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Minireview

Molecules and Cells

Reactive Oxygen Species in TNFα-Induced Signaling and Cell Death

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TNFα is a pleotropic cytokine that initiates many downstream signaling pathways, including NF-κB activation, MAP kinase activation and the induction of both apoptosis and necrosis. TNFα has shown to lead to reactive oxygen species generation through activation of NADPH oxidase, through mitochondrial pathways, or other enzymes. As discussed, ROS play a role in potentiation or inhibition of many of these signaling pathways. We particularly discuss the role of sustained JNK activation potentiated by ROS, which generally is supportive of apoptosis and "necrotic cell death" through various mechanisms, while ROS could have inhibitory or stimulatory roles in NF-κB signaling.

ROS and TNF

Reactive oxygen species, or ROS, are produced in the cell primarily by three sources. The mitochondria are by far the greatest source of ROS. The reactions that occur during oxidative phosphorylation processes by which ATP is generated in the mitochondria are not completely efficient and it has been estimated at between 2 to 5% of electons are lost as they are transferred between electron transport chain complexes (Boveris and Cadenas, 1975; Boveris and Chance, 1973). Secondly there are NADPH oxidases, which uses NADPH to reduce molecular oxygen resulting in superoxide (Brown and Griendling, 2009; Lambeth, 2004), which are used as a defense against infectious pathogens (Quinn et al., 2006). It has recently become apparent that many cytokines and growth factors that have previously been reported to generate ROS as second messengers in their signaling pathways do so through the activation of locally recruited NADPH oxidases (Ushio-Fukai, 2009). Lastly, there are other enzymes that cause the generation ROS in different ways, although usually less robustly than the NADPH oxidases, including, but not limited to, lipoxygenases, cyclooxygenases, myeloperoxidases, xanthine oxidase, heme oxygenase,, monoamine oxidases, and aldehyde oxidase, as well as cytochrome P450-based enzymes.

Although certain increases of ROS occur during signal transduction events, the low overall cellular levels of ROS are usually maintained by systems of antioxidant enzymes and their substrates, such as the glutathione and thioredoxin systems, superoxide dismutases, catalase, and peroxiredoxins, as well as other non-enzymatic antioxidants (Holmgren, 2000; Rhee et al., 2005; Sies, 1997) Nevertheless, ROS play a substantial role in many signal transduction pathways, and this is especially true of the TNFα signaling pathway.

In order to discuss the role of ROS in TNFα signaling, we must first briefly touch on the various pathways that are initiated by this prolific cytokine. The primary receptor for TNFα, TNFR1, is the prototypical member of the death receptor subfamily [see (Guicciardi and Gores, 2009; Wajant, 2003) for review] of the TNF Receptor superfamily, which mediate downstream signaling events through an intracellular "death domain". The death domain of the activated receptor interacts with the death domains of the adaptor proteins TRADD, and RIP1, which then recruit TRAF2 and other various cellular signaling machinery that initiate the downstream signals. TRADD is the primary adapter molecule and is required for virtually all of the downstream signaling pathways, including NF-κB activation, MAP kinase activation and both apoptosis and necrosis, though some weak signaling remains in its absence in some cell types with high RIP1 expression.

NF-κB

Perhaps the most important signaling event that occurs during TNFα signaling is the activation of the transcription factor NF-κB, which has a substantial role in innate immunity and inflammation (Beinke and Ley, 2004; Bonizzi and Karin, 2004; Hayden and Ghosh, 2008; Vallabhapurapu and Karin, 2009). RIP1 and TRAF2 play important roles in its activation, and though NF-κB activation occurs to some extent in the absence of one or the other (Wong et al., 2010; Yeh et al., 1997), likely due to some redundancies in IKK recruitment (Devin et al., 2000; 2001; Tada et al., 2001), a majority of the data in the literature support the notion that they are central molecules in the process (Devin et al., 2000; 2001; Ea et al., 2006; Kelliher et al., 1998; Poyet et al., 2000; Tada et al., 2001; Wu et al., 2006). The p105/*nfκb1* gene product is constitutively processed by the proteosome into an active p50 product, but is prevented from interacting with DNA through its interaction with IκBα, which has strong nuclear export signal and keeps it in the cytoplasm as a heterodimer with p65 (Beinke and Ley, 2004; Bonizzi

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and Karin, 2004; Hayden and Ghosh, 2008; Vallabhapurapu and Karin, 2009). NF-κB is activated by TNFα through recruitment of an IKK signaling complex consisting of two kinase subunits, IKKα and IKKβ, and a regulatory subunit, IKKγ. While TRAF2 is capable of interacting with IKKα and IKKβ directly (Devin et al., 2000; 2001), it also recruits cIAP1 and cIAP2, which polyubiquinate RIP1 (Bertrand et al., 2008; Mahoney et al., 2008; Varfolomeev et al., 2008). Polyubiquitinated RIP1 recruits the IKKγ subunit and stabilizes complex formation (Ea et al., 2006; Poyet et al., 2000; Wu et al., 2006). Likely recruited by RIP1 (Blonska et al., 2005; Lee et al., 2004), the TAK1 kinase appears to be required at this point for the activation of the IKK complex (Blonska et al., 2005; Liu et al., 2006; Sato et al., 2005; Shim et al., 2005; Takaesu et al., 2003), and MEKK3 may also be required as well (Blonska et al., 2005; Yang et al., 2001), but their exact roles are not yet completely clear. The activated complex leads to phosphorylation of IκBα, which is primarily mediated by IKKβ, and leads to the subsequent ubiquitination and degradation by the proteosome, allowing the p50/RelA heterodimer to translocate to the nucleus and activate transcription (Beinke and Ley, 2004; Bonizzi and Karin, 2004; Hayden and Ghosh, 2008; Vallabhapurapu and Karin, 2009).

MAPK pathways

In addition to NF-κB, TNFα signaling through TNFR1 leads to the activation of all three major Map Kinase signaling cascades, ERK, p38, and JNK. These kinases are activated by upstream MAPK kinase kinases (MAP2Ks), which are in turn activated by MAP3Ks and MAP4Ks. TRAF2 is required for efficient activation of each of these pathways (Devin et al., 2003; Lee et al., 1997; Yeh et al., 1997).

RIP1 is also required for efficient JNK activation, but the kinase activity of RIP1 is not essential (Devin et al., 2003). A significant number of MAP 3/4kinases have been implicated in the activation of JNK downstream of TNFα based on TNFα-induction of their activation and the use of overexpression or dominant negative molecules, including MEKK1 (Baud et al., 1999), ASK1 (Hoeflich et al., 1999; Liu et al., 2000; Nishitoh et al., 1998), TAK1 (Liedtke et al., 2002), MAP3K11 (Sathyanarayana et al., 2002), and the germinal center kinase family members, MAP4K2 (Yuasa et al., 1998), MAP4K5 (Shi and Kehrl, 1997; Shi et al., 1999), TNIK (Fu et al., 1999), NRK (Nakano et al., 2000), MAP4K3 (Diener et al., 1997), and MAP4K4 (Yao et al., 1999). MEKK1, ASK, TNIK, MAP3K11, MAP4K2, and MAP4K5 are all known interact with TRAF2 in a stimulation-dependent manner. TAK1 and MEKK1 and have both been proposed to be essential for TNFα-induction of JNK (Shim et al., 2005; Xia et al., 2000), based on signaling in deficient embryonic fibroblasts. However, there are conflicting reports based on similar studies that show that MEKK1 is not essential for TNFα -induced JNK activation (Devin et al., 2003; Yujiri et al., 2000), so it is possible that there is some redundancy of pathways. Deletion of MAP3K11 (MLK3) reduces, but does not elimate TNFα-stimulated JNK activation (Brancho et al., 2005). ASK1 is also not essential for transient TNFα-induced JNK activation, but a vital role for ASK1 in the second prolonged phase of TNFα-induced JNK and p38 activation is indicated by experiments in ASK1 deficient mice (Tobiume et al., 2001).

The upstream kinases involved in TNFα-induced p38 and ERK activation are also not well established. Unlike in JNK activation, TAK1 does not seem to play an important role in activation of these kinases, though there is slight reduction in p38 stimulation in TAK-/- cells (Shim et al., 2005). MEKK3-/- cells have been reported to have a defect in p38 activation in response to TNFα (Lee et al., 2003). However, another report found no difference in p38 activation in MEKK3 or MEKK1 knockout cells (Devin et al., 2003). Interestingly, these two reports are also at odd with respect to the essential role of TRAF2 in p38 activation, with one concluding that TRAF2 is required (Devin et al., 2003) and another concluding that the absence of TRAF2 does not effect p38 activation (Lee et al., 2003). However, it is agreed that RIP is absolutely essential for TNFα-induced p38 activation (Devin et al., 2003; Lee et al., 2003). As with JNK activation, the kinase activity of RIP appears to be dispensable for p38 activation (Devin et al., 2003; Lee et al., 2004), while it does seem to be important for ERK activation (Devin et al., 2003). Likewise, MADD, a splice variant of IG20, binds to TNFR1 directly and appears to be required for ERK activation, but not JNK or p38 activation (Kurada et al., 2009). As loss of MADD expression also results in reduced Grb2 and Sos1/2 recruitment to TNFR1 and decreased Ras and MEKK1/2 activation (Kurada et al., 2009), this may be the pathway by which TNFα activates ERK in some cell types. Alternatively, Syk is an upstream tyrosine kinase can be recruited to RIP1 and TRAF2 that can activate ERK through MAP3K8 (Tpl2/Cot) (Eliopoulos et al., 2006).

While the MAP2 kinases MKK7 and MKK4 are the immediate activators of JNK, only MKK7 is activated by TNFα (Moriguchi et al., 1997). An examination of TNFα-induced JNK activation in MKK4-/- and MKK7-/- cells confirmed the re-quirement for MKK7, while suggesting that a basal level of MKK4 is necessary for the maximal activation of JNK (Tournier et al., 2001). MKK3 appears to be essential for p38 activation in response to TNFα, but MKK3-/- cells have normal JNK activation (Wysk et al., 1999).

JNK, p38 and ERK activate several different transcription factors by phosphorylation. However, they also have a variety of other cellular targets, and their activation may initiate both pro-death and pro-survival effects, depending on the context of the signal. Of the three map kinases, ERK usually acts in a prosurvival fashion.

Cell death

Cell death is initiated by TNFR1 under specific sets of circumstances, primarily when NF-κB signaling is decreased or blocked. Depending on the circumstances and the cell type, the characteristics of TNFα-initiated cell death may vary and TNFα may lead to apoptosis or to necrosis.

Apoptosis

Apoptosis is usually defined as a type of programmed cell death characterized by the activation of caspases, which are cysteine proteases that cleave cellular substrates and effect specific cellular damage and events. Usually these events involve cellular shrinkage, chromatin condensation and nuclear fragmentation, membrane blebbing, and the formation of membrane-bounded bodies containing the cellular structures and organelles, which are then taken up by surrounding cells or by phagocytic cells of the immune system without inflammation (Fiers et al., 1999; Kroemer et al., 2005).

Caspase activation during TNFα-mediated apoptosis is achieved through the recruitment of Fas-associated death domain (FADD) protein to a secondary complex that is dissociated from the main complex (Micheau and Tschopp, 2003). FADD contains a death-effector domain, which recruits and causes the autocatalytic activation of the initiator caspases-8 and -10, which can directly cleave intracellular substrates or activate other caspases through their proteolytic processing.

