Leukemia (2002) 16, 1053–1068

www.nature.com/leu

REVIEW

Nuclear transcription factor-κB as a target for cancer drug development

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Nuclear factor kappa B (NF-κB) is a family of inducible transcription factors found virtually ubiquitously in all cells. Since its discovery by Sen and Baltimore in 1986, much has been discovered about its mechanisms of activation, its target genes, and its function in a variety of human diseases including those related to inflammation, asthma, atherosclerosis, AIDS, septic shock, arthritis, and cancer. Due to its role in a wide variety of diseases, NF-κB has become one of the major targets for drug development. Here, we review our current knowledge of NF-κB, the possible mechanisms of its activation, its potential role in cancer, and various strategies being employed to target the NF-κB signaling pathway for cancer drug development.
Leukemia (2002) **16,** 1053–1068. DOI: 10.1038/sj/leu/2402482
Keywords: nuclear factor kappa B (NF-κB); IκB kinase (IKK); inflammation; cancer; drug development; apoptosis

Introduction

Transcription is an important regulatory event in the pathway leading to gene expression. Transcription factors regulate transcription by binding to specific sequences present within the promoter, enhancer, or other regulatory regions of DNA. Hundreds of transcription factors with functionally separable domains, essential for DNA-binding and activation, have been identified and characterized in several organisms.[1] One such transcription factor, NF-κB, has been the subject of intense study based on the implications of its role as a key mediator of a wide variety of cellular responses.[2]

Nuclear Factor κB

NF-κB was first identified in the nuclei of mature B lymphocytes as a transcription factor that binds an 11-bp DNA sequence in the κ-light chain enhancer GGGACTTTCC.[3] Mammalian cells have five distinct NF-κB subunits based on a highly conserved 300 amino acid dimerization domain called the rel homology domain, which is required for binding DNA and mediating the transcription of over 180 target genes. These subunits may be classified into two functional groups, one containing the NF-κB1 (p105/p50) and NF-κB2 (p100/p52) subunits and the other containing the RelA (p65), Rel B, and c-Rel subunits. Members of the second group share a carboxy-terminal transactivation domain usually required within the Rel/NF-κB structure to promote transcription. Members of the first group exist as precursor subunits p105 and p100 (105 000 and 100 000 daltons), which contain a series of five to seven ankyrin repeat domains that mask the nuclear localization signals (NLS) within the rel homology domain. They must be cleaved to the active p50 and p52 subunits (from p105 and p100, respectively) before allowing the translocation of the NF-κB complex from the cytoplasm to the nucleus, where transcription takes place.

Other inhibitory subunits that utilize a similar ankyrin repeat domain (but do not contain the rel homology domain of the five subunits above) include IκBα (most common), IkBβ, IkBγ (derived from the C-terminal of p100), IkB-ϵ, Bcl-3, pp40 (chicken homologue), and avian swine fever virus protein p28.2. More recently, another IκB-like subunit called IkB-ζ, with six ankyrin repeat domains, was discovered and was found to retain the NF-κB proteins in the nucleus instead of the cytoplasm.[4]

There are several different structural combinations of subunits in the cytoplasm that are called NF-κB, with the most common heterodimer consisting of a Rel A subunit (p65), a NF-κB1 subunit (p105/p50), and the IκBα inhibitory subunit.[5] On activation, degradation of IκBα exposes nuclear localization signals (NLS) on the p50-p65 heterodimer, leading to nuclear translocation and binding to a specific sequence in the DNA, which in turn results in gene transcription. This pathway is well conserved, both in structure and function, from Drosophila to humans.[6]

How is NF-κB activated?

A lot has been learned about NF-κB activation in the last decade. Cellular responses to a wide variety of diverse stimuli have been identified, and have shown to lead to the activation of NF-κB (see Figure 1). These stimuli reveal that NF-κB is a common pathway for cellular adaptation to stress.[2,7,8] The stimuli include inflammatory cytokines, immune-related stress such as bacterial infection of *S. aureus*[9] and their products such as lipopolysaccharide[3] (or LPS), viruses such as HIV-1[10] and their products such as hemagglutinin of the flu virus,[11] physiologic stress such as ischemia,[12,13] physical stress such as UV irradiation,[14] environmental hazards such as cigarette smoke,[15] many therapeutic drugs such as taxol[16] or haloperidol,[17] apoptotic mediators such as anti-Fas,[18] growth factors such as insulin,[19] physiologic mediators such as angiotensin II[20] or PAF,[21,22] oxidative stress such as exposure to hydrogen peroxide,[23] and many more (see Figure 1).

Depending on the stimulus, the mechanism of activation involves overlapping and nonoverlapping steps. Among all the stimuli, perhaps the most is known about the mechanism by which TNF activates NF-κB. This pathway involves the interaction of the ligand with its receptor at the cell surface (TNFR), which then recruits a protein called TNF receptor-associated death domain (TRADD). This protein binds to TNF receptor-associated factor (TRAF)-2, which activates receptor-interacting protein (RIP). RIP interacts with mitogen-activated protein kinase kinase kinase 3 (MEKK3) to phosphorylate and

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Received 19 September 2001; accepted 21 January 2002

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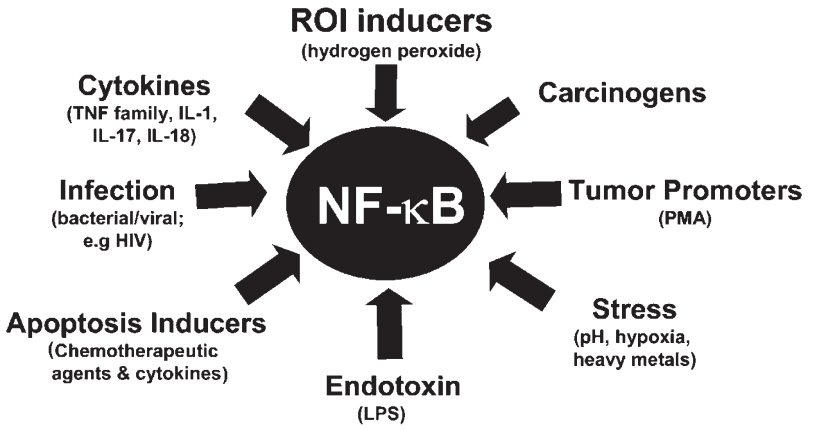

Figure 1 Activation of NF-κB by various agents reactive oxygen intermediates (ROIs), cytokines, infections, apoptotic inducers, endotoxin, physical stress, tumor promoters, and carcinogens.

activate the IκBα kinase complex (IKK).[60] The IKK complex phosphorylates IκBα at serines 32 and 36, which leads to ubiquitination at lysines 21 and 22, and this leads to the degradation of IκBα by the 26S proteosome, resulting in the translocation of NF-κB to the nucleus, where it binds to its consensus sequence (5′-GGGACTTTC-3′) and activates gene expression. It should be noted, however, that other mechanisms of NF-κB activation that do not require IκBα degradation have been identified, including those induced in response to such stimuli as oxidative stress and X-rays[24,25] (Figure 2). These mechanisms, although not precisely understood, likely involve tyrosine phosphorylation of IκBα instead of the traditional serine phosphorylation that causes its subsequent degradation.[26,27] Interestingly, it was recently found that erythropoietin (EPO) activates NF-κB through phosphorylation of tyrosine and serine residues of IκBα and this is mediated through Janus kinase-2[28] (JAK2) (see Figure 2), the only protein tyrosine kinase thus far implicated in the activation of NF-κB (see below).

The mechanism that activates the IKK complex is considered a common pathway for a number of different activation pathways (see below) and has been the subject of intense study.[29] An IKK complex consists of three subunits including IKKα, IKKβ, and IKKγ (also called NEMO). IKKβ is an inducible catalytic subunit that phosphorylates IκBα at serine 32 and 36 and causes the subsequent degradation of IκBα, leading to the activation of NF-κB. Physiologic roles of IKKβ via gene deletion studies have shown IKKβ to be integral in liver development and protection of T cells from TNF-α-induced apoptosis.[30,31] IKKα has recently been shown to be involved in the activation of NF-κB via an IκBα-independent pathway that involves the direct phosphorylation of NF-κB2 (p100 precursor) in response to upstream kinases.[32] Gene deletion studies of IKKα have shown that it plays an unexpected role in skin and skeletal development.[33,34] IKKγ (aka NEMO or IKKAP1) is a regulatory subunit without intrinsic kinase activity and was found to play an integral role in the activation of NF-κB as well via modulation of IκBα degra-

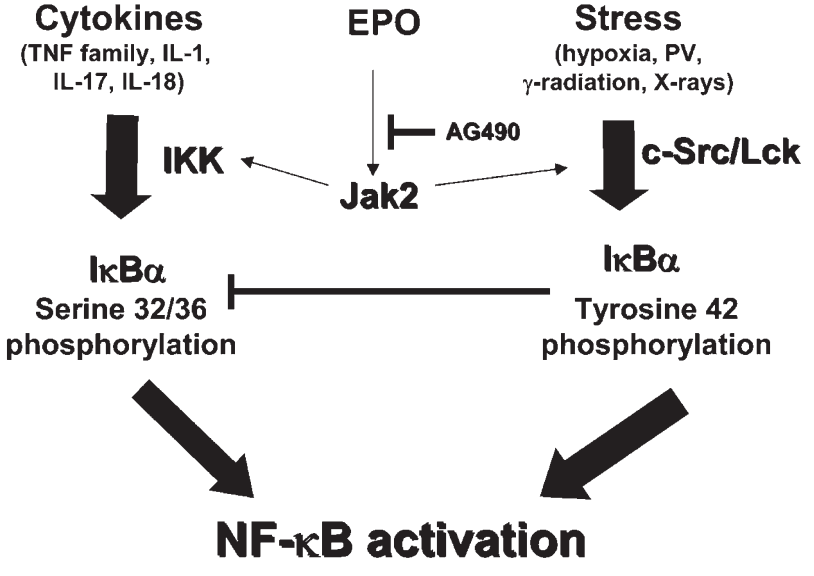

Figure 2 Various mechanisms leading to NF-κB activation. Depending on the inducing agents, NF-κB activation may follow different pathways. In response to pro-inflammatory cytokines, NF-κB activation follows IKK activation and subsequent IκBα phosphorylation at serine 32/36. In response to physical stress such as hypoxia and X-rays, c-Src/Lck is activated and causes the subsequent IκBα phosphorylation at tyrosine 42 which leads to NF-κB activation. Interestingly, the latter route inhibits the serine 32/36 phosphorylation of IκBα in response to TNF. Also, both pathways may be activated in response to erythropoietin (EPO) via the JAK2 kinase that can activate both IKK and C-Src/Lck.

dation pathways.[35,36] Gene deletion studies (X-linked) reveal that IKKγ is imperative for male survival in mice and important in lymphocyte development and persistence.[37] The novel IKK-related kinase called IKKε/IKKi is an LPS and PMA inducible kinase whose role in NF-κB activation is less well defined although likely involves the unique preferential phosphorylation of only serine 36 (and not serine 32) on IκBα. Its mechanism may involve interaction with the TRAF interacting protein/TRAF family member-associated NF-κB activation pathway as well as interactions with unidentified upstream and downstream kinases.[38–40] Additional research will clearly be needed to elucidate its precise mechanism.

Extensive research over the last few years indicates that NF-κB activation is highly complex and may involve as many as 20 different protein kinases (see Table 1). These kinases may form a cascade, and different cascades may be formed depending on the NF-κB activator. For instance, IKK can undergo phosphorylation by either NIK, MEKK or AKT. Although several signaling proteins and protein kinases have been recently identified that mediate IKK activation, the exact role of these kinases and their placement within the activation cascade is controversial. The activation of some of these kinases may be specific to cell type and to the stimulus employed to activate NF-κB.[81] For instance, NIK, while found critical for NF-κB activation by LT and CD40L, was found to have no role in TNF-induced NF-κB activation.

