

Review

Good cop, bad cop: the different faces of NF- κ B

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Abstract

Complexes formed from the nuclear factor κ B (NF- κ B) family of transcription factors are ubiquitously expressed and are induced by a diverse array of stimuli. This results in their becoming activated in a wide variety of different settings. While the functions of NF- κ B in many of these contexts have been the subject of intense research and are now well established, it is also clear that there is great diversity in the effects and consequences of NF- κ B activation. NF- κ B subunits do not necessarily regulate the same genes, in an identical manner, in all of the different circumstances in which they are induced. This review will discuss the different functions of NF- κ B, the pathways that modulate NF- κ B subunit activity and, in contrast to its more commonly thought of role as a promoter of cancer cell growth and survival, the ability of NF- κ B, under some circumstances, to behave as a tumor suppressor.

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Abbreviations: APC, adenomatous polyposis coli; ATM, ataxia telangiectasia mutated; ATR, ATM- and Rad3-related; BAFF, B-cell-activating factor of the TNF family; CDK, cyclin-dependent kinase; CK2, casein kinase II; CYLD, Cylindromatosis; EBV, Epstein–Barr virus; ER, Estrogen Receptor; HDAC, histone deacetylase; HMG, high mobility group; HTLV, human T-lymphotropic virus; I κ B, inhibitor of κ B; I κ B-SR, I κ B super-repressor; IAP, inhibitor of apoptosis protein; IKK, I κ B kinase; ING4, inhibitor of growth family member 4; IRF, interferon response factor; JNK, Jun N-terminal kinase; LMP-1, latent membrane protein 1; LPS, lipopolysaccharide; MAP, mitogen-activated protein; MnSOD, manganese-superoxide dismutase; IL, interleukin; NEMO, NF- κ B essential modifier; NF- κ B, nuclear factor κ B; NGF, nerve growth factor; NIK, NF- κ B-inducing kinase;

PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; MSK, mitogen- and stress-activated kinase; PKB, protein kinase B; PP4, protein phosphatase 4; PTEN, phosphatase and tensin homolog deleted on chromosome 10; RHD, Rel homology domain; ROS, reactive oxygen species; RSK1, ribosomal S6 kinase 1; SCC, small cell carcinoma; SCF, Skp1/Cul1/F-box protein; TNF, tumor necrosis factor; UV, ultraviolet; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor

Introduction

In the 20 years since the discovery of nuclear factor κ B (NF- κ B), a number of paradigms for its function have been established. These include its key roles in the inflammatory and immune responses. Within these contexts, NF- κ B stimulates immune cell function and acts in a proinflammatory manner by inducing the expression of cytokines, chemokines and their receptors.^{1–3} Moreover, when activated in these settings, NF- κ B inhibits programmed cell death by stimulating the transcription of antiapoptotic genes.⁴ These aspects of NF- κ B function are undoubtedly central to our understanding of the overall behavior of this family of transcription factors, and they also provide a foundation for therapeutic intervention in inflammatory diseases and cancer based on NF- κ B inhibition.^{5–7} However, a wider analysis of NF- κ B function reveals a more complicated picture. NF- κ B is not only involved in other biological processes, but can also exhibit apparently contradictory behaviors. For example, although many studies point to a tumor-promoting function for NF- κ B subunits, evidence also exists for tumor suppressor functions.⁸ This review will examine the mechanisms that can account for these differences and will discuss their implications for the role of NF- κ B in tumorigenesis and cancer therapy.

The Complexity of the NF- κ B Response

Although the term NF- κ B is often used to refer specifically to the ‘classical’ p50/RelA(p65) heterodimer, the term can also apply more generally to dimers composed of the other mammalian NF- κ B subunits p52(p100), c-Rel and RelB.¹ The characteristic feature of NF- κ B subunits is an approximately 300 amino-acid N-terminal domain, termed the Rel homology domain (RHD), that contains residues required for DNA binding, dimerization, nuclear localization and inhibitor (inhibitor of κ B (I κ B)) binding. The composition of the NF- κ B dimer can vary depending on cell type, the nature of the inducing stimulus or the time elapsed after initial exposure to the stimulus.^{1,9,10} Given that NF- κ B subunits are expressed in all cell types and there are hundreds of activators,³ the number of different contexts in which an NF- κ B dimer can become induced is enormous. Therefore, it is not surprising

that the functions of NF- κ B complexes will vary depending upon the setting in which they are found.

The canonical NF- κ B activation pathway

The rapid activation and nuclear translocation of cytoplasmic NF- κ B that ensues following degradation of its inhibitor I κ B α , as a result of phosphorylation of I κ B α by the activated I κ B kinase (IKK) complex, is often referred to as the 'classical' or 'canonical' pathway (Figure 1).^{1,11} In this pathway, the IKK complex consists primarily of two catalytic kinase subunits (IKK α and IKK β) and multiple copies of a regulatory subunit, IKK γ (aka NF- κ B essential modifier (NEMO)), which is required for the integration of signals from upstream kinases and receptor-associated proteins.^{1,11} Activation of the canonical pathway occurs in response to inflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukin-1, engagement of the T-cell receptor and in response to bacterial infection and exposure to agents such as lipopolysaccharide (LPS). This first leads to a series of modifications of components of the IKK complex, including ubiquitination and phosphorylation of IKK γ and phosphorylation of two serine residues in the activation loop of IKK β . Activated IKK β then phosphorylates I κ B α at serines 32 and 36, which causes I κ B α to become a substrate for the Skp1/Cul1/F-box protein- β -TrCP ubiquitin ligase complex, and ubiquitinated I κ B α is

rapidly degraded by the proteasome.¹ Release of the NF- κ B dimer allows it to relocate to the nucleus. However, at least under some circumstances, this process may be defined more accurately as a shifted equilibrium, in which the NF- κ B/I κ B α complex is shuttling between the cytoplasm and the nucleus, and degradation of I κ B α shifts the equilibrium of NF- κ B from primarily cytoplasmic to nuclear.¹² In most cell types, activation of the canonical pathway results in the nuclear localization of a p50/RelA complex within minutes. In addition, full activation of the canonical pathway may involve a number of post-translational modifications of the NF- κ B subunits, including phosphorylation, acetylation, and prolyl isomerization of RelA.^{11,13}

The canonical NF- κ B response can be terminated in several ways. One of the best characterized involves induction of I κ B α gene transcription by activated NF- κ B.³ Newly synthesized I κ B α can then enter the nucleus, bind NF- κ B, and return it to the cytoplasm.¹ In addition to I κ B α , two other mammalian I κ Bs, β and ϵ , appear to function through a similar mechanism of IKK-dependent phosphorylation and degradation.^{1,11} The transcription of I κ B β and I κ B ϵ is not regulated by NF- κ B, and these proteins dampen the oscillatory profile of NF- κ B activation seen with I κ B α alone.¹⁴ Other feedback loops also exist. The genes encoding c-Rel and RelB, together with the precursor proteins NF- κ B1 (p105/p50) and NF- κ B2 (p100/p52), contain κ B elements in their

