Dying for NF-κB? Control of cell death by transcriptional regulation of the apoptotic machinery

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The transcription factor nuclear factor κB (NF-κB) is a pleiotropic protein complex that is activated from a sequestered, cytoplasmic form by pro-inflammatory extracellular signals and cellular stress. Several hundred cellular genes have been shown to be regulated by NF-κB, including cytokines, chemokines and adhesion molecules. Nearly eight years ago, a flurry of publications showed that loss or suppression of NF-κB results in an enhanced sensitivity to apoptosis. In the ensuing years, activation of NF-κB has become almost synonymous with enhanced cell survival, although more recent data suggests that this transcription factor plays a more complex role in the regulation of cell death.

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Abbreviations

ASK1	apoptosis signaling kinase 1
c-IAP	cellular inhibitor of apoptosis
DD	death domain
DR	death receptor
Gadd45β	growth arrest and DNA damage inducible 45β
IAP	inhibitor of apoptosis
IKK	IκB kinase
IκB	inhibitor of NF-κB
IκB-α SD	superdominant IκB-α
JNK	c-jun N-terminal kinase
NF-κB	nuclear factor-κB
RHD	rel homology domain
SOD	superoxide dismutase
TNF	tumor necrosis factor
TNFR1	TNF type 1 receptor
TNFR2	TNF type 2 receptor
TRAIL	TNF-related apoptosis-inducing ligand
XIAP	X-linked inhibitor of apoptosis

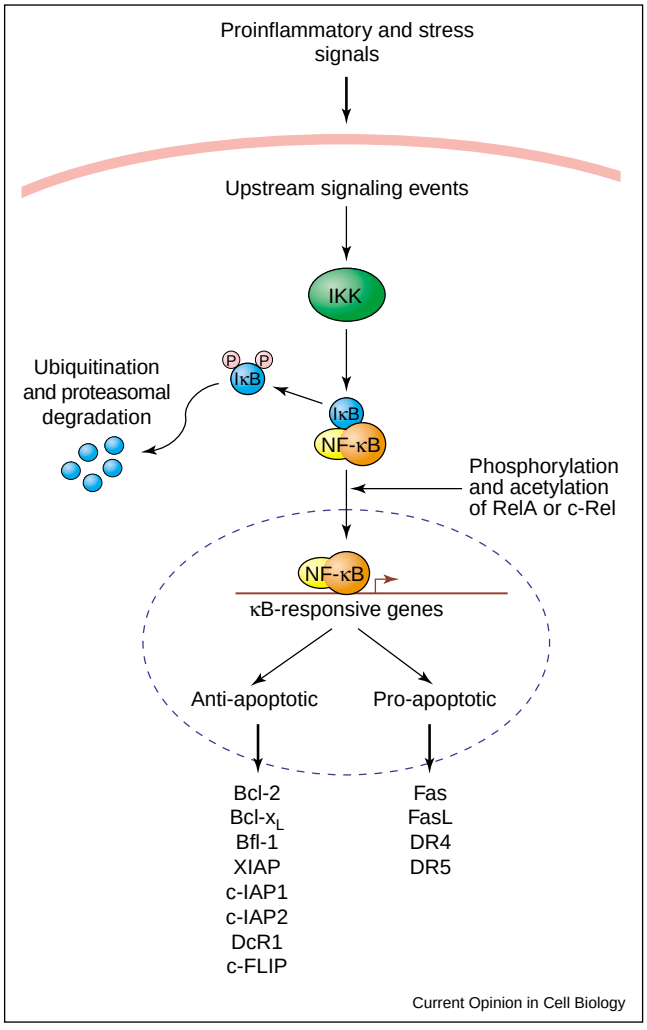
Introduction

Apoptosis is a fundamental phenomenon required for the homeostasis of multicellular organisms. From the early stages of development to the adult organism, cell populations are constantly regulated through this process. The mechanisms involved in apoptosis are evolutionarily conserved and involve the sequential activation of specialized proteases called caspases, which orchestrate and execute the cell death process [1]. Apoptosis is tightly regulated such that this event only takes place under appropriate circumstances; this regulation involves several endogenous cellular factors that counteract caspases or their activating signals. The relative expression of pro- or anti-apoptotic proteins can be regulated at the transcriptional level, and a role for the transcription factor NF-κB in the regulation of the apoptotic threshold has become apparent in the past decade.

