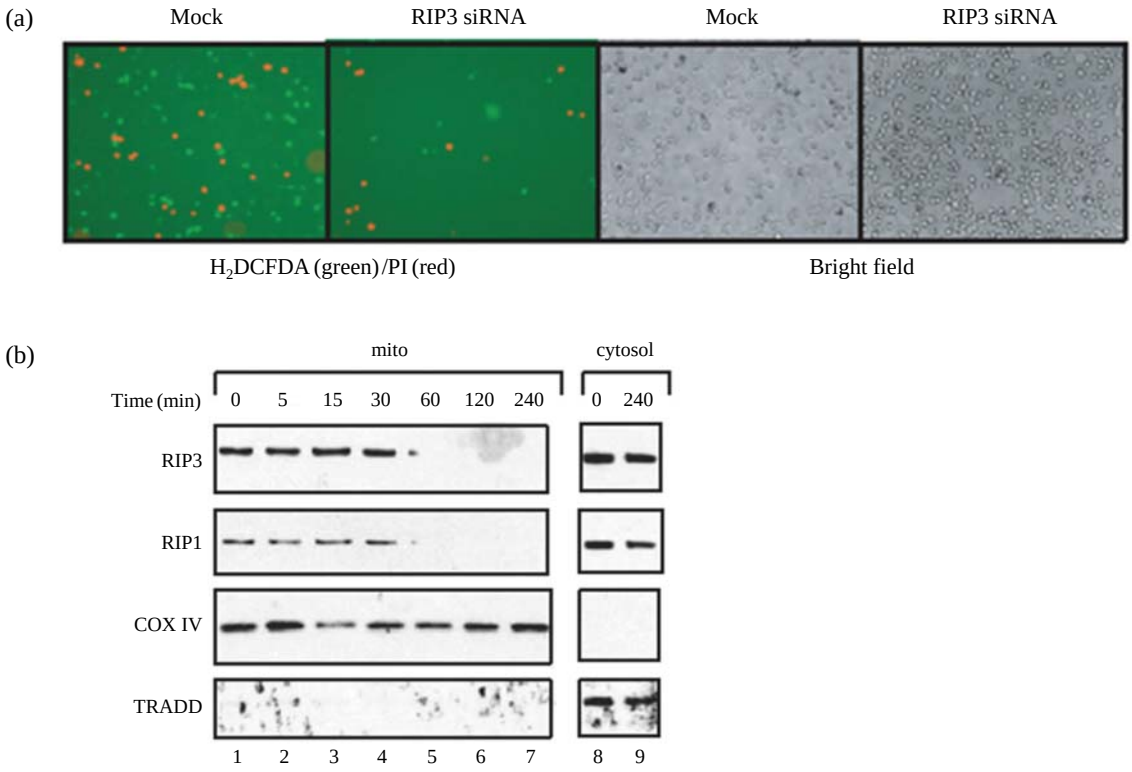

Fig. 4. RIP3 reuglates downstream ROS production. RIP3 acts upstream to regulate ROS generation during TNFα/zVAD-fmk-induced programmed necrosis. (a) Mock-transfected L929 cells or cells transfected with RIP3 siRNA were treated with TNFα and zVAD-fmk for 4 hours. The production of ROS and cell death was evaluated with H_2DCFDA (green) and PI (red) staining, respectively. Bright field images were shown on the right. (b) RIP1 and RIP3 dissociated from the mitochondria during TNFα-induced programmed necrosis. Mitochondrial and cytosolic extracts were purified as described in materials and methods at different times after TNFα/zVADfmk stimulation and analyzed by Western blots as indicated.

experiments using RIP1-deficient Jurkat cells, which exclusively undergo apoptosis in response to TNFα [11]. Following isolation of Complex II with FADD specific antibody, *in vitro* kinase assay was done in a mixture containing the complex II, [32P]-γ-ATP and the artificial substrate myelin basic protein (MBP) (Fig. 1). A dramatic induction of kinase activity was observed in TNFα-treated caspase-8 deficient Jurkat cells, but not in RIP1-deficient Jurkat cells (Fig. 1a, compare lanes 1-2 with 3-4). Hence, induction of Complex II kinase activity might be specific to cells undergoing programmed necrosis. In addition to MBP, we observed several other phosphorylated species that were presumably components of Complex II (Fig. 1a, lane 2). Significantly, reconstitution of RIP1-deficient Jurkat cells with wild type RIP1 restored the kinase activity of Complex II (Fig. 1b, lanes 4-6). Maximal kinase activity was attained when programmed necrosis was induced with TNFα in the presence of the broad caspase inhibitor zVAD-fmk (Fig. 1b, lane 6). In contrast to wild type RIP1, the kinase defective RIP1 (KD-RIP1) failed to restore Complex II kinase activity in RIP1-deficient Jurkat cells (Fig. 1b, lanes 7-9). Therefore, the induction of Complex II kinase activity requires an intact RIP1 kinase. The induction of RIP1-dependent kinase activity suggests that a cascade of kinases might regulate TNFα-induced programmed necrosis.