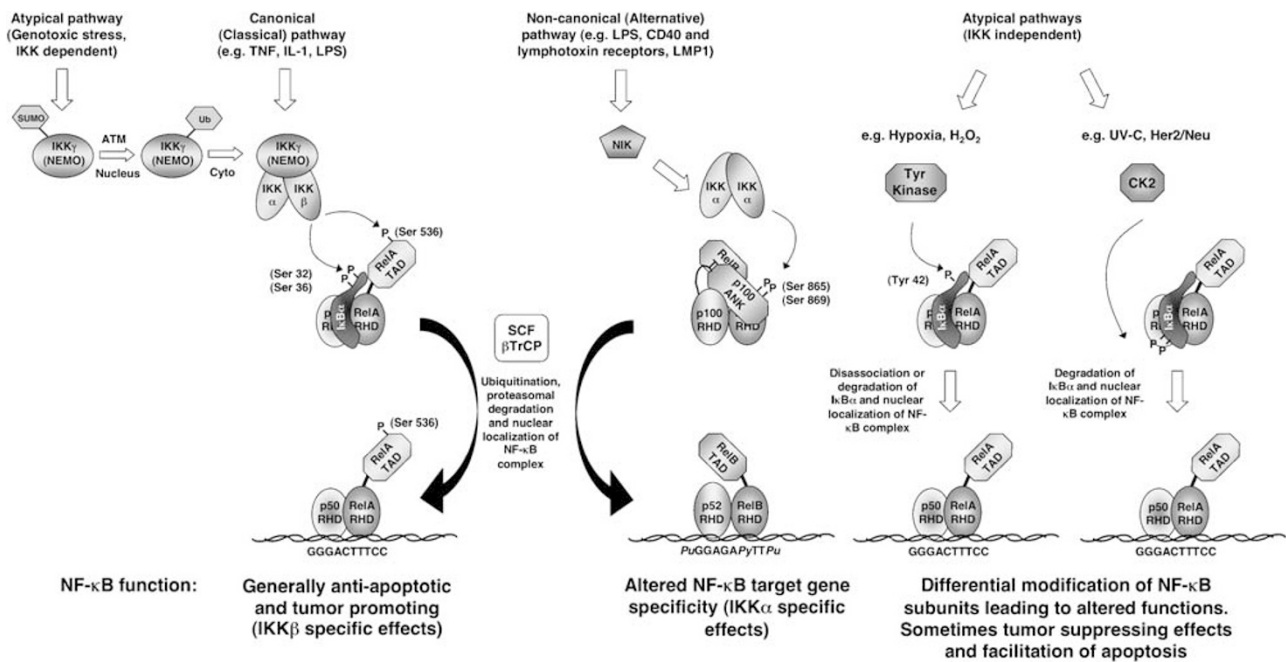


Figure 1 Pathways leading to activation of NF- κ B. NF- κ B complexes can be induced by a variety of mechanisms. The 'canonical' pathway is IKK β dependent and results in the phosphorylation of I κ B α at serines 32 and 36, leading to its ubiquitination by β -TrCP and subsequent degradation by the proteasome. The RelA (p65) NF- κ B subunit can also be directly phosphorylated by IKK β at Ser-536. IKK-dependent activation of NF- κ B can also occur following some genotoxic stresses. Here, however, IKK γ (NEMO) becomes sumoylated and nuclear localized, whereupon it is ubiquitinated in a manner dependent upon the ATM checkpoint kinase before relocating back to the cytoplasm. The noncanonical pathway results in the phosphorylation of the p100 NF- κ B subunit by IKK α . Similar to the canonical pathway, this results in its ubiquitination by β TrCP and C-terminal degradation by the proteasome, resulting in the generation of p52 NF- κ B. In addition, a number of IKK-independent, 'atypical' pathways have been described which still involve the phosphorylation of I κ B α . These include CK2- and tyrosine kinase-dependent pathways, which phosphorylate I κ B α at sites distinct from those seen with IKK. CK2- dependent activation of NF- κ B can result in degradation of I κ B α by calpain, while the Tyr-42 phosphorylation of I κ B α can result either in its degradation or merely disassociation from NF- κ B. Not shown are IKK β phosphorylation and subsequent processing of p105 NF- κ B, the targeting of other I κ B subunits and the induction of the wide variety of NF- κ B complexes that exist in the cell. Some of the consequences of differential activation of NF- κ B by these pathways are indicated. RHD = Rel homology domain; TAD = transcriptional activation domain; ANK = ankyrin repeat domain

promoters and are NF- κ B inducible.^{1,3} Consequently, the composition of the NF- κ B complex can change over time, resulting in temporal changes in the spectrum of genes induced and repressed.^{10,15}

The noncanonical NF- κ B pathway

Some activators of NF- κ B, such as stimulation of the CD40 and lymphotoxin- β receptors, B-cell-activating factor of the TNF family, LPS, and latent membrane protein (LMP)-1 of Epstein-Barr virus (EBV), also activate the 'noncanonical' (or alternative) NF- κ B pathway.^{1,2} The noncanonical pathway proceeds via activation of NF- κ B-inducing kinase (NIK), which in turn activates an IKK α dimer, which then phosphorylates the p100 (NF- κ B2) NF- κ B subunit, inducing its proteolytic processing to p52 (Figure 1).^{1,2} The C terminus of p100 contains the ankyrin repeats found in I κ B proteins and can thus function as a cytoplasmically localized I κ B protein. Processing of p100 to p52 results in activation of complexes containing this subunit, which in most circumstances consist of p52/RelB heterodimers.^{2,16} Different NF- κ B subunit combinations have overlapping but distinct DNA-binding specificities and p52/RelB heterodimers target distinct κ B elements.¹⁶ Thus, stimuli that activate both the canonical and noncanonical pathways lead to different mixtures of subunits in the nuclear NF- κ B complex, resulting in the targeting of a different spectrum of promoters and enhancers.

The p50 NF- κ B subunit is also derived from the proteolytic processing of a larger, ankyrin repeat-containing, precursor protein, p105 (NF- κ B1).¹ Processing of p105 does not occur through the noncanonical pathway, but results either from cotranslational processing or from IKK β -dependent phosphorylation and degradation.¹ Interestingly, a subfraction of p105 also binds the Tpl2 (Cot) kinase and activates the mitogen-activated protein (MAP) kinase signaling cascade;¹⁷ in this case, p105 processing releases and activates Tpl2, providing an example of how IKK activation can lead to crosstalk with other signaling pathways.

Atypical pathways of NF- κ B activation

Although the canonical and noncanonical pathways account for most of the physiological inducers of NF- κ B, as well as much of the research performed on NF- κ B activation, there is a growing list of alternative mechanisms leading to NF- κ B nuclear localization and DNA binding. These include IKK-independent mechanisms as well as utilization of IKK activity in a manner distinct from that found with the canonical and noncanonical pathways (Figure 1).

IKK-independent NF- κ B activation pathways include the casein kinase II (CK2)-dependent phosphorylation and degradation of I κ B α that occurs in response either to short-wavelength ultraviolet (UV-C) light or expression of the Her2/Neu oncogene.^{18,19} In these circumstances, I κ B α phosphorylation occurs at C-terminal sites, and not at serines 32/36.¹⁸ In the case of UV-C treatment, CK2 phosphorylation of I κ B α is also dependent on the p38 MAP kinase pathway¹⁸ while with Her2/Neu activation, I κ B α degradation is the result of the activity of calpain rather than the proteasome.¹⁹ It is not clear how widespread the CK2 pathway of NF- κ B activation is, but

other IKK-independent pathways have been identified. For example, activation of NF- κ B at late time points in response to the chemotherapeutic drug doxorubicin (adriamycin) has been shown to be IKK independent, although no alternative kinase has been identified for this form of NF- κ B induction.²⁰

Another well-established IKK-independent NF- κ B activation pathway involves phosphorylation of I κ B α at tyrosine residue 42. Stimuli associated with this pathway include hypoxia/reoxygenation, hydrogen peroxide stimulation, and treatment with nerve growth factor or the tyrosine phosphatase inhibitor pervanadate.²¹⁻²⁴ With some of these stimuli, Tyr-42 phosphorylation leads to degradation of I κ B α , possibly through a process requiring the PEST sequences near the C terminus of I κ B α .^{22,23} However, in other circumstances, Tyr-42-phosphorylated I κ B α is released from NF- κ B without degradation occurring.^{21,24} Curiously, in bone marrow macrophages, stimulation with TNF- α , which is one of the classical inducers of the canonical pathway, appears to proceed through the Tyr-42 phosphorylation pathway, underscoring the fact that NF- κ B regulatory mechanisms can differ in different cell types and contexts.²⁵ c-Src and Syk have been proposed as putative Tyr-42 I κ B α kinases.^{11,25}

Some mechanisms of NF- κ B induction can be IKK dependent but exhibit distinct functional differences to other IKK-dependent activation pathways. For example, in response to genotoxic stimuli such as UV-C and the chemotherapeutic drugs camptothecin and etoposide, NF- κ B induction can exhibit a requirement for the zinc-finger domain of the IKK γ subunit that is not seen with TNF- α .¹ Furthermore, etoposide also causes sumoylation of IKK γ . Sumoylated IKK γ is relocalized to the nucleus where it becomes ubiquitinated in an ataxia telangiectasia mutated (ATM)-dependent manner before being exported back to the cytoplasm.²⁶ (see also review by Janssens and Tschopp in this edition).