The transcription factor NF-κB is a dimeric complex formed by members of a highly conserved family of proteins that share a defining motif designated the Rel homology domain (RHD). In mammals, there are five genes that encode members of this family: *RelA*, *RelB*, *c-Rel*, *NF-κB1 and NF-κB2* [2]. Three of these genes, *RelA*, *c-Rel*, and *RelB*, encode proteins that contain RHD and transactivation domains. The *NF-κB1* and *NF-κB2* genes encode large precursor polypeptides known as p105 and p100; proteolytic cleavage of the C-terminal regions of p105 and p100 results in p50 and p52, respectively [3,4], and these subunits provide the bulk of the DNA binding specificity. Heterodimers of RelA and p50 are the most abundant NF-κB complexes in most cell types. In unstimulated cells, NF-κB complexes are ordinarily sequestered in the cytoplasm through their interaction with members of the inhibitor-of-κB (IκB) family. Phosphorylation of IκB by a multimeric kinase complex, IκB kinase (IKK), results in ubiquitination and proteasomal degradation of these proteins, allowing the nuclear translocation of NF-κB complexes (Figure 1). NF-κB dimers in the nucleus bind to cognate sequences of DNA present in an array of gene promoters, which leads to transcriptional activation. The transcriptional activity of nuclear-translocated NF-κB complexes is also regulated by post-translational modifications, such as phosphorylation and acetylation, that affect the transcriptional competence of the subunits or their rate of nuclear export [5–7].

NF-κB plays a role in several physiological and pathophysiological processes. It participates in the regulation of innate and adaptive immunity, as many pro-inflammatory gene products are controlled at the transcriptional level by NF-κB [8]. In addition, NF-κB has been shown to participate in the regulation of cell-cycle progression through its effects on cyclin D1 expression [9]. Deregulation of NF-κB is involved in the pathogenesis of various disorders; rearrangements and amplifications of

Figure 1



Schematic representation of the key steps in the activation of NF-κB. Upon encountering various pro-inflammatory and stress signals, and through various upstream signaling events, the IKK complex is activated. This leads to phosphorylation of IκB proteins, targeting them for ubiquitination and proteasomal degradation. Upon being released from IκB, the nuclear localization signals of NF-κB complexes are exposed allowing for nuclear translocation. Post-translational modifications of RelA and c-Rel, including phosphorylation and acetylation, can occur leading to enhanced transcriptional competence or decreased nuclear export of the complexes. Anti- and pro-apoptotic genes can be regulated by NF-κB, thus affecting apoptotic threshold of the cell.

various NF-κB genes have been detected in several malignancies and have been implicated in the resistance of cancer cells to therapy [10,11]. NF-κB also plays a role in the transcriptional regulation of various viral genomes, including that of HIV-1 [12–15]. Finally, NF-κB has been implicated in the regulation of cell death through its ability to regulate the expression of cellular factors that affect the apoptotic threshold. Here we survey some of the more notable NF-κB-induced target genes that have been proposed to confer these anti-apoptotic effects, and attempt to evaluate newer evidence that suggests a more complex role for NF-κB in the control of cell survival.