To identify additional kinases involved in the signaling pathway, we screened a 21-mer small interference RNA (siRNA) library consisting of 691 human kinase genes. Eventually, out of 10 siRNA clones that potently inhibited TNFα-induced programmed necrosis, but had no effects on staurosporine-induced apoptosis in FADD-deficient Jurkat cells, RIP3 siRNA has been identified as one of hits comparable to that of RIP1 siRNA [19,20]. Because of its homology to RIP1 in sequence, its reported interaction with RIP1 [21], we decided to further characterize the role of RIP3 in programmed necrosis. Primarily, we investigated whether RIP3 might similarly bind to TNFR-1. TNFα-dependent recruitment of RIP1 was comparable in wild type Jurkat cells undergoing apoptosis or programmed necrosis. However, we found little evidence of RIP3 binding to TNFR-

1. By contrast, RIP3 was recruited to Complex II in a ligand-dependent manner (Fig. 2a). Consistent with the sensitizing effect of zVAD-fmk on programmed necrosis [22], the kinetics of RIP1 and RIP3 binding to Complex II was greatly enhanced by zVAD-fmk treatment (Fig. 2a, compare lanes 2 and 5), possibly due to inhibition of caspase-8 mediated RIP1 cleavage [23]. In analyzing Complex II formation, we noticed that the Complex II-bound RIP1 and RIP3 consistently exhibited a slower mobility compared to the cellular RIP1 and RIP3 (Fig. 2a, compare lanes 6-7). We reasoned that this mobility shift might represent protein phosphorylation. To test this hypothesis, we labeled FADD-deficient Jurkat cells with [32P]-orthophosphate and then isolated RIP1 by immunoprecipitation. We found that TNFα stimulation led to a dramatic increase in protein phosphorylation (Fig. 2b, compare lanes 1and 2). A second immunoprecipitation with anti-RIP1 antibody confirmed that RIP1 was one of the proteins that underwent TNFα-induced phosphorylation (Fig. 2b, lane 4). When cells were treated with Nec-1, levels of the TNFα-induced protein phosphorylation were dramatically reduced (Fig. 2b, lane 3), indicating that RIP1 is a crucial kinase that regulates protein phosphorylations during programmed necrosis. Phosphatase treatment restored the mobility of Complex II-bound RIP3 to that of the total cellular RIP3 (Fig. 3a, compare lanes 1-3 with 4-6). Taken together, these results show that both RIP1 and RIP3 underwent phosphorylation in Complex II during programmed necrosis. Because RIP1 was critical for the phosphorylation of many Complex II components, we sought to address whether RIP3 might be a direct substrate of RIP1. Since the RIP1-specific inhibitor Nec-1 abolished Complex II kinase activity and RIP3 phosphorylation (Fig. 3b, compare lanes 1-3), the TNFα-induced RIP3 phosphorylation was likely due to RIP1 rather than RIP3 autophosphorylation. Because the production of reactive oxygen species (ROS) is a major effector mechanism for programmed necrosis [14,24,25], this result suggests that RIP1 and RIP3 might act upstream to regulate ROS production. Indeed, when RIP3 expression was silenced by siRNA in L929 cells, TNFα/zVAD-fmk-induced ROS production and necrotic cell death, which were respectively detected by staining with 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA, green) and propidium iodide (PI, red), were both dramatically reduced (Fig. 4a). Since the mitochondria are critical regulators of ROS, we examined whether RIP3 might reside in the mitochondria to regulate ROS production (Fig. 4b). The dissociation from the mitochondria was specific for RIP1 and RIP3, since the mitochondrial resident protein COX IV remained in the mitochondria throughout the stimulation. Thus, RIP1 and RIP3 might regulate ROS production through direct interaction with mitochondrial proteins.