In a mitochondrial amplification loop, cell death is amplified by regulated release of cytochrome c from the mitochondria,

which binds to the Apaf-1/caspase-9 complex, resulting in the activation of this caspase. Cytochrome c release is positively regulated by Bax and Bak, which are the main proapoptotic members of the Bcl-2 family, and inhibited antiapoptotic members of this family, such as Bcl-2 or Bcl-xL. Cleavage of the Bid protein by caspase-8 allows it to activate Bax/Bak, providing the main TNFα connection with the mitochondrial cell death pathway.

Necrosis

In addition to apoptosis, TNFα also is capable of activating a programmed necrotic-like cell death that is not dependent on the activity of caspases (Festjens et al., 2006b), and which is characterized by cellular swelling, organelle dysfunction, extensive mitochondrial damage, and plasma membrane rupture (Fiers et al., 1999; Kroemer et al., 2005). Necrotic cell death induced by TNFα requires the production of ROS (Festjens et al., 2006b; Fiers et al., 1999; Goossens et al., 1995; Lin et al., 2004; Sakon et al., 2003; Ventura et al., 2004). The RIP1 protein is required for TNFα-induced ROS production and is also required for TNFα-induced caspase-independent cell death (Festjens et al., 2007; Lin et al., 2004), making RIP1 a central player in the process. Unlike activation of NF-κB, JNK, and p38, the pronecrotic role of RIP1 requires its kinase activity. A novel drug that prevents programmed necrotic cell death has been recently developed, and appears to be an inhibitor of RIPK1 kinase activity (Degterev et al., 2005; 2008). While caspase inhibitors are known to prevent apoptosis, inhibition of caspases under necrotic cell death conditions potentiates cell death, possibly due, in part, to RIP1 cleavage by caspase-8 during apoptosis (Lin et al., 1999; Vandenabeele et al., 2006). In addition to RIP1 there are several other molecules suggested to play a role in necrotic cell death. RIPK3, (Cho et al., 2009; He et al., 2009; Zhang et al., 2009) cyclophilin D, (Li et al., 2004; Nakagawa et al., 2005) and possibly PARPs (Los et al., 2002; Xu et al., 2006) and lysosomal proteases, such as calpains and cathepsins (Luke et al., 2007; Sato et al., 2008), have been identified as being a part of the programmed necrotic execution system.

RIP3 is another member of the RIP kinase family identified by similarity between kinase domains. Using RNA interference screens and differential microarray analysis, RIP3 was independently identified by three separate groups as a downstream component of the TNF-induced necrotic pathway (Cho et al., 2009; He et al., 2009; Zhang et al., 2009). RIP3 lacks a death domain and is therefore recruited to a pronecrotic signaling complex through RIP1, which interacts with RIP3 through its homotypic interaction motif (RHIM). This interaction is associated with RIP1 kinase activity. The downstream effects of RIP3 association are not clear at this point, but one group found RIP3 to be associated with 7 different metabolic enzymes, including glycogen phosphorylase (PYGL), glutamate-ammonia ligase (GLUL), glutamate dehydrogenase 1 (GLUD1), fructose- 1,6-bisphosphatase 2 (FBP2), fumarate hydratase (FH), glycosyltransferase 25 domain containing 1 (GLT25D1), and isocitrate dehydrogenase 1 (IDH1) (Zhang et al., 2009). These associations may suggest that RIP3 functions in bioenergetic metabolic pathways that lead to ROS generation.

Cyclophilin D is a component of the mitochondrial permeability transition pore channel, the opening of which results in results in a loss in mitochondrial membrane potential. Despite the fact that Bcl-2 family member-dependent apoptosis is independent of cyclophilin D, fibroblasts and primary hepatocytes isolated from cyclophilin knockout mice are resistant to oxidative stress-mediated necrosis (Li et al., 2004; Nakagawa et al., 2005). This may suggest that at least in some situations, necrosis may depend on the mitochondrial permeability transition being opened in response to ROS.

PARP-1 also has been reported to being capable of causing mitochondrial dysfunction and JNK activation in a process dependent on RIP1 and TRAF2 (Xu et al., 2006). However, it is a nuclear enzyme, cleaved during apoptosis, that is activated by DNA damage and catalyzes the covalent attachment of poly (ADP-ribose) onto DNA-binding proteins using NAD+ as its substrate, making it unclear as to how it is connected with cytoplasmic RIP1 and TRAF2. It is, however, activated in L929 cells when treated with TNFα (Los et al., 2002), and its pharmacological inhibition blocksTNFα-induced necrotic death (Xu et al., 2006). PARP-2 was identified by an siRNA screen for inhibitors of TNF-induced necrotic cell death, and its knockdown causes some reduction of cell death (Hitomi et al., 2008).

Lysosomal calpains and cathepsins are proteases thought to be essential in some necrotic situations. They were identified as necrotic pathway targets in *C. elegans* (Luke et al., 2007), which were known to require lysosomal function for their necrotic pathways (Artal-Sanz et al., 2006). There is some evidence that this requirement for lysosomal proteases is conserved in mammalian systems (Sato et al., 2008), and lysosomal rupture may occur downstream of ROS damage to their lipids (Boya and Kroemer, 2008), and be important in TNFα-induced necrosis (Boya and Kroemer, 2008).

The source and nature of the ROS that are generated prior to TNFα-induced necrotic death is still currently a subject of some debate within the field. TNFα-stimulated ROS has been proposed on one hand to come from downstream events involving the mitochondria (Fiers et al., 1999; Goossens et al., 1995). However, that TNFα has been shown to cause the production of superoxide (Meier et al., 1989) through activation of NADPH oxidases (Anilkumar et al., 2008; Kim et al., 2007; Li et al., 2009a; 2010; Woo et al., 2006).

NADPH oxidases

NADPH oxidases are a family of enzymes specifically dedicated to ROS production (Brown and Griendling, 2009; Lambeth, 2004). Activated cells of the innate immune system, such as neutrophils and macrophages, activate the phagocytic form of NADPH oxidase, NOX2 (gp91phox), to produce extensive amounts of superoxide for defense against invading pathogens. Various kinds of nonphagocytic cells including endothelial cells, vascular smooth muscle cells, fibroblasts, and cardiac myocytes, are also known to produce superoxide by NADPH oxidases to regulate intracellular signaling events (Brown and Griendling, 2009; Lambeth, 2004).

Other forms of NADPH oxidase (NOX1, NOX3, and NOX4, NOX5, DUOX1, DUOX 2) have been described in phagocytic and non-phagocytic cell types (Brown and Griendling, 2009; Lambeth, 2004). These other NADPH oxidase family members typically produce small amounts of ROS by for use in intracellular signaling events Most NOX enzymes are heterodimers with, and require the presence of, a 22 kDa subunit (p22phox). In addition to this subunit, many oxidases require other subunits for activity. Nox2 requires a p47phox subunit, which upon phosphorylation, binds to membrane phospholipids, interacts with p22phox, and recruits the p67phox subunit. The p67phox activator binds the small GTPase, Rac1 or Rac2, and stabilizes its recruitment, which is necessary for an activity. Similar to p47phox and p67phox, respectively, the regulatory p41NOXO1 and p51NOXA1 subunits can function in other oxidase complexes, such as Nox1 (Banfi et al., 2003; Geiszt et al., 2003;

Takeya et al., 2003). NOXO1, unlike p47 phox, does not require phosphorylation for membrane trans-location and activity. Overexpression studies have shown that there is some functionality of the different oxidases when p47phox and/or p67phox are interchanged for NOXO1 and/or NOXA1, though efficiency of superoxide production can be reduced (Banfi et al., 2003; Geiszt et al., 2003; Takeya et al., 2003). This may indicate that there is some flexability or redundancy in subunit use by Nox enzymes depending on the expression of a given subunity in a specific cell type.

There are multiple ways that NADPH oxidases are regulated by TNFα. Many reports have shown that TNFα signaling initiates increased transcription of various NADPH oxidase components, which contributes to oxidase activity. Among the components that have been shown to be upregulated by TNF are Nox2, Nox3, Nox4, p22phox, p47phox, NOXO1, and p67phox (Condino-Neto and Newburger, 1998; De Keulenaer et al., 1998; Gauss et al., 2007; Kamizato et al., 2009; Li et al., 2010; Moe et al., 2006; Newburger et al., 1991; Yoshida and Tsunawaki, 2008). Other mechanisms for potentiation (or priming) of superoxide production by TNFa have been proposed such as the phosphorylation of p47phox by downstream kinases, including PKCzeta (Frey et al., 2002), tyrosine kinases (Akimaru et al., 1992; Dewas et al., 2003; Utsumi et al., 1992), or p38 MAPK (Dang et al., 2006), perhaps through mobilization of subcellular granules (Onnheim et al., 2008). TNFα has also been suggested to regulate NADPH oxidase activity though regulation of the associated hydrogen ion channels that maintain charge equilibrium due to the electronic charge translocated during Nox activation (Chenevier-Gobeaux et al., 2007).

We and others have also recently shown that TNFα is a direct activator, NADPH oxidases in various cell types (Kim et al., 2007). In L929 cells, Nox1, NOXO1, and Rac1 form a complex with TNF receptor signaling components in a TNF-dependent manner and lead to cell death (Kim et al., 2007). In this case the tyrosine kinase inhibitor genestein, the general PKC inhibitor, bisindolymaleimide, or a specific PKC zeta peptide inhibitor had no effect on the superoxide production or cell death in response to TNFα. One mechanism of activation of Nox1 by TNFα is suggested by protein interactions between the TNF-R1 adapter proteins RIP1 and TRADD, and NOXO1. RIP1 interacts strongly with NOXO1 protein though an as yet undefined domain, while the polyproline-rich region of TRADD interacts with the N-terminal SH3 domain of NOXO1. Consistent with the known requirement for RIP1 in TNFα-dependent ROS generation and necrotic cell death, RIP1 is absolutely required for the Nox1 and Rac1 recruitment to the signaling complex. Expression of a dominant negative TRADD protein with a mutation in its polyproline region eliminates binding to NOXO1 and diminishes superoxide formation and cell death in response to TNFα. Based on our estimation of the NOXO1 affinities for RIP1 and TRADD, we have proposed that RIP1 recruits NOXO1 and the other signaling components (Nox1/NOXA1/Rac1) to the complex, where the NOXO1-TRADD interaction promotes oxidase activation (Kim et al., 2007). TNFα-induced superoxide mediated by Nox1 contributes considerably to necrotic cell death since the siRNA knockdown of Nox1 prevents both superoxide generation and cell death in response to TNFα. Therefore, Nox1 appears to be a primary source of initial ROS involved in cell death in L929 cells by this stimulus.

Other groups have also shown that TNFα signaling is coupled with NADPH oxidase activation, albeit by different molecules and mechanism. While others have confirmed TNFα activation of Nox1 (Woo et al., 2006), Nox2 and Nox3 have also more recently been shown to be more directly activated by TNFα (Anilkumar et al., 2008; Li et al., 2008; 2009a). Nox4 has also been reported to be activated downstream of TNFα (St Hilaire et al., 2008), but since it is regulated differently than the other oxidases, this appears to be through a transcriptional mechanism (Moe et al., 2006) and TNFα-dependent ROS generation has been shown to be enhanced in Nox2-overexpressing cells, but is not further enhanced upon Nox4 overexpression (Anilkumar et al., 2008).