Lessons learned from NF-κB gene deletion

In the past 7 years, mouse models with a deletion of one or more of the genes that code for specific Rel/NF-κB proteins (termed 'knockout mice') have provided a valuable insight into the function and relevance of various NF-κB gene products. Overall, individual knockouts have caused either mild to severe immune-related deficiencies (eg p105/p50, p100/p52, Rel A, Rel C, IκBα), liver apoptosis (Rel A), or various other developmental abnormalities (eg IκBα, IKK). When p105/p50 is knocked out, there are functional defects in the immune system despite an otherwise normal development and phenotype.[82] More specifically, p105/p50 is essential for the survival of non-activated B cells but not essential for all B cell-activated pathways.[77,83,84] For example, p50-deficient mice are susceptible to *L. monocytogenes* and *S. pneumoniae* infections and do not proliferate in response to LPS but do respond to *Haemophilus influenzae* and *Escherichia coli*.[77] Knocking out the Rel A subunit causes embryonic lethality as a result of fetal liver cell apoptosis and granulopoiesis.[85] This implicates Rel A in cell survival, specifically in response to the cytotoxic effects of TNF-α via induction of IκBα. Also, Rel A has been shown to be important in induced lymphocyte proliferation and isotype switching but not basal transcription.[86]

C-rel knockout mice show normal development but B and T

Table 1 Protein kinases implicated in activation of NFκB

NF-κB activating kinases	*Ligand(s)*	*Refs*
IκB kinase α (IKKα)	Proinflammatory cytokines (eg TNF, IL-1, IL-6), LPS	42–45
IκB kinase β (IKKβ)	Proinflammatory cytokines (eg TNF, IL-1, IL-6), LPS	42–45
NEMO/IκB kinase γ (IKKγ)	Proinflammatory cytokines (eg TNF, IL-1, IL-6), LPS	35, 36
IκB kinase i (IKKi/IKKε)	TNF, PMA	38, 39
NF-κB inducing kinase (NIK)	TNF, CD95, and IL-1	41
NF-κB activating kinase (NAK; also called T2K, TBK1 or TRAF2 interacting kinase)	Phorbol esters, growth factors	61–63
Phosphotidylinositol 3 protein kinase (PI-3K)	IL-1	64
Protein kinase Bα (Akt 1)	TNF, TCR/CD28	53, 65
Hematopoietic protein kinase-1 (HPK-1)	Unknown	67
Protein kinase C-alpha (PKC-α)	TPA, TNF, Bimp1, Bcl10/MALT1	48–50
Protein kinase C-beta (PKC-β)	IgM receptor, Bimp1, Bcl10/MALT1	49, 51
Protein kinase C-theta (PkC-τ)	CD3-CD28 (TCR/CD28), Bimp1, Bcl10/MALT1	49, 52, 53
Protein kinase C-delta (PkC-δ)	TNF[a]	54
Protein kinase C-epsilon (PkC-ε)	Phorbol ester, Bimp1, Bcl10/MALT1	49, 55
Atypical protein kinase C (aPkC)	TNF, ras p21	46, 47
Mitogen activated protein kinase kinase kinase 1, 2 (MEKK1,2)	Proinflammatory cytokines	59, 43
Mitogen activated protein kinase kinase kinase 3 (MEKK3)	TNF, other proinflammatory cytokines	43, 60
TPL-2/Cot kinase	TNF	58
Ribosomal protein S6 kinase (pp90rsk)		
	p. aeruginosa	56
Raf-1 kinase	Serum growth factors, phorbol ester and PTK oncogenes	70, 71
RNA-dependent protein kinase (PKR)	dsRNA	57
TGF-β activated kinase-1 (TAK1/MAPKKK)	XIAP, TGF-β	68, 69
Mixed lineage kinase (MLK3)	CD3/CD28	66
p21 activated kinase (PAK1)	p21, LPS	72
Bruton's tyrosine kinase (BTK)	B-cell antigen receptor	73
Janus kinase-2 (JAK2)	Erythropoietin	28
Protein kinase A (PKA)	IL-1, LPS, many others	3, 74, 75
IL-1 receptor-associated kinase-1 (IRAK-1)	IL-1	76, 77
IL-1 receptor-associated kinase-2 (IRAK-2)	IL-1, LPS	78
IL-1 receptor-associated kinase-M (IRAK-M)	IL-1, LPS	78
P56 lck	Ceramide	79, 80

[a]Demonstrated in neutrophils. Type of kinase activated may vary with cell type.

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cell deficiencies.[87] Specifically, c-rel-deficient B cells cannot proliferate in response to immunogens due to a cell cycle block at G1 and more prevalent activation-induced apoptosis due to a failure to upregulate A1 (homologue of Bcl-2), a pro-survival protein.[78,88] C-rel has also been shown to cause a tissue-specific deficiency of various cytokines and growth factors in T cells and macrophages affecting both innate and humoral immune responses in the host.[89–92] Mice deficient in the NK-κB2 gene (p100/p52) mainly have defects in lymph node and splenic architecture although development is normal.[93,94] This leads to antigen presentation impairment from accessory cells such as dendritic cells and macrophages but does not affect B or T cells directly.[95] Knocking out the major inhibitory subunit IκBα produces severe runting (one-third of normal weight) despite normal development, death by day 8 of life due to widespread dermatitis and granulocytosis, scaly appearing skin with significant sloughing, extensive post-natal granulopoiesis, small spleen size caused by depletion of cells of erythroid and lymphoid lineages (not myeloid though), and elevated levels of NF-κB in hematopoietic tissues and some NF-κB-dependent target genes (implying that additional transcriptional factors are involved).[90,96,97]

Recently, IKKα and IKKβ knockouts have demonstrated that IKKβ is the major subunit involved in NF-κB activation in response to a majority of stimuli (ie pro-inflammatory cytokines).[98,99] Gene deletion studies have also revealed that IKKα plays little role in NF-κB activation but has proven to play an unexpected role in skin and skeletal development.[29,33]

Relevance of NF-κB to cancer

NF-κB is an ideal target for anticancer drug development for several reasons (see Figure 3). Cancer is a hyperproliferative disorder that involves transformation, initiation, promotion, angiogenesis, invasion, and metastasis. The diversity of its clinical presentation, aggressiveness, and current treatment strategies imply an equally diverse number of potential targets in the molecular pathways leading to its formation. NF-κB activation participates at multiple steps in these pathways shown below and its suppression may lead to the suppression of cancer development. First, NF-κB mediates the expression of genes that are involved in tumor promotion, angiogenesis, and metastasis.[100,101] Second, it has been shown that NF-κB is activated by hypoxia and acidic pH, both indigenous to the tumor microenvironment.[102] Third, the activation of NF-κB blocks apoptosis and promotes cell proliferation.[103–106] Fourth, several tumor types show a persistent constitutive nuclear activation of NF-κB. Fifth, NF-κB activation has been shown to induce resistance to various chemotherapeutic agents.[107,108]. Sixth, NF-κB gene products can be oncogenic when aberrantly expressed and are implicated in a number of tumor types.

NF-κB and tumor promotion

The development of cancer is generally categorized into three stages: tumor initiation, tumor promotion, and tumor metastasis. Besides regulating a number of genes involved in prolonged cell survival (see above), NF-κB regulates many genes involved in the promotion of cancer (ie clonal expansion, growth, diversification, angiogenesis, adhesion, extravasation, degradation of extracellular matrix, etc). For example, NF-κB may regulate the production of prostaglandins via the pro-inflammatory gene cyclooxygenase-2 (COX2), which has been shown to be overexpressed in a variety of cancers including colorectal cancer and mesothelioma.[109–111] Similar studies have been found for many other pro-inflammatory genes regulated by NF-κB including tumor necrosis factor[112] (TNF), interleukin-1[113] (IL-1), inducible NO-synthase[114] (iNOS), matrix metalloproteinase[115] (MMP-9), urokinase-type plasminogen activator[116] (uPA), and many other chemokines.[117–119]

Role of NF-κB in angiogenesis

Tumor cells, just like normal cells, need oxygen to survive and thus can be a limiting factor to progression of tumors. Vascularization of tumors requires the release of angiogenic growth factors (eg VEGF, MCP-1) from tumor cells and/or inflammatory cells such as macrophages and neutrophils or in response to pro-inflammatory cytokines (eg TNF).[120–122] NF-κB regulates the expression of such growth factors and cytokines (VEGF, TNF, MCP-1) necessary for angiogenesis providing another pathway for which inhibition of NF-κB may be justified in anti-cancer therapy.[123–126]

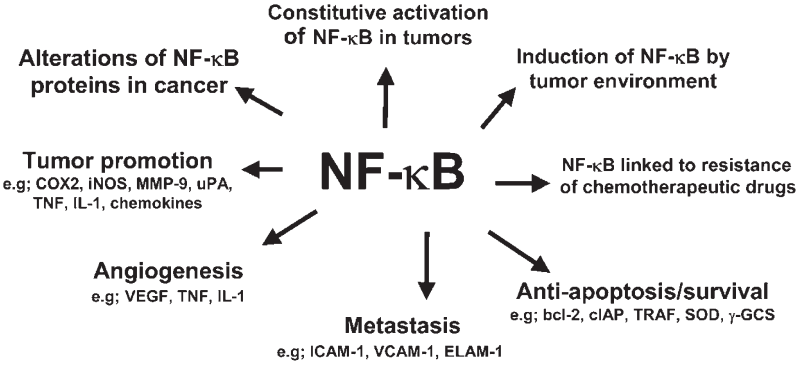

Figure 3 Potential mechanisms by which NF-κB activation can cause development of cancer. First, it regulates the transcription of many genes that are involved in tumor promotion, angiogenesis, metastasis, and increased cell survival. Second, NF-κB may be induced by the tumor microenvironment (eg hypoxia) and cause perpetuating tumorigenesis. Third, its activation is linked to increased resistance of tumors to chemotherapeutic drugs and radiation therapy. Fourth, the constitutive activation of NF-κB is present in a variety of tumors. Finally, aberrant expression of the NF-κB proteins themselves have shown to be linked with the development of cancer.

NF-κB and metastasis

The metastasis of cancer requires the migration of cancerous cells both into and out of the vessel walls that transport them to other parts of the body. The ability to cross vessel walls is mediated by specific molecules that are expressed in response to a number of signals from inflammatory cells, tumor cells, etc. Among those special molecules are ICAM-1, ELAM-1, and VCAM-1, all of which have been shown to be expressed in response to NF-κB activation.[127–129]

The induction of NF-κB by the tumor microenvironment

The stress of fluctuation in blood flow in the microenvironment of solid tumors and the resultant intermittent hypoxia has been shown to activate NF-κB.[130] Since oxygen is needed for a tumor to grow, the tumor must secrete chemotactic signals such as growth factors and cytokines in order to induce neovascularization.[131] Many of these growth factors and necessary signals for tumor progression (see below) are target genes of NF-κB and utilize its activation for their transcription. Once again, the cellular signals and precise mechanism of activation have not been elucidated, but the pathway of NF-κB activation by hypoxia is somewhat unique. The more 'traditional' pathway of activation in response to stimuli (eg TNF-α, IL-1) via IκB inhibitor phosphorylation, ubiquitination and degradation requires the phosphorylation of serines on IκB (see above), but Koong *et al*[130] have shown that hypoxia stimulates the phosphorylation of tyrosine groups instead. The addition of this pathway leading to NF-κB activation adds to the belief that NF-κB may involve several more pathways that are as yet undiscovered.

NF-κB in apoptosis, cell proliferation, and tumor initiation

In 1996, three separate reports appeared implicating NF-κB in the inhibition of apoptosis.[104,132,133] Since then, a flurry of reports, mostly supporting the original conclusions, have confirmed NF-κB's role as a mediator of inhibition of apoptosis in many cell types. Tumor initiation begins with the prolonged survival of a cell, and so, given its role in apoptosis, NF-κB has obvious implications for cancer.

An anti-apoptotic role of NF-κB has been linked to T cell lymphoma, osteoclasts,[134] melanoma,[135] pancreatic cancer,[136] bladder cancer,[137] and breast cancer.[138] Cell types (not necessarily oncogenes) that display an anti-apoptotic role for NF-κB include B cells,[139,140] T cells[141,142] granulocytes,[143] macrophages,[144] neuronal cells,[145,146] and smooth muscle cells.[147]

Although rare, there are systems in which NF-κB has been shown to play a pro-apoptotic role in addition to its more common anti-apoptotic role. Examples of its pro-apoptotic effects in cells include those found in B cells,[148] T cells,[149,150] neuronal cells,[151,152] and endothelial cells.[153]

The opposing effects of NF-κB are thought to be cell type specific and/or dependent on the inducing signal (eg IL-1, TNF-α, and UV radiation). Different activation pathways of NF-κB may cause the expression of proteins that promote apoptosis (eg Fas, c-myc, p53, IκBα) or inhibit apoptosis (eg TRAF2, IAP proteins, Bcl-2-like proteins).[154–156] In addition, NF-κB activation variably controls the regulation of cell cycle proteins (eg cyclin D1 and CDK2 kinase)[157–159] and the interaction with various cellular components (eg p300 and p53) that promote or induce apoptosis[160,161] (see Figure 3).

