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REVIEW

Current insights into the regulation of programmed cell death by NF-κB

J Dutta[1,2,4], Y Fan[1,4], N Gupta[1,2,4], G Fan[1,2,4] and C Gélinas[1,3]

[1]*Center for Advanced Biotechnology and Medicine, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway NJ, USA;* [2]*Graduate Program in Biochemistry and Molecular Biology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway NJ, USA and* [3]*Department of Biochemistry and Cancer Institute of New Jersey, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway NJ, USA*

The nuclear factor-kappaB (NF-κB) transcription factors have emerged as major regulators of programmed cell death (PCD) whether via apoptosis or necrosis. In this context, NF-κB's activity has important ramifications for normal tissue development, homoeostasis and the physiological functions of various cell systems including the immune, hepatic, epidermal and nervous systems. However, improper regulation of PCD by NF-κB can have severe pathologic consequences, ranging from neurodegeneration to cancer, where its activity often precludes effective therapy. Although NF-κB generally protects cells by inducing the expression genes encoding antiapoptotic and antioxidizing proteins, its role in apoptosis and necrosis can vary markedly in different cell contexts, and NF-κB can sensitize cells to death-inducing stimuli in some instances. This article describes our current knowledge of the role of NF-κB in apoptosis and necrosis, and focuses on the many advances since we last reviewed this rapidly evolving topic in *Oncogene* 3 years ago. There has been substantial progress in understanding NF-κB's mode of action in apoptosis and necrosis and the mechanisms that regulate its anti- vs proapoptotic activities. These recent developments shed new light on the role of NF-κB in many disease conditions including tumor development, tumor progression and anticancer treatment.
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Introduction

The signaling pathways that govern cell death are of critical importance for normal tissue development, homeostasis and function. Tremendous pathological implications are associated with dysregulation of the delicate balance between cell life and death including degeneration, immunodeficiency or infertility with increased cell death or at the other end of the spectrum, autoimmune diseases and cancer when too little cell death occurs (reviewed in Danial and Korsmeyer, 2004).

The nuclear factor-kappaB (NF-κB) transcription factors (p50/p105, p52/p100, RelA, c-Rel, RelB) and the pathways that control NF-κB activation are best known for their role in immune and inflammatory responses (see Hayden *et al*., 2006), but are also critical to many physiological and pathological processes and constitute attractive targets for therapy (see Gilmore and Herscovitch, 2006, this issue). The canonical NF-κB signaling cascade (i.e., Receptor- > adaptors- > inhibitor of NF-κB (Iκ B) kinase (IKK)- > Iκ B degradation- > nuclear translocation of NF-κB) is activated in response to injury, infection, inflammation and other stress conditions and rapidly leads to alterations in cellular gene expression (reviewed in Pahl, 1999; Scheidereit, 2006). A second, non-canonical pathway is activated by a more limited set of stimuli and involves the regulated processing of a p100/RelB dimer to p52/RelB, which can activate a distinct, but overlapping set of target genes (reviewed in Scheidereit, 2006). Among the many genes affected by NF-κB is an array of genes encoding factors that control the cell response to death-inducing stimuli. We recently reviewed the role of NF-κB in apoptosis (Kucharczak *et al*., 2003); however, the past 3 years have seen an impressive expansion in the number of studies investigating the role of NF-κB in programmed cell death (PCD) in different systems (> 2000 publications). Herein, we review recent progress regarding the anti- and proapoptotic effects of NF-κB and the mechanisms that regulate its activity, the interplay between the NF-κB and Jun kinase (JNK) signaling pathways and its role in apoptosis and necrosis, and the physiological and pathological impacts of NF-κB's role in cell death.

NF-κB's role in PCD is critical for normal development, homeostasis and function of different cell systems: recent progress

NF-κB is most commonly associated with inhibition of PCD and this has important physiological consequences

Correspondence: Professor C Gélinas, CABM, 679 Hoes Lane, Piscataway, New Jersey 08854-5638, USA.
E-mail: gelinas@cabm.rutgers.edu
[4]These authors contributed equally to this work.

for development and homeostasis of the immune, hepatic and nervous systems as well as for ectodermal appendages (e.g., hair follicles, exocrine glands and teeth) (reviewed in Li and Verma, 2002; Kucharczak *et al*., 2003). We provide here a brief update on recent progress in this area.

The embryonic lethality of RelA-deficient mice was one of the first indications that NF-κB contributes a crucial antiapoptotic effect during normal development and this embryonic death was attributed to extensive apoptosis of developing hepatocytes (Beg and Baltimore, 1996). A similar phenotype is seen in mice lacking both copies of IKKβ, lacking IKKβ along with IKKα or lacking the IKK regulator NEMO (Li *et al*., 1999, 2000; Tanaka *et al*., 1999; Rudolph *et al*., 2000). Because compound knockouts of RelA with tumor necrosis factor alpha (TNFα) or of IKKβ with TNF receptor 1 (TNF-R1) are viable, it is likely that RelA protects hepatocytes against the toxic effects of TNFα in developing embryos (Doi *et al*., 1999; Alcamo *et al*., 2001). Recent work has revealed that the overexpression of the NF-κB-regulated cell death inhibitor Bcl-2 is unable to rescue liver apoptosis in RelA-knockout mice (Gugasyan *et al*., 2006). This indicates that the protective activity of RelA must rely on the activation of other or additional antiapoptotic genes. This is perhaps consistent with the fact that many antiapoptotic targets of NF-κB show partial protective activity under conditions where NF-κB is inactivated: for example, it has been shown that co-expression of cellular inhibitor of apoptosis protein 1 (c-IAP1), c-IAP2, TNF receptor-associated factor 1 (TRAF1) and TRAF2 is required to effectively suppress TNF-induced apoptosis in RelA−/− mouse embryo fibroblasts (MEFs) (Wang *et al*., 1998). The finding that hepatocyte apoptosis induced by transforming growth factor β (TGF-β) is accompanied by repression of NF-κB-dependent antiapoptotic targets Bcl-xL, X-chromosome-linked IAP (XIAP) and α-fetoprotein also supports this hypothesis (Cavin *et al*., 2004).

Studies looking at the protective activity of NF-κB in immune cells highlighted a crucial role for RelA during early lymphopoiesis where RelA is required to block apoptosis induced by high levels of TNF, as illustrated by the markedly reduced survival of developing B cells following adoptive transfer of RelA−/− fetal liver hemopoietic precursor cells into Rag1−/− mice (Gerondakis and Strasser, 2003; Prendes *et al*., 2003; Siebenlist *et al*., 2005; reviewed in Claudio *et al*., 2006). This reduced viability of RelA-deficient B cells can be rescued by a knockout of the TNF-R1 gene and coincided with changes in the expression of the antiapoptotic NF-κB targets c-FLIP and Bcl-2, as overexpression of both could reduce TNF cytotoxicity following retroviral-mediated gene transfer. On the other hand, a crucial role for c-Rel in the survival and proliferation of mature B cells following B-cell receptor (BCR) activation was found to involve upregulation of Bcl-XL along with E2F3a and cyclin E (Feng *et al*., 2004). Data implicating c-Rel as a critical intermediary downstream of the B-cell adaptor for phosphoinositide 3-kinase (BCAP) are consistent with these findings (Yamazaki and Kurosaki, 2003).

Although it is clear that NF-κB protects developing and mature B and T lymphocytes from apoptosis (Gerondakis and Strasser, 2003; Kucharczak *et al*., 2003; Siebenlist *et al*., 2005; reviewed in Claudio *et al*., 2006), recent evidence indicates that de-regulated activation of NF-κB can be as detrimental to lymphocyte survival as is the absence of NF-κB activity. Indeed, mice with constitutive NF-κB activity due to compound inactivation of IκBα and IκBε die neonatally and lack B and T cells (Goudeau *et al*., 2003). Although these findings have not yet been explained at the molecular level, the mode of cell death differs dramatically between bone marrow (BM) progenitor cells lacking NF-κB and those in which NF-κB is constitutively active. Whereas apoptosis of BM progenitors from RelA−/− mice can be rescued by TNF-R deficiency or by co-transplantation with wild-type BM cells, the survival defect of BM progenitors from IκBα/IκBε double knockout mice appears to be intrinsic and cannot be rescued either by wild-type BM cells, by suppression of TNF or by treatment with a caspase inhibitor (Goudeau *et al*., 2003). These intriguing new findings underscore the importance of keeping NF-κB activity under strict control to achieve lymphocyte homeostasis and indicate that either too much or too little NF-κB activity can be detrimental to cell survival.

Opposite effects of NF-κB, namely either to promote or block apoptosis, have been observed in the nervous system, and the nature of NF-κB's effect appears to be influenced by the cell type and the death-inducing stimulus (reviewed in Mattson and Camandola, 2001; Kucharczak *et al*., 2003). Although activation of NF-κB in neurons is often protective, its activation in microglia tends to promote neuronal apoptosis, perhaps due to the production of proinflammatory cytokines, reactive oxygen species (ROS) and excitotoxins (John *et al*., 2003; Mattson and Meffert, 2006). Supporting the idea that NF-κB's well-documented role in inflammation can foster central nervous system (CNS) injury following trauma, targeted inhibition of NF-κB in astroglial cells by tissue-specific expression of the IκBα super-repressor noticeably ameliorated recovery of mice following spinal cord injury and this effect coincided with a reduced production of proinflammatory cytokines (Brambilla *et al*., 2005). Recently, increased levels of NF-κB have been shown to be associated with K+ loss and cortical neuron apoptosis following serum withdrawal and to correlate with elevated levels of the proapoptotic alternatively spliced product of the *bcl-x* gene, Bcl-xS (Tao *et al*., 2006). Whereas Bcl-xL is a well-known antiapoptotic transcriptional target of NF-κB, this effect in cortical neurons represents a rare example where upregulation of its alternatively-spliced proapoptotic variant Bcl-xS is observed.