Taken together, these reports demonstrate that there is much heterogeneity in the mechanisms that lead to activation of NF- κ B, with good evidence for some stimuli, such as UV-C, being able to activate NF- κ B by both IKK-dependent and -independent pathways. Importantly, induction of NF- κ B by these different mechanisms can have effects on the function of NF- κ B complexes once in the nucleus. Similar to other transcription factors, the function of NF- κ B subunits is also controlled by post-translational modifications, especially phosphorylation.^{11,13} It is now apparent that whether NF- κ B activation is IKK dependent or -independent can have a direct effect on subunit modifications. For example, IKK β has been shown to phosphorylate RelA at serine 536, a modification reported to stimulate NF- κ B transcriptional activity,^{11,13} decrease its affinity for I κ B α ²⁷ and promote its nuclear import.^{28,29} In addition, various stimuli in T cells can promote nuclear translocation of Ser-536-phosphorylated RelA, independent of I κ B α or the proteasome.²⁹ In macrophages, IKK α has also been shown to phosphorylate RelA at Ser-536; however, in this case, Ser-536 phosphorylation results in proteolytic degradation of RelA and termination of the NF- κ B response.³⁰ Moreover, proteasome-dependent degradation of RelA in the latter stages of its canonical induction cycle occurs in the nucleus and involves promoter-bound RelA.³¹ These contrasting effects emphasize the importance of cell or stimulus context when investigating NF- κ B function, and it is

probable that other factors determine the effect of Ser-536 phosphorylation on RelA. These effects are unlikely to occur with IKK-independent activation of NF- κ B. Consequently, IKK-independent pathways will result in differentially modified NF- κ B subunits with distinct functions.

NF- κ B-independent effects of IKK

Functions for the IKKs beyond the NF- κ B pathway have also been described. These include phosphorylation of histone H3 by IKK α ,¹¹ IKK α phosphorylation of the SMRT corepressor³² as well as estrogen receptor α and its coactivator SRC-3/AIB1,³³ IKK β phosphorylation of 14-3-3 β ³⁴ and Dok1,³⁵ and IKK β phosphorylation of the Forkhead transcription factor FOXO3a.³⁶ In the latter case, IKK-mediated phosphorylation of FOXO3a is inhibitory, resulting in its cytoplasmic localization and degradation, which enhances IKK-dependent induction of proliferation and tumorigenesis. Similarly, a mutant Dok1 protein that cannot be phosphorylated by IKK is unable to inhibit PDGF-induced cell growth.³⁵ Thus, such IKK signaling crosstalk will shape the nature of the cellular context within which the NF- κ B subunits operate, influencing their

function and the consequences of NF- κ B activation. Furthermore, these types of data suggest that the phenotypes of IKK knockout mice do not solely reflect the effects of the IKKs on NF- κ B. For example, studies of RelA $^{-/-}$ epidermis have revealed significant differences to IKK α or IKK β null tissue.³⁷

In addition, IKK α effects that are independent of both NF- κ B and its catalytic activity have been reported. These include IKK α regulation of keratinocyte differentiation³⁸ and tooth development.³⁹ IKK γ (NEMO) can also associate with and regulate hypoxia-inducible factor 2 α , independent of the catalytic IKK subunits.⁴⁰

Modulators of NF- κ B

Several cell stimuli or proteins do not obviously induce translocation of NF- κ B subunits to the nucleus, but nevertheless alter the activity of NF- κ B. These effects can involve post-translational modifications or cooperative interactions that result in targeting NF- κ B proteins to distinct promoters and enhancers (Figure 2). Modulators of NF- κ B transcriptional activity have not been investigated as thoroughly as inducers of its nuclear translocation, but understanding their

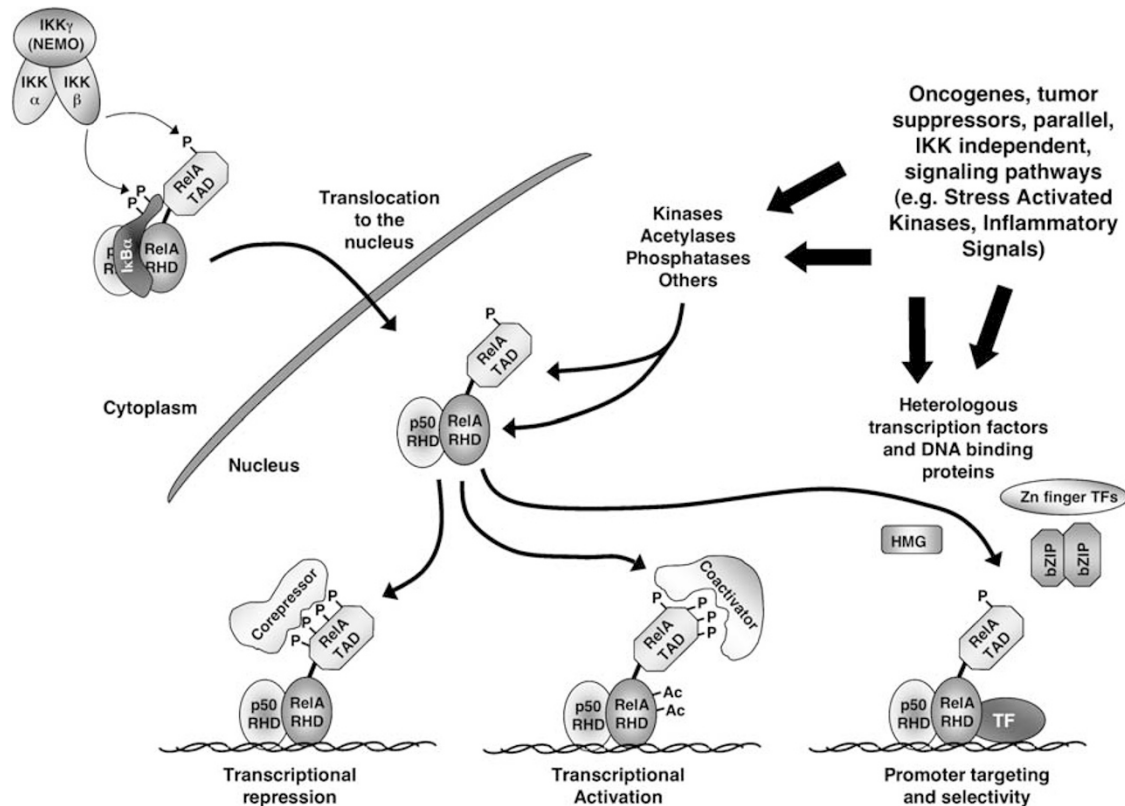


Figure 2 Modulation of NF- κ B transcriptional activity. A number of pathways can affect the activity of NF- κ B complexes in the nucleus. In addition to phosphorylation of NF- κ B subunits such as RelA (p65) by IKK β , modification by other, nuclear kinases can regulate its transactivation. These can result in transcriptional activation and repression as well as promoter-specific effects. Other modifications, such as acetylation, also regulate RelA transcriptional activity. In addition, cooperative interactions with heterologous transcription factors, which can frequently result in cooperative DNA binding, can target NF- κ B complexes to specific promoters, resulting in the selective activation of gene expression following cellular exposure to distinct stimuli. These pathways integrate NF- κ B activity with the function of oncogenes, tumor suppressors and parallel IKK-independent pathways. Although RelA is shown here, it is certain that similar processes can regulate the other NF- κ B subunits. Not shown are effects on chromatin, which also affect NF- κ B target gene specificity and function, together with modifications such as proline isomerization and ubiquitination. RHD = Rel homology domain; TAD = transcriptional activation domain; TF = transcription factor; bZIP = leucine zipper-containing transcription factors; HMG = HMG box containing transcription factors; Zn finger = zinc-finger containing transcription factors

role is, nonetheless, critical to understanding the function of NF- κ B subunits.

A number of signaling pathways not directly involved in I κ B phosphorylation target the NF- κ B subunits, either directly or indirectly. Investigations of nuclear NF- κ B subunit phosphorylation are still at a surprisingly early stage. While a number of modifications of RelA have been described, and are reviewed elsewhere,^{11,13} studies of the other subunits are limited and reagents for their study are not generally available. However, even with RelA it is apparent that much is still unknown about how phosphorylation controls its activity.