The paradigm: NF-κB mediates resistance to apoptosis

A role for NF-κB in resistance to cell death was initially apparent in several experimental systems, including cell death induced by tumor necrosis factor (TNF). TNF is a major physiologic mediator of inflammation and is the protoype member of a large family of >20 related proteins including lymphotoxin-α, CD30 ligand, CD40 ligand, Fas ligand and TNF-related apoptosis-inducing ligand (TRAIL) [16]. These molecules mediate their signals through members of the TNF receptor superfamily, which can be divided into two subfamilies on the basis of the intracellular signaling molecules recruited. The cytoplasmic domains of several receptors, including TNF type 1 receptor (TNFR1), Fas, and death receptors (DR) 3, 4 and 5, contain a conserved ~80 amino acid motif termed the death domain (DD). This element is capable of recruiting DD-containing adaptor molecules, such as TRADD and FADD, that are involved in the initiation of apoptotic cell death. Other members of the TNF receptor superfamily, such as TNF type 2 receptor (TNFR2), CD30 or CD40, lack the DD and associate with different types of adaptor molecules — most importantly, members of the TRAF (TNF receptor-associated factor) family. TNF mediates its signals through TNFR1 and TNFR2 and both receptors are capable of activating the IKK complex. In addition to NF-κB signaling, TNF induces activation of c-Jun N-terminal kinases (JNKs). Unlike TNFR2, TNFR1 contains a death domain in its cytoplasmic tail that is capable of activating caspase-8 and this event can ultimately lead to apoptotic cell death. Despite this pro-apoptotic pathway, most cell types are usually resistant to TNF-induced cell death.

Inhibition of cellular transcription or translation sensitizes most cells to the pro-apoptotic effects of TNF. This phenomenon suggested the possibility that the loss of certain cellular factor(s) were responsible for the sensitization observed. The possibility that NF-κB was a principal cellular factor responsible for resistance to TNF-mediated cell death was suggested by several different experimental models, including mouse strains deficient in NF-κB activity. Mice deficient in RelA have severely compromised NF-κB signaling, presumably because RelA/p50 dimers are the most abundant NF-κB complexes. These animals die *in utero* from massive hepatocyte cell death [17] and this phenotype can be rescued by concurrent deficiency of TNF or TNFR1 [18–20]. In addition, fibroblasts derived from these embryos demonstrate significant sensitivity to TNF-mediated apoptosis when cultured *in vitro* [21]. Similarly, deficiency of IKKβ, a subunit of the IKK complex that is necessary for TNF-mediated NF-κB activation, also results in *in utero* lethality in mice as a result of massive hepatocyte death.

Fibroblasts from these embryos are also significantly more sensitive to TNF [22–24]. Additional evidence for a role of NF-κB in regulating the sensitivity of cells to TNF-mediated apoptosis has been gleaned in other experimental systems. Expression of superdominant IκB-α (IκB-α SD), a mutant form that is abnormally stable due to loss of IKK phosphorylation sites, results in potent inhibition of NF-κB by sequestering complexes in the cytosol. Cells induced to express IκB-α SD by one of several transfection methods are sensitized to TNF-mediated cell death [25]. Similarly, the transduction of IκB-α SD into primary hepatocytes using adenoviral vectors also promotes the pro-apoptotic effects of TNF [26] and transgenic expression of IκB-α SD in hepatocytes has a similar effect [27•]. All of these studies point to the same conclusion: deficiency of basal NF-κB activity increases sensitivity to TNF-mediated cell death.

Many chemotherapeutic agents are capable of inducing apoptotic cell death. Although the mechanisms responsible for the activation of apoptosis by chemotherapeutic agents are not fully understood, in many cases these are known to involve mitochondrial destabilization and release of pro-apoptotic factors, such as cytochrome c, that lead to caspase-9 activation [28]. The sensitivity of different cell types to these pro-apoptotic effects is variable, and it has been noted that cancer cells can acquire relatively high tolerance to these agents. The mechanisms that cancer cells employ to acquire chemoresistance are multiple and complex [29], but a role for NF-κB activation has been suggested. Evidence for this includes the observation that expression of IκB-α SD as a means to block NF-κB can increase the sensitivity of several cancer cell lines to chemotherapeutic agents *in vitro* [30] and similar blockade can reverse chemoresistance in *in vivo* models [31].

NF-κB and the control of anti-apoptotic genes

Consistent with NF-κB having an anti-apoptotic effect, it has been observed that this transcription factor can regulate the expression of various anti-apoptotic proteins. The presence of κB sites in the promoters of these genes can affect the levels of these factors and the sensitivity of the cell to apoptotic stimuli.