The constitutive activation of NF-κB in cancer

Another potential mechanism through which NF-κB could play a role in tumorigenesis involves its constitutive activation. As explained above, the activation of NF-κB occurs as it is transported from the cytoplasm to the nucleus upon degradation of the inhibitory subunit. In the nucleus it binds to specific κB sites on the DNA and mediates the expression of a number of genes involved in the cellular response to various stresses. Thus, when NF-κB is found persistently in the nucleus, it is referred to as constitutive activation. Constitutive activation is shown in a wide variety of tumor types[162–184] (see Table 2) including those tumors induced in animal models. A higher level of NF-κB binding activity was found in 86% of nuclear extracts from mammary tumors that were induced in rats vs normal rat mammary glands and higher levels of NF-κB were found in estrogen receptor-negative breast cancer cell lines in rats and humans which correlated with tumorigenesis. Also, increased expression of NF-κB was found in papillary, anaplastic, and follicular thyroid cancer cell lines *vs* normal cells. Furthermore, the inhibition of p65 in these cancer cells led to a decrease in *c-myc* expression and a decrease in growth. Finally, constitutive activation was found in 83% of human pancreatic cancer cell lines. Taken together, these results, along with other experiments cited in Table 3, suggest a strong correlation between NF-κB expression and tumor formation, making the inhibition of NF-κB a valid therapeutic frontier. It should be noted, however, that constitutive activation of NF-κB is not limited exclusively to tumors as evidenced by the existence of normal cells which show constitutive activation.

The precise role of constitutive activation in tumors is not known but has been linked to resistance to apoptosis in human cutaneous T cell lymphoma cells.[105] It is tempting to believe that a similar mechanism accounts for the progression of all tumors that constitutively express NF-κB, but such a link has yet to be clearly identified. Normally, activation of NF-κB has been shown to be stimulus dependent, such that some

Table 2 Tumors that express constitutively active NF-κB

Tumor type	*Refs*
B cell lymphoma	162, 163
Hodgkin's disease	164–166
T cell lymphoma	167, 105
Adult T cell leukemia	168
Acute lymphoblastic leukemia	169
Breast	138, 106
Liver	170–172
Thyroid	173
Pancrease	174, 175
Prostate	176, 177
Melanoma	178
Head and neck SCC	179
Colon	180
Multiple myeloma	181
Ovarian cancer	182
Bladder cancer	137
Lung carcinoma	183, 184

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Table 3 Tumors with altered NF-κB proteins

NF-κB protein	*Cancer*	*Refs*
c-Rel	Diffuse lymphomas with large cell component	191–193
	Thymic B cell lymphoma, follicular large cell lymphoma	190, 184, 194
	Follicular lymphoma	195
	Human diffuse lymphoma cell line	195
	Non-small cell lung carcinoma	184
Rel A	Squamous head and neck carcinoma	188
	Adenocarcinomas of breast and stomach	133, 108
	Thyroid carcinoma cell line	173
	Multiple myeloma	190
	Non-small cell lung carcinoma	184
NF-κB1 (p105/p50)	Acute lymphoblastic leukemias	196
	T cell acute leukemias	197
	Non-small cell lung carcinoma	182, 189
	Colon, prostate, breast, brain, bone cell lines	189
NF-κB2 (p100/p52)	B cell non-Hodgkin's lymphoma	198–202
	Chronic lymphocytic leukemia, multiple myelomas, cutaneous T cell lymphomas	106, 184, 203
	Breast cancer, colon cancer	
IκBα	Hodgkin's lymphoma	166, 204, 205

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stimuli such as TNF-α cause its activation in certain cells whereas other stimuli such as IL-1 or hypoxia cause its activation in other cells. While many NF-κB stimuli have been identified, the stimulus responsible for constitutive activation of NF-κB in most cell types is not understood. Cells that express constitutively activated NF-κB are resistant to various chemotherapeutic agents.

NF-κB activation in chemotherapy and radiation therapy

The activation of NF-κB has been linked to cellular resistance of chemotherapeutic drugs and radiation treatment, making the development of anti-NF-κB drugs all the more promising.[132] Antagonizing NF-κB activity has also been shown to increase the efficacy of chemotherapeutic agents and radiation in some tumor cell lines.

In the case of radiotherapy, which has been a valuable tool in the treatment of several cancers including lymphomas and leukemias, NF-κB has been shown to be activated in response to therapy in tumor cell lines. Fibrosarcoma cells expressing genetically manipulated IκBα (an inhibitors of NF-κB not sensitive to phosphorylation, 'super repressor', see below) are more sensitive to radiation-induced apoptosis than in controls.[132] In addition, glioblastoma cell lines (A172, M054) that express the super repressor are more susceptible to radiation treatment.[185] While the precise mechanism leading to induction of radiation resistance is not clear, these examples make the development of inhibitors to NF-κB more promising.

Likewise, the precise mechanism for NF-κB's involvement in chemotherapeutic efficacy is not clear, but its enhanced activity has been shown to be linked to decreased apoptosis via expression of the anti-apoptotic gene A1/Bfl-1 and enhanced expression of the multiple-drug resistance gene product or MDR gene (prevents the intracellular accumulation of toxic drugs such as those used in chemotherapy), both likely factors in the progression of tumors.[108,186]

Alterations of NF-κB proteins in cancer

While the expression of a large number of genes involved in the development of cancer are regulated by NF-κB, the genes that code for individual NF-κB proteins themselves have also been implicated in the development of several types of cancers, both hematopoietic and solid tumors. These genes are expressed aberrantly, ie amplification of gene on chromosome, rearrangement, overexpression, substitution, mutation, truncation, etc.[187] Alterations in NF-κB proteins have been found in a wide variety of tumors (see Table 3). Thus, the altered expression of the NF-κB gene products may play a critical role in tumorigenesis.

Strategies to block NF-κB activation

Several strategies have been employed to block the activation of NF-κB. A wide variety of compounds (both natural and synthetic) have been screened for their ability to suppress NF-κB. These compounds block NF-κB activation through multiple mechanisms by intercepting various steps leading to NF-κB activation (see Figure 4). How specific some of these inhibitors are and whether they block other signaling pathways[206] remains to be elucidated. The following gives examples for each of the strategies shown to block NF-κB activation in response to one or more stimuli.

Block binding of NF-κB to the DNA

The most direct strategy for blocking activation of NF-κB is to block its binding to specific κB sites on DNA. This is assessed by assaying the amount of NF-κB protein that is able to bind to DNA after administration of inhibitor. One mechanism is the use of a transcription factor decoy (TFD) peptide, called double-stranded oligodeoxynucleotide (ODN), that binds the same complementary region of specific DNA sites, competitively inhibiting NF-κB binding.[207] Other examples of inhibi-

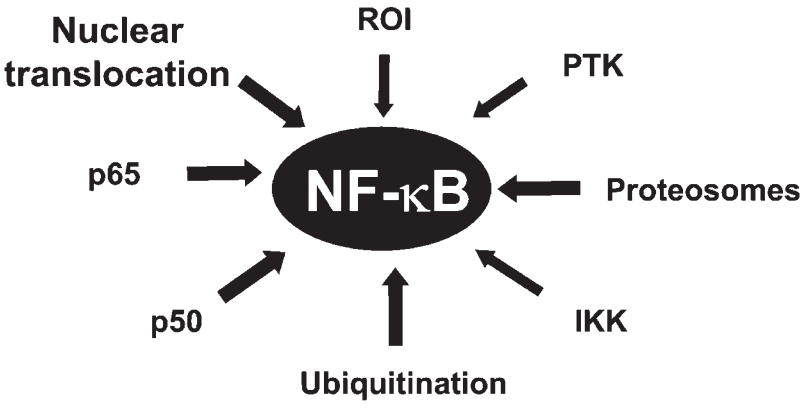

Figure 4 Potential target for inhibition in the pathway leading to NF-κB activation. These include the prevention of ROI formation, the inhibition of protein tyrosine kinases, proteosome inhibitors, the inhibition of IKK stimulation, the inhibition of ubiquitination of IκBα, the inhibition of direct activation via p50 and p65, and the blocking of nuclear translocation of the active complex to prevent DNA binding and transcription.

tors that block DNA binding of NF-κB either directly or indirectly include atrial natriuretic peptide[208] (ANP), IL-4,[209] metals such as chromium, cadmium, gold, mercury, and zinc,[210,211] ribavirin,[212] vascular endothelial growth factor[213,214] (VEGF), caffeine acid phenylether ester[215] (CAPE), and vasoactive intestinal peptide[216] (VIP).

Block proteasome from degrading IB inhibitory subunit

A more upstream strategy for blocking the activation of NF-κB is by affecting the inhibitory pathways in NF-κB activation. Proteasome inhibitors block the 26S proteasome necessary to degrade the IκB inhibitory subunit after its phosphorylation and ubiquitination in the cytoplasm and thus its release from the NF-κB complex.[217,218]. Examples of these include peptide aldehydes such as ALLnL, LLM, Z-LLnV, and Z-LLL,[111,119,220] lactacystine,[221] PS-341,[222] ubiquitin ligase inhibitors,[223] and cyclosporin A.[224] Other IκB degradation inhibitors, whose mechanisms are unknown, include capsaicin,[225] core protein hepatitis C virus,[226] fungal gliotoxin,[227] IL-13,[228] and pervanadate.[229]

Block phosphorylation of IκB

Because phosphorylation of IκBα is critical for NF-κB activation, compounds that block this phosphorylation prevent IκBα's ubiquitination and further degradation. Examples include aspirin,[230,231] (sodium salicylate), ibuprofen,[232] nitric oxide,[233–235] prostoglandin A1,[236] sanguinarine,[237] and YopJ (encoded by *Yersinia pseudotuberculosis*).[238] Recently, 4-hydroxy-2-nonenal, a lipid peroxidation product, has been shown to block phosphorylation by direct inhibition of IKK.[239] Also, a novel peptide that selectively blocks the association of IKK-γ (NEMO) with the rest of the IKK complex has been shown to inhibit NF-κB activation in response to pro-inflammatory cytokines in mice while preserving basal NF-κB activity.[240]

Up-regulate inhibitory subunit

Up-regulating the amount of IκBα is another strategy employed by various compounds to inhibit NF-κB activation. Examples include beta-amyloid (found in Alzheimer's),[145] glucocorticoids such as dexamethasone or prednisone,[241,242] IL-10,[243,244] and IL-13.[228]

Antioxidants

Various antioxidants have also been shown to inhibit NF-κB activation in response to a variety of stimulants (ie TNF-α, IL-1, phorbol ester, LPS, UV) through diverse and largely unknown mechanisms.[245–247] Examples include disulfiram,[248] curcumin,[249] glutathione,[215,250] and vitamin C.[251]

Block nuclear translocation

Another approach to inhibiting NF-κB activation is to use cell-permeable peptides that block the nuclear localization of the NF-κB complex. The mechanism works by mimicking the sequence of p50 responsible for transporting the NF-κB complex from the cytoplasm to the nucleus to block the normal import machinery.[252] Examples of these include SN-50[253] and o,o'-bismyristoyl thiamine disulfide.[254]

Suppression of NF-κB by gene transfer

Another strategy to block the activation of NF-κB is through the transfer of genes that code for proteins shown to suppress NF-κB activation. The most direct target is the IκBα gene. This entails the modification of IκBα at the specific phosphorylation sites (ser 32 and 36 switched with ala) and ubiquitination sites (lys 21 and 22 switched with arg) to prevent its degradation. This 'superrepressor' keeps the NF-κB complex in the cytoplasm indefinitely.[255–257] Recently, a nonphosphorylatable form of IκBα was shown to inhibit osteoclastogenesis and block bone resorption when injected into bone marrow macrophages.[258]

Another potential target for gene transfer has recently been shown to be HDAC3, a histone deacetylase that acts directly upon nuclear Rel A (part of the combined active p50/Rel A complex) enabling its association with IκBα and its subsequent export from the nucleus. Expression of HDAC3 in TNF-α-stimulated HeLa cells repressed both NF-κB DNA-binding and levels of Rel A with a corresponding increase in

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inactive cytoplasimic IκBα/NF-κB complexes.[259] This mechanism was shown to control the duration of NF-κB activation and thus may be a potential weapon against constitutive NF-κB activation.