One of the most important molecules appears to be that of riboflavin kinase, which has been shown to bind both to the death domains of TNFR1 and TRADD and also to p22phox (Yazdanpanah et al., 2009). Although its platform bridging between the complexes is in itself would be a possible potential mechanism of oxidase activation, and is necessary for the recruitment of either Nox1 or Nox2 and Rac1 to TNFR1 in HeLa cells, riboflavin kinase has an enzymatic function as well, which is likely its main mechanism of activation (Yazdanpanah et al., 2009). It converts riboflavin (vitamin B2) to flavin mononucleotide (FMN), which is then converted to Flavin Adenine Dinucleotide (FAD) by the enzyme FAD synthetase, FAD is an essential prosthetic group of NADPH oxidase, and is required for superoxide generation. Localization of the enzyme to the complex likely results in FAD generation in close proximity to the oxidase and allows for FAD loading of the enzyme.

One important concept in TNFα-induced NADPH oxidase activation is that of TNF-initiated endosomal formation. Increasingly, endocytosis of receptor components within endosomes has been identified as having a signaling role far beyond signal termination or the recycling of receptors. TNF receptor components have long been known to associate with specific lipid microdomains (Legler et al., 2003; Muppidi et al., 2004; Shen et al., 2004). This is important because NADPH oxidase components are also often associated with lipid rafts signaling platforms and caveolae (Ushio-Fukai, 2006), and these are often involved in trafficking with endosomes (Pelkmans et al., 2004). One study has shown that TNFα-induced, Nox1-mediated, ROS generation in HeLa cells requires TNFR1 internalization, and that treatment with internalization inhibitor monodansyl cadaverine or expression of a dominant negative dynamin mutant blocks ROS generation in response to TNFα (Woo et al., 2006). This is consistent with another study that reported that in smooth muscle cells where TNFα-stimulated a Nox1-dependent ROS generation in endosomes that was dependent on dynamin (Miller et al., 2010.). A dependence on endocytosis may explain the apparent requirement for gelsolin, an actin regulatory protein, in TNFα-dependent ROS generation (Li et al., 2009b), since actin is involved in endocytosis. Another study reported that Rac1 is specifically recruited to the endosomal compartment after TNFα stimulation and that TNFα-stimulated Nox2-mediated production of endosomal superoxide was dependent on receptor internalization (Li et al., 2009a). These studies suggest that endosomal formation in response to TNFα is important for ROS generation. However, it is important to remember that most of these studies measured the intracellular or endosomal ROS generation, and did not measure it extracellularly. In our study, which was measured total intracellular and extracellular superoxide, we found that no dependence on endocytosis for Nox1 activation, since neither cytochalasin D or latrunculin A inhibited superoxide formation (Kim et al., 2007). This may suggest either that the process of superoxide generation differs with cell type, or it may suggest that endosomal formation is not necessary for assembly of the oxidase machinery, but only be required for bringing the ROS in to an endosome where it can function within the cell.

Mitochondrial involvement

Cell death stimuli affect mitochondrial function in two ways; the first is the disruption of membrane potential and the initiation of the permeability transition (PT). This can lead to mitochondrial outer membrane permeability (MOMP), resulting in cytochrome c release, which activates caspase-9 in an Apaf-1 dependent mechanism and leads to apoptosis. The second contribution is through loss of energy production and the mitochondrial generation of ROS, which could potentially contribute to necrosis. However, oxidative stress itself causes the mitochondria to generate further ROS through mitochondrial protein damage, which can act as a positive feedback loop (Ott et al., 2007), which may explain apparently contradictory data on Bcl-2 function in antioxidant pathways through a conditioning effect (Kowaltowski and Fiskum, 2005). However, Bcl-2 is typically thought of in terms of inhibiting pro-oxidant-induced mitochondrial change and subsequent formation of ROS; thus the oxidative stress-induced response is suppressed by Bcl-2 in many cases (Ott et al., 2007). The thiol oxidant diamide, a crosslin-ker of thiols, mimics disulfide bond formation, and thus indu-ces mitochondrial membrane potential disruption and per-meability transition (Costantini et al., 1995), whereas mono- valent thiol-reactive compounds inhibit apoptosis (Marchetti et al., 1997).

While much recent data have established that the non-mitochondrial NADPH oxidases are involved in TNFα-initiated necrotic cell death, the source of the ROS is by no means exclusive. In fact, previous data point to the involvement in mitochondrial derived ROS (Fiers et al., 1999; Goossens et al., 1995; 1999; Schulze-Osthoff et al., 1992; 1993). In other experiments in less sensitive cell lines, our own group has detected what appears to be ROS derived from the mitochon-drial generated several hours after the addition of TNFα, and at a much later time point than we detected NADPH oxidase activity (unpublished data). While this could indicate the appearance of non-specific ROS due to the onset of cell death, it may also suggest that Nox1-produced superoxide and mitochondrial-produced ROS are linked together in programmed necrosis. It is possible that the superoxide produced by NADPH oxidases may lead to mitochondrial-produced ROS, or vice versa. One group has suggested that in necrotic cell death induced by serum withdrawal that Nox1 activation is actually downstream of the production of mitochondrial ROS (Lee et al., 2006). Indeed, another group, (which also agrees that Nox1 is critical for TNFα-induced necrosis) has suggested that Nox1 is present in both the plasma membrane and the mitochondria (Byun et al., 2008). However, the kinetics of complex formation suggests that the Nox1-produced superoxide generation is a fairly early event after TNFα treatment. Additionally, although it is clear that TNFα-induced ROS causes mitochondrial damage (Mariappan et al., 2009). ROS damage to the mitochondia does not seem to be the sole determinant of cell death as several groups have demonstrated that mitochondrial targeted antioxidants do not substantially protect from TNFα-induced ROS or necrosis (Goossens et al., 1999; Jarvis et al., 2007).

Additional evidence for a mitochondrial role in TNFα-induced ROS and its associated necrosis comes from the more recent suggestion that two BH3-only subunit of Bcl2-family proteins are important for this necrotic cell death. Over-expression of BNIP3 causes the mitochondrial permeability transition (Kim et al., 2002) leading to necrosis-like form of cell death independent of caspases (Vande Velde et al., 2000). A dominant negative mutant of BNIP3 (Bcl2/E1B 19kD interacting protein) prevented loss mitochondrial membrane potential, counteracted ROS-dependent lysosomal damage, and protected against TNFα-induced necrosis in L929 cells (Ghavami et al., 2009), while knockdown of Bmf using siRNA prevents TNFα-induced necrosis (Hitomi et al., 2008).

ROS and cell death

Reactive oxygen species play a role in both apoptotic and necrotic cell death. In general, moderate oxidative stress induces apoptosis, whereas necrotic cell death is triggered when cells have a higher exposure to ROS (Saito et al., 2006; Takeda et al., 1999; Teramoto et al., 1999). How then does ROS initiated by TNFα play a part in cell death? Some molecular targets for TNFα-induced ROS have been found, but much is yet to be discovered. ROS have been shown to induce apoptosis through the production of ceramide, the p53 activation, and the induction of the regulatory protein of the PI3-kinase, p85 (Andrieu-Abadie et al., 2001; Liu et al., 2008a; Yin et al., 1998). However, it is unclear as to whether these pathways have a significant impact on the TNF-ROS cell death pathways.

A problem with defining ROS targets is that ROS have a mixture of roles in cell death since ROS may directly oxidize cellular proteins, lipids, or nucleic acids and therefore cause general cellular damage, or ROS can initiate cell death through acting as initiators or second messengers in various signaling pathways (Morgan et al., 2007). Another problem with defining the role of ROS is when they may function at different points within at given pathway. For instance, ROS may act upstream or downstream of p53 in causing DNA damage and also affect the downsteam p53-mediated pathway (Liu et al., 2008a). Similarly, ROS may act upstream or downstream of JNK, the mitochondria, and caspase activition to lead to cell death (Festjens et al., 2006a; Kamata et al., 2005; Nakajima et al., 2008; Omori et al., 2008; Sidoti-de Fraisse et al., 1998; Ventura et al., 2004; Wicovsky et al., 2007).

One of the major ways that ROS is affects signaling is through ROS reaction with cysteine (Paulsen and Carroll, 2010), especially at the catalytic sites of enzymes. It has long been established that a classical protein tyrosine phosphatases can be inactivated by ROS oxidation of their catalytic cysteine (Groen et al., 2005; Nakashima et al., 2002; 2005). However ROS can also inactivate the dual specificity phosphatases (Kamata et al., 2005), which are capable of dephos-phorylating tyrosine and serine/threonine residues, as well as phospholipids. Oxidation of catalytic cysteines leads to reversible or irreversible inactivation of the phosphatases by ROS depending on the oxidation state of the cysteine (den Hertog et al., 2005; Groen et al., 2005). A prolonged phosphorylation status affects the activity of kinases within the cell, including the stress-activated MAP kinases, p38 and JNK (Kamata et al., 2005).

The TNFα activation of JNK has two phases that differ in how they affect cell death and also in their relationship to the generation of ROS. The first phase protects from cell death and is independent of ROS (Lamb et al., 2003; Ventura et al., 2006), while the second phase, sustained phase, is either dependent on caspase cleavage of MEKK1 (Cardone et al., 1997; Nakajima et al., 2008; Wicovsky et al., 2007) or the activation of ASK1 (see below), as well as ROS. This phase is the phase that usually contributes to both apoptosis and necrosis (Chang et al., 2006; Kamata et al., 2005; Sakon et al., 2003; Tobiume et al., 2001; Ventura et al., 2004; 2006).

Various Nox isoforms have been shown to mediate the TNFα-induced activation of JNK. We have showed that Nox1 is required for sustained activation of JNK by TNFα (Kim et al., 2007). JNK is augmented in Nox2 overexpressing 293 cells when further treated with TNFα (Anilkumar et al., 2008), while endogenous NOX3 has recently been shown to mediate the TNFα activation of JNK in HepG2 hepatocytes (Li et al., 2010).

The activation of JNK is upregulated by ROS in several ways. Firstly, JNK activity is maintained by the ROS-mediated inactivation of JNK phosphatases (Kamata et al., 2005). The glutathione S-transferase Pi (GSTπ) monomers have been reported to bind directly to the JNK C-terminus and inhibit its activity (Wang et al., 2001). Since ROS causes the oligomerization of GSTπ, this prevents monomeric GSTπ from JNK binding, resulting in JNK activity (Adler et al., 1999). As mentioned, ASK1 is a MAP3K that is important for sustained JNK phosphorylation under conditions when ROS is present (Tobiume et al., 2001). The reduced form of thioredoxin has been reported to bind to ASK1 and prevents the activation of its kinase activity. Activation occurs in a large complex with TRAFs and is designated as the ASK1 signalosome (Liu et al., 2000; Noguchi et al., 2005; Saitoh et al., 1998). When oxidized, thioredoxin is released and ASK1 is capable of binding to signaling proteins that activate it (Noguchi et al., 2005). Other reports suggest that thioredoxin inhibits ASK1 activation through an inducement of its ubiquitination and subsequent degradation (Liu and Min, 2002). Additionally, the SUMO-specific protease, SENP1 is also complexed with thioredoxin in resing endothelial cells. Upon ROS dependent oxidation of the complex, SENP1 translocates to the nucleus where it desumoylates HIPK1, which then translocates to the cytoplasm and potentiates ASK1 activation (Li et al., 2008). Since ASK1-/- MEFs are substantially resistant to the sustained JNK activity and apoptosis initiated by ROS such as H_2O_2 (Tobiume et al., 2001), the ROS-thioredoxin-ASK1 axis is believed to be an important molecular switch that may mediate second messenger ROS signals to JNK resulting in its activation.