An important parallel signaling pathway regulating RelA function is governed by the phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB)/Akt kinases. The PI3K/PKB pathway promotes cell survival and is induced by growth factors, cytokines and oncogenes. To some extent, these effects of PI3K/PKB on RelA function stimulate the antiapoptotic activity of NF- κ B.^{41–43} Although RelA Ser-536 phosphorylation is performed by IKK β in many circumstances,^{11,13} some reports indicate that this activity of IKK β also depends on PI3K/PKB activity.^{41–43} For example, one report demonstrated that while PKB was required for IKK β phosphorylation of RelA Ser-536 *in vitro*, PKB was not required for IKK β phosphorylation of I κ B α Ser-32/36.⁴² In contrast, following T-cell receptor stimulation, IKK β -dependent phosphorylation of RelA Ser-536 was found to be PI3K/PKB independent but to require Tpl2 (Cot), PKC θ and NIK.²⁸ IKK β is not the only Ser-536 kinase that has been identified: p53-induced ribosomal S6 kinase 1 (RSK1) has also been shown to be a RelA Ser-536 kinase,²⁷ and it is likely that other kinases are also able to phosphorylate Ser-536.

Interestingly, PI3K/PKB activity has also been shown to induce the p38 MAP kinase pathway.⁴¹ In numerous systems, p38 MAP kinase has been shown to regulate NF- κ B transcriptional activity without affecting its translocation to the nucleus.⁴⁴ p38 does not appear to phosphorylate RelA or other NF- κ B subunits directly. Rather, p38 has been suggested to function by stimulating the activity of the RelA-binding p300/CBP coactivator proteins⁴¹ or through regulating histone H3 Ser-10 phosphorylation,⁴⁵ probably through regulating the activity of the MSK1 and MSK2 kinases.⁴⁶ p38 affects the expression of only a subset of NF- κ B-regulated genes and H3 Ser-10 phosphorylation appeared to target NF- κ B to cryptic κ B elements in promoters.⁴⁵ Thus, NF- κ B modulators do not appear to be required for inducing transcription of all NF- κ B target genes, but rather act selectively to control the specificity of NF- κ B. A further example of this selectivity comes from Ras oncoprotein regulation of NF- κ B. Ras stimulates RelA transactivation, at least in part through stimulating p38 MAP kinase activity, and RelA together with c-Rel potentiate Ras-induced cell transformation.⁴⁷ Nevertheless, many of the genes induced by Ras-stimulated NF- κ B are different from those induced by stimulation with inflammatory cytokines, indicating that these represent different mechanistic pathways.⁴⁷

Kinases besides IKK have been identified that can directly phosphorylate RelA. These include ζ PKC, which phosphorylates RelA at Ser-311.¹¹ Moreover, the catalytic subunits of both protein kinase A and MSK1 have been reported to phosphorylate RelA at Ser-276.¹¹ These phosphorylations

appear to stimulate RelA transcriptional activity and its association with the p300/CBP coactivators. However, Ser-276 in RelA is also important for determining whether RelA can form homodimers or heterodimers.⁴⁸ In contrast, phosphorylation of RelA at Thr-254 allows Pin-1-induced proline isomerization to occur, resulting in increased RelA nuclear localization and stability.⁴⁹ Other sites of RelA phosphorylation identified include Ser-468 and Ser-529,^{11,13} although it is likely that others have yet to be identified. Interestingly, CK2 has been identified as a putative RelA Ser-529 kinase providing another mechanism in addition to I κ B α phosphorylation through which this kinase can regulate NF- κ B.¹¹ Such studies, of course, indicate that phosphatases also play an important regulatory role in NF- κ B subunit activity. Indeed, protein phosphatase 4 has recently been shown to dephosphorylate RelA at Thr-435 in response to cisplatin treatment.⁵⁰

In addition to stimulating RelA transactivation, modulators can also regulate RelA in negative ways. For example, the ARF tumor suppressor inhibits RelA transactivation (see below) as a result of Chk1-dependent phosphorylation of RelA at Thr-505.⁵¹ Furthermore, the anti-inflammatory actions of glucocorticoids are effected, at least in part, through repression of NF- κ B transactivation (reviewed in De Bosscher *et al.*⁵²). Genotoxic agents such as UV-C and the chemotherapeutic drugs daunorubicin/doxorubicin can induce RelA complexes that repress rather than activate transcription.^{53,54} A common effect of these modulators is that they promote an increased association between RelA and corepressor complexes containing histone deacetylases (HDACs). Negative modulators, therefore, do not simply inactivate NF- κ B, but can switch it from an activator to a repressor of transcription.

RelA is also modified by acetylation, which can occur at lysines 122, 123, 218, 221 and 310.^{13,55} RelA transactivation is stimulated by acetylation at lysines 218, 221 and 310, but inhibited by acetylation at lysines 122 and 123.^{13,55} Different histone acetyl transferases (HATs), such as p300 and PCAF, are responsible for inducing at least some of these acetylations while HDACs such as HDAC3 and SIRT1 can target their removal.^{13,55,56} Recently, phosphorylation of RelA at Ser-276 and Ser-536 has been shown to promote acetylation at Lys-310 and the assembly of RelA with coactivator p300.⁵⁷ RelA proteolysis is also induced by ubiquitination between residues 220 and 335.⁴⁹ This effect is involved in the termination of the NF- κ B response and is mediated by SOCS-1, a ubiquitin ligase, which competes with Pin-1 for binding to RelA. Whether ubiquitination of RelA is also linked to degradation induced by the IKK α phosphorylation of Ser-536³⁰ is not established.

While association with different coactivators and corepressors controls the transcriptional activity of NF- κ B complexes, the function of NF- κ B at specific promoters and enhancers is also dependent upon interactions with heterologous transcription factors. Through cooperative DNA binding and recruitment of coactivators, different transcription factors can more stably associate with their target sequences in chromatin and synergistically induce gene expression.^{9,58,59} That such promoter-specific effects are important is supported by data indicating that for endogenous genes, there is little correlation between the ability of subunits to regulate a promoter and the

sequence of the κ B element itself.^{10,15} One of the best examples of these cooperative effects occurs at the β -interferon enhancer. β -interferon expression is NF- κ B dependent, but is only induced upon viral infection and not by other NF- κ B inducers.⁵⁹ This effect results from the precise orientation and spacing of the transcription factor-binding sites in the enhancer, together with the positioning of a nucleosome over the TATA box. Through cooperative effects, NF- κ B, interferon response factors, c-Jun, ATF2 and high mobility group (HMG) I recruit coactivators and chromatin remodeling activities that result in sliding of this nucleosome to allow recruitment of the TATA-binding complex TFIID.⁶⁰ Many other interactions between NF- κ B subunits and heterologous transcription factors have been described. For example, a distinct subset of NF- κ B-dependent genes induced by LPS, also require the b-ZIP transcription factor JunB.⁶¹ Cooperative effects have also been described between NF- κ B and zinc-finger-containing transcription factors such as Sp1 as well as other b-ZIP factors such as C/EBP β .⁹ The range of such interactions is likely to be large and play a key role in determining the heterogeneity of NF- κ B-dependent gene expression.