Although many reports have highlighted a role for NF-κB in pathological conditions affecting the nervous system or in response to injury and stress, new data have uncovered a role for NF-κB in normal CNS physiology (reviewed in Meffert and Baltimore, 2005; Mattson and

Meffert, 2006). Mice lacking c-Rel exhibit defects in contextual fear memory (Levenson *et al*., 2004). On the other hand, RelA has been implicated in facilitating spatial memory formation and synaptic plasticity (Meffert *et al*., 2003), although it was suggested that inactivation of RelA might not be the only contributing factor to the memory defect of RelA−/−TNF-R1−/− mice, as TNF-R1 deficiency was previously described to alter synaptic plasticity in *in vitro* preparations (Albensi and Mattson, 2000). Further supporting a role for NF-κB in learning and memory, neuronal ablation of NF-κB by tetracycline-dependent expression of an IκBα super-repressor also gives rise to defects in spatial memory formation (Kaltschmidt *et al*., 2006). However, contrary to NF-κB's effects in response to stress or injury, its role in these physiological contexts seems to be independent of its effect on apoptosis, as RelA−/− TNF-R1-deficient mice or mice with targeted inhibition of NF-κB in the forebrain show normal CNS development and no defect in neuronal survival (Fridmacher *et al*., 2003; Meffert *et al*., 2003).

Update on NF-κB's protective activity following viral infection and its role in viral transformation

Successful infection and propagation of many viruses depends on their ability to activate the NF-κB signaling cascade, and activation of NF-κB target genes is often linked to their transforming activity. This is best exemplified by the oncoprotein v-Rel, encoded by the oncogene of the retroviral avian Reticuloendotheliosis virus strain T (Rev-T) that causes fatal leukemia/lymphomas in animal models (reviewed in Gilmore, 1999; Fan *et al*., 2006b). v-Rel and its cellular homologue c-Rel can block apoptosis triggered by different death-inducing stimuli and this activity is critical for their transforming activity, as is their ability to induce the expression of antiapoptotic genes of the Bcl-2 and IAP gene families (White and Gilmore, 1996; Gilmore *et al*., 2001; Kralova *et al*., 2002).

Several other viruses exploit the antiapoptotic property of NF-κB to enhance their replication and pathogenicity (reviewed in Hiscott *et al*., 2001; Santoro *et al*., 2003; and accompanying review by Hiscott *et al*., 2006). Among them, activation of NF-κB by the latent membrane protein 1 (LMP1) of the human Herpesvirus Epstein–Barr virus (EBV), implicated in Burkitt's lymphoma, is critical for transformation of human B cells (Cahir-McFarland *et al*., 2000; Feuillard *et al*., 2000; He *et al*., 2000; reviewed in Hiscott *et al*., 2006). Recent work implicates interleukin-1β receptor-associated kinase 1 (IRAK1) and TRAF6 in NF-κB activation via latent membrane protein 1 (LMP1) and in phosphorylation of the RelA transactivation domain (Luftig *et al*., 2003; Song *et al*., 2006; Wu *et al*., 2006). A second study has reported that activation of the non-canonical NF-κB pathway, leading to accumulation of nuclear p52/RelB complexes, is required for LMP-1-mediated transformation of human B cells (Luftig *et al*., 2004). The v-FLIP K13 protein of Human Herpesvirus 8/Kaposi's sarcoma associated Herpes virus (HHV8/KHSV), implicated in Kaposi sarcoma and primary effusion lymphoma (PEL), inhibits lytic replication and promotes survival of infected lymphoma cells by activating both the canonical and non-canonical NF-κB pathways resulting in establishment and maintenance of latency (Brown *et al*., 2003; Guasparri *et al*., 2004; Matta and Chaudhary, 2004). In support of these findings, the proteasome inhibitor Velcade/Bortezomib can inhibit both the canonical and non-canonical NF-κB pathways, can suppress PEL cell proliferation and can induce apoptosis (Matta and Chaudhary, 2005). As transgenic mice expressing v-FLIP remain sensitive to Fas-mediated apoptosis, the role of v-FLIP in oncogenesis was suggested to be independent of inhibition of Fas-mediated killing (Chugh *et al*., 2005). Thus, activation of NF-κB appears to be a critical mechanism by which EBV- and KHSV-infected lymphoma cells are spared from apoptosis and inhibition of NF-κB is associated with downregulation of various antiapoptotic targets (Keller *et al*., 2006).

The human T-cell leukemia virus type-1 (HTLV-1), associated with adult T-cell leukemia (ATL), also usurps the NF-κB signaling pathway via its oncoprotein Tax and leads to induction of the antiapoptotic proteins Bcl-xL, Bcl-2 and Bfl-1/A1 to suppress apoptosis and immortalize T cells (Harhaj *et al*., 1999; Tsukahara *et al*., 1999; Nicot *et al*., 2000). Recent studies have shown that Tax undergoes ubiquitination and sumoylation, and that these two modifications act coordinately to regulate Tax localization to nuclear bodies and recruitment of RelA and NEMO to activate NF-κB-dependent gene expression (Lamsoul *et al*., 2005). Together with many prior reports, these studies reinforce the notion that many viruses utilize the NF-κB signaling pathway to manipulate the host apoptotic response to favor viral transformation and pathogenicity.

How NF-κB's role in apoptosis and inflammation impacts its role in cancer: new insights

As described in greater detail in the accompanying review from Basseres and Baldwin (2006), NF-κB is a key contributing factor to the pathogenesis of many human tumors and their chemoresistance (reviewed in Rayet and Gélinas, 1999; Baldwin, 2001; Karin *et al*., 2002). A few examples include classical Hodgkin's lymphoma (cHL), multiple myeloma (MM), primary mediastinal B-cell lymphoma (PMBL), therapy-resistant diffuse large B-cell lymphoma (DLBCL), childhood acute lymphoblastic leukemia (c-ALL) and breast, prostate, ovarian, lung, colon and renal cell carcinoma (Bargou *et al*., 1997; Nakshatri *et al*., 1997; Sovak *et al*., 1997; Alizadeh *et al*., 2000; Cogswell *et al*., 2000; Kordes *et al*., 2000; Davis *et al*., 2001; Hinz *et al*., 2001; Kalaitzidis *et al*., 2002; Shipp *et al*., 2002; Munzert *et al*., 2004). Accumulating evidence suggests that NF-κB also contributes to brain cancer, as a novel trkAIII splice

variant of the neurotrophin tyrosine kinase receptor type 1 (TrkA) was shown to oppose growth-restricting nerve growth factor (NGF)/TrkAI signaling in neuroblastoma and to stimulate tumor-promoting signaling via PI3K/Akt and NF-κB (Tacconelli *et al*., 2004). The tumor suppressor inhibitor of growth family member isoform 4 (ING4), whose inactivation in brain tumors is associated with increased survival, growth and angiogenesis, reportedly interacts with RelA and leads to transcriptional repression of NF-κB-dependent genes (Garkavtsev *et al*., 2004). NF-κB activation has also been implicated along with Akt in high-grade glioma (Wang *et al*., 2004).

A common denominator among many human tumors is that NF-κB acts in an intrinsic fashion to confer resistance to cell death by activating the expression of antiapoptotic genes (see below). This is best exemplified by the fact that expression of a super-repressor IκBα mutant noticeably sensitizes many tumor-derived cells to chemotherapy and induces apoptosis (reviewed in Baldwin, 2001; Kucharczak *et al*., 2003; Lin and Karin, 2003). In many cases, NF-κB is persistently activated in tumors owing to constitutive IKK kinase activity, but there are several examples where it results from overexpression and nuclear accumulation of the c-Rel protein (Bargou *et al*., 1997; Nakshatri *et al*., 1997; Sovak *et al*., 1997; reviewed in Rayet and Gélinas, 1999; Alizadeh *et al*., 2000; Cogswell *et al*., 2000; Kordes *et al*., 2000; Davis *et al*., 2001; Hinz *et al*., 2001; Gilmore *et al*., 2002; Kalaitzidis *et al*., 2002; Karin and Lin, 2002; Shipp *et al*., 2002; Barth *et al*., 2003; Munzert *et al*., 2004; Feuerhake *et al*., 2005; Fan *et al*., 2006a, b). Recent work from Lou Staudt's group has shown that caspase recruitment domain family member 11 (CARD11)/card-membrane-associated guanylate kinase (MAGUK) protein 1 (CARMA1) drives constitutive activation of the IKK complex via MALT1 and B-cell lymphoma 10 (BCL-10) in therapy-resistant diffuse large B cell lymphoma (DLBCL) (Ngo *et al*., 2006). In mucosa-associated lymphoid tissue (MALT) lymphoma, the t(11;18)(q21;q21) chromosome translocation generates a chimeric protein between the antiapoptotic c-IAP2 factor and MALT1. The resulting c-IAP2/MALT1 fusion has the ability to self-oligomerize and directs constitutive activation of IKK via K63 ubiquitination of NEMO (Stoffel *et al*., 2004). Hence, c-IAP2/MALT1 exhibits both an oncogenic property by strongly promoting activation of p50/RelB dimers and tumor-suppressing activity, as it no longer downregulates BCL-10 as the c-IAP2 ubiquitin ligase does (Hu *et al*., 2006b; reviewed in Bertoni and Zucca, 2006).