The *bcl-2* gene family encodes a group of proteins that regulate the process of mitochondrial release of pro-apoptotic factors, such as cytochrome c and Smac/DIABLO [32,33]. Some members of this family, including Bax and Bad, promote mitochondrial release, whereas others, such as Bcl-2 and Bcl-x_L prevent this process and are generally anti-apoptotic. At least three anti-apoptotic members of this family, Bcl-2, Bcl-x_L and Bfl-1, have been shown to be induced by NF-κB [34–37], highlighting the strong link between NF-κB and cell survival.

Several factors encoded by the *inhibitor of apoptosis* (*iap*) family have been shown to regulate apoptosis through their ability to bind to and inhibit various caspases [38]. The best-characterized member of the family is XIAP (X-linked inhibitor of apoptosis), and this factor has the highest anti-apoptotic potency of all these molecules [39]. Several reports give evidence that XIAP expression can be affected by NF-κB [40,41], although this effect is probably highly tissue-specific. Two other members of this family, c-IAP1 and c-IAP2 (cellular inhibitor of apoptosis -1 and -2), associate with TNF receptors and are involved in TNF-mediated NF-κB activation [42]. When overexpressed in cells, c-IAP1 and -2 have been reported to possess anti-apoptotic activity, although their ability to directly inhibit caspases is at least 100-fold less than that of XIAP [43]. The expression of c-IAP1 and c-IAP2 is also responsive to NF-κB [44].

TNF-mediated JNK activation has also been implicated in the mechanism leading to TNF-induced cell death. Although the role of JNK-mediated signals in cell death can vary between different experimental systems, JNK-1 and -2 deficient cells are more resistant to several cell death stimuli [45]. Cells derived from RelA- or IKKβ-deficient mice, or normal cells in which NF-κB is blocked by transient expression of IκB-α S.D, display an abnormal pattern of JNK activation [40,46]. In these cells, TNF treatment results in intense and prolonged JNK activation. Inhibition of JNK partially restored resistance to cell death [47], although not all studies have found the same effect [48]. It has been proposed that decreased NF-κB activity results in decreased expression of *XIAP* and/or *gadd45β* (growth arrest and DNA damage inducible 45β) in these cells, and that these factors are involved in the regulation of JNK activation and cell-death sensitivity in these cells. Other lines of evidence suggest that the MAP3 kinase ASK1 (apoptosis signaling kinase 1) is the kinase responsible for the pro-apoptotic activation of JNK. Deficiency of ASK1 renders cells less sensitive to TNF-mediated cell death and prevents prolonged activation of JNK after TNF stimulation [49]. It is unclear whether XIAP or Gadd45β can regulate the activity of this kinase.

Various cell death pathways involve the generation of cytotoxic reactive oxygen species. Cellular mechanisms for scavenging these toxic metabolites include a group of enzymes named superoxide dismutases (SODs). The expression of one such SOD, MnSOD, is controlled by NF-κB and this has been implicated as a potential mechanism for mediating cell survival [50,51].

Against the paradigm: evidence that NF-κB plays a neutral or pro-apoptotic role

An anti-apoptotic role for NF-κB would predict that the inhibition of TNF-mediated NF-κB activation results in enhanced cell death. However, there are several examples

when this is not the case. A20 is a factor that was originally identified as a TNF-inducible cellular protein [52]. Functionally, A20 is capable of potently inhibiting TNF-mediated NF-κB activation, although at the same time this molecule has anti-apoptotic properties in various *in vitro* models, including TNF-mediated cell death [53]. Consistent with these dual functions, A20-deficient mice demonstrate a pro-inflammatory phenotype thought to be the result of their inability to regulate TNF-mediated NF-κB activation, while also showing increased sensitivity to TNF-mediated apoptosis [54]. An additional prediction of the proposed model in which NF-κB plays a generalized anti-apoptotic role in TNF-mediated cell death is that activation of NF-κB should be cytoprotective. However, NF-κB activation that results from stimulation of TNFR2 [55,56] or other homologous receptors such as CD30 [57] and CD40 [58] sensitizes cells to TNFR1-mediated apoptosis [57]. Thus, the functional consequences of NF-κB activation from different receptors vary, and not all signals that activate NF-κB are anti-apoptotic.

