Chapter 5

PRO-APOTOTIC AND ANTI-APOPTOTIC EFFECTS OF TUMOR NECROSIS FACTOR IN TUMOR CELLS

Role of Nuclear Transcription Factor NF-κB

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1. INTRODUCTION

An intricate balance between cell growth and cell death drives the proper growth, development, and function of most tissues [1]. A vast amount of information has accumulated regarding the molecular mechanisms governing cell growth, but the mechanisms by which cells regulate their own death still remain a matter of great intrigue and have recently begun to acquire great importance. One known mechanism, apoptosis, or programmed cell death, is a physiological process believed to be responsible for the deletion of unwanted cells during organ and tissue development, tissue homeostasis and removal of self-reactive immune cells and pathologically induced tissue damage. Virus-infected cells are eliminated by the interaction with cytotoxic T-lymphocytes that kill the virus infected cells by inducing apoptosis [2,3]. Cells that have DNA damage undergo apoptosis so as to eliminate cells that have accumulated genetic mutations and may become cancerous [4,5]. In addition to being activated during development-related cell reduction, apoptosis can be triggered in many cell types by various stresses, including chemotherapeutic agents, cytokines, ionizing radiation, osmotic stress, and expression of viral proteins such as E1A [6].

Extensive research within the last few years has revealed that cell death, whether at the single cell level, the tissue/organ level, or the organism level,

is as important to life as cell survival. The critical role of apoptosis has been recognized in a wide variety of situations including immunomodulation, autoimmunity, sepsis, arthritis, inflammatory bowel disease, chronic heart failure, periodontal diseases, allograft rejection, neovascularization, obesity, tumorigenesis, meningitis, and parturition [7].

NF-κB is a ubiquitously expressed transcription factor that plays a pivotal role in expression of various inducible target genes that regulate apoptosis among several other vital functions [8] it also controls, cell proliferation, differentiation, and immune and inflammatory responses. This factor is a member of the Rel family of proteins, which bind to specific DNA sequences. In non-stimulated cells, the heterodimeric NF-κB complexes are sequestered in the cytoplasm of most cell types by inhibitory proteins of the IκB family (**Figure 1**) [9].

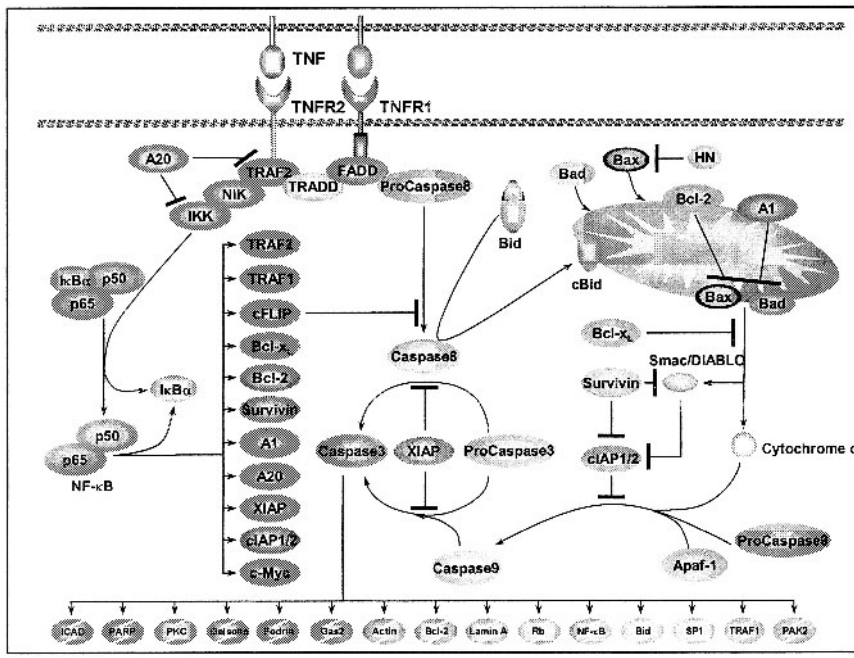

Figure 1. Negative regulation of apoptosis by the NF-κB-regulated gene products.

These inhibitors mask the NF-κB nuclear localization domain and inhibit its DNA-binding activity. In response to a large variety of stimuli, the IκB inhibitor is rapidly phosphorylated and degraded, thus allowing NF-κB nuclear translocation, DNA binding to specific recognition sequences in promoters, and transcription of the target genes [10,11]. Rel/NF-κB

transcription factors are induced in response to a large variety of stimuli and regulate a number of genes. The Rel/NF-κB transcription factor family is comprised of several structurally related proteins that exist in organisms from insects to humans. The vertebrate family includes five cellular proteins: c-Rel, RelA, RelB, p50/p105, and p52/p100. These proteins can form homodimers or heterodimers giving diverse combinations of dimeric complexes that bind to DNA target sites, collectively called κB sites, and directly regulate gene expression. The most common transcription factor of this family is called NF-κB and consists of a p50/RelA heterodimer. The different Rel/NF-κB proteins show distinct ability to form dimers, distinct preferences for different κB sites, and distinct abilities to bind to IκB inhibitor proteins [12]. Thus, different Rel/NF-κB complexes can be induced in different cell types and by distinct signals (**Figure 2**), can interact in distinct ways with other transcription factors and regulatory proteins, and can regulate the expression of distinct gene sets. Numerous kinases have been implicated in the activation of NF-κB induced by different agents (**Figure 3**). Furthermore, the activation of NF-κB is regulated both negatively and positively by other transcription factors and gene products (**Figure 4**).