The sequence of the κ B element itself can influence NF- κ B function at the promoter. For example, with the β -interferon enhancer, the κ B element contains a central core of five AT residues (GGGAAATTCC) which creates a binding site in the minor groove of the DNA for HMG I.⁵⁹ In addition, different NF- κ B subunits have distinct preferences for the κ B element half-sites.⁹ This creates subtle differences in NF- κ B complex DNA-binding specificity and affinity^{9,16} and also serves to orientate the NF- κ B complex on the DNA, promoting interactions with heterologous transcription factors.⁹ Furthermore, the sequence of the κ B element may affect the conformation of the NF- κ B complex bound to it, which in turn helps determine the recruitment of specific coactivator proteins.⁶²

Although NF- κ B DNA binding does not generally appear to be strongly affected by nucleosomes, chromatin structure at promoters can also influence NF- κ B function.⁵⁸ For example, the need to remodel promoter nucleosome structure, to allow binding of other transcription factors, basal factors or RNA polymerase, can affect the timing of NF- κ B-dependent gene expression.^{31,58,60} Furthermore, NF- κ B complexes bound to nucleosome-associated sites, might adopt distinct conformations (as is the case with variant κ B elements⁶²), with consequent effects on coactivator recruitment.⁵⁸

Taken together, it is clear that while I κ B phosphorylation and translocation of NF- κ B to the nucleus is the most obvious (and most studied) aspect of inducing an NF- κ B response, other modulatory pathways integrate NF- κ B function with other cellular signals to define the exact functional outcome of its activation. The context within which NF- κ B is activated, be it the cell type or the other stimuli to which the cell is exposed, is therefore a critical determinant of NF- κ B behavior. An understanding of these modulatory pathways will be required to obtain a complete picture of the NF- κ B pathway.

Other effects of NF- κ B

NF- κ B may also have effects at levels other than nuclear transcription. For example, NF- κ B can inhibit MyoD and Sox9

mRNA levels by affecting mRNA stability.⁶³ RelA, p50 and I κ B α have also been detected in mitochondria, where NF- κ B appears to repress the expression of mitochondrial genes encoding cytochrome *c* oxidase III and cytochrome *b*.⁶⁴ Furthermore, aspirin, UV-C and serum withdrawal can promote nucleolar localization of RelA in colorectal cancer cell lines, an effect that is associated with repression of NF- κ B transcriptional activity and induction of apoptosis.⁶⁵ These reports illustrate the complexity of NF- κ B function and the cell type- and context-dependent differences that can exist when investigating NF- κ B, and also point to the potential difficulties of interpreting and predicting the consequences of inhibiting NF- κ B activity.

NF- κ B can be Both a Tumor Promoter and a Tumor Suppressor

The physiological outcome of activation of NF- κ B, primarily through targeting different combinations of promoters and enhancers, differs according to the cell type or stimulus, and thus, NF- κ B can have apparently contradictory functions. This has important implications for our understanding of NF- κ B biology and its role in disease. An abundance of data indicates that the IKKs and NF- κ B subunits can act to promote tumorigenesis, and this subject has been extensively reviewed elsewhere^{8,66} (Figure 3). Briefly, the pro-oncogenic effect of NF- κ B can be thought of as arising from the overproduction of its normal target genes as a consequence of its chronic activation and nuclear localization in tumor cells. For example, NF- κ B can stimulate tumor cell survival through the continual induction of anti-apoptotic genes such as Bcl-xL, X-IAP, cIAP1 and 2, and A20.⁴ Through this antiapoptotic activity, NF- κ B can also reduce the effectiveness of many common cancer therapies, which themselves activate NF- κ B.^{8,66} By regulating gene expression, NF- κ B can also promote other oncogenic processes, including the following: tumor cell proliferation through its ability to induce proto-oncogenes such as cyclin D1 and c-Myc; metastasis through its ability to induce the expression of cellular adhesion molecules and matrix metalloproteinases; angiogenesis through regulation of vascular endothelial growth factor; and cell immortality through regulating telomerase.^{8,66} Finally, in some model systems, NF- κ B provides the critical link between tumor development and chronic inflammation, a process thought to be the basis for up to 20% of human cancers.⁶⁶

It is not difficult to see that pathways affecting the transcriptional activity of NF- κ B can also alter the oncogenic activity of NF- κ B. This can result in NF- κ B contributing to tumorigenesis in different ways depending on cell type. For example, in a model of ulcerative colitis-induced colon cancer in mice, NF- κ B activity was found to contribute to cell survival in colonic epithelial cells by inducing the expression of antiapoptotic genes.⁶⁷ NF- κ B also contributed to the tumorigenic process through its effects on infiltrating and non-cancerous macrophages, from which the secretion of NF- κ B-regulated cytokines and growth factors was shown to help drive tumor cell proliferation and size.⁶⁷

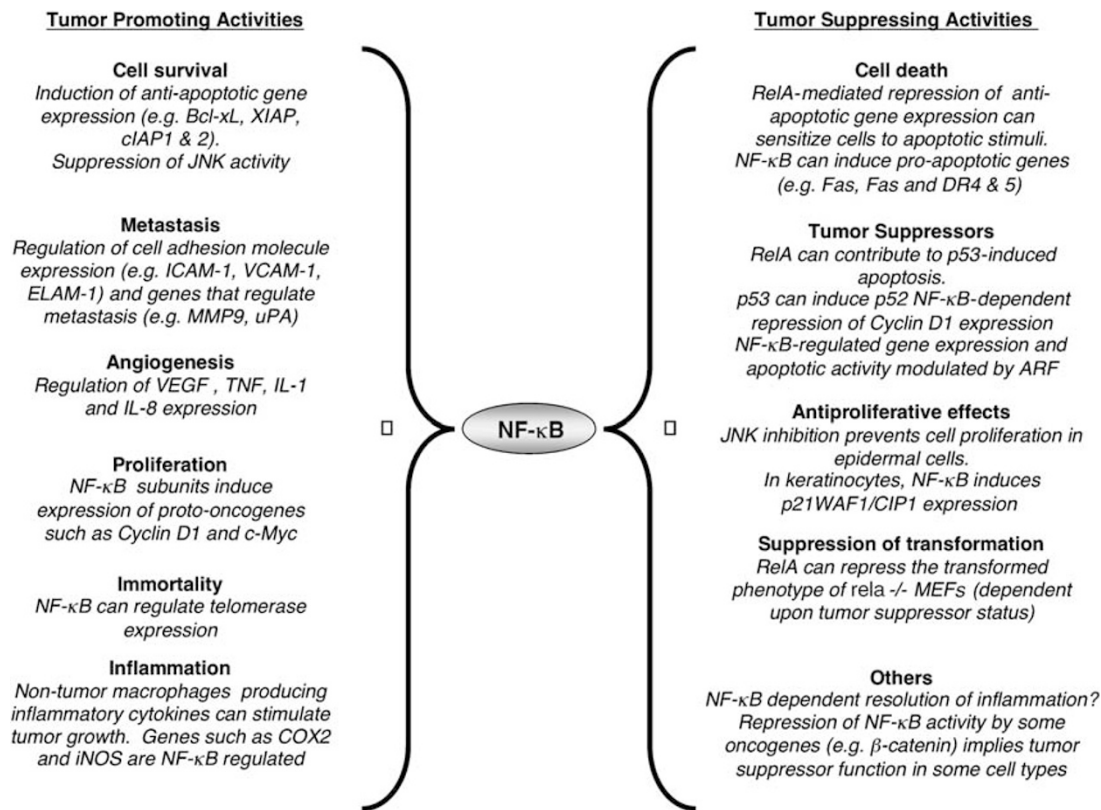


Figure 3 Different functions of NF- κ B can have either tumor-promoting or tumor suppressing effects. On the left is a summary of the different tumorigenic processes to which aberrantly active NF- κ B has been shown to contribute. In contrast, on the right is evidence indicating that under other circumstances active NF- κ B can act to inhibit tumor growth and survival

Mechanisms of NF- κ B-dependent tumor suppression

In contrast to its tumor-promoting effects, there is growing evidence that, in some circumstances, NF- κ B can function as a tumor suppressor (Figure 3).⁸ In many cases, this dramatically different role can be ascribed to alterations in NF- κ B functionality through pathways similar to those described above. Although inhibition of tumor cell growth and survival by NF- κ B is not as well understood as its tumor-promoting roles, this may reflect the fact that much work on NF- κ B has been performed in transformed cell lines. In such experiments, NF- κ B-dependent tumor suppressor mechanisms that stimulate cell death, for example, have probably been selected against and lost. Furthermore, in models of cancer development, the focus is inevitably on the role that NF- κ B plays in those cells that do develop into tumors and not what its function might have been in those cells that do not contribute to the tumor. Nonetheless, similar to its role in tumor promotion, several mechanisms through which NF- κ B can potentially inhibit tumor growth are emerging.