Discussion

RIP3 was originally identified as a NF-κB and apoptosis regulator [26-28]. However, RIP3-/- mice exhibited normal apoptosis and NF-κB responses to TNFα, TLR2, TLR4, T-cell receptor and B-cell receptor activation [29]. Previously, we identified a new role for RIP3 as a crucial mediator for TNFα-mediated programmed necrosis but not zVAD-fmk-induced autophagic cell death [30]. We show that RIP1 critically regulates the induction of Complex II kinase activity during programmed necrosis, most likely through phosphorylating and activating downstream substrates including RIP3. We cannot rule out the possibility that other kinases are involved, although our results are consistent with a model in which RIP1 directly interacts with RIP3 via the RHIM to mediate RIP3 phosphorylation. Our results indicate that RIP3 regulates programmed necrosis by controlling ROS production, possibly through its association with the mitochondria. The mitochondrial localization of RIP1 and RIP3 may be important for ROS generation during programmed necrosis. Several components of the mitochondria permeability transition pore (mPTP) are kinases substrates [31,32]. It is tempting to speculate that RIP1 or RIP3 may phosphorylate components of the mPTP to disrupt their functions and trigger an increase in ROS. Alternatively, ROS can be generated in a RIP3-independent manner. For instance, a recent report show that the plasma membrane associated Nox-1 mediates ROS production during programmed necrosis through binding to the TNFR-1 complex [33]. Hence, multiple pathways exist, perhaps in different cell types, to control ROS production during programmed necrosis.

Physiologically, necrotic cell death may have a broader role in regulating inflammatory processes through the release of “endogenous adjuvants” into the tissue environment [34]. For example, RIP3 expression was upregulated during wound healing [35], a biological process that shares some hallmarks of inflammation. In animal models of ischemic brain injury and cardiac infarction where “sterile inflammation” is involved, inhibition of programmed necrosis has indeed been efficacious in delaying tissue injury [1,10]. Finally, programmed necrosis can directly induce cancer cell death or promote cancer growth and metastasis through its pro-inflammatory effects [36].

Besides, programmed necrosis is able to play a key role in antiviral defense mechanism. When it comes to physiological consequences of host cell infected with virus, apoptosis is generally thought to limit viral replication by eliminating the viral factory. As such, many viruses have developed strategies to inhibit the host apoptosis machinery. In these situations, programmed necrosis may be an important anti-viral defense mechanism that circumvents viral inhibition on apoptosis [20]. In fact, it has been highlighted that RIP3 deficiency dramatically reduced virus-induced tissue necrosis in the visceral fat pad. Consistent with the notion that necrotic cell death is pro-inflammatory, VV-infected RIP3-/-mice exhibited greatly diminished tissue inflammation, which culminated in significantly higher viral load. RIP3 deficiency did not affect cellular responses to multiple TLR ligands [29]. Recombinant VV expressing TNFα reversed the lethality of infection in nude mice or sub-lethally irradiated mice, resulting in rapid clearance of the virus [37]. More recently, the M45 protein from mouse cytomegalovirus was shown to interact with RIP1 and RIP3 via the RHIM [12], suggesting that M45-like viral gene products may represent a novel class of viral inhibitors that specifically target the programmed necrosis machinery. Thus, RIP3-dependent programmed necrosis may be a significant component in determining trauma/injury-induced inflammation, cancers and the outcome of viral diseases.