In addition to its crucial protective activity within tumor cells, NF-κB plays an important role in the tumor microenvironment where it acts in a paracrine fashion to accelerate tumor cell growth. This was elegantly demonstrated in different mouse models of inflammation-associated cancer (Figure 1; Greten *et al*., 2004; Pikarsky *et al*., 2004; reviewed in de Visser and Coussens, 2005; Fan *et al*., 2006a; and the review by Basseres and Baldwin, 2006). While inhibition of NF-κB activity via targeted inactivation of IKKβ in intestinal epithelial cells dramatically reduces tumor incidence in a mouse model of colitis-associated cancer owing to increased apoptosis, its inactivation in myeloid cells also reduces tumor incidence, but in this case by interfering with tumor cell growth in a paracrine fashion, owing to reduced production of proinflammatory cytokines (Greten *et al*., 2004). A role for NF-κB in inflammation-associated tumor growth has also emerged in a mouse model of hepatocellular carcinoma (HCC), where production of proinflammatory cytokines by endothelial and inflammatory cells leads to chronic activation of NF-κB in hepatocytes and progression to carcinoma (Pikarsky *et al*., 2004).

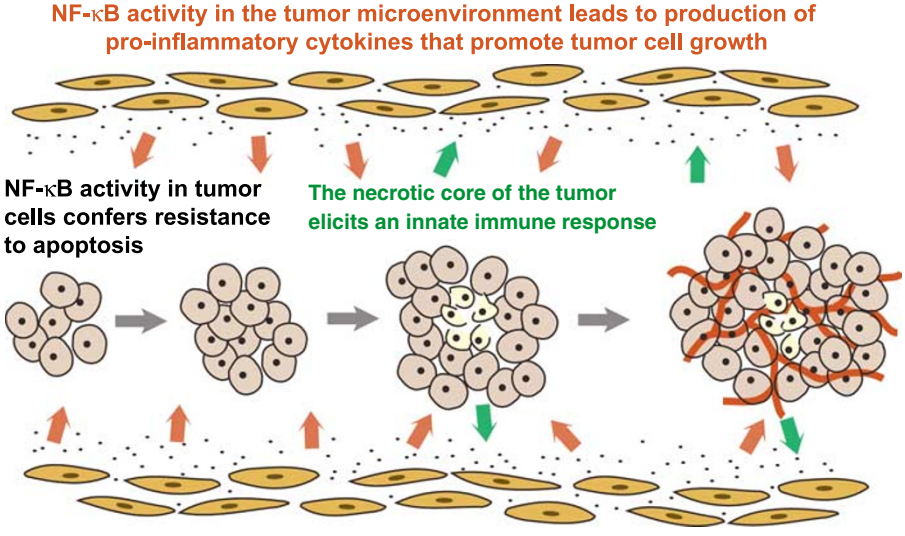

Figure 1 Intrinsic and paracrine involvement of NF-κB in tumor cell survival and proliferation. Activation of NF-κB in tumor cells confers resistance to apoptosis. Cells at the core of rapidly growing tumors can undergo necrosis when ATP is depleted. Necrotic tumor cells release potent proinflammatory factors. These factors activate an innate immune response in the tumor microenvironment that leads to NF-κB-dependent synthesis of inflammatory cytokines that foster tumor cell growth.

Although NF-κB is generally implicated in cancer by virtue of its ability to drive cell proliferation and survival, the inhibition of NF-κB in epidermal cells is associated with increased proliferation and hyperplasia (Seitz *et al*., 1998; van Hogerlinden *et al*., 1999; Seitz *et al*., 2000). Furthermore, inhibition of NF-κB activity combined with expression of oncogenic Ras in epidermal keratinocytes leads to invasive neoplasia with features similar to those of squamous cell carcinoma (Dajee *et al*., 2003). More recent studies have revealed that RelA-deficient skin manifests hyperplasia in absence of apoptosis, inflammation or abnormal differentiation and that this depends on TNF-R1-dependent activation of the JNK signaling pathway and activation of the cell cycle kinase CDK4 (Zhang *et al*., 2004, 2005). Consequently, it seems that NF-κB can adopt tumor-promoting or tumor-suppressor roles in different cell types.

Implication of NF-κB in apoptosis and necrosis

PCD mediated by apoptosis is usually a caspase-dependent process that is characterized by plasma membrane blebbing, cytoplasm contraction, chromatin condensation and DNA fragmentation. Apoptotic cells corpses are engulfed by macrophages, a process that does not generate an inflammatory response (reviewed in Leist and Jaattela, 2001; Edinger and Thompson, 2004). In contrast, PCD via necrosis is independent of caspases and is typically associated with breakdown of the plasma membrane, organelle swelling and nuclear degradation that is accompanied by the release of nuclear factors like high mobility group box 1 (HMGB1) that trigger a potent inflammatory response (reviewed in Lotze and Tracey, 2005; Zeh and Lotze, 2005). NF-κB is commonly cytoprotective toward PCD caused by apoptosis or necrosis and its prosurvival activity has been observed in response to a variety of death-inducing stimuli like the proinflammatory cytokine TNFα, ultraviolet (UV) radiation, anticancer agents and B-cell receptor crosslinking (Wu *et al*., 1996; Grumont *et al*., 1998; Wang *et al*., 1998; van Antwerp *et al*., 1998; Owyang *et al*., 2001). However, NF-κB can promote cell death in response to certain stimuli and in certain cells (reviewed in Baldwin, 2001; Karin and Lin, 2002; Kucharczak *et al*., 2003). Analysis of its activity in these different contexts has revealed important clues regarding NF-κB's mode of action and how its activity is regulated, as outlined below.

How the NF-κB, caspase and JNK cascades oppose each other to determine survival, apoptosis or necrosis downstream of TNF-R1 activation

There has been significant progress in the past few years in elucidating the molecular details that enable cells subjected to the death-inducing cytokine TNFα to survive, or conversely, to succumb to cell death via apoptosis or necrosis. NF-κB has emerged as a decisive factor in this choice, as highlighted in several excellent reviews on the interplay between the JNK and NF-κB signaling pathways (Luo *et al*., 2005; Nakano *et al*., 2006; Papa *et al*., 2006). Binding of TNFα to its receptor TNF-R1 activates the NF-κB, caspase and JNK signaling cascades that compete with one another to determine the fate of the cell (Figure 2; Micheau and Tschopp, 2003; reviewed in Jaattela and Tschopp, 2003). Interaction of activated TNF-R1 with the TNFR-associated death domain (TRADD) adaptor along with receptor interacting protein kinase 1 (RIP1) and TNF receptor-associated factor 2 (TRAF2) activates the canonical IKK kinase complex. This triggers degradation of IκBα, activation of NF-κB and expression of antiapoptotic genes (e.g., Fas-associating protein with death domain (FADD)-homologous interleukin-1β-converting enzyme (ICE)-like protease (FLICE)/caspase-8 inhibitor protein (cFLIP)) and antioxidant molecules (e.g., manganese superoxide dismutase (MnSOD)) that promote cell survival (Figure 2, center). On the other hand, recruitment of TRADD to the activated TNF-R1 complex, in the absence of RIP1, allows initiation of caspase-dependent apoptotic signaling via recruitment of FADD along with caspase-8 and -10 (Figure 2, left). This series of events promotes cleavage of the BH3-only protein Bid into tBid that relocalizes to mitochondria to activate the proapoptotic Bax and Bak factors, the release of mitochondrial factors such as cytochrome *c* and Smac/Diablo, and the activation of effector caspases to culminate in cell death via apoptosis.

A third pathway that is initiated by the binding of TNFα to TNF-R1 involves activation of JNK that can lead to PCD via apoptosis or necrosis (Figure 2, right). Sustained activation of JNK involves TNF-R1-mediated engagement of RIP1, but in this case the complex does not require TRADD, and relies upon the kinase activity of RIP1 and the generation of ROS (Leist and Jaattela, 2001; Jaattela and Tschopp, 2003; Papa *et al*., 2006; Zheng *et al*., 2006; and in the review by Bubici *et al*., 2006, this issue). ROS can contribute to prolonged JNK activity (Sakon *et al*., 2003) via inactivation of mitogen-activated protein kinase (MAPK) phosphatases (MKPs) (Kamata *et al*., 2005) and also via activation of apoptosis signal-regulating kinase 1 (ASK1)/mitogen-activated protein kinase/extra cellular signal-regulated kinase kinase 5 (MEKK5) (Davis, 2000; Matsuzawa and Ichijo, 2005). Although the details of how activation of the JNK pathway leads to apoptosis or necrosis have yet to be worked out, the metabolic state of the cell seems to be a key factor in this decision (reviewed in Papa *et al*., 2006). Actively dividing cells that depend on glycolysis appear to be more susceptible to undergoing necrosis, whereas quiescent cells that rely on oxidative phosphorylation appear to be better candidates for cell death via apoptosis (reviewed in Edinger and Thompson, 2004).