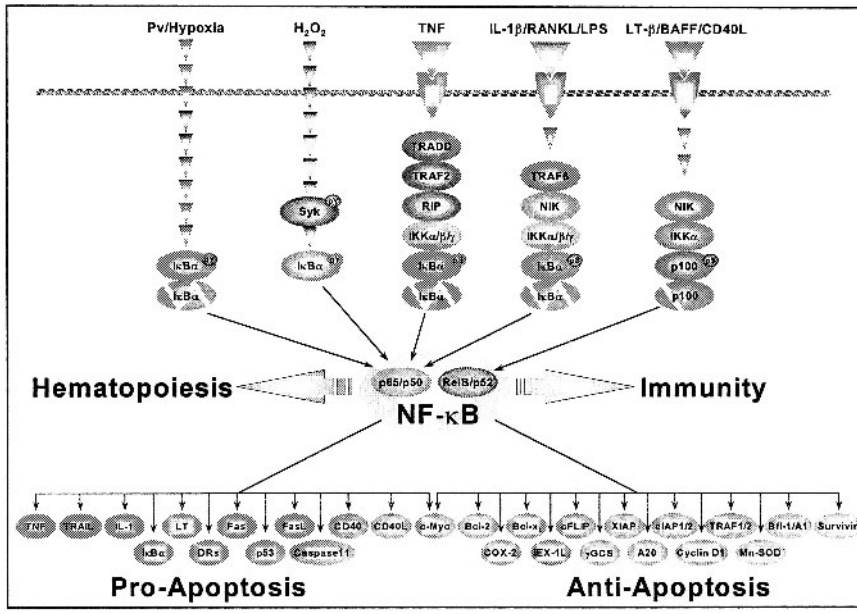

Figure 2. Positive regulation of apoptosis by the NF-κB-regulated gene products.

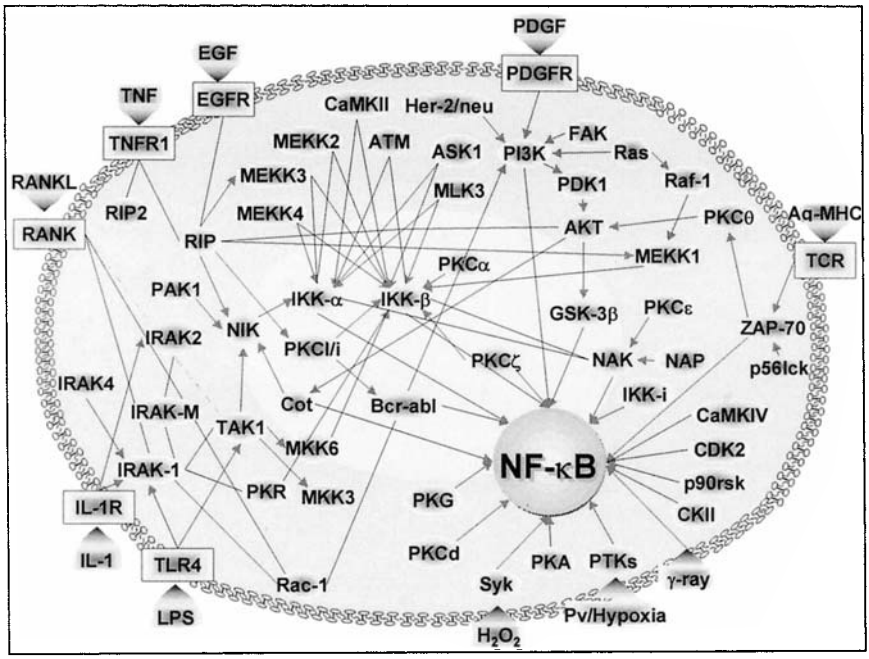

Figure 3. Regulation of NF-κB activation by various protein kinases.

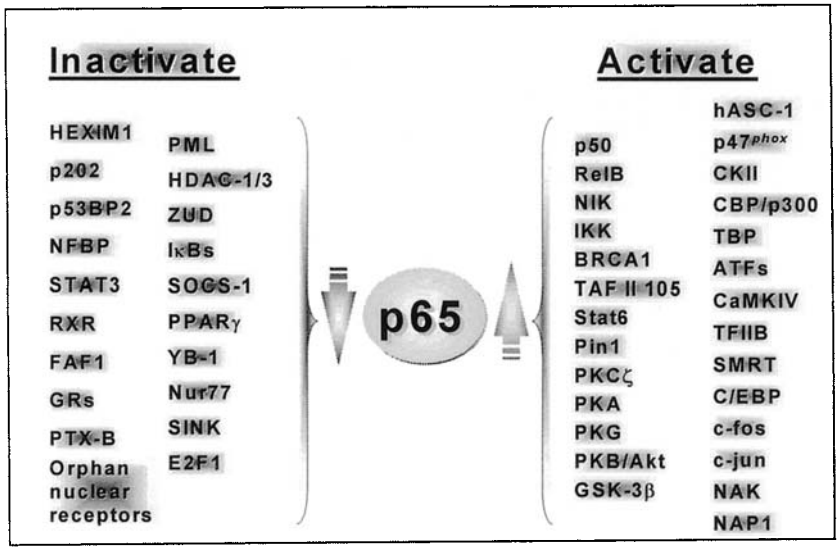

Figure 4. Regulation of NF-κB activation by p65-binding proteins

Mechanism of apoptosis: Besides dying by necrosis, multicellular organisms can initiate a series of events that activate intracellular proteases and ultimately result in the destruction of the cell. These are collectively known as apoptosis. Apoptotic cells undergo an orderly series of biochemical or morphological events including cell shrinkage, mitochondrial breakdown, and nuclear DNA fragmentation [13]. The dying cell degrades into subcellular membrane-bound vesicles called apoptotic bodies, which are ultimately removed by phagocytosis. Apoptosis is a molecular suicide program characterized by cytoplasmic shrinkage, nuclear condensation, and DNA fragmentation into 200-base pair fragments [14-17]. It is a genetically regulated mechanism, and its deregulation can result in multistep carcinogenesis [18-20].