References

1. Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, et al. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nat Chem Biol 2005; 1:112-119.
2. Golstein P, Kroemer G. Cell death by necrosis: towards a molecular definition. Trends Biochem Sci 2007;32:37-43.
3. Festjens N, Vanden Berghe T, Vandenabeele P. Necrosis, a well-orchestrated form of cell demise: signalling cascades, important mediators and concomitant immune response. Biochim Biophys Acta 2006;1757:1371-1387.
4. Zong WX, Thompson CB. Necrotic death as a cell fate. Genes Dev 2006;20:1-15.
5. Cho YS, Park SY, Shin HS, Chan FK. Physiological consequences of programmed necrosis, an alternative form of cell demise. Mol Cells 2010;29:327-332.
6. Wu W, Liu P, Li J. Necroptosis: an emerging form of programmed cell death. Crit Rev Oncol Hematol 2012;82:249-258.
7. Kono H, Rock KL. How dying cells alert the immune system to danger. Nat Rev Immunol 2008;8:279-289.
8. Mareninova OA, Sung KF, Hong P, Lugea A, Pandol SJ, Gukovsky I, et al. Cell death in pancreatitis: caspases protect from necrotizing pancreatitis. J Biol Chem 2006;281:3370-3381.
9. Cauwels A, Janssen B, Waeytens A, Cuvelier C, Brouckaert P. Caspase inhibition causes hyperacute tumor necrosis factor-induced shock via oxidative stress and phospholipase A2. Nat Immunol 2003;4:387-393.
10. Smith CC, Davidson SM, Lim SY, Simpkin JC, Hothersall JS, Yellon DM. Necrostatin: a potentially novel cardioprotective agent? Cardiovasc Drugs Ther 2007;21:227-233.
11. Chan FK, Shisler J, Bixby JG, Felices M, Zheng L, Appel M, et al. A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses. J Biol Chem 2003;278:51613-51621.
12. Upton JW, Kaiser WJ, Mocarski ES. Cytomegalovirus M45 cell death suppression requires receptor-interacting protein (RIP) homotypic interaction motif (RHIM)-dependent interaction with RIP1. J Biol Chem 2008;283:16966-16970.
13. Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, et al. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 2000;1:489-495.
14. Lin Y, Choksi S, Shen HM, Yang QF, Hur GM, Kim YS, et al. Tumor necrosis factor-induced nonapoptotic cell death requires receptor-interacting protein-mediated cellular reactive oxygen species accumulation. J Biol Chem 2004;279:10822-10828.
15. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 2003; 114:181-190.
16. Benedict CA, Norris PS, Ware CF. To kill or be killed: viral evasion of apoptosis. Nat Immunol 2002;3:1013-1018.
17. Li M, Beg AA. Induction of necrotic-like cell death by tumor necrosis factor alpha and caspase inhibitors: novel mechanism for killing virus-infected cells. J Virol 2000;74:7470-7477.
18. Festjens N, Vanden Berghe T, Cornelis S, Vandenabeele P. RIP1, a kinase on the crossroads of a cell's decision to live or die. Cell Death Differ 2007;14:400-410.
19. He S, Wang L, Miao L, Wang T, Du F, Zhao L, et al. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell 2009;137:1100-1111.
20. Cho YS, Challa S, Moquin D, Genga R, Ray TD, Guildford M, et al. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 2009;137:1112-1123.
21. Sun X, Yin J, Starovasnik MA, Fairbrother WJ, Dixit VM. Identification of a novel homotypic interaction motif required for the phosphorylation of receptor-interacting protein (RIP) by RIP3. J Biol Chem 2002;277:9505-9511.
22. Zheng L, Bidere N, Staudt D, Cubre A, Orenstein J, Chan FK, et al. Competitive control of independent programs of tumor necrosis factor receptor-induced cell death by TRADD and RIP1. Mol Cell Biol 2006;26:3505-3513.

23. Lin Y, Devin A, Rodriguez Y, Liu ZG. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. Genes Dev 1999;13:2514-2526.
24. Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S, et al. Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. Science 2004;304:1500-1502.
25. Liu Z, Lenardo MJ. Reactive oxygen species regulate autophagy through redox-sensitive proteases. Dev Cell 2007;12:484-485.
26. Meylan E, Burns K, Hofmann K, Blancheteau V, Martinon F, Kelliher M, et al. RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kappa B activation. Nat Immunol 2004; 5:503-507.
27. Sun X, Lee J, Navas T, Baldwin DT, Stewart TA, Dixit VM. RIP3, a novel apoptosis-inducing kinase. J Biol Chem 1999;274: 16871-16875.
28. Kasof GM, Prosser JC, Liu D, Lorenzi MV, Gomes BC. The RIP-like kinase, RIP3, induces apoptosis and NF-kappaB nuclear translocation and localizes to mitochondria. FEBS Lett 2000; 473:285-291.
29. Newton K, Sun X, Dixit VM. Kinase RIP3 is dispensable for normal NF-kappa Bs, signaling by the B-cell and T-cell receptors, tumor necrosis factor receptor 1, and Toll-like receptors 2 and 4. Mol Cell Biol 2004;24:1464-1469.
30. Park S, Shin H, Cho Y. Shikonin induces programmed necrosis-like cell death through the formation of receptor interacting protein 1 and 3 complex. Food Chem Toxicol 2013;55:36-41.
31. Bera AK, Ghosh S, Das S. Mitochondrial VDAC can be phosphorylated by cyclic AMP-dependent protein kinase. Biochem Biophys Res Commun 1995;209:213-217.
32. Le Mellay V, Troppmair J, Benz R, Rapp UR. Negative regulation of mitochondrial VDAC channels by C-Raf kinase. BMC Cell Biol 2002;3:14.
33. Kim YS, Morgan MJ, Choksi S, Liu ZG. TNF-induced activation of the Nox1 NADPH oxidase and its role in the induction of necrotic cell death. Mol Cell 2007;26:675-687.
34. Rock KL, Hearn A, Chen CJ, Shi Y. Natural endogenous adjuvants. Springer Semin Immunopathol 2005;26:231-246.
35. Adams S, Pankow S, Werner S, Munz B. Regulation of NF-kappaB activity and keratinocyte differentiation by the RIP4 protein: implications for cutaneous wound repair. J Invest Dermatol 2007;127:538-544.
36. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420:860-867.
37. Sambhi SK, Kohonen-Corish MR, Ramshaw IA. Local production of tumor necrosis factor encoded by recombinant vaccinia virus is effective in controlling viral replication in vivo. Proc Natl Acad Sci USA 1991;88:4025-4029.