The recent demonstration that recruitment of TRADD to TNF-R1 is necessary for activation of the NF-κB survival pathway and for the caspase-dependent apoptotic cascade, but not for RIP1-dependent necrosis following activation of JNK raises speculations that the common or exclusive engagement of TRADD and RIP1

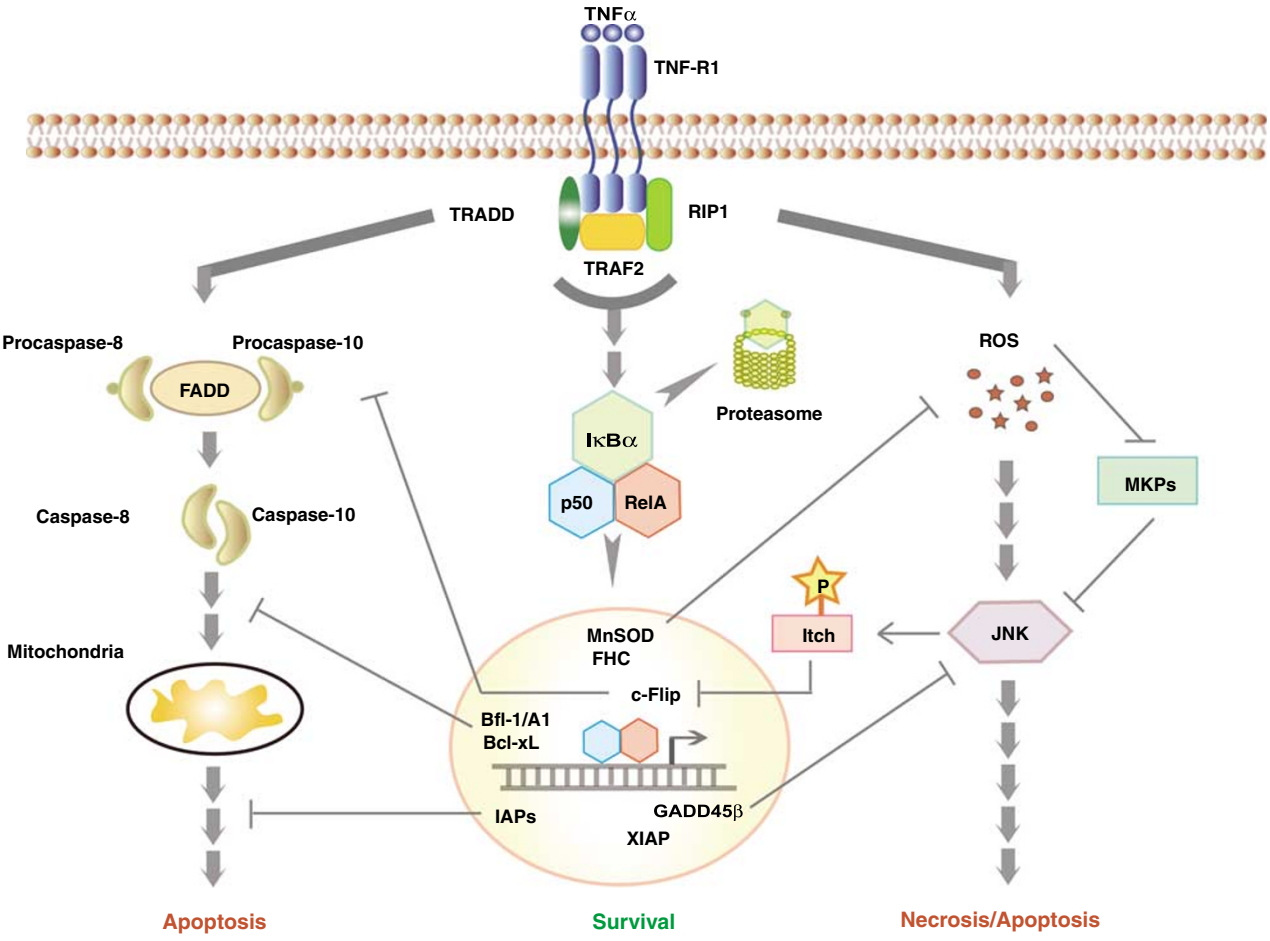

Figure 2 The interplay between the NF-κB, caspase and JNK signaling cascades determines the outcome of cells treated with TNFα. (**Left**) Binding of TNFα to TNF-R1 promotes activation of the caspase-dependent apoptotic cascade by engaging the adaptor protein TRADD, followed by recruitment of FADD, pro-caspase-8 and pro-caspase-10. Activated caspase-8 and -10 lead to activation of proapoptotic Bcl-2 family members that trigger the release of mitochondrial factors and activation of downstream effector caspases, resulting in cell death. (**Right**) Alternatively, recruitment of RIP1 to the activated TNF-R1 complex, in the absence of TRADD, promotes ROS generation and activation of the JNK signaling pathway that can lead to necrosis or apoptosis. (**Center**) However, the joint recruitment of TRADD along with RIP1 and TRAF2 to the activated TNF-R1 leads to activation of the IKK kinase complex, proteasome-dependent destruction of the NF-κB inhibitor IκBα, nuclear translocation of NF-κB dimers (e.g., p50/RelA) and synthesis of antiapoptotic factors and antioxidant molecules to suppress apoptotic or necrotic cell death induced by the caspase and JNK signaling pathways.

also weighs in the cell's decision to survive via NF-κB activation, undergo apoptosis via the caspase-dependent cascade, or die by necrosis via activation of JNK (Zheng *et al*., 2006). Although the extent to which NF-κB-mediated inhibition of the JNK pathway suppresses apoptosis as opposed to necrosis remains an open question (Ventura *et al*., 2004); reviewed in Papa *et al*., 2006), it is clear that efficient NF-κB-dependent synthesis of antiapoptotic factors and antioxidant molecules is necessary to block death signaling induced by the caspase and JNK cascades. An interesting new addition to the interplay between NF-κB and JNK is the recent finding that JNK-dependent phosphorylation and stabilization of the E3 ligase ITCH promotes TNF-induced killing via degradation of the antiapoptotic NF-κB target c-FLIP(L) (Chang *et al*., 2006). Clearly, cross-talk between the NF-κB, caspase and JNK signaling pathways is critical to dictate the outcome for a cell and explains why TNFα-induced PCD is only observed under conditions where NF-κB is inhibited (reviewed in Papa *et al*., 2004b; Luo *et al*., 2005; Papa *et al*., 2006; see below).

Molecular mechanisms whereby NF-κB suppresses or promotes PCD: an update

How NF-κB manages to act in either an anti- or a proapoptotic manner has been a subject of intense investigation. To achieve these effects, NF-κB employs several different strategies. While most of them rely on its transcriptional activity, significant progress in recent years has pinpointed additional new ways in which NF-κB operates in this context.

Suppression of apoptosis and necrosis

Transcriptional activation of antiapoptotic and antioxidant molecules. By and large, the protective activity of NF-κB depends on its ability to transactivate gene expression, although there are some exceptions to this rule (reviewed in Kucharczak *et al*., 2003; Luo *et al*., 2005; Papa *et al*., 2006). To suppress apoptosis, NF-κB relies on activating a collection of transcriptional target genes whose products can block different steps of the extrinsic and/or intrinsic death-signaling cascades (Table 1). These targets include the inhibitor of apoptosis proteins XIAP, c-IAP1 and c-IAP2 that have been implicated in preventing activation of pro-caspase-9 and blocking caspase-3 and -7 activity, although XIAP appears to be the most potent in this regard (reviewed in Deveraux *et al*., 1998; Liston *et al*., 2003; Wright and Duckett, 2005). More recently, a new caspase-independent mechanism has emerged to explain the anti-apoptotic effects of IAPs, as the baculovirus-derived OpIAP protein has been shown to induce ubiquitination and degradation of the IAP antagonist Smac/Diablo (Wilkinson *et al*., 2004; Duckett, 2005). This is consistent with other recent work suggesting that c-IAP1 and c-IAP2 bind to, but do not inhibit, caspases (Eckelman and Salvesen, 2006). XIAP has also been proposed to block cell killing due to persistent activation of JNK (Tang *et al*., 2001), and recent work indicates that XIAP inhibits JNK activation and apoptosis induced by TGF-β1, by inducing ubiquitination and degradation of the kinase TAK1 (Kaur *et al*., 2005). Survivin is a unique member of the IAP family that recently joined the ranks of NF-κB-regulated antiapoptotic targets (Kawakami *et al*., 2005; Shishodia *et al*., 2006).