Apoptosis is brought about by activation of the family of proteins known as caspases (cysteinyl, aspartate-specific proteases) [21,22]. There are about 14 caspases involved in the process of apoptosis. Caspases are synthesized as proenzymes that are activated by proteolysis at two or three sites to remove an N-terminal peptide and divide the proenzyme into large and small subunits, which in some cases are joined by a linker domain. The mature caspase is a heterotetramer of two large and two small subunits [23,24]. All caspases are activated by cleavage at a specific aspartate residue and act in a cascade. They are ultimately responsible for the proteolysis of the cellular subtrates responsible for apoptosis.

Poly (ADP-ribose) polymerase (PARP) is the most well characterized substrate for several caspase in many cell systems. Intact PARP (116 kDa) is cleaved into two fragments (89 kDa and 24 kDa) during apoptosis [25,26]. Cleavage of PARP is a valuable indicator of apoptosis, but its biological relevance is not known. Caspase-activated deoxyribonuclease (CAD) is a cytoplasmic endonuclease whose activation is thought to be responsible for generating the oligonucleosomal DNA fragments that are the hallmark of apoptosis [27].

DNA-dependent protein kinase (DNA-PK) is a DNA repair enzyme that is degraded during apoptosis by caspase-3 [28]. Degradation of DNA-PK will result in a decrease in the capacity of the cell to repair damage of nuclear DNA, thus facilitating the breakdown of DNA that is associated with apoptosis. Caspase-6 is responsible for degradation of lamin, which are the major structural components of the nuclear envelope [29]. Cleavage of the cytoskeletal proteins fodrin [30], Gas 2 [31], and actin [32] during apoptosis may induce cell shrinkage and membrane blebbing and alter cell signaling pathways. U1-70kDa, a small ribonucleosomal particle that functions in the splicing of mRNA transcripts, is cleaved during apoptosis (**Figure 1 and Figure 2**) [33]. Caspases also cleave the initiation factors [34]. This may inhibit translation during apoptosis. Caspases also cleave certain cell-signaling

proteins, e.g., and MEKK-1, which are rendered constitutively active and pro-apoptotic. In contrast, protein kinase B, which is involved in the anti-apoptotic pathway, is cleaved and inactivated by caspases [35].

A cell is induced to undergo apoptosis either by internal signals arising within the cells or external signals triggered by death activators that bind to receptors located at the cell surface. Internal signals initiate apoptosis in the mitochondria with the release of cytochrome c [36,37]. The mitochondrial pathway is controlled by the Bcl-2 family of proteins [38]. There are 15 members of the Bcl-2 protein family that share homology in at least one of three conserved domains (BH1–BH4) and these may either promote survival e.g., Bcl-2, Bcl-x_L or promote apoptosis, e.g., Bax, or Bak [39]. The Bcl-2 family of proteins register both positive and negative stimuli and integrate them to determine whether the mitochondrial apoptotic pathway is turned on or off. Oncogenes encode mutated versions of the signaling proteins that control normal cell proliferation e.g., Ras signaling. Another, the Raf oncoprotein eventually initiates apoptosis when the cell receives an abnormal proliferative signal [40].

The apoptotic program can also be initiated by the action of extracellular messengers, termed death ligands. These bind to the cell surface receptors, termed death receptors, that activate intracellular signaling events that begin an apoptotic cascade [41,42]. Death receptors belong to the TNF receptor superfamily that is characterized by a cysteine-rich extracellular ligand-binding domain [43]. Death receptors contain a consensus module known as the death domain that is found in the intracellular portion of the molecule and is involved in transducing the apoptotic signal [6]. Fas and the TNF receptor are the two best-characterized death receptors, the cognate ligands for which are FasL and TNF, respectively.

Among all the known physiological inducers of apoptosis in mammalian cells, tumor necrosis factor (TNF) is perhaps the most potent and well studied. Many other members of the TNF superfamily also induce apoptosis, including LT (lymphotoxin), FasL (fibroblast-associated ligand), TRAIL (TNF-related apoptosis-inducing ligand), DR3L (for death receptor 3 ligand or also known as TWEAK for a weak homologue of TNF), THANK (TNF homologue that activates apoptosis, NF-κB and JNK), and VEGI (vascular endothelial cell growth inhibitor) [44,45]. Whether all these TNF family members induce apoptosis by the same mechanism as TNF is not known. Besides killer cytokines outlined above, apoptosis is also induced by various chemotherapeutic agents.

Within the last few years, a series of biochemical steps have been identified in the apoptotic pathway induced by cytokines and chemotherapeutic agents. For instance in TNF-induced apoptosis the TNF

receptor is activated, which, through its cytoplasmic death domain, recruits a protein called TNF receptor-associated death domain (TRADD), which in turn sequentially recruits Fas-associated death domain (FADD) and FADD-like ICE (FLICE, also called caspase-8) [46-48]. The last activates caspase-9, which in turn activates caspase-3 (the executioner protease), resulting in apoptosis.

In contrast to cytokines, chemotherapeutic agents induce cellular apoptosis by inducing formation of mitochondrial transition pores, a rapid decrease in the mitochondrial transmembrane potential, and release of cytochrome c. The latter, in the presence of the protein Apaf-1, activates caspase-9, which then activates caspase-3. Several recent studies, however, have suggested that these two receptor-mediated and non-receptor-mediated pathways initiated by cytokines and chemotherapeutic agents, respectively, are not exclusive of each other and share similar steps.