Finally, the presence of pro-apoptotic cellular proteins have been shown to inhibit the anti-apoptotic function of NF-κB as described earlier, serving as potential targets for gene transfer. Erg-1, a transcription factor that is activated by similar stimuli as NF-κB, has recently been shown to block NF-κB activation both *in vitro* and *in vivo*. Erg-1 dimerizes with the p65 (Rel A) subunit of NF-κB via a specific zinc-finger domain and prevents NF-κB from binding to its promotor regions on DNA.[260,261] The RAI (Rel A-associated inhibitor) gene also encodes a protein that associates with p65 (Rel A) and inhibits the anti-apoptotic activity of NF-κB. RAI shares a homologous region with 53BP2, a protein involved in apoptosis regulation.[262] Par-4 (prostate apoptosis response-4) is another recently identified inhibitor of NF-κB at the level of IKK and activator of the Fas apoptotic pathway.[263,264] In addition, Par-4 inhibits Bcl-2, a well known anti-apoptotic oncogene.[265] E2F-1 is another transcription factor whose expression has been correlated with increased apoptotic activity via mechanisms including the inhibition of NF-κB. E2F-1 promotes cell cycle progression and its aberrant expression is present in most tumors.[266] Finally, p53 (the classic gatekeeper of cell cycle progression) and cyclin E-cdk2 may inhibit the anti-apoptotic action of NF-κB via the transcriptional co-activator protein CRB/p300.[267–270] Before NF-κB binds to its promotor regions on DNA, it is acetylated by CRB/p300 which maintains its presence in the nucleus. P53 and cyclin E-cdk2 compete for the finite CRB/p300 complexes and prevent its interaction with NF-κB. It should be noted, however, that the classic antagonism between the pro-apoptotic p53 and the anti-apoptotic NF-κB has been the subject of debate after p53 was found to activate NF-κB and correlate with the ability of p53 to induce apoptosis.[271]

Further genetic manipulation of proteins involved in the activation cascade

It has been possible to block the activation of NF-κB by manipulating the genes that encode proteins (eg TRAF2, TRAF6, I-TRAF, NIK, MEKK1, and IKK) found directly in the known activation pathways.[206] TRAF2 (TNF receptor-associated factor) and TRAF6 interact with TNF receptors and serve as adapters for the activation of NF-κB. Dominant negative mutants of TRAF2 and TRAF6 have been shown to repress NF-κB activity in response to TNF-α and IL-1, respectively.[272,273] I-TRAF interacts with TRAF2, and its overexpression inhibits TRAF2 activation of NF-κB.[274] NIK (NF-κB-inducing kinase) is induced by several proteins in the activation pathway, including TRAF-2 and TRAF-6, and activates IKKα (as does MEKK1). Therefore it has been shown that a dominant-negative mutant of NIK (and MEKK1) represses NF-κB activation.[41,275,276] The IKKs (IκB kinases, alpha and beta) phosphorylate IκBα to subsequently cause its ubiquitination and ultimate degradation. Thus, alterations in the ATP-binding site of the IKK complex (IKKβ more so than IKKα) or its activation loop have been shown to block the activation of NF-κB as well.[42–44,277–279]

Physiologic consequence of NF-κB inhibition and future direction

The inhibition of NF-κB with the methods illustrated above represents a theoretical approach to the more complicated issue of creating drug therapies that are effective in preventing or attenuating tumorigenesis. So far, several agents have been shown to utilize the modulation and/or inhibition of NF-κB to carry out some part of their therapeutic purpose such as glucocorticoids, nonsteroidal anti-inflammatory agents (NSAID), vitamin E, curcumin, thiols, cyclosporin, rifampicin, dithiocarbamates, methotrexate, thalidomide, leflunomide and various fungal and bacterial metabolites.[280] Could these agents and a number of others mediate an anti-carcinogenic role? It is too early to tell because our knowledge of their precise mechanisms of action, specificity, and even toxicity with respect to NF-κB is still incomplete. We do know, however, that many of these drugs influence the NF-κB pathway among others, and the interactions between these different pathways *in vivo* may play a role in their characteristic effects.

While we show that NF-κB is potentially capable of causing much distress, the elements in its pathways that we have targeted for inhibition above (eg ubiquitin ligase inhibitors, proteasome inhibitors, I-κBα super repressors, etc) may be valuable parts for other pathways that are required for the normal functioning of the body. For example, the turnover of the outer layers of skin requires the function of a specific ubiquitin ligase complex, SCFβ-TrCP, which is also part of the traditional activation cascade for NF-κB.[281] Ironically, the consequences of its inhibition include oncogenesis. Furthermore, the complete inhibition of NF-κB via genetic knockout may produce severe toxicity as evidenced from mice studies using dominant-negative Rel A among others[282] (see above 'Lessons to be learned from NF-κB knockouts'). These Rel A-deficient mice die during embryogenesis due to pathologic apoptosis of the liver. Other gene knockouts that involve the NF-κB complex and its inhibitors have shown us that its complete deficiency likely results in severe immunodeficiencies and accelerated apoptosis.

Thus it is clear that for effective drug development, targeting NF-κB must focus on its partial and specific inhibition with respect to toxicity. This level of specificity is achievable given our current progress, as in the example of glucocorticoids,[283] which target the inhibition of NF-κB in lymphoid cells making it the most effective, although not ideal anti-asthmatic therapy.

Conclusion

From this description, it is clear that during the last decade there have been major developments in our understanding of how NF-κB is activated and also how it may contribute to the development of cancer. Activation of NF-κB is emerging as one of the major mechanisms of tumor cell resistance to cytokines and chemotherapeutic agents. Until now most of our knowledge about NF-κB and its role in cellular physiology has been based on *in vitro* experimentation. Future *in vivo* studies may demonstrate the true importance of NF-κB and allow that knowledge to be used in clinical medicine. The development of specific inhibitors that can block NF-κB activation will have great potential in improving cancer therapy.

Acknowledgements

This research was supported by grants from The Clayton Foundation for Research, from National Institute of Health (P01 CA91844), and from Department of Defence to one of us (BBA). We would like to thank Walter Pagel for his critical comments.

References

1 Latchman DS. Cell stress genes and neuronal protection. *Neuropathol Appl Neurobiol* 1995; **21**: 475–477.
2 Mercurio F, Manning A. NF-kB as a primary regulator of the stress response. *Oncogene* 1999; **18**: 6163–6171.
3 Sen R, Baltimore D. Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. *Cell* 1986; **47**: 921–928.
4 Yamazaki S, Muta T, Takeshige K. A novel IκB protein, IκB-ζ, induced by proinflammatory stimuli, negatively regulates nuclear factor-κB in the nuclei. *J Biol Chem* 2001; **276**: 27657–27662.
5 Verma IM, Stevenson JK, Schwarz EM, Van Antwerp D, Miyamoto S. Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. *Genes Dev* 1995; **9**: 2723–2735.
6 Chen F, Ghosh G. Regulation of DNA binding by Rel/NF-kB transcription factors: structural views. *Oncogene* 1999; **18**: 6845–6852.
7 Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene* 1999; **18**: 6853–6866.
8 Li N, Karin M. Is NF-kappaB the sensor of oxidative stress?. *FASEB J* 1999; **13**: 1137–1143.
9 Busam K, Gieringer C, Freudenberg M, Hohmann HP. Staphylococcus aureus and derived exotoxins induce nuclear factor kappa B-like activity in murine bone marrow macrophages. *Infect Immunol* 1992; **60**: 2008–2015.
10 Bachelerie F, Alcami J, Arenzana-Seisdedos F, Virelizier JL. HIV enhancer activity perpetuated by NF-kappa B induction on infection of monocytes. *Nature* 1991; **350**: 709–712.
11 Pahl HL, Baeuerle PA. Expression of influenza virus hemagglutinin activates transcription factor NF-kappa B. *J Virol* 1994; **69**: 1480–1484.
12 Gabriel C, Justicia C, Camins A, Planas AM. Activation of nuclear factor-kappaB in the rat brain after transient focal ischemia. *Brain Res Mol Brain Res* 1999; **65**: 61–69.
13 Li C, Browder W, Kao RL. Early activation of transcription factor NF-kappaB during ischemia in perfused rat heart. *Am J Physiol* 1999; **276**: H543–552.
14 Stein B, Kramer M, Rahmsdorf HJ, Ponta H, Herrlich P. UV-induced transcription from the human immunodeficiency virus type 1 (HIV-1) long terminal repeat and UV-induced secretion of an extracellular factor that induces HIV-1 transcription in non-irradiated cells. *J Virol* 1989; **63**: 4540–4544.
15 Nishikawa M, Kakemizu N, Ito T, Kudo M, Kaneko T, Suzuki M, Udaka N, Ikeda H, Okubo T. Superoxide mediates cigarette smoke-induced infiltration of neutrophils into the airways through nuclear factor-kappaB activation and IL-8 mRNA expression in guinea pigs *in vivo*. *Am J Respir Cell Mol Biol* 1999; **20**: 189–198.
16 Hwang S, Ding A. Activation of NF-kappa B in murine macrophages by taxol. *Cancer Biochem Biophys* 1995; **14**: 265–272.
17 Post A, Holsboer F, Behl C. Induction of NF-kappaB activity during haloperidol-induced oxidative toxicity in clonal hippocampal cells: suppression of NF-kappaB and neuroprotection by antioxidants. *J Neurosci* 1989; **18**: 8236–8246.
18 Rensing-Ehl A, Hess S, Ziegler-Heitbrock HW, Riethmuller G, Engelmann H. Fas/Apo-1 activates nuclear factor kappa B and induces interleukin-6 production. *J Inflam* 1995; **45**: 161–174.
19 Bertrand F, Philippe C, Antoine PJ, Baud L, Groyer A, Capeau J, Cherqui G. Insulin activates nuclear factor kappa B in mammalian cells through a Raf-1-mediated pathway. *J Biol Chem* 1995; **270**: 24435–24441.
20 Li J, Brasier AR. Angiotensinogen gene activation by angiotensin II is mediated by the rel A (nuclear factor-kappaB p65) transcription factor: one mechanism for the renin angiotensin system positive feedback loop in hepatocytes. *Mol Endocrinol* 1996; **10**: 252–264.
21 Smith CS, Shearer WT. Activation of NF-kappa B and immunoglobulin expression in response to platelet-activating factor in a human B cell line. *Cell Immunol* 1994; **155**: 292–303.
22 Mutoh H, Ishii S, Izumi T, Kato S, Shimizu T. Platelet-activating factor (PAF) positively auto-regulates the expression of human PAF receptor transcript 1 (leukocyte-type) through NF-kappa B. *Biochem Biophys Res Commun* 1994; **205**: 1137–1142.
23 Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO J* 1991; **10**: 2247–2258.
24 Canty TG Jr, Boyle EM Jr, Farr A, Morgan EN, Verrier ED, Pohlman TH. Oxidative stress induces NF-kappaB nuclear translocation without degradation of IkappaBalpha. *Circulation* 1999; **100** (19 Suppl.): II361–II364.
25 Raju U, Gumin GJ, Noel F, Tofilon PJ. IkappaBalpha degradation is not a requirement for the X-ray-induced activation of nuclear factor kappaB in normal rat astrocytes and human brain tumour cells. *Int J Rad Biol* 1998; **74**: 617–624.
26 Singh S, Darnay BG, Aggarwal BB. Site-specific tyrosine phosphorylation of IκBα negatively regulates its inducible phosphorylation and degradation. *J Biol Chem* 1996; **271**: 31049–31054.
27 Imbert V, Rupec RA, Livolsi A, Pahl HL, Traenckner EB, Mueller-Dieckmann C, Farahifar D, Rossi B, Auberger P, Baeuerle PA, Peyron JF. Tyrosine phosphorylation of I kappa B-alpha activates NF-kappa B without proteolytic degradation of I kappa B-alpha. *Cell* 1996; **86**: 787–798.
28 Digicaylioglu M, Lipton SA. Erythropoietin-mediated neuroprotection involves cross-talk between JAK2 and NF-kB signalling cascades. *Nature* 2001; **412**: 641–647.
29 Karin M. How NF-kappaB is activated: the role of the IkappaB kinase (IKK) complex. *Oncogene* 1999; **18**: 6867–6874.
30 Li Q, Van Antwerp D, Mercurio F, Lee KF, Verma IM. Severe liver degeneration in mice lacking the IkappaB kinase 2 gene. *Science* 1999; **284**: 321–325.
31 Senftleben U, Li ZW, Baud V, Karin M. IKKbeta is essential for protecting T cells from TNFalpha-induced apoptosis. *Immunity* 2001; **14**: 217–230.
32 Senftleben U, Cao Y, Xiao G, Greten FR, Krahn G, Bonizzi G, Chen Y, Hu Y, Fong A, Sun SC, Karin M. Activation by IKKalpha of a second, evolutionary conserved, NF-kappa B signaling pathway. *Science* 2001; **293**: 1495–1499.
33 Hu Y, Baud V, Delhase M, Zhang P, Deerinck T, Ellisman M, Johnson R, Karin M. Abnormal morphogenesis but intact IKK activation in mice lacking the IKKalpha subunit of IkappaB kinase. *Science* 1999; **284**: 316–320.
34 Takeda K, Takeuchi O, Tsujimura T, Itami S, Adachi O, Kawai T, Sanjo H, Yoshikawa K, Terada N, Akira S. Limb and skin abnormalities in mice lacking IKKalpha. *Science* 1999; **284**: 313–316.
35 Li J, Peet GW, Balzarano D, Li X, Massa P, Barton RW, Marcu KB. Novel NEMO/IkappaB kinase and NF-kappa B target genes at the pre-B to immature B cell transition. *J Biol Chem* 2001; **276**: 18579–18590.
36 Rothwarf DM, Zandi E, Natoli G, Karin M. IKK-gamma is an essential regulatory subunit of the IkappaB kinase complex. *Nature* 1998; **395**: 297–300.
37 Schmidt-Supprian M, Bloch W, Courtois G, Addicks K, Israel A, Rajewsky K, Pasparakis M. NEMO/IKK gamma-deficient mice model incontinentia pigmenti. *Mol Cell* 2000; **5**: 981–992.
38 Shimada T, Kawai T, Takeda K, Matsumoto M, Inoue J, Tatsumi Y, Kanamaru A, Akira S. IKK-i, a novel lipopolysaccharide-inducible kinase that is related to IkappaB kinases. *Int Immunol* 1999; **11**: 1357–1362.
39 Peters RT, Liao SM, Maniatis T. IKKepsilon is part of a novel PMA-inducible IkappaB kinase complex. *Mol Cell* 2000; **5**: 513–522.
40 Nomura F, Kawai T, Nakanishi K, Akira S. NF-kappaB activation through IKK-i-dependent I-TRAF/TANK phosphorylation. *Genes Cells* 2000; **5**: 191–202.
41 Malinin NL, Boldin MP, Kovalenko AV, Wallach D. MAP3K-related kinase involved in NF-kappaB induction by TNF, CD95 and IL-1. *Nature* 1997; **385**: 540–544.
42 DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E, Karin M. A cytokine-responsive IkappaB kinase that activates the transcription factor NF-kappaB. *Nature* 1997; **388**: 548–557.
43 Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, Li J, Young DB, Barbosa M, Mann M, Manning A, Rao A, IKK-1 and IKK-2: cytokine-activated IkappaB kinases essential for NF-kappaB activation. *Science* 1997; **278**: 860–866.
44 Zandi E, Rothwarf DM, Delhase M, Hayakawa M, Karin M. The IkappaB kinase complex (IKK) contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. *Cell* 1997; **91**: 243–252.