Other important NF-κB target genes include ones encoding the antiapoptotic Bcl-2 family proteins Bcl-xL, Bfl-1/A1, NR13 and Bcl-2, all of which suppress discharge of mitochondrial cytochrome *c* and Smac/Diablo by antagonizing the activity of proapoptotic Bcl-2 family members. Upregulation of these proapoptotic Bcl-2 family members prevents activation of effector caspases and cell death. The adaptor molecules TRAF1 and TRAF2 suppress TNF-induced apoptosis along with c-IAP1 and c-IAP2 by augmenting activation of the NF-κB signaling cascade, essentially creating a positive feedback loop (Wang *et al*., 1998). The zinc-finger protein A20 is well documented to block both NF-κB signaling and TNF-induced cell death (reviewed in Beyaert *et al*., 2000). Recent work has uncovered that A20 promotes the elimination of RIP1 in the TNF-R1 complex by replacing K63 ubiquitin chains with K48 polyubiquitin, thereby acting as both a deubiquitinating enzyme and as an E3 ligase to downregulate NF-κB signaling to suppress chronic inflammation and cell death (Wertz *et al*., 2004). cFLIP potently suppresses death receptor-induced apoptosis, as it blunts activation of pro-caspase-8 and -10 (Micheau *et al*., 2001; Kreuz *et al*., 2004). It has recently been shown that cFLIP also participates along with caspase-8 in promoting NF-κB activation via BCL-10, mucosa-associated-lymphoid-tissue lymphoma-translocation gene 1 (MALT1) and RIP1 and thus plays a key role in T-cell survival and proliferation following T-cell-receptor stimulation (Abraham and Shaham, 2004; Zhou *et al*., 2004; Launay *et al*., 2005, reviewed in Budd *et al*., 2006). There has been some debate regarding the anti- vs proapoptotic activities of the stress-inducible NF-κB-regulated gene IEX-1 (reviewed in Kucharczak *et al*., 2003). Possibly relevant developments in this area include the description of distinct regions of IEX-1 that are involved in its anti- vs proapoptotic effects and the finding that IEX-1's protective activity is correlated with regulation of intracellular ROS production (Shen *et al*., 2006). NF-κB-mediated induction of decoy TNF-related apoptosis-inducing ligand (TRAIL) receptor 1 (DcR1) has been implicated in suppressing apoptosis induced by TRAIL (Bernard *et al*., 2001b), whereas induction of the cathepsin B inhibitor serine protease inhibitor 2A (Spi2A) reduces TNF cytotoxicity by impeding lysosome-mediated PCD via necrosis (Liu *et al*., 2003, reviewed in Papa *et al*., 2006).

Myd118/GADD45β can oppose JNK signaling by blocking the activity of the JNK-activating kinase MKK7/JNKK2, hence suppressing cell death (De Smaele *et al*., 2001; Papa *et al*., 2004a, b), although *gadd45β*-deficient mice do not show persistent activation of JNK akin to those lacking *xiap* (Harlin *et al*., 2001; Amanullah *et al*., 2003). Furthermore, GADD45β has recently been implicated in protecting T cells from activation induced cell death (AICD) following activation of RelA-containing NF-κB complexes (Mittal *et al*., 2006). In keeping with the idea that other NF-κB-regulated factors are also important for suppressing proapoptotic JNK signaling, NF-κB controls the expression of the antioxidant enzyme MnSOD, and of ferritin heavy chain (FHC) that prevents the buildup of ROS via iron sequestration (Bernard *et al*., 2001a; Bernard *et al*., 2002; Delhalle *et al*., 2002; Tanaka *et al*., 2002; Pham *et al*., 2004; reviewed in Papa *et al*., 2004b). It should be noted that many of the genes illustrated in Table 1 show partial protective activity under conditions where NF-κB is inactivated, suggesting that in many cases transcriptional activation of multiple cell death inhibitors are necessary for manifestation of NF-κB's full protective activity.

Other means by which NF-κB enhances cell survival. In addition to its ability to transactivate the expression of death-antagonizing genes, NF-κB has evolved several other ways to restrain PCD (Table 1). One mechanism involves the transcriptional repression of death-inducing genes, as exemplified by RelA-dependent suppression of caspase-8, and TRAIL receptors DR4 and DR5 gene expression that confers survival in response to the death-inducing ligand TRAIL. This is accompanied by induction of the antiapoptotic factors c-IAP1 and c-IAP2 (Chen *et al*., 2003b). Transcriptional repression of the hypoxia-inducible protein BNIP3, which was implicated in cell death, has recently been associated with the protective activity of NF-κB in ventricular myocytes (Baetz *et al*., 2005). An alternative means by which

Table 1 NF-κB target genes involved in the regulation of programmed cell death (PCD)

Inhibition of PCD	*Function in PCD*	*References*
Transactivation of antiapoptotic genes		
IAPs:		
c-IAP1, c-IAP2, XIAP, Survivin	Prevents activation of pro-caspase-9 Inhibits caspase-3 and -7, and also induces degradation of Smac/Diablo XIAP inhibits persistent JNK activation	Liston *et al*. (1996); Chu *et al*. (1997); You *et al*. (1997); Deveraux *et al*. (1998); Stehlik *et al*. (1998); Takahashi *et al*. (1998); Wang *et al*. (1998); Tang *et al*. (2001); Wilkinson *et al*. (2004); Duckett (2005); Kawakami *et al*. (2005); Shishodia *et al*. (2006)
Bcl-2 family:		
Bcl-2, Bcl-xL, Bfl-1/A1, NR13	Antagonizes proapoptotic Bcl-2 family members	Grumont *et al*. (1999); Lee *et al*. (1999a); Lee *et al*. (1999b); Tamatani *et al*. (1999); Wang *et al*. (1999); Zong *et al*. (1999); Chen *et al*. (2000); Grossmann *et al*. (2000); Catz and Johnson (2001)
Adaptor molecules;		
TRAF-1, TRAF-2	Augments NF-κB activation	Wang *et al*. (1998); Schwenzer *et al*. (1999)
Others:		
A20	Promotes degradation of RIP1 via DUB and E3 ligase activities	Krikos *et al*. (1992); Malewicz *et al*. (2003); Wertz *et al*. (2004)
c-FLIP	Interferes with activation of pro-caspase-8 and -10 and works with caspase-8 to activate NF-κB via Bcl-10/MALT1/RIP1	Kreuz *et al*. (2001); Micheau *et al*. (2001); Su *et al*. (2005)
IEX-1	Regulates ROS production	Shen *et al*. (2006)
Spi2A	Cathepsin B inhibitor, blocks lysosome-mediated cell death	Liu *et al*. (2003)
DcR1 (decoy receptor 1)	Decoy Trail death receptor	Bernard *et al*. (2001b)
GADD45β	Inhibits TNFα-induced JNK Inhibits AICD in T cells	De Smaele *et al*. (2001); Jin *et al*. (2002); Zazzeroni *et al*. (2003a); Zazzeroni *et al*. (2003b); Mittal *et al*. (2006)
α-fetoprotein	Blocks TGFβ-induced apoptosis	Cavin *et al*. (2004)
Transactivation of antioxidant molecules		
MnSOD	Clears reactive oxygen species	Bernard *et al*. (2001a); Bernard *et al*. (2002); Delhalle *et al*. (2002); Tanaka *et al*. (2002)
FHC	Suppresses ROS accumulation through iron sequestration	Pham *et al*. (2004)
Activation of E3 ligases		
Hdm2, Mdm2	Induces p53 degradation	Tergaonkar *et al*. (2002); Kashatus *et al*. (2006)
Transactivation and regulation of alternative splicing		
TERT	Maintains telomere length	Hrdlicková *et al*. (2006)
Transcriptional repression of pro-death molecules		
BNIP3	Hypoxia-inducible death factor	Baetz *et al*. (2005)
Caspase 8	Apical caspase	Chen *et al*. (2003a)
DR4 (TRAIL-R1), DR5 (TRAIL-R2)	Death receptors	Chen *et al*. (2003a)
Transactivation of proapoptotic genes		
Ligands:		
FasL (CD95L), TNFα, TRAIL	Death receptor ligands	Collart *et al*. (1990); Shakhov *et al*. (1990); Kasibhatla *et al*. (1998); Matsui *et al*. (1998); Chan *et al*. (1999); Kasibhatla *et al*. (1999); Baetu *et al*. (2001); Rivera-Walsh *et al*. (2001); Siegmund *et al*. (2001)
Receptors:		
Fas (CD95), DR4 (TRAIL-R1), DR5 (TRAIL-R2), DR6	Death receptor	Kasibhatla *et al*. (1998); Chan *et al*. (1999); Kasof *et al*. (2001); Ravi *et al*. (2001)
Bcl-2 family:		
Bcl-xS	Antagonizes prosurvival Bcl-2 proteins	Chen *et al*. (2003b)
Bax	Multi-domain proapoptotic Bcl-2 protein	Grimm *et al*. (2005)
B7-H1 (PCD ligand1/pdcd1)	Induces apoptosis of T lymphocytes	Dong *et al*. (2002); Yu *et al*. (2004); Lee *et al*. (2005)
Caspase-11	Can activate caspase-3	Kang *et al*. (2000); Schauvliege *et al*. (2002)

Table 1 *(continued)*

Inhibition of PCD	*Function in PCD*	*References*
Transactivation of tumor suppressors		
CYLD	Deubiquitinase for TRAF2/6 and NEMO	Jono *et al.* (2004)
P53	Acts with NF-κB and IRF-1 to stimulate production of iNOS	Wu and Lozano (1994); Denk *et al.* (2000)
Transcriptional repression of antiapoptotic genes		
Bcl-xL	Antagonizes proapoptotic Bcl-2 proteins	Campbell *et al.* (2004); Jacque *et al.* (2005)
XIAP	Inhibits caspase-3 and caspase-7, prevents activation of pro-caspase-9	Campbell *et al.* (2004)
TRAF2	Augments NF-κB activation	Hu *et al.* (2006a)
Transactivation and regulation of alternative splicing		
Bcl-xS	Alternatively spliced form of *bcl-x* Antagonizes prosurvival Bcl-2 proteins	Chen *et al.* (2005)

Abbreviations: AICD, activation induced cell death; BNIP3, Bcl-2/adenovirus E1B 19KDa interacting protein 3; cFLIP, FLICE/caspase-8 inhibitor protein; FHC, ferritin heavy chain; GADD45beta, growth arrest and DNA damage 45beta; IEX-1, immediate early response gene X-1; iNOS, inducible nitric oxide synthase; IAP, inhibitor of apoptosis protein; JNK, janus kinase; MALT, mucosa-associated lymphoid tissue; MnSOD, manganese superoxide dismutase; RIP1, receptor interacting protein kinase 1; ROS, reactive oxygen species; Spi2A, serine protease inhibitor 2A; NFα, tumor necrosis factor alpha; TRAF, TNF receptor-associated factor; TRAIL, TNF-related apoptosis-inducing ligand; XIAP, X-chromosome-linked IAP.