Most agents that induce apoptosis also activate NF-κB. Thus it is not too surprising that almost all cytokines of the TNF superfamily and chemotherapeutic agents activate NF-κB. TNF-induced activation of NF-κB (primarily consisting of p50 and p65 subunits) involves recruitment of TNF receptor-associated factor (TRAF)-2 by TRADD, which then binds to NIK. TRADD also binds to receptor-interacting protein (RIP). Either NIK or RIP then activate a kinase called IκBα kinase (IKK), which in turn leads to the phosphorylation, ubiqutination, and degradation of IκBα (the inhibitory subunit of NF-κB), leading to NF-κB activation [48]. Some recent studies exclude NIK from a role in TNF-induced NF-κB activation. How chemotherapeutic agents activate NF-κB is not fully understood, but most likely it also involves phosphorylation, ubiqitination, and degradation of IκBα. How NF-κB activation is linked with induction of apoptosis by TNF and chemotherapeutic agents is the subject of this review.

Anti-apoptotic effects of NF-κB: Almost five years ago it was shown that TNF-induced apoptosis can be blocked by NF-κB activation [49-52]. Rel/NF-κB transcription factors exercise their anti-apoptotic effects in a wide variety of cells to protect them from various apoptotic agents. They promote cell survival by inducing the transcription of anti-apoptotic genes (**Figure 1**). Activation of NF-κB either upregulates the activity of anti-apoptotic genes or downregulates the activity of apoptotic genes. Inhibition of NF-κB nuclear translocation enhances apoptotic killing by cytokines that belong to the TNF superfamily, ionizing radiation, overexpression of oncoproteins, chemotherapeutic agents, cytokines, phorbol esters, hyperoxia, hormones, and micro-organisms (**Table 1**, at the end of this chapter).

Some earlier studies showed that the oncogene v-rel from the avian retrovirus reticuloendotheliosis virus strain can block apoptosis [53] in chickens. Similarily, v-rel rendered chicken B-cells resistant to radiation-

induced apoptosis [54]. A large number of reports have demonstrated the anti-apoptotic effect of NF-κB in a wide variety of cell types. The protective role of NF-κB has now been shown in a large variety of cell types, including human breast carcinoma [50], T-cells [51,55,56], fibroblasts and macrophages [49], endothelial cells [57], EBV-infected lymphoblastoid cells [58], non-small lung cancer cells [59], glomerular mesangial cells [60], human ovarian cancer cells [61], human pancreatic cancer cell lines [62], Ewing sarcoma cells [63], cardiomyocytes [64], mouse embryos [65], and HT1080 fibrosarcoma [52].

Treatment of RelA-deficient (the transcriptionally active subunit of NF-κB) mouse fibroblasts and macrophages with TNF significantly reduced cell viability, whereas $RelA^{+/+}$ cells were unaffected. In addition, reintroduction of RelA into $RelA^{-/-}$ fibroblasts enhanced survival, demonstrating that Rel A is required for protection from TNF [49]. Another report showed that activation of the NF-κB by TNF, ionizing radiation, or daunorubicin protects cells from apoptosis, whereas inhibition of NF-κB enhanced apoptotic killing by these reagents but not by apoptotic stimuli that do not activate NF-κB [52]. Van Antwerp *et al.*, however, showed that the sensitivity and kinetics of TNF-induced apoptosis are enhanced in a number of cell types expressing a dominant-negative IκBα (an inhibitor of NF-κB) [51]. Continued expression of v-Rel is necessary to maintain the viability of transformed lymphoid cells and enables primary spleen cells to escape apoptosis in culture [66].

Liu *et al.* used the signaling proteins and showed that recruitment of FADD to the TNFR1 complex mediates apoptosis, that recruitment of RIP and TRAF2 mediate NF-κB activation, and that activation of the latter protects cells against TNF-induced apoptosis [50]. Substoichiometric TFIID subunit TAFII105 is essential for activation of anti-apoptotic genes in response to TNF-α, serving as a transcriptional co-activator for NF-κB [67].

Adenovirus E1A protein has inhibited activation of NF-κB and rendered cells more sensitive to TNF-induced apoptosis. This inhibition was brought about through suppression of IκB kinase (IKK) activity and IκB phosphorylation [68]. NF-κB can attenuate TNF-α-induced apoptosis without de novo protein synthesis in the human pancreatic cancer cell lines MIA PaCa-2 and Capan-2. TNF-α-induced apoptosis was blocked by IL-1β, a potent inducer of NF-κB activation [62]. These findings suggest that de novo protein synthesis is dispensible for anti-apoptotic effects of NF-κB and support the possibility that NF-κB exerts its anti-apoptotic action through protein-protein interaction.

The NF-κB cascade is important in Bcl-x_L expression and for the anti-apoptotic effects of the CD28 receptor in primary human $CD4^+$ lymphocytes [56]. HuT-78, a lymphoblastoid T-cell line with constitutive NF-κB activity, contains elevated levels of Bcl-x_L protein and, similar to proliferating $CD4^+$

T-cells, is resistant to apoptotic stimuli such as anti-Fas and TNFα. In contrast, the same stimuli readily induced apoptosis in Jurkat cells without producing any detectable Bcl-x_L expression.