NF- κ B and tumor suppressors

Several reports have linked NF- κ B function to the p53 tumor suppressor pathway.⁸ p53 forms one of the first lines of defense against oncogene- or DNA damage-induced tumor-

igenesis: p53 induction by these events can result in cell-cycle arrest in order to allow DNA damage to be repaired or cell death through the induction of proapoptotic genes. In either case, these effects protect the organism from potentially tumorigenic events. Loss of p53 is very frequent in many forms of cancer and suppression of p53 activity in those cells that retain a wild-type p53 gene is almost certainly obligatory during tumor cell development. Consistent with their roles as tumor promoters, IKK and NF- κ B can contribute to suppression of p53 activity through, for example, inducing the expression of Hdm2 (Mdm2 in mice), an inhibitor of p53.⁶⁸ Furthermore, NF- κ B-mediated induction of antiapoptotic genes can counteract p53's proapoptotic activity.

In contrast to these observations, several lines of evidence point to NF- κ B subunits actually being recruited to the p53 tumor suppressor pathway. In some cell systems, RelA can enhance p53-induced cell death⁶⁹ and, as discussed above, p53-induced RSK1 kinase phosphorylates RelA at Ser-536.²⁷ p53 can also induce increased association of the p52 NF- κ B subunit with HDAC1, resulting in repression of cyclin D1 expression, an effect which will contribute towards cell-cycle arrest.⁷⁰ In addition to p53, the ARF tumor suppressor also regulates RelA activity.^{51,71} ARF expression, which is induced by oncogenes, results in activation of p53 through binding to and inactivation of Hdm2/Mdm2. As described above, ARF expression can also result in activation of the ATM- and Rad3-related (ATR)/Chk1 checkpoint kinases, leading to Thr-505

phosphorylation of RelA.⁵¹ This results in repression of Bcl-xL expression and sensitization of cells to TNF-induced apoptosis.^{51,71} The effect of this will also be to reduce RelA's ability to oppose p53's proapoptotic function. Interestingly, ARF-induced ATR activity can also regulate p53, which provides a mechanism to coordinately regulate and integrate NF- κ B and p53 function.⁵¹ Surprisingly, not all activators of ATR/Chk1 activity regulate RelA through Thr-505, suggesting a requirement for Chk1 adaptors or scaffold proteins (K Campbell, S Rocha and N Perkins, unpublished observation).^{51,53}

Crosstalk between NF- κ B and ARF/p53 may also affect cellular tumorigenicity. Some immortalized mouse fibroblasts lacking RelA have a weakly transformed phenotype and can form tumors in mice that subsequently regress.⁷² Re-expression of wild-type RelA can reverse the transformed phenotype of these cells, providing direct evidence for a RelA tumor suppressor function. However, different isolates of *rela*-/- fibroblasts have different characteristics and the transformed phenotype appears to depend, at least in part, on which components of the p53 pathway were altered upon immortalization.⁷³ That is, *rela*-/- cells with a transformed phenotype have low or mutant levels of p53, high levels of ARF, and low levels of Mdm2.⁷³ However, these correlations between the transformed phenotype, the absence of RelA, and the status of the p53 pathway are based on only four established cell lines and need to be further investigated using additional isolates. Nevertheless, these different effects could explain why, in some studies, loss of RelA in mouse fibroblasts enhances tumorigenesis induced by oncoproteins such as E1A and Ras⁷⁴ while, in other studies,^{47,73} RelA activity assists Ras-induced transformation. This is also consistent with the finding that the NF- κ B transcriptional response to the Bcr-Abl oncoprotein is dependent upon the ARF status of the cell: in the absence of ARF, Bcr-Abl-induced NF- κ B functions as an activator whereas in the presence of ARF NF- κ B functions as a repressor.⁷¹

Other tumor suppressors have also been implicated as regulators of RelA transcriptional activity. These include the p16^{INK4A} cyclin-dependent kinase (CDK) 4/6 inhibitor, which shares an overlapping gene locus with ARF. p16 can interact directly with RelA and overexpression of wild-type p16 inhibits NF- κ B transcriptional activity.⁸ In contrast, overexpressed melanoma-associated mutants of p16 do not inhibit RelA,⁷⁵ and the loss of p16 has been correlated with acquisition of aberrantly active RelA in primary melanomas and metastases.⁷⁶ These observations suggest that for NF- κ B to contribute to melanoma, certain key tumor suppressor functions must first be lost. Given that many deletions of p16 also inactivate ARF, this result could provide further supporting data for the link between ARF and RelA described above, as well as the differing phenotypes of *rela*-/- fibroblasts.

Similar to p16, the inhibitor of growth family member 4 (ING4) tumor suppressor, which inhibits brain tumor growth and angiogenesis, interacts with RelA and represses transcription of NF- κ B target genes.⁷⁷ Furthermore, overexpression of the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) tumor suppressor protein (the natural antagonist for PI3-Kinase) can inhibit the RelA transactivation domain;⁷⁸ however, this effect might simply

result from inhibition of PKB/Akt activity, which, as discussed above, is a positive modulator of NF- κ B transactivation. Whether p16, ING4 or PTEN actively modulate NF- κ B function, in the manner of ARF and p53, or merely repress its activity is not currently known.

In other contexts, the loss of a tumor suppressor can lead directly to increased NF- κ B activity and result in tumor promotion. For example, the *CYLD* tumor suppressor gene is inactivated in familial cylindromatosis (tumors of hair follicles), and these tumors have high NF- κ B activity. This elevated NF- κ B activity in the absence of CYLD protein occurs because CYLD is a deubiquitinating enzyme that acts as a negative regulator of NF- κ B signaling, through deubiquitination of IKK γ , TRAF2 and TRAF6.¹

NF- κ B and oncogenes

Consistent with its role as a tumor promoter, NF- κ B is required for several oncoproteins, including Bcr-Abl and Ras, to induce cellular transformation *in vitro*.⁸ Interestingly, these oncoproteins appear to increase NF- κ B transactivation and not its translocation to the nucleus. On the other hand, certain viral oncoproteins, such as Tax of HTLV-1 and LMP-1 of EBV, induce nuclear NF- κ B DNA-binding activity.⁷⁹

β -Catenin is a downstream component of the Wnt signaling pathway, which is controlled by the adenomatous polyposis coli (APC) tumor suppressor. Loss of APC and disruption of the Wnt pathway leads to aberrant β -catenin activation, which is particularly associated with colorectal cancer. The β -catenin proto-oncoprotein can associate indirectly with p50 and RelA, and this association inhibits NF- κ B DNA binding, transactivation, and target gene expression.⁸⁰ Interestingly, re-expression of APC in some colon cancer cell lines can restore NF- κ B activity.⁸¹ A link between β -catenin and NF- κ B signaling is also supported by the finding that deregulated β -catenin binds to p50, resulting in repression of selective NF- κ B target genes, including the metastasis repressor gene KAI1.⁸² These results are consistent with a tumor suppressor role for NF- κ B, at least in some colorectal cancers. It is interesting to note that an unusual nucleolar localization of RelA has also been reported in colorectal cancer cells in response to aspirin and UV-C, suggesting unique functions for NF- κ B in these cells (see above).⁶⁵

Similar to β -catenin, the HSCO protein, which is overexpressed in hepatocellular carcinomas, has been reported to bind RelA through its RHD and sequester it in the cytoplasm, an effect that can prevent the induction of apoptosis by doxorubicin and etoposide, but not TNF.⁸³

NF- κ B and apoptosis

The antiapoptotic functions of NF- κ B, and RelA in particular, are well established and have been reviewed extensively elsewhere.⁴ Since resistance to cell death is a key characteristic of many tumor cells, the antiapoptotic function of NF- κ B/RelA likely contributes to much of its tumor-promoting abilities. However, there are now numerous reports that NF- κ B can perform a proapoptotic role in some circumstances.⁴ Most directly, NF- κ B/RelA can stimulate the expression of apoptosis-inducing genes, such as Fas, Fas-ligand and death

receptors 4 and 5.⁴ In other cases, RelA can repress (rather than induce) the expression of certain antiapoptotic genes, such as Bcl-xL, in response to atypical NF- κ B inducers such as UV-C and daunorubicin or as a result of the action of the ARF tumor suppressor (see above).^{51,53,71} In these cases, activation of NF- κ B appears to sensitize cells to programmed cell death, since the actual apoptotic stimulus needs to come from another source, such as TNF stimulation, DNA damage, or p53 activity. In these situations, the death-inducing abilities of NF- κ B are more consistent with a tumor suppressor function. It should also be acknowledged that some reports failed to observe any effect of inhibiting NF- κ B on chemotherapy-induced apoptosis.⁸⁴