NF-κB's transcriptional activity is associated with improved cell survival is via enhanced degradation of tumor suppressor p53 caused by NF-κB-dependent transcriptional upregulation of Mdm2, the E3 ligase for p53. The consequent destabilization of p53 has been implicated in IKKβ's ability to promote the resistance of MEFs to killing by the chemotherapeutic drug doxorubicin (Tergaonkar *et al.*, 2002). In keeping with these findings, the IκB-related Bcl-3 protein, which acts as a transcriptional coactivator with p50 or p52 homodimers, has recently been shown to suppress proapoptotic activation of p53 in response to DNA damage by inducing expression of Hdm2, thereby provoking p53 degradation (Kashatus *et al.*, 2006). However, it remains to be determined if this is subject to cell-type specificity, as others have found that activation of p53 in IKKβ-deficient intestinal epithelial cells following radiation is not associated with decreased expression of *mdm2* (Egan *et al.*, 2004). Recently, transcriptional activation of the telomerase reverse transcriptase gene TERT by the NF-κB oncoprotein v-Rel has been associated with suppression of apoptosis in v-Rel-transformed lymphoid cells, along with alternative splicing to generate full-length TERT splice variants that encode the active form of the enzyme (Hrdlicková *et al.*, 2006). Together with data showing that NF-κB can lead to the production of alternatively spliced *bcl-x* gene products Bcl-xL and Bcl-xS, this raises the possibility that the regulation of alternative splicing constitutes a novel mode of apoptosis regulation by NF-κB.

Mechanisms implicated in NF-κB's proapoptotic activity

Despite undisputed evidence that NF-κB suppresses PCD, it is clear that under certain circumstances, NF-κB activation is associated with or required for cell death (reviewed in Kucharczak *et al.*, 2003). A few proapoptotic NF-κB transcriptional target genes appear to be involved in this effect. These proapoptotic targets include the death receptors Fas(CD95), TRAIL receptors DR4, DR5 and DR6, the death-inducing ligands FasL, TNFα and TRAIL, tumor suppressor p53, the proapoptotic multidomain Bcl-2 family member Bax and the proapoptotic alternatively spliced form of Bcl-xL, Bcl-xS (Collart *et al.*, 1990; Shakhov *et al.*, 1990; Wu and Lozano, 1994; Kasibhatla *et al.*, 1998; Matsui *et al.*, 1998; Chan *et al.*, 1999; Kasibhatla *et al.*, 1999; Baetu *et al.*, 2001; Kasof *et al.*, 2001; Rivera-Walsh *et al.*, 2001; Siegmund *et al.*, 2001; Zheng *et al.*, 2001; Kim *et al.*, 2002; Shou *et al.*, 2002; Kucharczak *et al.*, 2003; Chen *et al.*, 2003b; Grimm *et al.*, 2005). A recent example of a proapoptotic NF-κB activity is the apoptosis of cortical neurons that occurs following serum withdrawal and K+ loss, which is associated with increased levels of NF-κB and the resultant upregulation of Bcl-xS (Tao *et al.*, 2006). NF-κB-dependent transcriptional activation of the *bcl-x* promoter along with accumulation of alternatively spliced Bcl-xS transcripts suggests that NF-κB plays a role in alternative splicing as a new way to regulate cell fate.

Two signals that emanate from endoplasmic reticulum (ER) stress have highlighted an important role for NF-κB in ER stress-induced apoptosis, by establishing a link with the TNF-R death receptor pathway. Studies have shown that ER stress promotes NF-κB-dependent activation of TNFα expression via association of IKK with the ER stress sensor IRE1α and TRAF2, whereas simultaneous downregulation of TRAF2

impairs activation of NF-κB and JNK greatly potentiates the toxic effect of TNFα (Hu *et al*., 2006a).

Another way in which NF-κB can enhance cell death is via upregulation of the tumor suppressor CYLD that acts as a deubiquitinase to remove K63 polyubiquitin chains from TRAF2/6 and NEMO, leading to disassembly of the IKK complex, termination of NF-κB signaling and enhanced cell death (Brummelkamp *et al*., 2003; Kovalenko *et al*., 2003; Jono *et al*., 2004; Reiley *et al*., 2004). CYLD also deubiquitinates the p50 and p52 coactivator Bcl-3 to suppress activation of genes that promote cell proliferation and tumor growth (Ikeda and Dikic, 2006; Massoumi *et al*., 2006). Loss of CYLD in cylindromatosis is associated with tumor development of hair follicles and sweat and scent glands, which is coincident with increased NF-κB signaling. CYLD has recently been shown to also be important for T-cell development and has been implicated to promote deubiquitination of the T-cell-specific tyrosine kinase Lck (Reiley *et al*., 2006). NF-κB-induced cell death has also been linked to increased expression of the pro-death factors B7-H1 (PCD ligand 1, pdcd1), which induces apoptosis of T lymphocytes (Dong *et al*., 2002; Yu *et al*., 2004; Lee *et al*., 2005), and caspase-11, which can activate the death effector caspase-3 (Kang *et al*., 2000; Schauvliege *et al*., 2002). Additionally, NF-κB can function indirectly to activate genes that regulate cell death via upregulation of other transcription factors like p53 or IRF-1, which acts along with NF-κB to stimulate production of the death-promoting inducible nitric oxide synthase after ischemic injury (Wu and Lozano, 1994; reviewed in Denk *et al*., 2000).

Mechanisms that help determine NF-κB's protective vs sensitizing effect toward apoptosis: new insights

Several mechanisms can influence the protective activity of NF-κB. Some of these alter NF-κB's transcriptional properties, its recruitment to promoters or its subcellular localization and involve post-translational modification of NF-κB components, its interaction with tumor suppressors or other cellular factors (Table 2). New developments are briefly reviewed in this section.

Alteration of NF-κB's transcriptional activity

Important advances in our understanding of how NF-κB is activated in response to genotoxic stress include the discoveries that the IKK regulator NEMO translocates to the nucleus in response to DNA damage, and in the nucleus NEMO associates with the kinase RIP1 and the p53-inducible death domain-containing protein (PIDD) to undergo sumoylation (Huang *et al*., 2003; Janssens *et al*., 2005). In this complex, PIDD constitutes a critical link between DNA damage and the cell death response. Ataxia telangiectasia mutated (ATM)-dependent phosphorylation of NEMO promotes replacement of SUMO with ubiquitin, and enables relocalization of NEMO to the cytoplasm where it engages in active IKK complexes with IKKα/IKKβ to promote NF-κB signaling (Huang *et al*., 2003). Interestingly, the outcome of the DNA damage response appears to be dictated by the inducing stimulus. Whereas the topoisomerase I inhibitor camptothecin and the topoisomerase II inhibitor etoposide enhance NF-κB's transcriptional activity to promote expression of antiapoptotic genes and cell survival, other agents like doxorubicin and daunorubicin are somewhat poor inducers of NF-κB-mediated transcription and can actually repress expression of NF-κB-dependent antiapoptotic genes in certain cells, and thereby sensitize them to apoptosis (Huang *et al*., 2000; Campbell *et al*., 2004).

How some of these agents shift the transactivating property of RelA into that of a promoter-specific transcriptional repressor has recently come to light (Figure 3; reviewed in Perkins and Gilmore, 2006). Atypical activators of NF-κB such as UV-C radiation, the chemotherapeutic drugs daunorubicin, doxorubicin and cisplatin, or the tumor suppressor protein ARF that is activated in response to DNA damage have all been found to abolish the protective activity of RelA by converting it into a transcriptional repressor of antiapoptotic genes. However, these different inducers utilize slightly different mechanisms to achieve this proapoptotic outcome. Both cisplatin and alternative reading frame protein (ARF) activate ataxia telangiectasia (AT) and rad-related (ATR)-/checkpoint kinase 1 (Chk1)-dependent phosphorylation of RelA on Thr-505, enabling recruitment of histone deacetylase 1 (HDAC1) to actively repress expression of the antiapoptotic Bcl-xL protein and sensitize cells to apoptosis (Figure 3; Rocha *et al*., 2003a; Rocha *et al*., 2005; Campbell *et al*., 2006b). This effect appears to be gene- and stimulus-specific, as expression of the cell death inhibitor XIAP is unaffected by cisplatin, and cell stimulation with the DNA damage-inducing drug etoposide promotes RelA's transactivation property and enhances expression of both Bcl-xL and XIAP to suppress apoptosis (Campbell *et al*., 2006b).