The quinone reductase inhibitors dicoumarol and menadione block SAPK/JNK and NF-κB and thereby potentiate apoptosis [69]. Javelaud and Besancon have demonstrated that the repression of JNK activation by NF-κB is involved in the anti-apoptotic effect of this transcription factor in TNFα-treated Ewing sarcoma cells [63]. Also, NF-κB exercises its anti-apoptotic effects through NF-κB-inducing kinases (NIK). NIK induces PC12 cell differentiation and prevents apoptosis [70]. Cardiomyocytes utilize transcription factor NF-κB to activate survival factors in the context of TNF-α stimulation. As locally increased levels of TNFα have been detected in heart failure, NF-κB activity is essential for cellular homeostasis in the heart [64].

NF-κB is required for TNF-mediated induction of the gene encoding human cIAP2. When overexpressed in mammalian cells, cIAP2 activates NF-κB and suppresses TNF cytotoxicity. Both of these cIAP2 activities are blocked in vivo by coexpression of a dominant form of IκB that is resistant to TNF-induced degradation [55]. Functional coupling of NF-κB and cIAP2 during the TNF response may provide signal amplification loop that promotes cell survival rather than death. The *IAP* genes function to protect the cell from undergoing apoptotic death in response to a variety of stimuli. The *IAP* genes *hIAP1, hIAP2,* and *XIAP* were found to be strongly upregulated upon treatment of endothelial cells with the inflammatory cytokines TNFα, IL-1β and LPS, which in turn lead to activation of NF-κB. This suggests that xiap represents one of the NF-κB-regulated genes that counteracts the apoptotic signals elicited by TNFα and thereby prevents endothelial cells from undergoing apoptosis during inflammation [57].

Treatment of WEH1 231 cells with N-tosyl-L-phenylalanine chloromethyl ketone, a protease inhibitor that prevents degradation of IκBα, or with low doses of pyrrolidine dithiocarbamate selectively inhibited NF-κB activation and induced apoptosis [71]. Similarly, microinjection of WEHI 231 cells with either IκBα-GST protein or a c-Rel affinity-purified antibody induced apoptosis [71].

Arlt *et al.* have shown that under certain conditions the resistance of pancreatic carcinoma cells to chemotherapy is due to their constitutive NF-κB rather than the transient induction of NF-κB by some anti-cancer drugs [72]. Exposure of normal keratinocytes to IFN-γ plus TPA produced a synergistic activation of NF-κB. They acquired a resistance to UV-light-induced apoptosis that was dependent on NF-κB because expression of a dominant negative form of IκBα overcame the resistance [73]. There is enough evidence to suggest that activation and proper regulation of NF-κB

is essential for acquisition of an apoptotic–resistant phenotype for epidermal-derived keratinocytes. Kolenko *et al.* have demonstrated that inhibition of NF-κB activity by cell permeable SN50 peptide in human T lymphocytes induces caspase-dependent apoptosis [74]. Kawai *et al.* have shown that p53 is involved in NF-κB inactivation and is required for X-ray-induced apoptosis in thymic lymphoma cells and normal thymocytes [75].

Oxidative stress induces apoptosis in human aortic endothelial cells through the downregulation of Bcl-2, translocation of bax, and upregulation of p53, probably through NF-κB activation. Oxidative stress may play an important role in endothelial apoptosis mediated by hypoxia, through the activation of NF-κB [76]. NF-κB is a redox-sensitive transcription factor that is activated by oxidative insult, and NF-κB activation can protect cells from apoptosis. When human alveolar epithelial (A549) cells were exposed to hyperoxia, NF-κB was activated and within minutes was translocated to the nucleus [77]. Reactive oxygen species could act synergistically with TNFα in causing cytotoxicity via inhibition of a cytoprotective branch of TNFα signaling pathways that starts with NF-κB activation. Ginis *et al.* have demonstrated that H_2O_2 inhibited TNFα-induced accumulation of p65 in the nucleus, although it had no effect on degradation of IκB in the cytoplasm [78].

It is known that adenovirus protein E1B blocks TNF-induced apoptosis, whereas E1A enhances TNF-induced apoptosis through unknown mechanisms. Recent evidence indicates the effect of these proteins is mediated through modulation of NF-κB activation [61].

The growth arrest–specific 6 gene product (Gas6) is a growth and survival factor related to protein S. Gas6 induces a rapid and transient increase in nuclear NF-κB binding activity coupled to transcription activation. This plays a central role in promoting survival in NIH 3T3 cells [79]. MKK6 activates myocardial cell NF-κB and inhibits apoptosis in a p38 mitogen–activated protein kinase dependent manner [80]. Limb girdle muscular dystrophy type 2A results in decreased production of calpain 3. Calpain 3 is responsible for IκBκ turnover. Over expression of IκBα results in sequestration of NF-κB outside the nucleus. Myonuclear apoptosis occurred because of the downregulation of NF-κB [81].

The stimulation of the CD95- and TRAIL-resistant human pancreatic adenocarcinoma cell line Panc TuI with an agonistic anti-CD95 antibody or TRAIL activates of protein kinase C and NF-κB. The activation of PKC operates directly in a death receptor dependent manner in PancTuI cells and pancreatic tumor cells, protecting them from anti-CD95 and TRAIL-mediated apoptosis by preventing the loss of $\Delta\psi$ and cytochrome c release as well as by induction of NF-κB [82]. Pharmacologic or molecular inhibition of the NF-κB pathway blocked cell survival in MCF-7 APO+ cells, while only

molecular inhibition induced cytotoxicity in the APO- cells [40]. TGF-α protected gastric mucosal cells against apoptosis induced by serum depletion or sodium butyrate in a dose-dependent manner. This anti-apoptotic effect of TGF-α was blocked by pre-treatment with reagents that can potentially inhibit NF-κB activation. This suggests that TGF-α plays an antiapoptotic role in gastric mucosal cells via the NF-κB-dependent pathway [83].