JNK and p38 are the two main MAP kinases that are readily activated in response to stress and these two kinases play significant roles in the processes determining cellular fate during cellular stress. The activation of JNK has been shown to play central roles in many ROS-dependent apoptotic processes (Liu and Lin, 2005; Nakano et al., 2006; Shen and Liu, 2006). For instance, the suppression of JNK activity using genetic or pharmacological approaches substantially protects cells from ROS-induced apoptosis (Shen and Liu, 2006). JNK mediates phosphorylation and activation of the Itch E3 ubiquitin ligase, which ubiquitinates c-FLIP and causes its proteasomal degradation, thus preventing its inhibition of caspase-8 (Chang et al., 2006). The main mechanistic site of action for JNK in apoptosis is likely the mitochondria. The most conclusive evidence of this is from a study using JNK1-/-JNK2-/- primary MEFs which were significantly resistant to UV radiation due to protection from cytochrome c release or mitochondrial depolarization (Tournier et al., 2000). Further evidence comes from the observation that most members of the Bcl-2 family, including Bcl-2 itself, are phosphorylation targets of JNK. This suggests that the proapoptotic activity of JNK can be executed via phosphorylative regulation of Bcl-2 family members (Bogoyevitch and Kobe, 2006; Nakano et al., 2006). For instance, the phosphorylation of Bax by JNK and p38 leads to Bax activation and its translocation to mitochondria (Kim et al., 2006). This could indeed be the main molecular links between JNK and the mitochondria apoptotic machinery, as Bax and Bak are required for JNK-dependent apoptosis (Lei et al., 2002). In some reports, ROS have been shown to be downstream of JNK (Ventura et al., 2004), or dependent on caspase activation of JNK (Nakajima et al., 2008), and this may be likely due to JNK-dependent induction of mitochondrial dysfunction. ROS have also been shown to lead to the p38/JNK dependent upregulation of TNFα in macrophages (Nakao et al., 2008), which, if true as in other cell types where p38 or JNK upregulate TNF (Lee et al., 1994; 2005; Yao et al., 1997) would act as an amplification loop of the TNF signal through further production of the more potent membrane form of membrane TNFα (Weingartner et al., 2002).

In addition to its role in apoptosis, significant evidence supports the role of ROS-mediated JNK activation in necrotic cell death (Shen and Liu, 2006). Exogenous ROS induce necrotic cell death via JNK activation (Shen et al., 2004) therefore JNK is now suggested to be one essential mediator of necrotic cell death pathways (Shen and Pervaiz, 2006). ROS are crucial coactivators in TNFR1-mediated sustained JNK activation, and sustained JNK activation represents an important event in both apoptotic and necrotic TNFα-induced cell death (Chang et al., 2006; Kamata et al., 2005; Ventura et al., 2004; 2006). In L929 cells, which undergo default necrotic cell death in response to TNFα, the sustained JNK directly correlates with superoxide generation and necrotic cell death**,** and inhibition of JNK completely blocks TNFα-induced necrotic cell death of these cells (Kim et al., 2007). Other reports have shown similar results from the inhibition of sustained JNK activation using genetic or pharmacological manipulation (Kamata et al., 2005; Ventura et al., 2004; 2006), though ROS may also have JNK independent effects (Wicovsky et al., 2007).

Reactive Oxygen Species from sources other than TNFα-initiated ROS potentiates TNFα cytotoxicity in many situations. For instance, sustained JNK activation in human skin fibroblasts that is mediated by substantial ROS derived from integrin-mediated signaling potentiates TNFα/CHX-induced apoptosis without perturbation of NFkB signaling. Moreover, in HeLa and 293 cells exogenous oxidative stress promotes ligand-independent TNFα signaling and also enhances ligand-mediated TNF signaling (Ozsoy et al., 2008). It is unclear whether endogenous ROS can function in the same way, but overexpression of Nox1, NoxO1, and NoxA1 potentiates TNFR1-dependent activation of JNK but not NF-κB, and also induces TNFR1- and ASK1- dependent cell death (Pantano et al., 2007).

Cell death, NF-KB, JNK, and ROS

Typically, treatment with TNFα does not actually result in cell death. This largely due to the activation of NF-κB, which initiates transcription of pro-survival proteins (Karin and Lin, 2002), such as the cellular inhibitor of apoptosis proteins (cIAPs), the caspase-8 inhibitory protein cFLIP, the ubiquitin-editing enzyme TNFAIP3 (A20), and the antiapoptotic Bcl2 family members A1 and Bcl-xL. These proteins directly or indirectly inhibit apoptosis through their various functions. Crosstalk from NF-κB to JNK is known to prevent sustained JNK activation and thus prevent cell death (Reuther-Madrid et al., 2002; Tang et al., 2002). Specific NF-κB controlled gene targets have been shown to affect JNK activation, such as GADD45β and XIAP, (Papa et al., 2004; Tang et al., 2001). In addition, NF-κB activation induced by TNFα can directly affect ROS via increased expression of the antioxidant proteins, such as ferritin heavy chain (FHC) and manganese superoxide dismutase (MnSOD) (Jones et al., 1997; Pham et al., 2004). Therefore, NF-κB regulates genes that suppress ROS and decrease sustained JNK activation, thus preventing both apoptotic and necrotic cell death (Nakano et al., 2006; Papa et al., 2004; Reuther-Madrid et al., 2002; Tang et al., 2002).

ROS and other TNF signals

While the connection between JNK and ROS seem to be very well established, the affect of ROS on the other TNFα-induced

pathways has been much more debated. Given that ROS often target the same phosphatases that dephosphorylate JNK, the p38 and ERK pathways are often simultaneously activated with JNK. Initially, reactive oxygen species were proposed to also be involved in NF-κB activation based on the observed inhibition of TNFα-induced NF-κB by the antioxidants N-acetyl-L-cysteine (NAC) and pyrrolidine dithiocarbamate (PDTC) (Schreck et al., 1992; Staal et al., 1990). NF-κB activation was also reduced by overexpression of catalase (Schmidt et al., 1995). It was later shown that the effect of these NAC and PDTC eliminated the binding of the TNFα ligand to its receptor and by inhibiting IκBα ubiquitin ligase activity, respectively (Hayakawa et al., 2003). It is since been recognized that ROS better serve to modulate NF-κB rather than activate what NF-κB has already been activated by other mechanisms (Oliveira-Marques et al., 2009). Nevertheless, reports continue to abound as to both inhibitory and stimulatory effects of ROS on NF-κB in TNFα signaling.

Just as the effect of ROS vary between the different cell types, so the mechanisms vary widely in the prostimulatory or inhibitory effects on NF-κB. Some of these reports suggest that TNFα-induced Nox-derived ROS affects NF-κB signaling one way or another.

The stimulatory effects on NF-κB activation are less reported and characterized than the inhibitory effects. TNFα treatment was reported downregulate catalase expression in MCF-7, Caco-2 and Hct-116 cancer cells, leading to increased duration of NF-κB signal (Lupertz et al., 2008). In MCF-7 cells, Nox2 is reported to be required for endosomal superoxide that potentiates the TRAF2 recruitment to endosomal TNFR1, and is thus is required for efficient NF-κB activation (Li et al., 2009a). Rac1 (Rac1N17) suppressed TNF-induced ROS generation and subsequent NF-κB activation in HUVEC cells (Deshpande et al., 2000). Likewise, DPI, an inhibitor of NADPH oxidases, as well as dominant-negative Rac1 reduce NF-κB due to TNFα in A549 cells (Kim et al., 2008). TNFα-induced ROS is apparently involved in the pre-transcriptional activation of RelA by phosphorylation at Ser276 through a PKA dependent event in U937 cells (Jamaluddin et al., 2007). Conversely, the phosphorylation of RelA at serine 536, which contributes to its DNA-binding activity, is induced by NAC in HeLa, Hep3B, and A549 cells (Liu et al., 2008b).

The effects of ROS on inhibition of NF-κB components are more characterized. For instance, NF-κB has been shown to be glutathionylated causing less transcriptional activity (Klatt and Lamas, 2000). Likewise, IκBα is glutathionylated at cysteine 189, preventing its phosphorylation and degradation (Kil et al., 2008). Another major target for ROS is the catalytic subunit of IKK, IKKβ which is oxidized by ROS at cysteine 179 (Reynaert et al., 2006), thus subduing the NF-κB signaling by TNF. Thus ROS attenuate the NFkB signal primarily through oxidation of the many pathway components.

CONCLUSION

In conclusion, ROS affects TNFα signaling in many and diverse ways. ROS plays a role in MAP kinase signaling, NF-κB activation, apoptosis and necrosis. One of the more characterized roles of ROS is in the sustained activation of JNK, which often acts on different targets to potentiate apoptosis and necrosis. Due to the potential for ROS to act at various points and in multiple pathways during TNFα signaling, the ultimate effects of ROS are quite complex. Though we have learned much about the roles of ROS, doubtless there is still much to learn.

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REFERENCES

Adler, V., Yin, Z., Fuchs, S.Y., Benezra, M., Rosario, L., Tew, K.D., Pincus, M.R., Sardana, M., Henderson, C.J., Wolf, C.R., et al. (1999). Regulation of JNK signaling by GSTp. EMBO J. *18*, 1321-1334.

Akimaru, K., Utsumi, T., Sato, E.F., Klostergaard, J., Inoue, M., and Utsumi, K. (1992). Role of tyrosyl phosphorylation in neutrophil priming by tumor necrosis factor-alpha and granulocyte colony stimulating factor. Arch. Biochem. Biophys. *298*, 703-709.

Andrieu-Abadie, N., Gouaze, V., Salvayre, R., and Levade, T. (2001). Ceramide in apoptosis signaling: relationship with oxidative stress. Free Radic. Biol. Med. *31*, 717-728.

Anilkumar, N., Weber, R., Zhang, M., Brewer, A., and Shah, A.M. (2008). Nox4 and nox2 NADPH oxidases mediate distinct cellular redox signaling responses to agonist stimulation. Arterioscler. Thromb. Vasc. Biol. *28*, 1347-1354.

Artal-Sanz, M., Samara, C., Syntichaki, P., and Tavernarakis, N. (2006). Lysosomal biogenesis and function is critical for necrotic cell death in Caenorhabditis elegans. J. Cell Biol. *173*, 231-239.

Banfi, B., Clark, R.A., Steger, K., and Krause, K.H. (2003). Two novel proteins activate superoxide generation by the NADPH oxidase NOX1. J. Biol. Chem. *278*, 3510-3513.

Baud, V., Liu, Z.G., Bennett, B., Suzuki, N., Xia, Y., and Karin, M. (1999). Signaling by proinflammatory cytokines: oligomerization of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain. Genes Dev. *13*, 1297-1308.

Beinke, S., and Ley, S.C. (2004). Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. Biochem. J. *382*, 393-409.