SPOTLIGHT

45 Zandi E, Chen Y, Karin M. Direct phosphorylation of IkappaB by IKKalpha and IKKbeta: discrimination between free and NF-kappaB-bound substrate. *Science* 1998; **281**: 1360–1363.
46 Sanz L, Sanchez P, Lallena MJ, Diaz-Meco MT, Moscat J. The interaction of p62 with RIP links the atypical PKCs to NF-kappaB activation. *EMBO J* 1999; **18**: 3044–3053.
47 Diaz-Meco MT, Berra E, Municio MM, Sanz L, Lozano J, Dominguez I, Diaz-Golpe V, Lain de Lera MT, Alcami J, Paya CV. A dominant negative protein kinase C zeta subspecies blocks NF-kappaB activation. *Mol Cell Biol* 1993; **13**: 4770–4775.
48 Chen CC, Sun YT, Chen JJ, Chiu KT. TNF-alpha-induced cyclooxygenase-2 expression in human lung epithelial cells: involvement of the phospholipase C-gamma 2, protein kinase C-alpha, tyrosine kinase, NF-kappa B-inducing kinase, and I-kappa B kinase 1/2 pathway. *J Immunol* 2000; **165**: 2719–2728.
49 Vertegaal AC, Kuiperij HB, Yamaoka S, Courois G, van der Eb AJ, Zantema A. Protein kinase C-alpha is an upstream activator of the IkappaB kinase complex in the TPA signal transduction pathway to NF-kappaB in U20S cells. *Cell Signal* 2000; **12**: 759–768.
50 McAllister-Lucas LM, Inohara N, Lucas PC, Ruland J, Benito A, Li Q, Chen S, Chen FF, Yamaoka S, Verma IM, Mak TW, Nunez G. Bimp1, a MAGUK family member linking protein kinase C activation to Bc110-mediated NF-kappaB induction. *J Biol Chem* 2001: **276**: 30589–30597.
51 Leitges M, Schmedt C, Guinamard R, Davoust J, Schaal S, Stabel S, Tarakhovsky A. Immunodeficiency in protein kinase cbeta-deficient mice. *Science* 1996; **273**: 788–791.
52 Lin X, O'Mahony A, Mu Y, Geleziunas R, Greene WC. Protein kinase C-theta participates in NK-kappaB activation induced by CD3-CD28 costimulation through selective activation of IkappaB kinase beta. *Mol Cell Biol* 2000; **20**: 2933–2940.
53 Bauer B, Krumbock N, Fresser F, Hochholdinger F, Spitaler M, Simm A, Uberall F, Schraven B, Baier G. Complex formation and cooperation of protein kinase C theta and Akt1/protein kinase B alpha in the NF-kappa B transactivation cascade in Jurkat T cells. *J Biol Chem* 2001; **276**: 31627–31634.
54 Vancurova I, Miskolci V, Davidson D. NF-kappa B activation in tumor necrosis factor alpha-stimulated neutrophils is mediated by protein kinase Cdelta. Correlation to nuclear Ikappa Balpha. *J Biol Chem* 2001; **276**: 19746–19752.
55 Diaz-Guerra MJ, Bodelon OG, Velasco M, Whelan R, Parker PJ, Bosca L. Up-regulation of protein kinase C-epsilon promotes the expression of cytokine-inducible nitric oxide synthase in RAW 264.7 cells. *J Biol Chem* 1996; **271**: 32028–32033.
56 Li JD, Feng W, Gallup M, Kim JH, Gum J, Kim Y, Basbaum C. Activation of NF-kappaB via a Src-dependent Ras-MAPK-pp90rsk pathway is required for Pseudomonas aeruginosa-induced mucin overproduction in epithelial cells. *Proc Natl Acad Sci USA* 1998; **95**: 5718–5723.
57 Kumar A, Haque J, Lacoste J, Hiscott J, Williams BR. Double-stranded RNA-dependent protein kinase activates transcription factor NF-kappa B by phosphorylating I kappa B. *Proc Natl Acad Sci USA* 1994; **91**: 6288–6292.
58 Belich MP, Salmeron A, Johnston LH, Ley SC. TPL-2 kinase regulates the proteolysis of the NF-kappaB-inhibitory protein NF-kappaB1 p105. *Nature* 1999; **397**: 363–368.
59 Zhao Q, Lee FS. Mitogen-activated protein kinase/ERK kinase kinases 2 and 3 activate nuclear factor-kappaB through IkappaB kinase-alpha and IkappaB kinase-beta. *J Biol Chem* 1999; **274**: 8355–8358.
60 Yang J, Lin Y, Guo Z, Cheng J, Huang J, Deng L, Liao W, Chen Z, Liu Z, Su B. The essential role of MEKK3 in TNF-induced NF-kappaB activation. *Nat Immunol* 2001; **7**: 620–624.
61 Tojima Y, Fujimoto A, Delhase M, Chen Y, Hatakeyama S, Nakayama K, Kaneko Y, Nimura Y, Motoyama N, Ikeda K, Karin M, Nakanishi M. NAK is an IkappaB kinase-activating kinase. *Nature* 2000; **404**: 778–782.
62 Pomerantz JL, Baltimore D. NF-kappaB activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase. *EMBO J* 1999; **18**: 6694–6704.
63 Bonnard M, Mirtsos C, Suzuki S, Graham K, Huang J, Ng M, Itie A, Wakeham A, Shahinian A, Henzel WJ, Elia AJ, Shillinglaw W, Mak TW, Cao Z, Yeh WC. Deficiency of T2K leads to apoptotic liver degeneration and impaired NF-kappaB-dependent gene transcription. *EMBO J* 2000; **19**: 4976–4985.
64 Reddy SA, Huang JH, Liao WS. Phosphatidylinositol 3-kinase in interleukin 1 signaling. Physical interaction with the interleukin 1 receptor and requirement in NFkappaB and AP-1 activation. *J Biol Chem* 1997; **272**: 29167–29173.
65 Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. *Nature* 1999; **401**: 82–85.
66 Hehner SP, Hofmann TG, Ushmorov A, Dienz O, Wing-Lan Leung I, Lassam N, Scheidereit C, Droge W, Schmitz ML. Mixed-lineage kinase 3 delivers CD3/CD28-derived signals into the IkappaB kinase complex. *Mol Cell Biol* 2000; **20**: 2556–2568.
67 Hu MC, Wang Yp, Qiu WR, Mikhail A, Meyer CF, Tan TH. Hematopoietic progenitor kinase-1 (HPK1) stress response signaling pathway activates IkappaB kinases (IKK-alpha/beta) and IKK-beta is a developmentally regulated protein kinase. *Oncogene* 1999; **18**: 5514–5524.
68 Wang W, Zhou G, Hu MC, Yao Z, Tan TH. Activation of the hematopoietic progenitor kinase-1 (HPK1)-dependent, stress-activated c-Jun N-terminal kinase (JNK) pathway by transforming growth factor beta (TGF-beta)-activated kinase (TAK1), a kinase mediator of TGF beta signal transduction. *J Biol Chem* 1997; **272**: 22771–22775.
68 Wang W, Zhou G, Hu MC, Yao Z, Tan TH. Activation of the hematopoietic progenitor kinase-1 (HPK1)-dependent, stress-activated c-Jun N-terminal kinase (JNK) pathway by transforming growth factor beta (TGF-beta)-activated kinase (TAK1), a kinase mediator of TGF beta signal transduction. *J Biol Chem* 1997; **272**: 22771–22775.
70 Finco TS, Baldwin AS Jr. Kappa B site-dependent induction of gene expression by diverse inducers of nuclear factor kappa B requires Raf-1. *J Biol Chem* 1993; **268**: 17676–17679.
71 Troppmair J, Hartkamp J, Rapp UR. Activation of NF-kappa B by oncogenic Raf in HEK 293 cells occurs through autocrine recruitment of the stress kinase cascade. *Oncogene* 1998; **17**: 685–690.
72 Frost JA, Swantek JL, Stippec S, Yin MJ, Gaynor R, Cobb MH. Stimulation of NFkappa B activity by multiple signaling pathways requires PAK1. *J Biol Chem* 2000; **275**: 19693–19699.
73 Petro JB, Rahman SM, Ballard DW, Khan WN. Bruton's tyrosine kinase is required for activation of IkappaB kinase and nuclear factor kappaB in response to B cell receptor engagement. *J Exp Med* 2000; **191**: 1745–1754.
74 Zhong H, Voll RE, Ghosh S. Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. *Mol Cell* 1998; **1**: 661–671.
75 Zhong H, Su Yang H, Erdjument-Bromage H, Tempst P, Ghosh S. The transcriptional activity of NF-kappaB is regulated by the IkappaB-associated PKAc subunit through a cyclic AMP-independent mechanism. *Cell* 1997; **89**: 413–424.
76 Li YL, Guo FK, Wu SG. Effects of antisense IRAK-2 oligonucleotides on PGI2 release induced by IL-1 and TNF. *Acta Pharmacol Sin* 2000; **21**: 646–648.
77 Jefferies C, Bowie A, Brady G, Cooke EL, Li X, O'Neill LA. Transactivation by the p65 subunit of NF-kappaB in response to interleukin-1 (IL-1) involves MyD88, IL-1 receptor-associated kinase 1, TRAF-6, and Rac1. *Mol Cell Biol* 2001; **21**: 4544–4552.
78 Wesche H, Gao X, Li X, Kirschning CJ, Stark GR, Cao Z. IRAK-M is a novel member of the Pelle/interleukin-1 receptor-associated kinase (IRAK) family. *J Biol Chem* 1999; **274**: 19403–19410.
79 Manna SK, Sah NK; Aggarwal BB. Protein tyrosine kinase p561ck is required for ceramide-induced by not TNF-induced cellular responses: effect on activation of NF-kB, AP-1, JNK and apoptosis. *J Biol Chem* 2000; **275**: 13297–13306.
80 Manna SK, Aggarwal BB. Differential requirement for p561ck in HIV-TAT vs. TNF-induced cellular responses: effects on NF-kB, AP-1, JNK and apoptosis. *J Immunol* 2000; **164**: 5166.
81 Chen F, Demers LM, Vallyathan V, Ding M, Lu Y, Castranova V, Shi X. Vanadate induction of NF-kappaB involves IkappaB kinase beta and SAPK/ERK kinase 1 in macrophages. *J Biol Chem* 1999; **274**: 20307–20312.
82 Sha WC, Liou HC, Tuomanen EI, Baltimore D. Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses. *Cell* 1995; **80**: 321–330.