Mice deficient in the NF-κB2 gene were challenged with the intracellular parasite *Toxoplasma gondii*. During the chronic phase of the infection, susceptibility of NF-κB knockout mice to toxoplasmic encephalitis was associated with a reduced capacity of their splenocytes to produce IFN-γ associated with a loss of $CD4^+$ and $CD8^+$ T-cells. This loss of T-cells correlated with increased levels of apoptosis and with elevated expression of the pro-apoptotic molecule Fas by T-cells from infected NF-κB knockout mice. This suggests a role of NF-κB in maintenance of T-cell responses required for long-term resistance to *Toxoplasma gondii* [84].

How NF-κB suppresses apoptosis? Although it is clear that NF-κB activation plays a role in suppressing TNF-induced apoptosis, just how is only now beginning to emerge. Several genes that may play a role in blocking apoptosis and whose expression is regulated by NF-κB have been identified, including cellular inhibitors of apoptosis (cIAP)-1 and cIAP-2, TRAF-1, and TRAF-2 [55,57,85]. cIAP-1, cIAP-2, and TRAF-1 are known to bind to TRAF-2 and TRAF-2 is required for NF-κB activation. Thus, how these proteins block apoptosis is not clear. Other reports show that TNF induces manganous superoxide dismutase (SOD), whose expression is also regulated by NF-κB, and the overexpression of SOD induces resistance to TNF-induced apoptosis [86]. Also, altered SOD expression in HeLa cells after low dose γ-irradiation is responsible for NF-κB-mediated cisplatin resistance [87]. Insulin manifests its antiapoptotic signaling though the activation of the NF-κB-dependent survival genes encoding TRAF-2 and SOD [88]. The TNF-inducible zinc finger protein A20 is regulated by NF-κB, and the role of this protein in induction of resistance to TNF-induced apoptosis has been demonstrated [89,90]. The expression of a protein critical in the regulation of the cell cycle, cyclin D1, is also regulated by NF-κB, and this activity may contribute to the cell growth and differentiation function assigned to NF-κB [91,92].

The prosurvival Bcl-2 homolog Bfl-1/A1 is another gene whose transcription is regulated by NF-κB and blocks TNF-induced apoptosis [93,94]. There are other studies which show that Bcl-2 activates NF-κB through the degradation of the inhibitor IκBα [95]. Crawford *et al.* have demonstrated that Bcl-2 overexpression protects photooxidative stress-induced apoptosis of photoreceptor cells through NF-κB preservation. It has been known that the Ras/PI-3K/Akt pathway plays a critical role in cell survival. It now appears

that this pathway is linked to the activation of IKK, the kinase needed for IκBα phosphorylation and NF-κB activation. Akt may also play a cytoprotective role through activation of NF-κB [96,97]. An NF-κB-independent cytoprotective pathway has also been described. The NF-κB activation induced by overexpression of TRAF2 was found to be insufficient to protect cells from apoptosis induced by TNF and cycloheximide together, thus indicating an essential role for additional components in the cytoprotective response [98].

While NF-κB activation blocks apoptosis, it seems that activation of apoptosis also blocks NF-κB activation, suggesting a feedback loop. For instance, endothelial cells undergo apoptosis when deprived of growth factors. The surviving viable cells exhibit increased activity of NF-κB, whereas apoptotic cells show caspase-mediated cleavage of the NF-κB p65/ReIA subunit, resulting in loss of carboxy-terminal transactivation domains and a transcriptionally inactive p65 molecule, which itself acts as a dominant-negative inhibitor of NF-κB, promoting apoptosis. In contrast an uncleavable, caspase-resistant p65 protects the cells from apoptosis. The generation of a dominant-negative fragment of p65 during apoptosis may be an efficient pro-apoptotic feedback mechanism between caspase activation and NF-κB inactivation [99]. Similarly apoptosis has been shown to promote a caspase-induced amino-terminal truncation of IκBα that functions as a stable inhibitor of NF-κB [100], thus further enhancing apoptosis. And Fas, another member of the TNF receptor family, was found to induce caspase-3-mediated proteolysis of both p50 and p65 subunits of NF-κB in T Jurkat cells, thus sensitizing the cells to apoptosis [101].

Pro-apoptotic activity of NF-κB: The decision of life or death in response to an inducing signal within a cell is dependent upon a delicate balance of positive and negative influences. While there are several reports that NF-κB activation protects cells from undergoing apoptosis induced by TNF or chemotherapeutic agents, there are also reports suggesting that NF-κB activation mediates apoptosis in response to a variety of inducers in a number of cell types (**Table 2**, at end of the chapter). For instance, in murine clonal osteoblasts NF-κB activation mediated TNF-induced apoptosis [102]. The suppression of growth of $CD34^+$ myeloid cells by TNF also correlated with NF-κB activation [103]. Apart from this, Fas activates NF-κB and induces apoptosis in T-cell lines by signaling pathways distinct from those induced by TNFα [104]. Human melanoma cells are protected against UV-induced apoptosis through downregulation of NF-κB activity and Fas expression [105]. Oxidative stress induced apoptosis in human aortic endothelial cells through the downregulation of Bcl-2, translocation of bax, and upregulation of p53 probably takes place through NF-κB activation.