Bertrand, M.J., Milutinovic, S., Dickson, K.M., Ho, W.C., Boudreault, A., Durkin, J., Gillard, J.W., Jaquith, J.B., Morris, S.J., and Barker, P.A. (2008). cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. Mol. Cell *30*, 689-700.

Blonska, M., Shambharkar, P.B., Kobayashi, M., Zhang, D., Sakurai, H., Su, B., and Lin, X. (2005). TAK1 is recruited to the tumor necrosis factor-alpha (TNF-alpha). receptor 1 complex in a receptor-interacting protein (RIP).-dependent manner and cooperates with MEKK3 leading to NF-kappaB activation. J. Biol. Chem. *280*, 43056-43063.

Bogoyevitch, M.A., and Kobe, B. (2006). Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. Microbiol. Mol. Biol. Rev. *70*, 1061-1095.

Bonizzi, G., and Karin, M. (2004). The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol. *25*, 280-288.

Boveris, A., and Chance, B. (1973). The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem. J. *134*, 707-716.

Boveris, A., and Cadenas, E. (1975). Mitochondrial production of superoxide anions and its relationship to the antimycin insensitive respiration. FEBS Lett. *54*, 311-314.

Boya, P., and Kroemer, G. (2008). Lysosomal membrane permeabilization in cell death. Oncogene *27*, 6434-6451.

Brancho, D., Ventura, J.J., Jaeschke, A., Doran, B., Flavell, R.A., and Davis, R.J. (2005). Role of MLK3 in the regulation of mitogen-activated protein kinase signaling cascades. Mol. Cell. Biol. *25*, 3670-3681.

Brown, D.I., and Griendling, K.K. (2009). Nox proteins in signal transduction. Free Radic. Biol. Med. *47*, 1239-1253.

Byun, H.S., Won, M., Park, K.A., Kim, Y.R., Choi, B.L., Lee, H., Hong, J.H., Piao, L., Park, J., Kim, J.M., et al. (2008). Prevention of TNF-induced necrotic cell death by rottlerin through a Nox1 NADPH oxidase. Exp. Mol. Med. *40*, 186-195.

Cardone, M.H., Salvesen, G.S., Widmann, C., Johnson, G., and Frisch, S.M. (1997). The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. Cell *90*, 315-323.

Chang, L., Kamata, H., Solinas, G., Luo, J.L., Maeda, S., Venuprasad, K., Liu, Y.C., and Karin, M. (2006). The E3 ubiquitin li-

gase itch couples JNK activation to TNFalpha-induced cell death by inducing c-FLIP(L) turnover. Cell *124*, 601-613.

Chenevier-Gobeaux, C., Simonneau, C., Therond, P., Bonnefont-Rousselot, D., Poiraudeau, S., Ekindjian, O.G., and Borderie, D. (2007). Implication of cytosolic phospholipase A2 (cPLA2) in the regulation of human synoviocyte NADPH oxidase (Nox2) activity. Life Sci. *81*, 1050-1058.

Cho, Y.S., Challa, S., Moquin, D., Genga, R., Ray, T.D., Guildford, M., and Chan, F.K. (2009). Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell *137*, 1112-1123.

Condino-Neto, A., and Newburger, P.E. (1998). NADPH oxidase activity and cytochrome b558 content of human Epstein-Barr-virus-transformed B lymphocytes correlate with expression of genes encoding components of the oxidase system. Arch. Biochem. Biophys. *360*, 158-164.

Costantini, P., Chernyak, B.V., Petronilli, V., and Bernardi, P. (1995). Selective inhibition of the mitochondrial permeability transition pore at the oxidation-reduction sensitive dithiol by monobromobimane. FEBS Lett. *362*, 239-242.

Dang, P.M., Stensballe, A., Boussetta, T., Raad, H., Dewas, C., Kroviarski, Y., Hayem, G., Jensen, O.N., Gougerot-Pocidalo, M.A., and El-Benna, J. (2006). A specific p47phox -serine phosphorylated by convergent MAPKs mediates neutrophil NADPH oxidase priming at inflammatory sites. J. Clin. Invest. *16*, 2033-2043.

De Keulenaer, G.W., Alexander, R.W., Ushio-Fukai, M., Ishizaka, N., and Griendling, K.K. (1998). Tumour necrosis factor alpha activates a p22phox-based NADH oxidase in vascular smooth muscle. Biochem. J. *329*, 653-657.

Degterev, A., Huang, Z., Boyce, M., Li, Y., Jagtap, P., Mizushima, N., Cuny, G.D., Mitchison, T.J., Moskowitz, M.A., and Yuan, J. (2005). Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nat. Chem. Biol. *1*, 112-119.

Degterev, A., Hitomi, J., Germscheid, M., Ch'en, I.L., Korkina, O., Teng, X., Abbott, D., Cuny, G.D., Yuan, C., Wagner, G., et al. (2008). Identification of RIP1 kinase as a specific cellular target of necrostatins. Nat. Chem. Biol. *4*, 313-321.

den Hertog, J., Groen, A., and van der Wijk, T. (2005). Redox regulation of protein-tyrosine phosphatases. Arch. Biochem. Biophys. *434*, 11-15.

Deshpande, S.S., Angkeow, P., Huang, J., Ozaki, M., and Irani, K. (2000). Rac1 inhibits TNF-alpha-induced endothelial cell apoptosis: dual regulation by reactive oxygen species. FASEB J. *14*, 1705-1714.

Devin, A., Cook, A., Lin, Y., Rodriguez, Y., Kelliher, M., and 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., Yamaoka, S., Li, Z., Karin, M., and Liu, Z. (2001). The alpha and beta subunits of IkappaB kinase (IKK) mediate TRAF2-dependent IKK recruitment to tumor necrosis factor (TNF) receptor 1 in response to TNF. Mol. Cell. Biol. *21*, 3986-3994.

Devin, A., Lin, Y., and Liu, Z.G. (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.

Dewas, C., Dang, P.M., Gougerot-Pocidalo, M.A., and El-Benna, J. (2003). TNF-alpha induces phosphorylation of p47(phox) in human neutrophils: partial phosphorylation of p47phox is a common event of priming of human neutrophils by TNF-alpha and granulocyte-macrophage colony-stimulating factor. J. Immunol. *171*, 4392-4398.

Diener, K., Wang, X.S., Chen, C., Meyer, C.F., Keesler, G., Zukowski, M., Tan, T.H., and Yao, Z. (1997). Activation of the c-Jun N-terminal kinase pathway by a novel protein kinase related to human germinal center kinase. Proc. Natl. Acad. Sci. USA *94*, 9687-9692.

Ea, C.K., Deng, L., Xia, Z.P., Pineda, G., and Chen, Z.J. (2006). Activation of IKK by TNFalpha requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. Mol. Cell *22*, 245-257.

Eliopoulos, A.G., Das, S., and Tsichlis, P.N. (2006). The tyrosine kinase Syk regulates TPL2 activation signals. J. Biol. Chem. *281*, 1371-1380.

Festjens, N., Kalai, M., Smet, J., Meeus, A., Van Coster, R., Saelens, X., and Vandenabeele, P. (2006a). Butylated hydroxyanisole is more than a reactive oxygen species scavenger. Cell Death Differ. *13*, 166-169.

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

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

Fiers, W., Beyaert, R., Declercq, W., and Vandenabeele, P. (1999). More than one way to die: apoptosis, necrosis and reactive oxygen damage. Oncogene *18*, 7719-7730.

Frey, R.S., Rahman, A., Kefer, J.C., Minshall, R.D., and Malik, A.B. (2002). PKCzeta regulates TNF-alpha-induced activation of NADPH oxidase in endothelial cells. Circ. Res. *90*, 1012-1019.

Fu, C.A., Shen, M., Huang, B.C., Lasaga, J., Payan, D.G., and Luo, Y. (1999). TNIK, a novel member of the germinal center kinase family that activates the c-Jun N-terminal kinase pathway and regulates the cytoskeleton. J. Biol. Chem. *274*, 30729-30737.

Gauss, K.A., Nelson-Overton, L.K., Siemsen, D.W., Gao, Y., DeLeo, F.R., and Quinn, M.T. (2007). Role of NF-kappaB in transcriptional regulation of the phagocyte NADPH oxidase by tumor necrosis factor-alpha. J. Leukoc. Biol. *82*, 729-741.

Geiszt, M., Lekstrom, K., Witta, J., and Leto, T.L. (2003). Proteins homologous to p47phox and p67phox support superoxide production by NAD(P)H oxidase 1 in colon epithelial cells. J. Biol. Chem. *278*, 20006-20012.

Ghavami, S., Eshraghi, M., Kadkhoda, K., Mutawe, M.M., Maddika, S., Bay, G.H., Wesselborg, S., Halayko, A.J., Klonisch, T., and Los, M. (2009). Role of BNIP3 in TNF-induced cell death--TNF upregulates BNIP3 expression. Biochim. Biophys. Acta *1793*, 546-560.

Goossens, V., Grooten, J., De Vos, K., and Fiers, W. (1995). Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. Proc. Natl. Acad. Sci. USA *92*, 8115-8119.

Goossens, V., De Vos, K., Vercammen, D., Steemans, M., Vancompernolle, K., Fiers, W., Vandenabeele, P., and Grooten, J. (1999). Redox regulation of TNF signaling. BioFactors *10*, 145-156.

Groen, A., Lemeer, S., van der Wijk, T., Overvoorde, J., Heck, A.J., Ostman, A., Barford, D., Slijper, M., and den Hertog, J. (2005). Differential oxidation of protein-tyrosine phosphatases. J. Biol. Chem. *280*, 10298-10304.

Guicciardi, M.E., and Gores, G.J. (2009). Life and death by death receptors. FASEB J. *23*, 1625-1637.

Hayakawa, M., Miyashita, H., Sakamoto, I., Kitagawa, M., Tanaka, H., Yasuda, H., Karin, M., and Kikugawa, K. (2003). Evidence that reactive oxygen species do not mediate NF-kappaB activation. EMBO J. *22*, 3356-3366.

Hayden, M.S., and Ghosh, S. (2008). Shared principles in NF-kappaB signaling. Cell *132*, 344-362.

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

Hitomi, J., Christofferson, D.E., Ng, A., Yao, J., Degterev, A., Xavier, R.J., and Yuan, J. (2008). Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. Cell *135*, 1311-1323.

Hoeflich, K.P., Yeh, W.C., Yao, Z., Mak, T.W., and Woodgett, J.R. (1999). Mediation of TNF receptor-associated factor effector functions by apoptosis signal-regulating kinase-1 (ASK1). Oncogene *18*, 5814-5820.

Holmgren, A. (2000). Antioxidant function of thioredoxin and glutaredoxin systems. Antioxid. Redox. Signal. *2*, 811-820.

Jamaluddin, M., Wang, S., Boldogh, I., Tian, B., and Brasier, A.R. (2007). TNF-alpha-induced NF-kappaB/RelA Ser(276) phosphorylation and enhanceosome formation is mediated by an ROS-dependent PKAc pathway. Cell. Signal. *19*, 1419-1433.

Jarvis, R.M., Gottert, J., Murphy, M.P., and Ledgerwood, E.C. (2007). Mitochondria-targeted antioxidants do not prevent tumour necrosis factor-induced necrosis of L929 cells. Free Radic. Res. *41*, 1041-1046.