Other atypical NF-κB activators, like UV-C and the anthracycline daunorubicin, prompt RelA to actively repress expression of antiapoptotic factors Bcl-xL, XIAP and A20 in U2OS osteosarcoma cells and sensitize them to apoptosis by promoting recruitment of HDAC1. However, this occurs without the involvement of ATR/Chk1 or Thr-505 phosphorylation of RelA (Figure 3; Campbell *et al*., 2004). Others found that doxorubicin's effect is independent of RelA's association with HDACs, and rather reduces the recruitment of RelA to promoters (Ho *et al*., 2005). Therefore, cell context might be an important contributor to the outcome, as some have found that NF-κB confers protection from cell killing by doxorubicin (Baldwin, 2001; Nakanishi and Toi, 2005), whereas others have shown that activation of NF-κB by doxorubicin is important for its cytotoxic effect in colon cancer cells (Ashikawa *et al*., 2004). New data have revealed that the ability of different topoisomerase II inhibitors to promote transcriptional repression by NF-κB depends on their ability to intercalate into DNA, but not on their ability to inhibit topoisomerase

Table 2 Mechanisms that influence NF-κB's anti- and pro-death activities

Effector	*Mechanism*	*Output*	*References*
Mechanisms that alter NF-κB's transcriptional activity			
ARF, cisplatin	ATR/Chk1-dependent phosphorylation of RelA (Thr505)	Converts RelA into a transcriptional repressor via recruitment of HDAC1	Rocha *et al*. (2003a); Campbell *et al*. (2006b)
UV-C, daunorubicin, doxorubicin	Unknown	Converts RelA into a transcriptional repressor via recruitment of HDAC1 or reduces RelA recruitment to promoter	Campbell *et al*. (2004); Ho *et al*. (2005)
DNA damage via PIDD	Sumoylation and ubiquitination of NEMO	Activation of the IKK complex	Huang *et al*. (2003); Janssens *et al*. (2005)
BRCA1	Associates with NF-κB	Enhances NF-κB-mediated transcription of Fas	Benezra *et al*. (2003)
Mechanisms that affect NF-κB's recruitment to promoters			
PRMT2	Blocks nuclear export of IκBα	Increases nuclear IκBα and reduces NF-κB DNA binding	Ganesh *et al*. (2006)
Murr1/COMMD1	Associates with NF-κB	Blocks NF-κB recruitment to chromatin	Burstein *et al*. (2005)
PPARγ	Sumoylation of PPARγ	Promotes retention of corepressor complexes on NF-κB target genes	Pascual *et al*. (2005)
ING4	Associates with RelA	Inhibits RelA DNA binding	Garkavtsev *et al*. (2004)
GSK3β	Phosphorylates Ser-468 in RelA or other unknown mechanism	Promotes recruitment of RelA to gene-specific promoters	Steinbrecher *et al*. (2005)
Mechanisms that alter NF-κB's subcellular localization			
Fas-associated factor 1 (FAF1)	Interacts with RelA	Inhibits nuclear translocation of RelA	Park *et al*. (2004)
Aspirin; serum deprivation; UV-C	Unknown	Sequesters RelA in the nucleolus	Stark and Dunlop (2005)
Pin1	Binds to RelA and blocks its interaction with IκBα and with SOCS-1	Prevents nuclear export of RelA and prevents its ubiquitin-dependent degradation	Ryo *et al*. (2003)
Notch-IC	Interacts with NF-κB	Competes with IκBα and increases nuclear accumulation of NF-κB	Shin *et al*. (2006)
Other means of regulating NF-κB activity			
INK4	Associates with RelA	Suppresses RelA's transcriptional activity	Wolff and Naumann (1999)
c-Myc	Blocks NF-κB-dependent activation	Blocks transactivation of antiapoptotic Bfl-1	You *et al*. (2002)
Degradation of promoter-bound RelA	Ubiquitin/proteasome-dependent degradation of RelA	Terminates NF-κB-mediated transcription	Saccani *et al*. (2004)
Exchange of subunits within NF-κB dimers	Formation of RelA/RelB heterodimer	Represses the expression of Bcl-xL	Jacque *et al*. (2005)
p27	Associates with RelA	Enhances RelA's transcriptional activity	Wolff and Naumann (1999)

Abbreviations: ARF, alternate reading frame protein; ATR/Chk1, ataxia telangiectasia and rad-related/checkpoint kinase 1; GSK3BETA, glycogen synthase kinase-3 beta; ING4, inhibitor of growth family member 4 isoform; INK4, inhibitor of cyclin-dependent kinase 4; HDAC1, histone deacetylase 1; Murr1/COMMD1, mouse U2af1/rs1 region/copper metabolism (Murr1) domain containing 1; PIDD, p53-inducible death domain-containing protein; PPARgamma, peroxisome proliferator-activated receptor gamma; PRMT2, protein arginine methyltransferase 2; SOCS-1, suppressor of cytokine signaling 1; UV, ultraviolet.

II or on production of oxygen-free radicals (Campbell *et al*., 2006a). Others found that certain types of DNA lesions are correlated with production of TNFα and that this influences the outcome of the cells (Strozyk *et al*., 2006). Although much remains to be understood at the molecular level, these findings begin to elucidate why atypical activators like etoposide promote RelA's transcriptional and antiapoptotic activity while others repress it. These findings should be an important consideration for the chemotherapeutic treatment of tumors in which NF-κB is involved (see below).

In addition to the effects of ARF on RelA's transcriptional and protective activities, several reports have found that association of NF-κB with other tumor suppressors either facilitates cell death or suppresses its proproliferative effect. One example is the interaction of RelA with BRCA1, which magnifies NF-κB-mediated transcription of the death-inducing receptor Fas (CD95) to enhance apoptosis (Benezra *et al*., 2003). In another example, tumor suppressor p53 was shown to suppress expression of Bcl-3, which acts as a transcriptional coactivator with p52 (Rocha *et al*., 2003b). In this scenario, p53 is thought to promote the association of p52 with HDAC1 to repress expression of cyclin D1. When analysed in the context of ARF activity, the latter finding suggests that the net effect of ARF is to

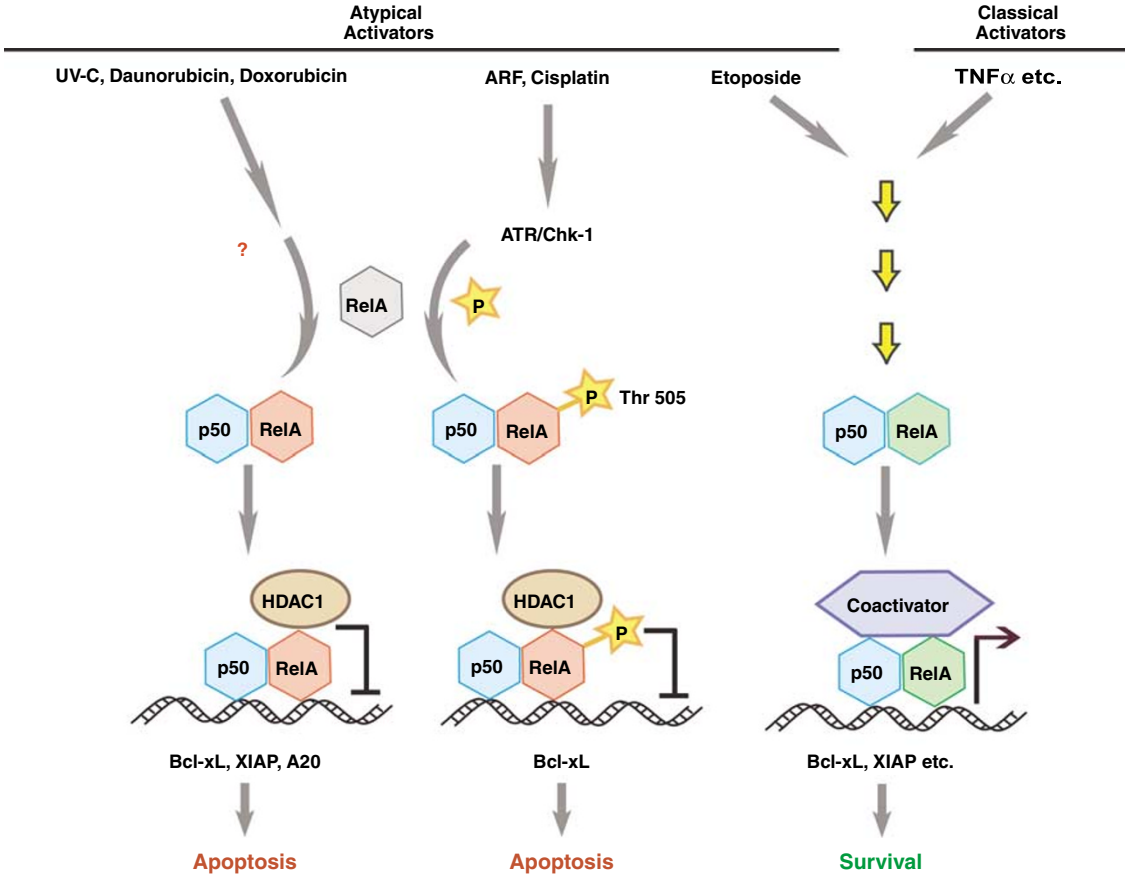

Figure 3 Atypical activators of NF-κB can convert RelA from a transcriptional activator into a transcriptional repressor of antiapoptotic genes. (**Center**) Tumor suppressor ARF and cisplatin can promote ATR/Chk1-dependent phosphorylation of RelA (Thr505) to promote recruitment of HDAC1 and gene-specific transcriptional repression of Bcl-xL, resulting in cell death. (**Left**) UV-C, daunorubicin and doxorubicin can similarly provoke association of RelA with HDAC1, but independent of RelA phosphorylation, to actively repress expression of Bcl-xL, XIAP and A20, leading to apoptosis. (**Right**) In contrast, activation of NF-κB by etoposide or classical activators like TNFα promotes RelA-dependent transcriptional activation of Bcl-xL and XIAP, resulting in cell survival.

neutralize both NF-κB's antiapoptotic and growth-promoting activities, given that ARF activates p53 by inhibiting Mdm2.