Oxidative stress may play an important role in endothelial apoptosis mediated by hypoxia, through the activation of NF-κB [76]. That the activation of NF-κB is rather required for apoptosis has also been shown for other inducers such as H_2O_2 [106,107]. Similarly, H_2O_2-induced apoptosis was not suppressed by hyperoxia-induced NF-κB activation [77]. In pancreatic islets, A20 inhibited both apoptosis and NF-κB activation induced by cytokines, suggesting that NF-κB may actually mediate apoptosis [108]. Apoptosis in HL-60 cells induced by chemotherapeutic agents such as etoposide or 1-beta-D-arabinofuranosylcytosine was also found to require NF-κB activation, inasmuch as suppression of NF-κB by PDTC also blocked apoptosis [109].

Recently, Stark *et al.* demonstrated that aspirin induces cell death by an active apoptotic process that involves nuclear translocation of NF-κB preceding cell death [110]. *Helicobacter pylori* induces NF-κB-mediated apoptosis in chronic gastritis [111]. The apoptosis induced by alphavirus was also found to require the activation of NF-κB, since the thiol agents and Bcl-2 blocked both activities [112]. During adenoviral infection, NF-κB mediates apoptosis through transcriptional activation of Fas [113]. Apoptosis in Ca^{++} reperfusion injury of cultured astrocytes was also found to be mediated through NF-κB activation [114]. The cell death-promoting role of NF-κB has also been demonstrated in focal cerebral malaria [115], as it has for induction of apoptosis by double-stranded-RNA-dependent protein-kinase (PKR) [116]. Lin *et al.* showed that NF-κB can be proapoptotic or antiapoptotic depending on the timing of modulating NF-κB activity relative to the death stimulus [117]. How NF-κB may mediate apoptosis is not clear, but the role of p53 and c-myc induction through NF-κB has been demonstrated [118]. In addition, NF-κB is required for the anti-CD3-mediated apoptosis of double-positive thymocytes through a pathway that involves the regulation of the antiapoptotic gene Bcl-x_L [119]. c-myc has also been implicated in survival of certain cells such as hepatocytes [120]. These observations suggest that NF-κB activation not only negatively, but also positively regulates apoptosis. This idea has been further strengthened by studies on NMRI mice, Wistar rats and WI-38 fibroblasts in which aging induced a strong and consistent increase in the nuclear binding activity of NF-κB [121].

We recently showed that doxorubicin and its structural analogues WP631 and WP744, activate NF-κB, and this activation is essential for apoptosis in myeloid (KBM-5) and lymphoid (Jurkat) cells (138). Because the anthracycline analogue (WP744), most active as a cytotoxic agent, was also most active in inducing NF-κB activation and the latter preceded the cytotoxic effects, suggests that NF-κB activation may mediate cytotoxicity. Second, receptor-interacting protein-deficient cells, which did not respond to doxorubicin-induced NF-κB activation, were also protected from the cytotoxic effects of all the three anthracyclines. Third, suppression of NF-

κB activation by pyrrolidine dithiocarbamate, also suppressed the cytotoxic effects of anthracyclines. Fourth, suppression of NF-κB activation by NEMO-binding domain peptide, also suppressed the cytotoxic effects of the drug. Overall our results clearly demonstrated that NF-κB activation and IκBα degradation are early events activated by doxorubicin and its analogues and that they play a critical pro-apoptotic role.

Evidence that apoptosis is unaffected by NF-κB: There are increasing reports that NF-κB activation plays little or no role in apoptosis. For instance, Cai *et al.* showed that overexpression of IκBα, an inhibitor of NF-κB, in human breast carcinoma MCF7 cells inhibits NF-κB activation but not TNF-induced apoptosis. Similarly, in endothelial cells A20 inhibited NF-κB activation without enhancing TNF-induced apoptosis [122]. LPS- and IL-1- induced prolongation in survival of endothelial cells did not require NF-κB activation [123]. The pro- and anti-apoptotic role of NF-κB appears to be determined more by the nature of the death stimulus than by the origin of the tissue [113]. Bone morphogenetic protein (BMP)-2 and –4 inhibited TNF-mediated apoptosis by inhibiting caspase-8 activation in C2C12 cells, a pluripotent mesenchymal cell line that has potential to differentiate into osteoblasts depending on BMP stimulation. The BMP/Smad signaling pathway can inhibit TNF-mediated apoptosis independently of the pro-survival activity of NF-κB. This suggests that BMPs not only stimulate osteoblast differentiation but also promote cell survival during the induction of bone formation, offering new insight into the biological functions of BMPs [124]. There are proteins that associate with cytokine receptors such as SODD (for silencer of death domain) [125], sentris [126], and c-FLIP [127], that can also negatively regulate apoptosis, again independently of NF-κB.

The redox-sensitive transcription factor Ref-1 plays a critical role in the survival of endothelial cells in response to hypoxia and cytokines including TNFα. Upregulation of Ref-1 promotes endothelial cell survival in response to hypoxia and TNF through NF-κB–independent and NF-κB-dependent signaling cascades [128]. It has been observed in human non-small-cell lung carcinoma that apoptosis induced by topoisomerase poisons, e.g. Etoposide, is not mediated by NF-κB but can be manipulated by proteasome inhibitors [129]. Why NF-κB plays a role in apoptosis induced by some agents and not others is not clear but suggests that the apoptotic pathway varies from one inducer to another and also perhaps from one cell type to another.

Conclusion: It is clear that apoptosis is regulated by mitochondria-dependent and -independent pathways involving a series of proteins that preexist in the cells. Most agents that induce apoptosis, also activate NF-κB and the latter suppresses apoptosis in most cases. While it may appear paradoxical that the same agent could perform both functions, in reality it is

not. The same stress that induces cells to die provokes a self-defense response in the cell. How NF-κB plays an antiapoptitic role in some cells, pro-apoptotic in others and no role in some requires further understanding. It is possible that activation of NF-κB alone is not sufficient to regulate apoptosis and that other transcription factors are involved (141). Most NF-κB-regulated genes (such as cyclooxygenase-2) play critical roles in inflammation, suggesting that inflammation can also negatively regulate apoptosis.