Jones, P.L., Ping, D., and Boss, J.M. (1997). Tumor necrosis factor alpha and interleukin-1beta regulate the murine manganese superoxide dismutase gene through a complex intronic enhancer

involving C/EBP-beta and NF-kappaB. Mol. Cell. Biol. *17*, 6970-6981.
Kamata, H., Honda, S., Maeda, S., Chang, L., Hirata, H., and Karin, M. (2005). Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell *120*, 649-661.
Kamizato, M., Nishida, K., Masuda, K., Takeo, K., Yamamoto, Y., Kawai, T., Teshima-Kondo, S., Tanahashi, T., and Rokutan, K. (2009). Interleukin 10 inhibits interferon gamma- and tumor necrosis factor alpha-stimulated activation of NADPH oxidase 1 in human colonic epithelial cells and the mouse colon. J. Gastroenterol. *44*, 1172-1184.
Karin, M., and Lin, A. (2002). NF-kappaB at the crossroads of life and death. Nat. Immunol. *3*, 221-227.
Kelliher, M.A., Grimm, S., Ishida, Y., Kuo, F., Stanger, B.Z., and Leder, P. (1998). The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. Immunity *8*, 297-303.
Kil, I.S., Kim, S.Y., and Park, J.W. (2008). Glutathionylation regulates IkappaB. Biochem. Biophys. Res. Commun. *373*, 169-173.
Kim, J.Y., Cho, J.J., Ha, J., and Park, J.H. (2002). The carboxy terminal C-tail of BNip3 is crucial in induction of mitochondrial permeability transition in isolated mitochondria. Arch. Biochem. Biophys. *398*, 147-152.
Kim, B.J., Ryu, S.W., and Song, B.J. (2006). JNK- and p38 kinase-mediated phosphorylation of Bax leads to its activation and mitochondrial translocation and to apoptosis of human hepatoma HepG2 cells. J. Biol. Chem. *281*, 21256-21265.
Kim, Y.S., Morgan, M.J., Choksi, S., and Liu, Z.G. (2007). TNF-induced activation of the Nox1 NADPH oxidase and its role in the induction of necrotic cell death. Mol. Cell *26*, 675-687.
Kim, H., Hwang, J.S., Woo, C.H., Kim, E.Y., Kim, T.H., Cho, K.J., Kim, J.H., Seo, J.M., and Lee, S.S. (2008). TNF-alpha-induced up-regulation of intercellular adhesion molecule-1 is regulated by a Rac-ROS-dependent cascade in human airway epithelial cells. Exp. Mol. Med. *40*, 167-175.
Klatt, P., and Lamas, S. (2000). Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. Eur. J. Biochem. *267*, 4928-4944.
Kowaltowski, A.J., and Fiskum, G. (2005). Redox mechanisms of cytoprotection by Bcl-2. Antioxid. Redox. Signal. *7*, 508-514.
Kroemer, G., El-Deiry, W.S., Golstein, P., Peter, M.E., Vaux, D., Vandenabeele, P., Zhivotovsky, B., Blagosklonny, M.V., Malorni, W., Knight, R.A., et al. (2005). Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ. *12*, 1463-1467.
Kurada, B.R., Li, L.C., Mulherkar, N., Subramanian, M., Prasad, K.V., and Prabhakar, B.S. (2009). MADD, a splice variant of IG20, is indispensable for MAPK activation and protection against apoptosis upon tumor necrosis factor-alpha treatment. J. Biol. Chem. *284*, 13533-13541.
Lamb, J.A., Ventura, J.J., Hess, P., Flavell, R.A., and Davis, R.J. (2003). JunD mediates survival signaling by the JNK signal transduction pathway. Mol. Cell *11*, 1479-1489.
Lambeth, J.D. (2004). NOX enzymes and the biology of reactive oxygen. Nat. Rev. Immunol. *4*, 181-189.
Lee, J.C., Laydon, J.T., McDonnell, P.C., Gallagher, T.F., Kumar, S., Green, D., McNulty, D., Blumenthal, M.J., Heys, J.R., Landvatter, S.W., et al. (1994). A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature *372*, 739-746.
Lee, S.Y., Reichlin, A., Santana, A., Sokol, K.A., Nussenzweig, M.C., and Choi, Y. (1997). TRAF2 is essential for JNK but not NF-kappaB activation and regulates lymphocyte proliferation and survival. Immunity *7*, 703-713.
Lee, T.H., Huang, Q., Oikemus, S., Shank, J., Ventura, J.J., Cusson, N., Vaillancourt, R.R., Su, B., Davis, R.J., and Kelliher, M.A. (2003). The death domain kinase RIP1 is essential for tumor necrosis factor alpha signaling to p38 mitogen-activated protein kinase. Mol. Cell. Biol. *23*, 8377-8385.
Lee, T.H., Shank, J., Cusson, N., and Kelliher, M.A. (2004). The kinase activity of Rip1 is not required for tumor necrosis factor-alpha-induced IkappaB kinase or p38 MAP kinase activation or for the ubiquitination of Rip1 by Traf2. J. Biol. Chem. *279*, 33185-33191.
Lee, D.C., Cheung, C.Y., Law, A.H., Mok, C.K., Peiris, M., and Lau, A.S. (2005). p38 mitogen-activated protein kinase-dependent hyperinduction of tumor necrosis factor alpha expression in response to avian influenza virus H5N1. J. Virol. *79*, 10147-10154.
Lee, S.B., Bae, I.H., Bae, Y.S., and Um, H.D. (2006). Link between mitochondria and NADPH oxidase 1 isozyme for the sustained production of reactive oxygen species and cell death. J. Biol. Chem. *281*, 36228-36235.
Legler, D.F., Micheau, O., Doucey, M.A., Tschopp, J., and Bron, C. (2003). Recruitment of TNF receptor 1 to lipid rafts is essential for TNFalpha-mediated NF-kappaB activation. Immunity *18*, 655-664.
Lei, K., Nimnual, A., Zong, W.X., Kennedy, N.J., Flavell, R.A., Thompson, C.B., Bar-Sagi, D., and Davis, R.J. (2002). The Bax subfamily of Bcl2-related proteins is essential for apoptotic signal transduction by c-Jun NH(2)-terminal kinase. Mol. Cell. Biol. *22*, 4929-4942.
Li, Y., Johnson, N., Capano, M., Edwards, M., and Crompton, M. (2004). Cyclophilin-D promotes the mitochondrial permeability transition but has opposite effects on apoptosis and necrosis. Biochem. J. *383*, 101-109.
Li, Q., Spencer, N.Y., Oakley, F.D., Buettner, G.R., and Engelhardt, J.F. (2009a). Endosomal Nox2 facilitates redox-dependent induction of NF-kappaB by TNF-alpha. Antioxid. Redox. Signal. *11*, 1249-1263.
Li, Q., Ye, Z., Wen, J., Ma, L., He, Y., Lian, G., Wang, Z., Wei, L., Wu, D., and Jiang, B. (2009b). Gelsolin, but not its cleavage, is required for TNF-induced ROS generation and apoptosis in MCF-7 cells. Biochem. Biophys. Res. Commun. *385*, 284-289.
Li, L., He, Q., Huang, X., Man, Y., Zhou, Y., Wang, S., Wang, J., and Li, J. (2010). NOX3-derived reactive oxygen species promote TNF-alpha-induced reductions in hepatocyte glycogen levels via a JNK pathway. FEBS Lett. *584*, 995-1000.
Liedtke, C., Plumpe, J., Kubicka, S., Bradham, C.A., Manns, M.P., Brenner, D.A., and Trautwein, C. (2002). Jun kinase modulates tumor necrosis factor-dependent apoptosis in liver cells. Hepatology *36*, 315-325.
Lin, Y., Devin, A., Rodriguez, Y., and Liu, Z.G. (1999). Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. Genes Dev. *13*, 2514-2526.
Lin, Y., Choksi, S., Shen, H.M., Yang, Q.F., Hur, G.M., Kim, Y.S., Tran, J.H., Nedospasov, S.A., and Liu, Z.G. (2004). Tumor necrosis factor-induced nonapoptotic cell death requires receptor-interacting protein-mediated cellular reactive oxygen species accumulation. J. Biol. Chem. *279*, 10822-10828.
Liu, Y., and Min, W. (2002). Thioredoxin promotes ASK1 ubiquitination and degradation to inhibit ASK1-mediated apoptosis in a redox activity-independent manner. Circ. Res. *90*, 1259-1266.
Liu, J., and Lin, A. (2005). Role of JNK activation in apoptosis: a double-edged sword. Cell. Res. *15*, 36-42.
Liu, H., Nishitoh, H., Ichijo, H., and Kyriakis, J.M. (2000). Activation of apoptosis signal-regulating kinase 1 (ASK1) by tumor necrosis factor receptor-associated factor 2 requires prior dissociation of the ASK1 inhibitor thioredoxin. Mol. Cell. Biol. *20*, 2198-2208.
Liu, H.H., Xie, M., Schneider, M.D., and Chen, Z.J. (2006). Essential role of TAK1 in thymocyte development and activation. Proc. Natl. Acad. Sci. USA *103*, 11677-11682.
Liu, B., Chen, Y., and St Clair, D.K. (2008a). ROS and p53: a versatile partnership. Free Radic. Biol. Med. *44*, 1529-1535.
Liu, J., Yoshida, Y., and Yamashita, U. (2008b). DNA-binding activity of NF-kappaB and phosphorylation of p65 are induced by N-acetylcysteine through phosphatidylinositol (PI) 3-kinase. Mol. Immunol. *45*, 3984-3989.
Los, M., Mozoluk, M., Ferrari, D., Stepczynska, A., Stroh, C., Renz, A., Herceg, Z., Wang, Z.Q., and Schulze-Osthoff, K. (2002). Activation and caspase-mediated inhibition of PARP: a molecular switch between fibroblast necrosis and apoptosis in death receptor signaling. Mol. Biol. Cell *13*, 978-988.
Luke, C.J., Pak, S.C., Askew, Y.S., Naviglia, T.L., Askew, D.J., Nobar, S.M., Vetica, A.C., Long, O.S., Watkins, S.C., Stolz, D.B., et al. (2007). An intracellular serpin regulates necrosis by inhibiting the induction and sequelae of lysosomal injury. Cell *130*, 1108-1119.
Lupertz, R., Chovolou, Y., Kampkotter, A., Watjen, W., and Kahl, R. (2008). Catalase overexpression impairs TNF-alpha induced NF-kappaB activation and sensitizes MCF-7 cells against TNF-alpha. J. Cell Biochem. *103*, 1497-1511.
Mahoney, D.J., Cheung, H.H., Mrad, R.L., Plenchette, S., Simard, C., Enwere, E., Arora, V., Mak, T.W., Lacasse, E.C., Waring, J., et al. (2008). Both cIAP1 and cIAP2 regulate TNFalpha-mediated NF-kappaB activation. Proc. Natl. Acad. Sci. USA *105*,