83 Grumont RJ, Rourke IJ, O'Reilly LA, Strasser A, Miyake K, Sha W, Gerondakis S. B lymphocytes differentially use the Rel and nuclear factor kappaB1 (NF-kappaB1) transcription factors to regulate cell cycle progression and apoptosis in quiescent and mitogen-activated cells. *J Exp Med* 1998; **187**: 663–674.
84 Snapper CM, Zelazowski P, Rosas FR, Kehry MR, Tian M. Baltimore D, Sha WC. B cells from p50/NF-kappa B knockout mice have selective defects in proliferation, differentiation, germ-line CH transcription, and Ig class switching. *J Immunol* 1996; **156**: 183–191.
85 Beg AA, Baltimore D. An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science* 1996; **274**: 782–784.
86 Doi TS, Takahashi T, Taguchi O, Azuma T, Obata Y. NF-kappa B RelA-deficient lymphocytes: normal development of T cells and B cells, impaired production of IgA and IgG1 and reduced proliferative responses. *J Exp Med* 1997; **185**: 953–961.
87 Kontgen F, Grumont RJ, Strasser A, Metcalf D, Li R, Tarlinton D, Gerondakis S. Mice lacking the c-rel proto-oncogene exhibit defects in lymphocyte proliferation, humoral immunity, and interleukin-2 expression. *Genes Dev* 1995; **9**: 1965–1977.
88 Grumont RJ, Rourke IJ, Gerondakis S, Rel-dependent induction of A1 transcription is required to protect B cells from antigen receptor ligation-induced apoptosis. *Genes Dev* 1999; **13**: 400–411.
89 Gerondakis S, Grumont R, Rourke I, Grossmann M. The regulation and roles of Rel/NF-kappa B transcription factors during lymphocyte activation. *Curr Opin Immunol* 1998; **10**: 353–359.
90 Gerondakis S, Strasser A, Metcalf D, Grigoriadis G, Scheerlinck JY, Grumont RJ. Rel-deficient T cells exhibit defects in production of interleukin 3 and granulocyte-macrophage colony-stimulating factor. *Proc Natl Acad Sci USA* 1996; **93**: 3405–3409.
91 Grigoriadis G, Zhan Y, Grumont RJ, Metcalf D, Handman E, Cheers C, Gerondakis S. The Rel subunit of NF-kappaB-like transcription factors is a positive and negative regulator of macrophage gene expression: distinct roles for Rel in different macrophage populations. *EMBO J* 1996; **15**: 7099–7107.
92 Gilmore TD, Cormier C, Jean-Jacques J, Gapuzan ME. Malignant transformation of primary chicken spleen cells by human transcription factor c-Rel. *Oncogene* 2001; **20**: 7098–7103.
93 Caamano JH, Rizzo CA, Durham SK, Barton DS, Raventos-Suarez C, Snapper CM, Bravo R. Nuclear factor (NF)-kappa B2 (p100/p52) is required for normal splenic microarchitecture and B cell-mediated immune responses. *J Exp Med* 1998; **187**: 185–196.
94 Franzoso G, Carlson L, Poljak L, Shores EW, Epstein S, Leonardi A, Grinberg A, Tran T, Scharton-Kersten T, Anver M, Love P, Brown K, Siebenlist U. Mice deficient in nuclear factor (NF)-kappa B/p52 present with defects in humoral responses, germinal center reactions, and splenic microarchitecture. *J Exp Med* 1998; **187**: 147–159.
95 Gerondakis S, Grossmann M, Nakamura Y, Pohl T, Grumont R. Genetic approaches in mice to understand Rel/NF-kappaB and IkappaB function: transgenics and knockouts. *Oncogene* 1999; **18**: 6888–6895.
96 Beg AA, Sha WC, Bronson RT, Baltimore D. Constitutive NF-kappa B activation, enhanced granulopoiesis, and neonatal lethality in I kappa B alpha-deficient mice. *Genes Dev* 1995; **9**: 2736–2746.
97 Klement JF, Rice NR, Car BD, Abbondanzo SJ, Powers GD, Bhatt PH, Chen CH, Rosen CA, Stewart CL. IkappaBalpha deficiency results in a sustained NF-kappaB response and severe widespread dermatitis in mice. *Mol Cell Biol* 1996; **16**: 2341–2349.
98 Li ZW, Chu W, Hu Y, Delhase M, Deerinck T, Ellisman M, Johnson R, Karin M. The IKKbeta subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. *J Exp Med* 1999; **189**: 1839–1845.
99 Tanaka M, Fuentes ME, Yamaguchi K, Durnin MH, Dalrymple SA, Hardy KL, Goeddel DV. Embryonic lethality, liver degeneration, and impaired NF-kappa B activation in IKK-beta-deficient mice. *Immunity* 1999; **10**: 421–429.
100 Sharma HW, Narayanan R. The NF-kappaB transcription factor in oncogenesis. *Anticancer Res* 1996; **16**: 589–596.
101 Waddick KG, Uckun FM. Innovative treatment programs against cancer: II. Nuclear factor-kappaB (NF-kappaB) as a molecular target. *Biochem Pharmacol* 1999; **57**: 9–17.
102 Dong G, Chen Z, Kato T, Van Waes C. The host environment promotes the constitutive activation of nuclear factor-kappaB and proinflammatory cytokine expression during metastatic tumor progression of murine squamous cell carcinoma. *Cancer Res* 1999; **59**: 3495–3504.
103 Wang TH, Wang HS. p53, apoptosis and human cancers. *J Formos Med Assoc* 1996; **95**: 509–521.
104 Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM. Suppression of TNF-alpha-induced apoptosis by NF-kappaB. *Science* 1996; **274**: 787–789.
105 Giri DK, Aggarwal BB. Constitutive activation of NF-kappaB causes resistance to apoptosis in human cutaneous T cell lymphoma HuT-78 cells. Autocrine role of tumor necrosis factor and reactive oxygen intermediates. *J Biol Chem* 1998; **273**: 14008–14014.
106 Nakshatri H, Bhat-Nakshatri P, Martin DA, Goulet RJ Jr, Sledge GW Jr. Constitutive activation of NF-kappaB during progression of breast cancer to hormone-independent growth. *Mol Cell Biol* 1997; **17**: 3629–3639.
107 Wang CY, Cusack JC Jr, Liu R, Baldwin AS Jr. Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-kappaB. *Nat Med* 1999; **5**: 412–417.
108 Wang CY, Guttridge DC, Mayo MW, Baldwin AS Jr. NF-kappaB induces expression of the Bcl-2 homologue A1/Bfl-1 to preferentially suppress chemotherapy-induced apoptosis. *Mol Cell Biol* 1999; **19**: 5923–5929.
109 Kalgutkar AS, Zhao Z. Discovery and design of selective cyclooxygenase-2 inhibitors as non-ulcerogenic, anti-inflammatory drugs with potential utility as anti-cancer agents. *Curr Drug Targets* 2001; **2**: 79–106.
110 Marrogi A, Pass HI, Khan M, Metheny-Barlow LJ, Harris CC, Gerwin BI. Human mesothelioma samples overexpress both cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (NOS2: *in vitro* antiproliferative effects of a COX-2 inhibitor. *Cancer Res* 2000; **60**: 3696–3700.
111 Toyota M, Shen L, Ohe-Toyota M, Hamilton SR, Sinicrope FA, Issa JP. Aberrant methylation of the cyclooxygenase 2 CpG island in colorectal tumors. *Cancer Res* 2000; **60**: 4044–4048.
112 Noguchi Y, Makino T, Yoshikawa T, Nomura K, Fukuzawa K, Matsumoto A, Yamada T. The possible role of TNF-alpha and IL-2 in inducing tumor-associated metabolic alterations. *Surg Today* 1996; **26**: 36–41.
113 Tomimatsu S, Ichikura T, Mochizuki H. Significant correlation between expression of interleukin-1alpha and liver metastasis in gastric carcinoma. *Cancer* 2001; **91**: 1272–1276.
114 Klotz T, Bloch W, Jacobs G, Niggemann S, Engelmann U, Addicks K. Immunolocalization of inducible and constitutive nitric oxide synthases in human bladder cancer. *Urology* 1999; **54**: 416–419.
115 Dong Z. Nemeth JA. Cher ML. Palmer KC. Bright RC. Fridman R. Differential regulation of matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 (TIMP-1) and TIMP-2 expression in co-cultures of prostate cancer and stromal cells. *Int J Cancer* 2001; **93**: 507–515.
116 Pacheco MM, Nishimoto IN, Mourao Neto M, Mantovani EB, Brentani MM. Prognostic significance of the combined expression of matrix metalloproteinase-9, urokinase type plasminogen activator and its receptor in breast cancer as measured by Northern blot analysis. *Int J Biol Markers* 2001; **16**: 62–68.
117 Strieter RM. Chemokines: not just leukocyte chemoattractants in the promotion of cancer. *Nat Immunol* 2001; **2**: 285–286.
118 Scotton CJ, Wilson JL, Milliken D, Stamp G, Balkwill FR. Epithelial cancer cell migration: a role for chemokine receptors? *Cancer Res* 2001; **61**: 4961–4965.
119 Palmer K, Hitt M, Emtage PC, Gyorffy S, Gauldie J. Combined CXC chemokine and interleukin-12 gene transfer enhances antitumor immunity. *Gene Ther* 2001; **8**: 282–290.
120 Loch T, Michalski B, Mazurek U, Graniczka M. Vascular endothelial growth factor (VEGF) and its role in neoplastic processes. *Postepy Hig Med Dosw* 2001; **55**: 257–274.
121 Ueno T, Toi M, Saji H, Muta M, Bando H, Kuroi K, Koike M, Inadera H, Matsushima K. Significance of macrophage chemoat-

tractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer. *Clin Cancer Res* 2000; **6**: 3282–3289.
122 Oyama T, Sakuta T, Matsushita K, Maruyama I, Nagaoka S, Torii M. Effects of roxithromycin on tumor necrosis factor-alpha-induced vascular endothelial growth factor expression in human periodontal ligament cells in culture. *J Periodontol* 2000; **71**: 1546–1553.
123 Chilov D, Kukk E, Taira S, Jeltsch M, Kaukonen J, Palotie A, Joukov V, Alitalo K. Genomic organization of human and mouse genes for vascular endothelial growth factor C. *J Biol Chem* 1997; **272**: 25176–25183.
124 Shakhov AN, Collart MA, Vassalli P, Nedospasov SA, Jongeneel CV. Kappa B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor alpha gene in primary macrophages. *J Exp Med* 1990; **171**: 35–47.
125 Collart MA, Baeuerle P, Vassalli P. Regulation of tumor necrosis factor alpha transcription in macrophages: involvement of four kappa B-like motifs and of constitutive and inducible forms of NF-kappa B. *Mol Cell Biol* 1990; **10**: 1498–1506.
126 Ueda A, Okuda K, Ohno S, Shirai A, Igarashi T, Matsunaga K, Fukushima J, Kawamoto S, Ishigatsubo Y, Okubo T. NF-kappa B and Sp1 regulate transcription of the human monocyte chemoattractant protein-1 gene. *J Immunol* 1994; **153**: 2052–2063.
127 van de Stolpe A, Caldenhoven E, Stade BG, Koenderman L, Raaijmakers JA, Johnson JP, van der Saag PT. 12-O-tetradecanoylphorbol-13-acetate- and tumor necrosis factor alpha-mediated induction of intercellular adhesion molecule-1 is inhibited by dexamethasone. Functional analysis of the human intercellular adhesion molecular-1 promoter. *J Biol Chem* 1994; **269**: 6185–6192.
128 Whelan J, Ghersa P, Hooft van Huijsduijnen R, Gray J, Chandra G, Talabot F, DeLamarter JF. An NF kappa B-like factor is essential but not sufficient for cytokine induction of endothelial leukocyte adhesion molecule 1 (ELAM-1) gene transcription. *Nucleic Acids Res* 1991; **19**: 2645–2653.
129 Iademarco MF, McQuillan JJ, Rosen GD, Dean DC. Characterization of the promoter for vascular cell adhesion molecule-1 (VCAM-1). *J Biol Chem* 1992; **267**: 16323–16329.
130 Koong AC, Chen EY, Giaccia AJ. Hypoxia causes the activation of nuclear factor kappa B through the phosphorylation of I kappa B alpha on tyrosine residues. *Cancer Res* 1994; **54**: 1425–1430.
131 Folkman J. Diagnostic and therapeutic applications of angiogenesis research. *C R Acad Sci III* 1993; **316**: 909–918.
132 Beg AA, Baltimore D .An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science* 1996; **274**: 782–784.
133 Wang CY, Mayo MW, Baldwin AS Jr. TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappaB. *Science* 1996; **274**: 784–787.
134 Jimi E, Nakamura I, Ikebe T, Akiyama S, Takahashi N, Suda T. Activation of NF-kappaB is involved in the survival of osteoclasts promoted by interleukin-1. *J Biol Chem* 1998; **273**: 8799–8805.
135 Bakker TR, Reed D, Renno T, Jongeneel CV. Efficient adenoviral transfer of NF-kappaB inhibitor sensitizes melanoma to tumor necrosis factor-mediated apoptosis. *Int J Cancer* 1999; **80**: 320–323.
136 McDade TP, Perugini RA, Vittimberga FJ Jr, Callery MP. Ubiquitin-proteasome inhibition enhances apoptosis of human pancreatic cancer cells. *Surgery* 1999; **126**: 371–377.
137 Sumitomo M, Tachibana M, Ozu C, Asakura H, Murai M, Hayakawa M, Nakamura H, Takayanagi A, Shimizu N, Induction of apoptosis of cytokine-producing bladder cancer cells by adenovirus-mediated IkappaBalpha overexpression. *Hum Gene Ther* 1999; **10**: 37–47.
138 Sovak MA, Bellas RE, Kim DW, Zanieski GJ, Rogers AE, Traish AM, Sonenshein GE. Aberrant nuclear factor-kappaB/Rel expression and the pathogenesis of breast cancer. *J Clin Invest* 1997; **100**: 2952–2960.
139 Arsura M, Wu M, Sonenshein GE. TGF beta 1 inhibits NF-kappa B/Rel activity inducing apoptosis of B cells: transcriptional activation of I kappa B alpha. *Immunity* 1996; **5**: 31–40.
140 Lee H, Wu M, La Rosa FA, Duyao MP, Buckler AJ, Sonenshein GE. Role of the Rel-family of transcription factors in the regulation of c-myc gene transcription and apoptosis of WEHI 231 murine B-cells. *Curr Top Microbiol Immunol* 1995; **194**: 247–255.
141 Boothby MR, Mora AL, Scherer DC, Brockman JA, Ballard DW. Perturbation of the T lymphocyte lineage in transgenic mice expressing a constitutive repressor of nuclear factor (NF)-kappaB. *J Exp Med* 1997; **185**: 1897–1907.
142 Esslinger CW, Jongeneel CV, MacDonald HR. Survival-independent function of NF-kappaB/Rel during late stages of thymocyte differentiation. *Mol Immunol* 1998; **35**: 847–852.
143 Ward C, Chilvers ER, Lawson MF, Pryde JG, Fujihara S, Farrow SN, Haslett C, Rossi AG. NF-kappaB activation is a critical regulator of human granulocyte apoptosis *in vitro*. *J Biol Chem* 1999; **274**: 4309–4318.
144 von Knethen A, Callsen D, Brune B. Superoxide attenuates macrophage apoptosis by NF-kappa B and AP-1 activation that promotes cyclooxygenase-2 expression. *J Immunol* 1999; **163**: 2858–2866.
145 Bales KR, Du Y, Dodel RC, Yan GM, Hamilton-Byrd E, Paul SM. The NF-kappaB/Rel family of proteins mediates Abeta-induced neurotoxicity and glial activation. *Brain Res Mol Brain Res* 1998; **57**: 63–72.
146 Maggirwar SB, Sarmiere PD, Dewhurst S, Freeman RS. Nerve growth factor-dependent activation of NF-kappaB contributes to survival of sympathetic neurons. *J Neurosci* 1998; **18**: 10356–10365.
147 Erl W, Hansson GK, de Martin R, Draude G, Weber KS, Weber C. Nuclear factor-kappa B regulates induction of apoptosis and inhibitor of apoptosis protein-1 expression in vascular smooth muscle cells. *Circ Res* 1999; **84**: 668–677.
148 Abbadie C, Kabrun N, Bouali F, Smardova J, Stehelin D, Vandenbunder B, Enrietto PJ. High levels of c-rel expression are associated with programmed cell death in the developing avian embryo and in bone marrow cells *in vitro*. *Cell* 1993; **75**: 899–912.
149 Dumont A, Hehner SP, Hofmann TG, Ueffing M, Droge W, Schmitz ML. Hydrogen peroxide-induced apoptosis is CD95-independent, requires the release of mitochondria-derived reactive oxygen species and the activation of NF-kappaB. *Oncogene* 1999; **18**: 747–757.
150 Kasibhatla S, Brunner T, Genestier L, Echeverri F, Mahboubi A, Green DR. DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kappa B and AP-1. *Mol Cell* 1998; **1**: 543–551.
151 Qin ZH, Chen RW, Wang Y, Nakai M, Chuang DM, Chase TN. Nuclear factor kappaB nuclear translocation upregulates c-Myc and p53 expression during NMDA receptor-mediated apoptosis in rat striatum. *J Neurosci* 1999; **19**: 4023–4033.
152 Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, Schwaninger M. NF-kappaB is activated and promotes cell death in focal cerebral ischemia. *Nat Med* 1999; **5**: 554–559.
153 DeMeester SL, Qiu Y, Buchman TG, Hotchkiss RS, Dunnigan K, Karl IE, Cobb JP. Nitric oxide inhibits stress-induced endothelial cell apoptosis. *Crit Care Med* 1998; **26**: 1500–1509.
154 Chan H, Bartos DP, Owen-Schaub LB. Activation-dependent transcriptional regulation of the human Fas promoter requires NF-kappaB p50-p65 recruitment. *Mol Cell Biol* 1999; **19**: 2098–2108.
155 Qin JZ, Chaturvedi V, Denning MF, Choubey D, Diaz MO, Nickoloff BJ. Role of NF-kappaB in the apoptotic-resistant phenotype of keratinocytes. *J Biol Chem* 1999; **274**: 37957–37964.
156 Stehlik C, de Martin R, Binder BR, Lipp J. Cytokine induced expression of porcine inhibitor of apoptosis protein (iap) family member is regulated by NF-kappa B. *Biochem Biophys Res Commun* 1998; **243**: 827–832.
157 Guttridge DC, Albanese C, Reuther JY, Pestell RG, Baldwin AS Jr. NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol Cell Biol* 1999; **19**: 5785–5799.
158 Hinz M, Krappmann D, Eichten A, Heder A, Scheidereit C, Strauss M. NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. *Mol Cell Biol* 1999; **19**: 2690–2698.
159 Bash J, Zong WX, Gelinas C. c-Rel arrests the proliferation of HeLa cells and affects critical regulators of the G1/S-phase transition. *Mol Cell Biol* 1997; **17**: 6526–6536.

160 Ravi R, Mookerjee B, van Hensbergen Y, Bedi GC, Giordano A, El-Deiry WS, Fuchs EJ, Bedi A. p53-mediated repression of nuclear factor-kappaB RelA via the transcriptional integrator p300. *Cancer Res* 1998; **58**: 4531–4536.
161 Yang JP, Hori M, Takahashi N, Kawabe T, Kato H, Okamoto T. NF-kappaB subunit p65 binds to 53BP2 and inhibits cell death induced by 53BP2. *Oncogene* 1999; **18**: 5177–5186.
162 Miyamoto S, Chiao PJ, Verma IM. Enhanced I kappa B alpha degradation is responsible for constitutive NF-kappa B activity in mature murine B-cell lines. *Mol Cell Biol* 1994; **14**: 3276–3282.
163 Wu M, Lee H, Bellas RE, Schauer SL, Arsura M, Katz D, FitzGerald MJ, Rothstein TL, Sherr DH, Sonenshein GE. Inhibition of NF-kappaB/Rel induces apoptosis of murine B cells. *EMBO J* 1996; **15**: 4682–4690.
164 Bargou RC, Leng C, Krappmann D, Emmerich F, Mapara MY, Bommert K, Royer HD, Scheidereit C, Dorken B. High-level nuclear NF-kappa B and Oct-2 is a common feature of cultured Hodgkin/Reed–Sternberg cells. *Blood* 1996; **87**: 4340–4347.
165 Bargou RC, Emmerich F, Krappmann D, Bommert K, Mapara MY, Arnold W, Royer HD, Grinstein E, Greiner A, Scheidereit C, Dorken B. Constitutive nuclear factor-kappaB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells. *J Clin Invest* 1997; **100**: 2961–2969.
166 Wood KM, Roff M, Hay RT. Defective IkappaBalpha in Hodgkin cell lines with constitutively active NF-kappaB. *Oncogene* 1998; **16**: 2131–2139.
167 O'Connell MA, Cleere R, Long A, O'Neill LA, Kelleher D. Cellular proliferation and activation of NF kappa B are induced by autocrine production of tumor necrosis factor alpha in the human T lymphoma line HuT 78. *J Biol Chem* 1995; **270**: 7399–7404.
168 Mori N, Nunokawa Y, Yamada Y, Ikeda S, Tomonaga M, Yamamoto N. Expression of human inducible nitric oxide synthase gene in T-cell lines infected with human T-cell leukemia virus type-I and primary adult T-cell leukemia cells. *Blood* 1999; **94**: 2862–2870.
169 Kordes U, Krappmann D, Heissmeyer V, Ludwig WD, Scheidereit C. Transcription factor NF-kappaB is constitutively activated in acute lymphoblastic leukemia cells. *Leukemia* 2000; **14**: 399–402.
170 FitzGerald MJ, Webber EM, Donovan JR, Fausto N. Rapid DNA binding by nuclear factor kappa B in hepatocytes at the start of liver regeneration. *Cell Growth Differ* 1995; **6**: 417–427.
171 Iimuro Y, Nishiura T, Hellerbrand C, Behrns KE, Schoonhoven R, Grisham JW, Brenner DA. NFkappaB prevents apoptosis and liver dysfunction during liver regeneration. *J Clin Invest* 1998; **101**: 802–811.
172 Arsura M, Mercurio F, Oliver AL, Thorgeirsson SS, Sonenshein GE. Role of the IkappaB kinase complex in oncogenic Ras- and Raf-mediated transformation of rat liver epithelial cells. *Mol Cell Biol* 2000; **20**: 5381–5391.
173 Visconti R, Cerutti J, Battista S, Fedele M, Trapasso F, Zeki K, Miano MP, de Nigris F, Casalino L, Curcio F, Santoro M, Fusco A. Expression of the neoplastic phenotype by human thyroid carcinoma cell lines requires NFkappaB p65 protein expression. *Oncogene* 1997; **15**: 1987–1994.
174 Wang W, Abbruzzese JL, Evans DB, Chiao PJ. Overexpression of urokinase-type plasminogen activator in pancreatic adenocarcinoma is regulated by constitutively activated RelA. *Oncogene* 1999; **18**: 4554–4563.
175 Want W, Abbruzzese JL, Evans DB, Larry L, Cleary KR, Chiao PJ. The nuclear factor-kappa B RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. *Clin Cancer Res* 1999; **5**: 119–127.
176 Palayoor ST, Youmell MY, Calderwood SK, Coleman CN, Price BD. Constitutive activation of IkappaB kinase alpha and NF-kappaB in prostate cancer cells in inhibited by ibuprofen. *Oncogene* 1999; **18**: 7389–7394.
177 Sumitomo M, Tachibana M, Nakashima J, Murai M, Miyajima A, Kimura F, Hayakawa M, Nakamura H. An essential role for nuclear factor kappa B in preventing TNF-alpha-induced cell death in prostate cancer cells. *J Urol* 1999; **161**: 674–679.
178 Shattuck-Brandt RL, Richmond A. Enhanced degradation of I-kappaB alpha contributes to endogenous activation of NF-kappaB in Hs294T melanoma cells. *Cancer Res* 1997; **57**: 3032–3039.
179 Ondrey FG, Dong G, Sunwoo J, Chen Z, Wolf JS, Crowl-Bancroft CV, Mukaida N, Van Waes C. Constitutive activation of transcription factors NF-(kappa)B, AP-1, and NF-IL6 in human head and neck squamous cell carcinoma cell lines that express pro-inflammatory and pro-angiogenic cytokines. *Mol Carcinog* 1999; **26**: 119–129.
180 Cadoret A, Bertrand F, Baron-Delage S, Levy P, Courtois G, Gespach C, Capeau J, Cherqui G. Down-regulation of NF-kappaB activity and NF-kappaB p65 subunit expression by ras and polyoma middle T oncogenes in human colonic Caco-2 cells. *Oncogene* 1997; **14**: 1589–1600.
181 Feinman R, Koury J, Thames M, Barlogie B, Epstein J, Siegel DS. Role of NF-kappaB in the rescue of multiple myeloma cells from glucocorticoid-induced apoptosis by bcl-2. *Blood* 1999; **93**: 3044–2052.
182 Bours V, Dejardin E, Goujon-Letawe F, Merville MP, Castronovo V. The NF-kappa B transcription factor and cancer: high expression of NF-kappa B- and I kappa B-related proteins in tumor cell lines. *Biochem Pharmacol* 1994; **47**: 145–149.
183 Batra RK, Guttridge DC, Brenner DA, Dubinett SM, Baldwin AS, Boucher RC. IkappaBalpha gene transfer is cytotoxic to squamous-cell lung cancer cells and sensitizes them to tumor necrosis factor-alpha-mediated cell death. *Am J Respir Cell Mol Biol* 1999; **21**: 238–245.
184 Mukhopadhyay T, Roth JA, Maxwell SA. Altered expression of the p50 subunit of the NF-kappa B transcription factor complex in non-small cell lung carcinoma. *Oncogene* 1995; **11**: 999–1003.
185 Yamagishi N, Miyakoshi J, Takebe H. Enhanced radiosensitivity by inhibition of nuclear factor kappa B activation in human malignant glioma cells. *Int J Radiat Biol* 1997; **72**: 157–162.
186 Zhou G, Kuo MT. NF-kappaB-mediated induction of mdr1b expression by insulin in rat hepatoma cells. *J Biol Chem* 1997; **272**: 15174–15183.
187 Rayet B, Gelinas C. Aberrant rel/nfkb genes and activity in human cancer. *Oncogene* 1999; **18**: 6938–6947.
188 Mathew S, Murty VV, Dalla-Favera R, Chaganti RS. Chromosomal localization of genes encoding the transcription factors, c-rel, NF-kappa Bp50, NF-kappa Bp65, and lyt-10 by fluorescence *in situ* hybridization. *Oncogene* 1993; **8**: 191–193.
189 Motokura T, Arnold A. PRAD1/cyclin D1 proto-oncogene: genomic organization, 5′ DNA sequence, and sequence of a tumor-specific rearrangement breakpoint. *Genes Chromosomes Cancer* 1993; **7**: 89–95.
190 Trecca D, Guerrini L, Fracchiolla NS, Pomati M, Baldini L, Maiolo AT, Neri A. Identification of a tumor-associated mutant form of the NF-kappaB RelA gene with reduced DNA-binding and transactivating activities. *Oncogene* 1997; **14**: 791–799.
191 Barth TF, Dohner H, Werner CA, Stilgenbauer S, Schlotter M, Pawlita M, Lichter P, Moller P, Bentz M. Characteristic pattern of chromosomal gains and losses in primary large B-cell lymphomas of the gastrointestinal tract. *Blood* 1998; **91**: 4321–4330.
192 Houldsworth J, Mathew S, Rao PH, Dyomina K, Louie DC, Parsa N, Offit K, Chaganti RS. REL proto-oncogene is frequently amplified in extranodal diffuse large cell lyphoma. *Blood* 1996; **87**: 25–29.
193 Rao PH, Houldsworth J, Dyomina K, Parsa NZ, Cigudosa JC, Louie DC, Popplewell L, Offit K, Jhanwar SC, Chaganti RS. Chromosomal and gene amplification in diffuse large B-cell lymphoma. *Blood* 1998; **92**: 234–240.
194 Joos S, Otano-Joos MI, Ziegler S, Bruderlein S, du Manoir S, Bentz M, Moller P, Lichter P. Primary mediastinal (thymic) B-cell lymphoma is characterized by gains of chromosomal material including 9p and amplification of the REL gene. *Blood* 1996; **87**: 1571–1578.
195 Lu D, Thompson JD, Gorski GK, Rice NR, Mayer MG, Yunis JJ. Alterations at the rel locus in human lymphoma. *Oncogene* 1991; **6**: 1235–1241.
196 Liptay S, Schmid RM, Perkins ND, Meltzer P, Altherr MR, McPherson JD, Wasmuth JJ, Nabel GJ. Related subunits of NF-kappa B map to two distinct loci associated with translocations in leukemia, NFKB1 and NFKB2. *Genomics* 1992; **13**: 287–292.
197 Ferrier R, Nougarede R, Doucet S, Kahn-Perles B, Imbert J, Mathieu-Mahul D. Physical interaction of the bHLH LYL1 protein and NF-kappaB1 p105. *Oncogene* 1999; **18**: 995–1005.