Abbreviations used: NF-κB, nuclear factor κB; TNF, tumor necrosis factor; IκB, inhibitor of NF-κB; TRADD, TNF receptor-associated death domain; NIK, NF-κB-inducing kinase; TRAF2, TNF receptor-associated factor 2; SOD, superoxide dismutase; RIP, receptor interacting proteins; SODD, silencer of death domain; FADD, Fas-associated death domain; FLICE, FADD-like ICE; c-FLIP, cellular FLICE inhibitory protein; LT, lymphotoxin; FasL, fibroblast associated ligand; TRAIL, TNF-related apoptosis-inducing ligand; DR3L, death receptor 3 ligand; TWEAK, weak homologue of TNF; THANK, TNF homologue that activates apoptosis, NF-κB and JNK; JNK, c-jun N-terminal kinase; VEGI, vascular endothelial cell growth inhibitor; cIAP, cellular inhibitors of apoptosis; PKR, double-stranded-RNA-dependent protein kinase; MEKK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase

Table 1. Anti-apoptotic activity of NF-κB

Apoptosis Inducing Agent	Cell Type	Reference
TNF	Rel A-/- fibroblasts and macrophages	49
TNF	MCF-7	50
TNF	HEF, Jurkat, T24	51
TNFα, radiation, daunorubicin	HT1080	52
TNF	Jurkat	55
TNF	CD4+ T lymphocytes	56
TNF	Endothelial cells	57
TNF	EBV infected lymphoblastoid cells	58
TNF	A549, MCF-7	59
TNF	Glomerular mesangial cells	60
γ-radiation	(SK-OV-3.ipl) cells	61
TNF	MIAPaCa-2, Capan-2	62
TNF	Ewing sarcoma cells	63
TNF	Cardiomyocytes	64
TNF, IL-1	Mouse embryos	65
v-Rel inducers	HeLa cells, spleen cells	66
TNF	293	67
TNF	SK-OV-3.ipl	68
TNF	Human pulmonary macrophages	69

Apoptosis Inducing Agent	Cell Type	Reference
NIK suppression	PC12	70
TPCK, PDTC (NF-κB blockers)	WEHI 231	71
TPA and IFN-γ	Kerationocytes	73
SN50 (NF-κB blocker)	T Lymphocytes	74
X-ray irradiation	Lymphoma cells, Thymocytes	75
Hyperoxia	A549	77
TNFα and ROI	Brain capillary endothelial cells	78
Gas 6 suppression	NIH 3T3	79
Anisomycin	Myocardial cells	80
Calpain 3 deficiency	Myogenic satellite cells	81
Anti-CD95	Panc TuI	82
Serum depletion, sodium butyrate	GSM 06	83
Toxoplasma gondii	T-cells	84
Insulin	CHP overexpressing insulin receptor	88
TNF	Prostate carcinoma cells	130
TGF-β, serum withdrawal, anoikis, TNF-α	Mv1Lu and MDCK	131
Growth factor deprivation	Hematopoietic cells	132
v-Rel	Spleen cells, fibroblasts, C4-1	133
TRAIL	Renal Cell carcinoma	134
Hyperoxia, TNF-α	Lung epithelial cells	135
TNF	Endothelial cells	136

MCF-7, human breast carcinoma; Panc TuI, human pancreatic adenocarcinoma; A549, nonsmall cell lung cancer; SKOV3ipl, human ovarian cancer cell line was generated from ascites developed in *nu/nu* mouse by administering an intraperitoneal injection of SK-OV-3, a human ovarian carcinoma cell line; MIAPaCa-2 and Capan-2, human pancreatic cancer cell lines; HT1080, fibrosarcoma; Mv1Lu and MDCK, epithelial cells; C4-1 and WEHI 231, B-cells; PC12, rat adrenal pheochromocytoma; GSM o6, gastric mucosal cell line.

Table 2. Pro-Apoptotic Activity of NF-κB

Inducing Agent	Cell Type	Reference
Oxidative stress	Aortic endothelial cells	76
TNFα, HTLV-1 Tax/TNFα	Osteoblast cell line	102
TNFα	Myeloid leukemic cell lines	103
Fas/TNFα	CEM-C7	104
UV light	Human melanoma	105
H2O2	Jurkat, CEM C7, Oligodendrocytes	106, 107
Etoposide	HL-60 and thymocytes	109
Aspirin	Colon cancer cells	110
Helicobacter pylori	Gastric epithelial cells	111
Sindbis-virus induction	AT-3	112, 117
Adenovirus	Hepatocytes	113

Inducing Agent	Cell Type	Reference
RO1	Astrocytes	114
Focal cerebral ischemia	Neurons (Mice Ischemic model)	115
PKR	BSC-40, 3T3	116
Kainic acid	Rat striatum	118
α-CD3	Thymocytes from mIκBα mice	119
Constitutive enhanced by etoposide	Immature Rat thymocytes	137
Doxorubicin	KBM-5, SH-SY5Y, IMR32	138, 139
Mullerian Inhibiting substance	T47D, MDA-MB-231	140

Jurkat, CEM-C7, human T-cells; Hl-60, human promyelocytic leukemia; KBM-5, human myeloid; SH-SY5Y, IMR32, N-type neuroblastoma cells; T47D, MDA-MB-231, numan breast: BSC-40, African green monkey kidney cells; AT-3, prostrate carcinoma cell line; PKR, doublestranded-RNA-dependent protein pinase.

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