In many cases, the proapoptotic effects of NF- κ B occur in response to atypical inducers⁸⁴ and this is consistent with the hypothesis that it is the mechanism of induction of NF- κ B that determines its physiological function. Nevertheless, there are still apparent contradictions. For instance, the chemotherapeutic drugs daunorubicin and doxorubicin, which are structurally similar anthracyclines, have been described as inducing NF- κ B that can both stimulate and inhibit cell death.^{4,84} It is possible that experimental conditions might account for some of these differences. For example, the methods used to inhibit NF- κ B, such as proteasome inhibitors, are quite nonspecific. Moreover, the frequently used nondegradable super-repressor form of I κ B, I κ B super-repressor (I κ B-SR), can also affect non-NF- κ B pathways such as nuclear import/export.⁸ In addition, overexpressed I κ B κ can interact with non-NF- κ B proteins such as CDK4 and p53.⁸ However, it is unlikely that these contradictory results

are due to one group of studies being correct and the others being completely wrong. More likely, these results reflect the inherent variability of NF- κ B function, which depends on the context of its activation. In particular, the genetic background of the transformed and immortalized cells used for these studies can profoundly influence the outcome of these experiments. This observation has enormous implications for cancer therapy. If differences in the NF- κ B response to a chemotherapeutic drug also occur in different tumors in patients or between patients with apparently the same type of cancer, the ability to more accurately diagnose NF- κ B status could profoundly affect treatment choice and outcome (Figure 4).

Jun N-terminal kinase (JNK) signaling

NF- κ B regulation of JNK signaling pathways can also have divergent effects on tumorigenesis. A number of mechanisms have now been identified whereby NF- κ B activation results in suppression of JNK activity.⁸⁵ These include induction of Gadd45 β (Myd118) expression, which functions as an inhibitor of the JNK upstream kinase MKK7 (JNKK2) and induction of XIAP, which in addition to inhibiting caspase-3 and -7 activity can also inhibit JNK through an undefined mechanism. However, in some circumstances, loss of Gadd45 β or XIAP does not appear to affect JNK signaling,^{85,86} and reactive oxygen species (ROS) have been shown to play a role in NF- κ B/JNK crosstalk: stimulation with TNF, under conditions where NF- κ B is inhibited, results in the generation of ROS, which leads to JNK activation and

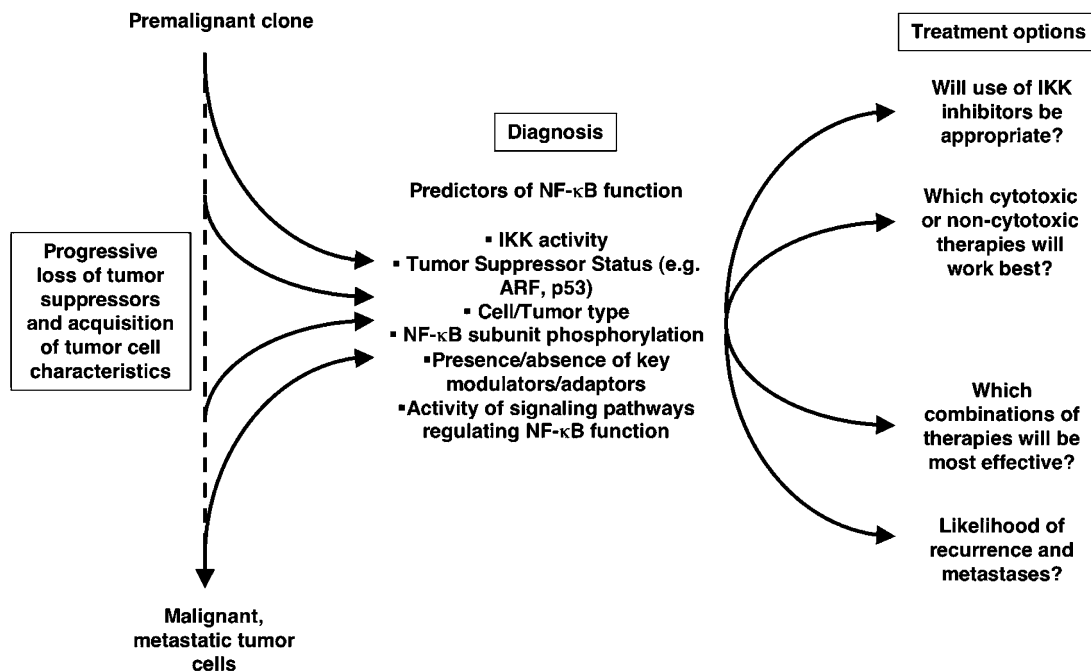


Figure 4 Clinical implications of multifunctional NF- κ B during tumor development. Distinct patterns of genetic changes are found during tumor progression, between different tumor types and even between patients initially diagnosed with the same form of cancer. These are likely to affect NF- κ B function in these tumor cells, whether it be aberrantly active NF- κ B or NF- κ B induced by cancer therapy. Introduction of the diagnostic procedures shown should lead to better prediction of NF- κ B function in individual patients, which could in turn influence the use of IKK inhibitors in therapy as well as the choice of existing anticancer drugs, which are known to affect or be affected by NF- κ B activity. Furthermore, NF- κ B function and the expression of NF- κ B target genes could lead to better predictions of the likelihood of tumor recurrence and metastasis

apoptosis.^{85,86} However, when NF- κ B is activated by TNF, ROS build-up is suppressed through the induction of genes encoding antioxidant enzymes such as manganese-superoxide dismutase and Ferritin heavy chain.^{85,86} Therefore, whether the JNK or NF- κ B pathway is dominant will depend on the signal context and cell type.

In most cell types, the consequence of NF- κ B inhibition of JNK and suppression of ROS production is the prevention of apoptosis. This pathway, therefore, is an important basis for the tumor-promoting activity of NF- κ B.^{85,86} But there are also exceptions to this rule. Some of the best evidence for NF- κ B functioning as a tumor suppressor comes from work demonstrating that NF- κ B inhibition in the epidermis results in hyperplasia and the development of squamous cell carcinomas.^{87–89} In addition, RelA is frequently excluded from the nucleus in human sporadic small cell carcinomas (SCC), and coexpression of the I κ B-SR and activated Ras in human keratinocytes transplanted into mice results in neoplasia resembling invasive SCC.⁸⁷ Many of these effects appear to derive from the absence of RelA-mediated inhibition of TNF-induced JNK activity, mediated through TNF receptor 1.^{37,89} The key difference here is that the consequence of JNK activation in the epidermis is different than in other cell types. In the epidermis, deregulated JNK activity leads to the upregulation of CDK4 protein, which drives cell proliferation and results in hyperplasia.⁹⁰ This is an indication of how the consequence of the same NF- κ B-driven effect, such as JNK inhibition, may result in profoundly different effects. In this case, it is also possible that other cell type-specific effects are involved. For example, in keratinocytes but not in fibroblasts, NF- κ B can induce the expression of the CDK inhibitor p21^{WAF1/CIP1}.⁹¹

NF- κ B-dependent suppression of JNK signaling has also been determined to play a role in hepatocarcinogenesis, where a conditional, hepatocyte-specific knockout of IKK β was used in conjunction with a model for chemical carcinogenesis.⁹² In this case, however, the increase in ROS and JNK activities following diethylnitrosamine treatment were found to result in the increased death of hepatocytes, in keeping with the usual role of the JNK pathway. This increase in cell death then resulted in a compensatory proliferation of surviving cells, which in turn provided the driving force for carcinogenesis. These studies further indicate the difficulty of interpreting NF- κ B activation and function in different settings. Even if activation of NF- κ B is apparently having the same effect at the level of gene expression and pathway activation/inhibition, the consequences of this can be profoundly different depending, once again, on the context and cell type.