11778-11783.
Marchetti, P., Decaudin, D., Macho, A., Zamzami, N., Hirsch, T., Susin, S.A., and Kroemer, G. (1997). Redox regulation of apoptosis: impact of thiol oxidation status on mitochondrial function. Eur. J. Immunol. *27*, 289-296.
Mariappan, N., Elks, C.M., Fink, B., and Francis, J. (2009). TNF-induced mitochondrial damage: a link between mitochondrial complex I activity and left ventricular dysfunction. Free Radic. Biol. Med. *46*, 462-470.
Meier, B., Radeke, H.H., Selle, S., Younes, M., Sies, H., Resch, K., and Habermehl, G.G. (1989). Human fibroblasts release reactive oxygen species in response to interleukin-1 or tumour necrosis factor-alpha. Biochem. J. *263*, 539-545.
Miller, F.J., Jr., Chu, X., Stanic, B., Tian, X., Sharma, R.V., Davisson, R.L., and Lamb, F.S. (2010). A differential role for endocytosis in receptor-mediated activation of Nox1. Antioxid. Redox. Signal. *12*, 583-593.
Moe, K.T., Aulia, S., Jiang, F., Chua, Y.L., Koh, T.H., Wong, M.C., and Dusting, G.J. (2006). Differential upregulation of Nox homologues of NADPH oxidase by tumor necrosis factor-alpha in human aortic smooth muscle and embryonic kidney cells. J. Cell. Mol. Med. *10*, 231-239.
Morgan, M.J., Kim, Y.S., and Liu, Z. (2007). Lipid rafts and oxidative stress-induced cell death. Antioxid. Redox Signal. *9*, 1471-1483.
Moriguchi, T., Toyoshima, F., Masuyama, N., Hanafusa, H., Gotoh, Y., and Nishida, E. (1997). A novel SAPK/JNK kinase, MKK7, stimulated by TNFalpha and cellular stresses. EMBO J. *16*, 7045-7053.
Muppidi, J.R., Tschopp, J., and Siegel, R.M. (2004). Life and death decisions: secondary complexes and lipid rafts in TNF receptor family signal transduction. Immunity *21*, 461-465.
Nakagawa, T., Shimizu, S., Watanabe, T., Yamaguchi, O., Otsu, K., Yamagata, H., Inohara, H., Kubo, T., and Tsujimoto, Y. (2005). Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. Nature *434*, 652-658.
Nakajima, A., Kojima, Y., Nakayama, M., Yagita, H., Okumura, K., and Nakano, H. (2008). Downregulation of c-FLIP promotes caspase-dependent JNK activation and reactive oxygen species accumulation in tumor cells. Oncogene *27*, 76-84.
Nakano, K., Yamauchi, J., Nakagawa, K., Itoh, H., and Kitamura, N. (2000). NESK, a member of the germinal center kinase family that activates the c-Jun N-terminal kinase pathway and is expressed during the late stages of embryogenesis. J. Biol. Chem. *275*, 20533-20539.
Nakano, H., Nakajima, A., Sakon-Komazawa, S., Piao, J. H., Xue, X., and Okumura, K. (2006). Reactive oxygen species mediate crosstalk between NF-kappaB and JNK. Cell Death Differ. *13*, 730-737.
Nakashima, I., Kato, M., Akhand, A.A., Suzuki, H., Takeda, K., Hossain, K., and Kawamoto, Y. (2002). Redox-linked signal transduction pathways for protein tyrosine kinase activation. Antioxid. Redox Signal. *4*, 517-531.
Nakashima, I., Takeda, K., Kawamoto, Y., Okuno, Y., Kato, M., and Suzuki, H. (2005). Redox control of catalytic activities of membrane-associated protein tyrosine kinases. Arch. Biochem. Biophys. *434*, 3-10.
Newburger, P.E., Dai, Q., and Whitney, C. (1991). In vitro regulation of human phagocyte cytochrome b heavy and light chain gene expression by bacterial lipopolysaccharide and recombinant human cytokines. J. Biol. Chem. *266*, 16171-16177.
Nishitoh, H., Saitoh, M., Mochida, Y., Takeda, K., Nakano, H., Rothe, M., Miyazono, K., and Ichijo, H. (1998). ASK1 is essential for JNK/SAPK activation by TRAF2. Mol. Cell *2*, 389-395.
Noguchi, T., Takeda, K., Matsuzawa, A., Saegusa, K., Nakano, H., Gohda, J., Inoue, J., and Ichijo, H. (2005). Recruitment of tumor necrosis factor receptor-associated factor family proteins to apoptosis signal-regulating kinase 1 signalosome is essential for oxidative stress-induced cell death. J. Biol. Chem. *280*, 37033-37040.
Oliveira-Marques, V., Marinho, H.S., Cyrne, L., and Antunes, F. (2009). Role of hydrogen peroxide in NF-kappaB activation: from inducer to modulator. Antioxid. Redox Signal. *11*, 2223-2243.
Omori, E., Morioka, S., Matsumoto, K., and Ninomiya-Tsuji, J. (2008). TAK1 regulates reactive oxygen species and cell death in keratinocytes, which is essential for skin integrity. J. Biol. Chem. *283*, 26161-26168.
Onnheim, K., Bylund, J., Boulay, F., Dahlgren, C., and Forsman, H. (2008). Tumour necrosis factor (TNF)-alpha primes murine neutrophils when triggered via formyl peptide receptor-related sequence 2, the murine orthologue of human formyl peptide receptor-like 1, through a process involving the type I TNF receptor and subcellular granule mobilization. Immunology *125*, 591-600.
Ott, M., Gogvadze, V., Orrenius, S., and Zhivotovsky, B. (2007). Mitochondria, oxidative stress and cell death. Apoptosis *12*, 913-922.
Ozsoy, H.Z., Sivasubramanian, N., Wieder, E.D., Pedersen, S., and Mann, D.L. (2008). Oxidative stress promotes ligand-independent and enhanced ligand-dependent tumor necrosis factor receptor signaling. J. Biol. Chem. *283*, 23419-23428.
Pantano, C., Anathy, V., Ranjan, P., Heintz, N.H., and Janssen-Heininger, Y.M. (2007). Nonphagocytic oxidase 1 causes death in lung epithelial cells via a TNF-RI-JNK signaling axis. Am. J. Respir. Cell. Mol. Biol. *36*, 473-479.
Papa, S., Zazzeroni, F., Bubici, C., Jayawardena, S., Alvarez, K., Matsuda, S., Nguyen, D.U., Pham, C.G., Nelsbach, A.H., Melis, T., et al. (2004). Gadd45 beta mediates the NF-kappa B suppression of JNK signalling by targeting MKK7/JNKK2. Nat. Cell. Biol. *6*, 146-153.
Paulsen, C.E., and Carroll, K.S. (2010). Orchestrating redox signaling networks through regulatory cysteine switches. ACS Chem. Biol. *5*, 47-62.
Pham, C.G., Bubici, C., Zazzeroni, F., Papa, S., Jones, J., Alvarez, K., Jayawardena, S., De Smaele, E., Cong, R., Beaumont, C., et al. (2004). Ferritin heavy chain upregulation by NF-kappaB inhibits TNFalpha-induced apoptosis by suppressing reactive oxygen species. Cell *119*, 529-542.
Poyet, J.L., Srinivasula, S.M., Lin, J.H., Fernandes-Alnemri, T., Yamaoka, S., Tsichlis, P.N., and Alnemri, E.S. (2000). Activation of the Ikappa B kinases by RIP via IKKgamma/NEMO-mediated oligomerization. J. Biol. Chem. *275*, 37966-37977.
Quinn, M.T., Ammons, M.C., and Deleo, F.R. (2006). The expanding role of NADPH oxidases in health and disease: no longer just agents of death and destruction. Clin. Sci. *111*, 1-20.
Reuther-Madrid, J.Y., Kashatus, D., Chen, S., Li, X., Westwick, J., Davis, R.J., Earp, H.S., Wang, C.Y., and Baldwin Jr, A.S., Jr. (2002). The p65/RelA subunit of NF-kappaB suppresses the sustained, antiapoptotic activity of Jun kinase induced by tumor necrosis factor. Mol. Cell. Biol. *22*, 8175-8183.
Reynaert, N.L., van der Vliet, A., Guala, A.S., McGovern, T., Hristova, M., Pantano, C., Heintz, N.H., Heim, J., Ho, Y.S., Matthews, D.E., et al. (2006). Dynamic redox control of NF-kappaB through glutaredoxin-regulated S-glutathionylation of inhibitory kappaB kinase beta. Proc. Natl. Acad. Sci. USA *103*, 13086-13091.
Rhee, S.G., Yang, K.S., Kang, S.W., Woo, H.A., and Chang, T.S. (2005). Controlled elimination of intracellular H(2).O(2).: regulation of peroxiredoxin, catalase, and glutathione peroxidase via post-translational modification. Antioxid. Redox Signal. *7*, 619-626.
Saito, Y., Nishio, K., Ogawa, Y., Kimata, J., Kinumi, T., Yoshida, Y., Noguchi, N., and Niki, E. (2006). Turning point in apoptosis/necrosis induced by hydrogen peroxide. Free Radic. Res. *40*, 619-630.
Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K., Sawada, Y., Kawabata, M., Miyazono, K., and Ichijo, H. (1998). Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J. *17*, 2596-2606.
Sakon, S., Xue, X., Takekawa, M., Sasazuki, T., Okazaki, T., Kojima, Y., Piao, J.H., Yagita, H., Okumura, K., Doi, T., et al. (2003). NF-kappaB inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. EMBO J. *22*, 3898-3909.
Sathyanarayana, P., Barthwal, M.K., Kundu, C.N., Lane, M.E., Bergmann, A., Tzivion, G., and Rana, A. (2002). Activation of the Drosophila MLK by ceramide reveals TNF-alpha and ceramide as agonists of mammalian MLK3. Mol. Cell *10*, 1527-1533.
Sato, S., Sanjo, H., Takeda, K., Ninomiya-Tsuji, J., Yamamoto, M., Kawai, T., Matsumoto, K., Takeuchi, O., and Akira, S. (2005). Essential function for the kinase TAK1 in innate and adaptive immune responses. Nat. Immunol. *6*, 1087-1095.
Sato, T., Machida, T., Takahashi, S., Murase, K., Kawano, Y., Hayashi, T., Iyama, S., Takada, K., Kuribayashi, K., Sato, Y., et al.

(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.

Schmidt, K.N., Amstad, P., Cerutti, P., and Baeuerle, P.A. (1995). The roles of hydrogen peroxide and superoxide as messengers in the activation of transcription factor NF-kappa B. Chem. Biol. *2*, 13-22.

Schreck, R., Meier, B., Mannel, D.N., Droge, W., and Baeuerle, P.A. (1992). Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. J. Exp. Med. *175*, 1181-1194.

Schulze-Osthoff, K., Bakker, A.C., Vanhaesebroeck, B., Beyaert, R., Jacob, W.A., and Fiers, W. (1992). Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. J. Biol. Chem. *267*, 5317-5323.

Schulze-Osthoff, K., Beyaert, R., Vandevoorde, V., Haegeman, G., and Fiers, W. (1993). Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene-inductive effects of TNF. EMBO J. *12*, 3095-3104.

Shen, H.M., and Liu, Z.G. (2006). JNK signaling pathway is a key modulator in cell death mediated by reactive oxygen and nitrogen species. Free Radic. Biol. Med. *40*, 928-939.

Shen, H.M., and Pervaiz, S. (2006). TNF receptor superfamily-induced cell death: redox-dependent execution. FASEB J. *20*, 1589-1598.