Decreased basal NF-κB activity that results from RelA deficiency sensitizes cells to TNF-mediated cell death. If NF-κB plays a general role in setting the apoptotic threshold, it would be expected that these cells might be sensitive to other pro-apoptotic stimuli; similarly, deficiencies of other NF-κB subunits could result in similar phenotypes. However, c-Rel deficient fibroblasts are not sensitized to TNF-mediated cell death. Moreover, c-Rel deficient cells are completely resistant to cell death mediated by TRAIL, a molecule similar to TNF that signals through DR4 and DR5; in addition, RelA-deficient fibroblasts are only slightly more sensitive to this stimulus than wild-type fibroblasts [59]. NF-κB subunits that contain c-Rel are involved in the expression of DR4 and DR5 [60], and cells lacking c-Rel are therefore deficient in these receptors and resistant to TRAIL-mediated cell death.

NF-κB has also been implicated in the regulation of cell death mediated by several endogenous cellular factors. p53 can mediate apoptosis in response to several cellular stresses through its ability to induce mitochondrial release of pro-apoptotic factors [61•]. A separate report found that inducible expression of p53 in cultured cells resulted in nuclear translocation of NF-κB complexes, and that this event was necessary for p53-mediated apoptosis [62]. In this study, blockade of NF-κB translocation via expression of IκB-α SD protected cells from p53-mediated cell death while sensitizing the same cells to TNF-mediated apoptosis. These data suggest that inhibition of NF-κB signaling can change the apoptotic threshold in different directions depending on the apoptotic stimulus. Similarly, HSCO, a novel NF-κB inhibitor that promotes nuclear export of the NF-κB complex through its association with RelA, was found to sensitize cells to TNF-mediated cell death, while at the same time it prevented p53-mediated apoptotic death [63••]. In other studies, decreased NF-κB baseline activity as a result of deficiencies in IKKα and IKKβ resulted in decreased Mdm2 expression and enhanced p53 stabilization in response to chemotherapeutic agents [64]. These data raise the possibility that NF-κB activation could play a protective role against p53-mediated cell death. Indeed, cells deficient in IKKα and IKKβ were more sensitive to cell death induced by chemotherapy, consistent with prior data showing that NF-κB inhibition by expression of IκB-α SD sensitizes cells to death induced by these agents.

Various members of the TNF receptor superfamily contain a conserved motif within their cytoplasmic domains known as the death domain [65]. When stimulated, these so-called death receptors can induce apoptosis via oligomerization with adaptor proteins that recruit and induce the activation of several upstream caspases, including caspase-8 and -10. As discussed above, NF-κB has been found to regulate the expression of DR4 and DR5 and can also regulate other death receptors such as Fas [66]. NF-κB can also regulate the expression of Fas ligand. In this context, NF-κB has been found to play a pro-apoptotic role in activation-induced cell death and chemotherapy-induced cell death in T cells by inducing expression of Fas ligand [67,68]. On the other hand, cellular factors that inhibit apoptosis mediated by death receptors can also be regulated by NF-κB. These include decoy death receptors, which are capable of binding to ligand but lack cytoplasmic death effector domains [69], and c-FLIP, a molecule that can block caspase-8 recruitment and activation [70].

Conclusions

NF-κB is clearly capable of controlling the expression of several anti- and pro-apoptotic factors. The inhibition of basal levels of NF-κB can lead to increased sensitivity to certain apoptotic stimuli. However, this has been interpreted as evidence that NF-κB activation resulting from cell stimulation is also anti-apoptotic. This interpretation assumes that the transcriptional activity and gene specificity of basal and stimulated NF-κB complexes are identical. In addition, this model presupposes that the simultaneous initiation of death signals and NF-κB activation can result in a protective effect in this setting, which has yet to be comprehensively addressed. More recent data suggest that, under certain circumstances, NF-κB activation can render cells more sensitive to certain pro-apoptotic stimuli and that inhibition of certain NF-κB complexes can render cells more resistant to certain forms of cell death. These findings are consistent with the observations that both pro- and anti-apoptotic gene products are regulated by NF-κB. Given the cell-type- and stimulus-dependent effects of NF-κB on the regulation of apoptosis, it will be of great importance to further evaluate these pathways as NF-κB inhibitors are developed for therapeutic use.

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