NF- κ B and inflammation

The proinflammatory effects of NF- κ B and IKK activity are well established.^{1,2,7} However, there is evidence that NF- κ B activity can also be required for the resolution of an inflammatory response. NF- κ B activity in the later stages of inflammation has been associated with induction of anti-inflammatory genes and the induction of cell death.⁹³ Moreover, inhibition of this late-stage NF- κ B activity extended the length of the inflammatory response, inhibited the expression of p53 and Bax, and prevented apoptosis.⁹³ An inhibitory role

for p53 and RelA was also observed with LPS-induced shock in mice.⁹⁴ Given the link between chronic inflammation and tumorigenesis, one might consider that the role NF- κ B plays in resolving the inflammatory response also fulfills a tumor suppressor-like function. Whether such late-stage NF- κ B effects involve pathways similar to those described above, resulting in repression of NF- κ B target genes or altered promoter specificity, remains to be determined.

Clinical Implications of NF- κ B-Mediated Tumor Suppression

Given that aberrant activation of IKK and NF- κ B is associated with so many human diseases, inhibiting this pathway is an obvious strategy for drug development. Indeed, the effectiveness of many current anti-inflammatory treatments, such as glucocorticoids and anti-TNF antibodies, probably relies, at least partly, on NF- κ B inhibition. Despite this, there is a strong argument for the development of new, more effective NF- κ B pathway inhibitors. Current drug treatments that target NF- κ B have many other effects, leading to NF- κ B-independent clinical side effects.

To date, the favored therapeutic target of researchers and the pharmaceutical industry has been IKK β , the kinase principally responsible for I κ B α phosphorylation in response to inflammatory and many other stimuli.^{5,6} However, IKK α drugs might also have clinical efficacy, especially considering its likely role in breast cancer.^{33,95} Although IKK β inhibitors have clear clinical potential, the issue of potential side effects has long been a subject of concern. The long-standing argument against such inhibitors is that given the essential role that IKK activation of NF- κ B plays in the immune system, suppression of IKK β activity could have unwanted effects. It is possible that such issues can be resolved by careful dosage regimens, given that complete inhibition of NF- κ B might not be required for significant clinical effect.

Many of the issues described in this article raise another potential problem with side effects. Because NF- κ B can perform a tumor suppressor function in some tissues, will its inhibition actually promote cancer in some situations? This is almost certainly a minor issue in the treatment of most cancers, wherein treatment is relatively short term, i.e., there would probably not generally be sufficient time for the concomitant inhibition of NF- κ B tumor suppressor effects to manifest themselves in, for example, skin cancer. Moreover, given the frequent severity of the primary disease, side effects are better tolerated as part of clinical process. Another concern arises from the different effects of chemotherapeutic drugs on NF- κ B (see above). If NF- κ B is assisting the therapeutic activity of these drugs under some circumstances, then NF- κ B inhibition could actually hinder certain therapies rather than assist them. However, it is likely that these beneficial effects of NF- κ B are selected against during tumor development and in malignant, metastatic and chemo-resistant tumors, inhibiting NF- κ B will most often be a suitable therapeutic approach. This does not mean that these issues should be ignored. Indeed, there is great opportunity for exploiting NF- κ B status as a means of improved tumor diagnosis (Figure 4).

Side effects of NF- κ B inhibition might be more of an issue if such agents were used for the long-term treatment of chronic inflammatory diseases such as rheumatoid arthritis (where NF- κ B has a well-established role⁷). Under these circumstances, continuous suppression of NF- κ B activity over a number of years could manifest itself in, for example, squamous cell carcinoma, an effect seen with inhibition of NF- κ B DNA binding in mice.^{88,89} This might not be a problem for IKK inhibitors, however, because inhibiting IKK may not be identical to blocking nuclear NF- κ B DNA-binding activity. IKK β null keratinocytes display a number of differences to RelA null keratinocytes (discussed in Zhang *et al.*³⁷): *rela*-/- keratinocytes are hyperproliferative *in vitro* while *ikkb*-/- keratinocytes are hypoproliferative,^{37,96} and NF- κ B DNA-binding activity remains TNF inducible in both IKK α or β null keratinocytes, albeit at a significantly reduced level in the latter case.^{38,96} These latter effects may reflect redundancy between IKK α and β in some cells or a compensatory effect of one of the atypical pathways discussed above. Interestingly, and consistent with these cell type-specific effects, IKK β null hepatocytes do not show increased sensitivity to TNF-induced cell death although IKK γ null cells do.⁹⁷ It is also possible that the tumor suppressor effects of NF- κ B manifest themselves largely through atypical activation pathways and consequently, the inhibition of IKK might not disturb this aspect of NF- κ B function to any great degree. Therefore, while concerns about side effects of IKK inhibitors should be considered, they may not prove as harmful as first thought.

Physiological differences between the loss of IKK subunit activity and the direct inhibition of NF- κ B DNA-binding activity suggest that there are other routes to exploiting NF- κ B function for therapeutic purposes. The NF- κ B-independent effects of IKKs, together with the fact that small-molecule inhibitors are rarely 100% specific for their targets at usable concentrations, means that drugs that directly target NF- κ B in IKK-independent ways might have very different therapeutic potentials. Other strategies could target NF- κ B subunits more directly. One approach is to use gene therapy vectors to express the I κ B-SR protein. This has been used in animal model systems⁹⁸ and has the advantage that the therapy could be targeted specifically to the diseased tissues, thus avoiding the potential side effects resulting from inappropriate inhibition of NF- κ B. Of course, I κ B-SR-based therapy is not without its potential drawbacks: the problems of vector design and delivery are an issue for all gene therapy-based treatments. In addition, I κ B-SR proteins might also be expected to exert NF- κ B-independent effects (see above), although there is no reason to suppose that these effects need be deleterious, especially in a tumor cell that has already lost p53 function. Of note, there are also several natural products and natural product derivatives that can simultaneously inhibit both IKK and NF- κ B DNA binding by affecting reactive Cys residues in their targets (Liang *et al.*⁹⁹ and references therein) and such dual-specific inhibitors may have clinical relevance.¹⁰⁰

The IKK-independent signaling pathways that directly modify the NF- κ B subunits themselves represent another potential drug discovery route. Inhibition or activation of such kinases would provide a route to modulate NF- κ B directly, without totally inhibiting its function. There are advantages to such an approach. For example, converting RelA from its

oncogenic to its tumor suppressor form, where antiapoptotic genes are actively repressed rather than induced, might be expected to be a more potent strategy than merely inhibiting RelA activity. Owing to functional redundancy at promoters, merely removing an activator can be compensated for by other proteins. Furthermore, when such an activator is modified and in its tumor suppressor form, other genes might be induced that would otherwise remain inactive. The effects of ARF, UV-C and daunorubicin on RelA transactivation are dominant over those of TNF, which increases the chances of such an approach being effective.^{51,53} Targeting the NF- κ B subunit kinases directly would not be a specific method of modulating NF- κ B since these proteins certainly phosphorylate many other cellular targets, although this does not mean that such compounds would not be clinically effective. Peptides, siRNAs or molecules that target the docking of kinases to RelA and other NF- κ B proteins might be more specific but these approaches are not commonly used in current drug development. In addition, not enough is known about the basic biology of NF- κ B subunit modification to be able to accurately predict the effects of modulating these pathways at the organismal level.

An understanding of modulators of NF- κ B might have a more immediate and practical impact on the use of current cancer therapies. It has recently become apparent that the effects of cytotoxic drugs on NF- κ B function are quite diverse and may differ between cell and tumor types or even the different stages of tumor development. Better prediction of the effect of a drug or radiation treatment on NF- κ B at the tumor diagnosis stage might help match the type of therapy to the patient. Furthermore, such approaches might help predict when inhibiting NF- κ B in a tumor will also inhibit, rather than stimulate, tumor growth and help in the efficacious use of IKK inhibitors, once they are more commonly available (Figure 4). Overall, the diversity of NF- κ B functions should not be viewed as reasons for why therapies based on its function will not work. Rather, an increased understanding of the different roles and regulations of NF- κ B will create opportunities for the development of techniques that will allow existing and new therapies to be more effective. This knowledge could result in novel approaches to modulating NF- κ B function for the treatment of cancer and many other diseases.

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