Altered NF-κB recruitment to promoters

Phosphorylation of RelA by GSK3β has emerged as another important regulatory mechanism for NF-κB's protective activity, as GSK3β knockout mice die from massive liver apoptosis similar to that seen in mice deficient for RelA, IKKβ or NEMO (Bonnard *et al*., 2000; Hoeflich *et al*., 2000). Interestingly, GSK3β appears to regulate NF-κB's protective activity by promoting efficient recruitment of RelA to gene-specific promoters (Steinbrecher *et al*., 2005). Although inhibition of glycogen synthase kinase-3 beta (GSK3β) has little effect on NF-κB-dependent expression of genes like IκBα or macrophage inflammatory protein 2 (MIP-2), it adversely affects expression of interleukin (IL-6) and monocyte chemoattractant protein 1 (MCP-1). Although it remains to be determined whether GSK3β affects other pathways or factors that contribute to NF-κB-dependent gene transactivation, the fact that expression of the antiapoptotic *c-iap2* gene is dependent on GSK3β supports the idea that GSK3β plays a role in NF-κB-mediated suppression of cell death. This effect of GSK3β on NF-κB would explain the acute sensitivity of GSK3β-deficient hepatocytes to apoptosis induced by TNFα.

Conversely, a recently published study shows that protein arginine methyltransferase 2 (PRMT2) facilitates PCD when induced by TNF or the DNA-damaging drug etoposide by inhibiting export of IκB from the nucleus, hence reducing NF-κB DNA binding (Ganesh *et al*., 2006). MURR1/COMMD1, an XIAP-interacting factor known for its involvement in copper homeostasis (Burstein *et al*., 2004), has recently been shown to inhibit NF-κB-mediated transcription by suppressing the association of RelA with chromatin, as observed on the promoter for the antiapoptotic NF-κB target gene *c-iap2* (Burstein *et al*., 2005). As XIAP promotes degradation of MURR1/COMMD1 via K48 ubiquitin ligation (Burstein *et al*., 2004), these data raise the possibility of another way in which XIAP promotes cell survival, that is, by indirectly relieving MURR1-dependent inhibition of NF-κB-driven transcription of antiapoptotic genes like *c-iap2*. Peroxisome proliferator-activated receptor PPARgamma (PPARγ) is another

example of a factor that suppresses NF-κB-mediated gene expression. Whereas PPARγ is commonly involved in activating transcription, its sumoylation promotes retention of repressor complexes on NF-κB-dependent proinflammatory genes like those encoding TNF and inducible nitric oxide synthase (iNOS) (Bailey and Ghosh, 2005; Pascual *et al*., 2005). The candidate tumor suppressor ING4 physically associates with RelA and regulates brain tumor growth and angiogenesis by suppressing NF-κB DNA binding to antagonize expression of NF-κB-dependent expression of the proangiogenic *interleukin-8* gene (Garkavtsev *et al*., 2004). Future studies will determine whether ING4 exerts similar effects on anti- or proapoptotic genes that are under NF-κB's transcriptional control.

Altered subcellular localization of NF-κB

Several factors and stimuli influence NF-κB's protective activity by altering its subcellular localization. These include the death receptor Fas-associated factor 1 (FAF1) that inhibits nuclear accumulation of NF-κB (Park *et al*., 2004). Targeting of RelA to the nucleolus appears to be a novel means to reduce RelA's transcriptional and protective activities, as observed following treatment of colorectal cancer cells with aspirin, serum deprivation or UV-C radiation (Stark and Dunlop, 2005). It should be noted, however, that others have found that aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDS) inhibit activation of NF-κB by interfering with the activity of the IKK kinase complex (Kopp and Ghosh, 1994; Yin *et al*., 1998; Yamamoto *et al*., 1999). It remains to be determined whether differences in experimental conditions account for these different observations.

In contrast to factors that antagonize NF-κB's transcriptional and protective activities, the peptidyl prolyl-isomerase Pin1 enhances nuclear accumulation of RelA by blocking its association with IκBα and by interfering with suppressor of cytokine signalling 1 (SOCS-1)-dependent ubiquitination and degradation of RelA (Ryo *et al*., 2003). Although direct evidence is still lacking about whether Pin1 enhances the protective activity of NF-κB, Pin1 is frequently upregulated in breast cancer and in mouse mammary tumors (Ryo *et al*., 2003; Currier *et al*., 2005). A recently published report indicates that the intracellular domain of Notch (Notch-IC) interacts with NF-κB to compete with IκBα, consequently enhancing NF-κB's nuclear retention and sustained NF-κB activity (Shin *et al*., 2006). As with Pin1, further studies will be necessary to determine whether Notch positively impacts NF-κB's antiapoptotic activity. An interesting counterpoint to the effect of Notch-IC on NF-κB activity is evidence that IκBα is recruited to the promoter of the Notch1 target gene *hes1*, and is associated with its transcriptional repression (Aguilera *et al*., 2004).

Other means of regulation of NF-κB's effects on PCD

INK4, best known as a CDK4 inhibitor, has been reported to associate with RelA and suppress its transcriptional activity, whereas the cell-cycle inhibitor p27 reportedly has the opposite effect (Wolff and Naumann, 1999). c-Myc, on the other hand, can block RelA's transactivation potential including activation the NF-κB-regulated cell death inhibitor Bfl-1A1, and can sensitize reconstituted c-Myc-null MEFs to TNF-induced killing (You *et al*., 2002). Other mechanisms that regulate the transcriptional activity and specificity of NF-κB subunits might also indirectly impact the outcome of the cell. These mechanisms include proteasome-mediated degradation of promoter-bound RelA that rapidly terminates the NF-κB response (Saccani *et al*., 2004) and the exchange of subunits within NF-κB dimers. Indeed, RelA can dampen the transcriptional activity of p52/RelB complexes to repress expression of the antiapoptotic Bcl-xL protein in TNF-treated MEFs, resulting in cell lethality (Jacque *et al*., 2005). This was shown to involve formation of RelA/RelB heterodimers devoid of DNA-binding activity.

Conclusions and outlook for therapy

Work in the last few years has reaffirmed the pivotal role of the NF-κB pathway in the regulation of PCD and in tumor cell survival and proliferation (Figure 1) and has shown how interplay of the NF-κB pathway with the caspase and JNK signaling cascades determines whether a cell will live or undergo suicide via apoptosis or necrosis (Figure 2). It is now clear that the ability of NF-κB to suppress cell demise has far-reaching consequences for normal development and homeostasis in the liver, the immune system, the nervous system and ectodermal appendages. NF-κB also impinges on the outcome of viral infections where it often helps to establish viral transformation and pathogenicity. However, in certain cell- and/or stimulus-dependent scenarios, NF-κB has the opposite effect where it can visibly exert a tumor suppressor activity to antagonize cell death. Notable examples include NF-κB activity in the skin epidermis, in certain cells following activation of tumor suppressor ARF or upon treatment with DNA-intercalating anticancer agents like cisplatin (Figure 3).

There has been significant progress in understanding NF-κB's mode of action in contexts where it either suppresses or sensitizes cells to PCD. Although this most commonly involves transcriptional activation of genes that promote or suppress PCD, other means have surfaced including NF-κB-dependent transcriptional repression, alternative splicing and the regulation of ubiquitin ligases and deubiquitinases that act in an indirect fashion to modulate NF-κB activity (Table 1). Deregulated NF-κB activity is well documented in many diseases, including conditions associated with too much or too little cell death. As described in this review, excessive NF-κB activity can be as detrimental as not enough activity. This phenomenon emphasizes the need for mechanisms to tightly regulate NF-κB activity, perhaps within a narrow window. In addition to the negative feedback mechanisms that regulate the NF-κB signaling cascade, there has been significant progress

since we last reviewed this topic (in 2003) in uncovering new mechanisms that control NF-κB's activity in PCD. These include methods of regulating NF-κB's transcriptional activity, its recruitment to promoters, its subcellular localization and its interaction with cellular factors (Table 2).

The NF-κB signaling cascade is an appealing target for therapeutic intervention. The recent demonstration that inducible RNA interference screens can identify previously unsuspected candidates responsible for the constitutive activation of NF-κB is likely to help identify other molecular targets for the treatment of NF-κB-associated cancers (Ngo *et al*., 2006). In the past several years, there has been a major effort to discover compounds that can act at various steps in the pathway to restrict NF-κB activation, as detailed in the accompanying review by Gilmore and Herscovitch (2006, this issue). These strategies offer hopeful prospects to enhance the effectiveness of existing therapies. However, the fact that NF-κB can exhibit tumor suppressor activities in certain contexts should not be overlooked. Efforts to better understand how NF-κB functions to suppress or promote PCD and the mechanisms that dictate its anti- vs pro-death activities will undoubtedly continue to bring invaluable insights for this worthwhile pursuit.

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