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

Shi, C.S., and Kehrl, J.H. (1997). Activation of stress-activated protein kinase/c-Jun N-terminal kinase, but not NF-kappaB, by the tumor necrosis factor (TNF) receptor 1 through a TNF receptor-associated factor 2- and germinal center kinase related-dependent pathway. J. Biol. Chem. *272*, 32102-32107.

Shi, C.S., Leonardi, A., Kyriakis, J., Siebenlist, U., and Kehrl, J.H. (1999). TNF-mediated activation of the stress-activated protein kinase pathway: TNF receptor-associated factor 2 recruits and activates germinal center kinase related. J. Immunol. *163*, 3279-3285.

Shim, J.H., Xiao, C., Paschal, A.E., Bailey, S.T., Rao, P., Hayden, M.S., Lee, K.Y., Bussey, C., Steckel, M., Tanaka, N., et al. (2005). TAK1, but not TAB1 or TAB2, plays an essential role in multiple signaling pathways *in vivo*. Genes Dev. *19*, 2668-2681.

Sidoti-de Fraisse, C., Rincheval, V., Risler, Y., Mignotte, B., and Vayssiere, J.L. (1998). TNF-alpha activates at least two apoptotic signaling cascades. Oncogene *17*, 1639-1651.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. Exp. Physiol. *82*, 291-295.

St Hilaire, C., Koupenova, M., Carroll, S. H., Smith, B.D., and Ravid, K. (2008). TNF-alpha upregulates the A2B adenosine receptor gene: The role of NAD(P)H oxidase 4. Biochem. Biophys. Res. Commun. *375*, 292-296.

Staal, F.J., Roederer, M., and Herzenberg, L.A. (1990). Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA *87*, 9943-9947.

Tada, K., Okazaki, T., Sakon, S., Kobarai, T., Kurosawa, K., Yamaoka, S., Hashimoto, H., Mak, T.W., Yagita, H., Okumura, K., et al. (2001). Critical roles of TRAF2 and TRAF5 in tumor necrosis factor-induced NF-kappa B activation and protection from cell death. J. Biol. Chem. *276*, 36530-36534.

Takaesu, G., Surabhi, R.M., Park, K.J., Ninomiya-Tsuji, J., Matsumoto, K., and Gaynor, R.B. (2003). TAK1 is critical for IkappaB kinase-mediated activation of the NF-kappaB pathway. J. Mol. Biol. *326*, 105-115.

Takeda, M., Shirato, I., Kobayashi, M., and Endou, H. (1999). Hydrogen peroxide induces necrosis, apoptosis, oncosis and apoptotic oncosis of mouse terminal proximal straight tubule cells. Nephron *81*, 234-238.

Takeya, R., Ueno, N., Kami, K., Taura, M., Kohjima, M., Izaki, T., Nunoi, H., and Sumimoto, H. (2003). Novel human homologues of p47phox and p67phox participate in activation of superoxide-producing NADPH oxidases. J. Biol. Chem. *278*, 25234-25246.

Tang, G., Minemoto, Y., Dibling, B., Purcell, N.H., Li, Z., Karin, M., and Lin, A. (2001). Inhibition of JNK activation through NF-kappaB target genes. Nature *414*, 313-317.

Tang, F., Tang, G., Xiang, J., Dai, Q., Rosner, M.R., and Lin, A. (2002). The absence of NF-kappaB-mediated inhibition of c-Jun N-terminal kinase activation contributes to tumor necrosis factor alpha-induced apoptosis. Mol. Cell. Biol. *22*, 8571-8579.

Teramoto, S., Tomita, T., Matsui, H., Ohga, E., Matsuse, T., and Ouchi, Y. (1999). Hydrogen peroxide-induced apoptosis and necrosis in human lung fibroblasts: protective roles of glutathione. Jpn. J. Pharmacol. *79*, 33-40.

Tobiume, K., Matsuzawa, A., Takahashi, T., Nishitoh, H., Morita, K., Takeda, K., Minowa, O., Miyazono, K., Noda, T., and Ichijo, H. (2001). ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. EMBO Rep. *2*, 222-228.

Tournier, C., Dong, C., Turner, T.K., Jones, S.N., Flavell, R.A., and Davis, R.J. (2001). MKK7 is an essential component of the JNK signal transduction pathway activated by proinflammatory cytokines. Genes Dev. *15*, 1419-1426.

Ushio-Fukai, M. (2009). Compartmentalization of redox signaling through NADPH oxidase-derived ROS. Antioxid. Redox Signal. *11*, 1289-1299.

Utsumi, T., Klostergaard, J., Akimaru, K., Edashige, K., Sato, E.F., and Utsumi, K. (1992). Modulation of TNF-alpha-priming and stimulation-dependent superoxide generation in human neutrophils by protein kinase inhibitors. Arch. Biochem. Biophys. *294*, 271-278.

Vallabhapurapu, S., and Karin, M. (2009). Regulation and function of NF-kappaB transcription factors in the immune system. Annu. Rev. Immunol. *27*, 693-733.

Vande Velde, C., Cizeau, J., Dubik, D., Alimonti, J., Brown, T., Israels, S., Hakem, R., and Greenberg, A.H. (2000). BNIP3 and genetic control of necrosis-like cell death through the mitochondrial permeability transition pore. Mol. Cell. Biol. *20*, 5454-5468.

Vandenabeele, P., Vanden Berghe, T., and Festjens, N. (2006). Caspase inhibitors promote alternative cell death pathways. Sci. STKE 2006, pe44.

Varfolomeev, E., Goncharov, T., Fedorova, A.V., Dynek, J.N., Zobel, K., Deshayes, K., Fairbrother, W.J., and Vucic, D. (2008). c-IAP1 and c-IAP2 are critical mediators of tumor necrosis factor alpha (TNFalpha)-induced NF-kappaB activation. J. Biol. Chem. *283*, 24295-24299.

Ventura, J.J., Cogswell, P., Flavell, R.A., Baldwin, A.S., Jr., and Davis, R.J. (2004). JNK potentiates TNF-stimulated necrosis by increasing the production of cytotoxic reactive oxygen species. Genes Dev. *18*, 2905-2915.

Ventura, J.J., Hubner, A., Zhang, C., Flavell, R.A., Shokat, K.M., and Davis, R.J. (2006). Chemical genetic analysis of the time course of signal transduction by JNK. Mol. Cell *21*, 701-710.

Wajant, H. (2003). Death receptors. Essays Biochem. *39*, 53-71.

Wang, T., Arifoglu, P., Ronai, Z., and Tew, K.D. (2001). Glutathione S-transferase P1-1 (GSTP1-1). inhibits c-Jun N-terminal kinase (JNK1) signaling through interaction with the C terminus. J. Biol. Chem. *276*, 20999-21003.

Weingartner, M., Siegmund, D., Schlecht, U., Fotin-Mleczek, M., Scheurich, P., and Wajant, H. (2002). Endogenous membrane tumor necrosis factor (TNF) is a potent amplifier of TNF receptor 1-mediated apoptosis. J. Biol. Chem. *277*, 34853-34859.

Wicovsky, A., Muller, N., Daryab, N., Marienfeld, R., Kneitz, C., Kavuri, S., Leverkus, M., Baumann, B., and Wajant, H. (2007). Sustained JNK activation in response to tumor necrosis factor is mediated by caspases in a cell type-specific manner. J. Biol. Chem. *282*, 2174-2183.

Wong, W.W., Gentle, I.E., Nachbur, U., Anderton, H., Vaux, D.L., and Silke, J. (2010). RIPK1 is not essential for TNFR1-induced activation of NF-kappaB. Cell Death Differ. *17*, 482-487.

Woo, C.H., Kim, T.H., Choi, J.A., Ryu, H.C., Lee, J.E., You, H.J., Bae, Y.S., and Kim, J.H. (2006). Inhibition of receptor internalization attenuates the TNFalpha-induced ROS generation in non-phagocytic cells. Biochem. Biophys. Res. Commun. *351*, 972-978.

Wu, C.J., Conze, D.B., Li, T., Srinivasula, S.M., and Ashwell, J.D. (2006). Sensing of Lys 63-linked polyubiquitination by NEMO is a key event in NF-kappaB activation [corrected]. Nat. Cell. Biol. *8*, 398-406.

Wysk, M., Yang, D.D., Lu, H.T., Flavell, R.A., and Davis, R.J. (1999). Requirement of mitogen-activated protein kinase kinase 3 (MKK3) for tumor necrosis factor-induced cytokine expression. Proc. Natl. Acad. Sci. USA *96*, 3763-3768.

Xia, Y., Makris, C., Su, B., Li, E., Yang, J., Nemerow, G.R., and Karin, M. (2000). MEK kinase 1 is critically required for c-Jun N-

terminal kinase activation by proinflammatory stimuli and growth factor-induced cell migration. Proc. Natl. Acad. Sci. USA *97*, 5243-5248.

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

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

Yao, J., Mackman, N., Edgington, T.S., and Fan, S.T. (1997). Lipopolysaccharide induction of the tumor necrosis factor-alpha promoter in human monocytic cells. Regulation by Egr-1, c-Jun, and NF-kappaB transcription factors. J. Biol. Chem. *272*, 17795-17801.

Yazdanpanah, B., Wiegmann, K., Tchikov, V., Krut, O., Pongratz, C., Schramm, M., Kleinridders, A., Wunderlich, T., Kashkar, H., Utermohlen, O., et al. (2009). Riboflavin kinase couples TNF receptor 1 to NADPH oxidase. Nature *460*, 1159-1163.

Yeh, W.C., Shahinian, A., Speiser, D., Kraunus, J., Billia, F., Wakeham, A., de la Pompa, J.L., Ferrick, D., Hum, B., Iscove, N., et al. (1997). Early lethality, functional NF-kappaB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. Immunity *7*, 715-725.

Yin, Y., Terauchi, Y., Solomon, G.G., Aizawa, S., Rangarajan, P.N., Yazaki, Y., Kadowaki, T., and Barrett, J.C. (1998). Involvement of p85 in p53-dependent apoptotic response to oxidative stress. Nature *391*, 707-710.

Yoshida, L.S., and Tsunawaki, S. (2008). Expression of NADPH oxidases and enhanced H(2)O(2)-generating activity in human coronary artery endothelial cells upon induction with tumor necrosis factor-alpha. Int. Immunopharmacol. *8*, 1377-1385.

Yuasa, T., Ohno, S., Kehrl, J.H., and Kyriakis, J.M. (1998). Tumor necrosis factor signaling to stress-activated protein kinase (SAPK)/Jun NH2-terminal kinase (JNK) and p38. Germinal center kinase couples TRAF2 to mitogen-activated protein kinase/ERK kinase kinase 1 and SAPK while receptor interacting protein associates with a mitogen-activated protein kinase kinase kinase upstream of MKK6 and p38. J. Biol. Chem. *273*, 22681-22692.

Yujiri, T., Ware, M., Widmann, C., Oyer, R., Russell, D., Chan, E., Zaitsu, Y., Clarke, P., Tyler, K., Oka, Y., et al. (2000). MEK kinase 1 gene disruption alters cell migration and c-Jun NH2-terminal kinase regulation but does not cause a measurable defect in NF-kappa B activation. Proc. Natl. Acad. Sci. USA *97*, 7272-7277.

Zhang, D.W., Shao, J., Lin, J., Zhang, N., Lu, B.J., Lin, S.C., Dong, M.Q., and Han, J. (2009). RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science *325*, 332-336.