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198 Fracchiolla NS, Lombardi L, Salina M, Migliazza A, Baldini L, Berti E, Cro L, Polli E, Maiolo AT, Neri A. Structural alterations of the NF-kappa B transcription factor lyt-10 in lymphoid malignancies. *Oncogene* 1993; **8**: 2839–2845.
199 Migliazza A, Lombardi L, Rocchi M, Trecca D, Chang CC, Antonacci R, Fracchiolla NS, Ciana P, Maiolo AT, Neri A. Heterogeneous chromosomal aberrations generate 3′ truncations of the NFKB2/lyt-10 gene in lymphoid malignancies. *Blood* 1994; **84**: 3850–3860.
200 Neri A, Chang CC, Lombardi L, Salina M, Corradini P, Maiolo AT, Chaganti RS, Dalla-Favera R. B cell lymphoma-associated chromosomal translocation involves candidate oncogene lyt-10, homologous to NF-kappa B p50. *Cell* 1991; **67**: 1075–1087.
201 Neri A, Fracchiolla NS, Roscetti E, Garatti S, Trecca D, Boletini A, Perletti L, Baldini L, Maiolo AT, Berti E. Molecular analysis of cutaneous B- and T-cell lymphomas. *Blood* 1995; **86**: 3160–3172.
202 Thakur S, Lin HC, Tseng WT, Kumar S, Bravo R, Foss F, Gelinas C, Rabson AB. Rearrangement and altered expression of the NFKB-2 gene in human cutaneous T-lymphoma cells. *Oncogene* 1994; **9**: 2335–2344.
203 Dejardin E, Bonizzi G, Bellahcene A, Castronovo V, Merville MP, Bours V. Highly-expressed p100/p52 (NFKB2) sequesters other NF-kappa B-related proteins in the cytoplasm of human breast cancer cells. *Oncogene* 1995; **11**: 1835–1841.
204 Cabannes E, Khan G, Aillet F, Jarrett RF, Hay RT. Mutations in the IkBa gene in Hodgkin's disease suggest a tumour suppressor role for IkappaBalpha. *Oncogene* 1999; **18**: 3063–3070.
205 Krappmann D, Emmerich F, Kordes U, Scharschmidt E, Dorken B, Scheidereit C. Molecular mechanisms of constitutive NF-kappaB/Rel activation in Hodgkin/Reed–Sternberg cells. *Oncogene* 1999; **18**: 943–953.
206 Epinat JC, Gilmore TD. Diverse agents act at multiple levels to inhibit the Rel/NF-kappaB signal transduction pathway. *Oncogene* 1999; **18**: 6896–6909.
207 D'Acquisto F, Ialenti A, Ianaro A, Di Vaio R, Carnuccio R. Local administration of transcription factor decoy oligonucleotides to nuclear factor-kappaB prevents carrageenin-induced inflammation in rat hind paw. *Gene Ther* 2000; **7**: 1731–1737.
208 Gerbes AL, Vollmar AM, Kiemer AK, Bilzer M. The guanylate cyclase-coupled natriuretic peptide receptor: a new target for prevention of cold ischemia-reperfusion damage of the rat liver. *Hepatology* 1998; **28**: 1309–1317.
209 Manna SK, Aggarwal BB. Interleukin-4 down-regulates both forms of tumor necrosis factor receptor and receptor-mediated apoptosis, NF-kappaB, AP-1, and c-Jun N-terminal kinase. Comparison with interleukin-13. *J Biol Chem* 1998; **273**: 33333–33341.
210 Shumilla JA, Wetterhahn KE, Barchowsky A. Inhibition of NF-kappa B binding to DNA by chromium, cadmium, mercury, zinc, and arsenite *in vitro*: evidence of a thiol mechanism. *Arch Biochem Biophys* 1998; **349**: 356–362.
211 Yang JP, Merin JP, Nakano T, Kato T, Kitade Y, Okamoto T. Inhibition of the DNA-binding activity of NF-kappa B by gold compounds *in vitro. FEBS Lett* 1995; **361**: 89–96.
212 Fiedler MA, Wernke-Dollries K, Stark JM. Inhibition of viral replication reverses respiratory syncytial virus-induced NF-kappaB activation and interleukin-8 gene expression in A549 cells. *J Virol* 1996; **70**: 9079–9082.
123 Oyama T, Ran S, Ishida T, Nadaf S, Kerr L, Carbone DP, Gabrilovich DI. Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-kappa B activation in hemopoietic progenitor cells. *J Immunol* 1998; **160**: 1224–1232.
214 Gabrilovich D, Ishida T, Oyama T, Ran S, Kravtsov V, Nadaf S, Carbone DP. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages *in vivo. Blood* 1998; **92**: 4150–4166.
215 Natarajan K, Singh S, Burke TR Jr, Grunberger D, Aggarwal BB. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa B. *Proc Natl Acad Sci USA* 1996; **93**: 9090–9095.
216 Delgado M, Munoz-Elias EJ, Kan Y, Gozes I, Fridkin M, Brenneman DE, Gomariz RP, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit tumor necrosis factor alpha transcriptional activation by regulating nuclear factor-kB and cAMP response element-binding protein/c-Jun. *J Biol Chem* 1998; **273**: 31427–31436.
217 Russo SM, Tepper JE, Baldwin AS Jr, Liu R, Adams J, Elliott P, Cusack JC Jr. Enhancement of radiosensitivity by proteasome inhibition: implications for a role of NF-kappaB. *Int J Radiat Oncol Biol Phys* 2001; **50**: 183–193.
218 Palombella VJ, Rando OJ, Goldberg AL, Maniatis T. The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* 1994; **78**: 773–785.
219 Grisham MB, Palombella VJ, Elliott PJ, Conner EM, Brand S, Wong HL, Pien C, Mazzola LM, Destree A, Parent L, Adams J. Inhibition of NF-kappa B activation *in vitro* and *in vivo*: role of 26S proteasome. *Methods Enzymol* 1999; **300**: 345–363.
220 Jobin C, Hellerbrand C, Licato LL, Brenner DA, Sartor RB. Mediation by NF-kappa B of cytokine induced expression of intercellular adhesion molecule 1 (ICAM-1) in an intestinal epithelial cell line, a process blocked by proteasome inhibitors. *Gut* 1998; **42**: 779–787.
221 Fenteany G, Schreiber SL. Lactacystin, proteasome function, and cell fate. *J Biol Chem* 1998; **273**: 8545–8548.
222 Cusack JC Jr, Liu R, Houston M, Abendroth K, Elliott PJ, Adams J, Baldwin AS Jr. Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: implications for systemic nuclear factor-kappaB inhibition. *Cancer Res* 2001; **61**: 3535–3540.
223 Yaron A, Gonen H, Alkalay I, Hatzubai A, Jung S, Beyth S, Mercurio F, Manning AM, Ciechanover A, Ben-Neriah Y. Inhibition of NF-kappa-B cellular function via specific targeting of the I-kappa-B-ubiquitin ligase. *EMBO J* 1997; **16**: 6486–6494.
224 Frantz B, Nordby EC, Bren G, Steffan N, Paya CV, Kincaid RL, Tocci MJ, O'Keefe SJ, O'Neill EA. Calcineurin acts in synergy with PMA to inactivate I kappa B/MAD3, an inhibitor of NF-kappa B. *EMBO J* 1994; **13**: 861–870.
225 Singh S, Natarajan K, Aggarwal BB. Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a potent inhibitor of nuclear transcription factor-kappa B activation by diverse agents. *J Immunol* 1996; **157**: 4412–4420.
226 Shrivastava A, Manna SK, Ray R, Aggarwal BB. Ectopic expression of hepatitis C virus core protein differentially regulates nuclear transcription factors. *J Virol* 1998; **72**: 9722–9728.
227 Pahl HL, Krauss B, Schulze-Osthoff K, Decker T, Traenckner EB, Vogt M, Myers C, Parks T, Warring P, Muhlbacher A, Czernilofsky AP, Baeuerle PA. The immunosuppressive fungal metabolite gliotoxin specifically inhibits transcription factor NF-kappaB. *J Exp Med* 1996; **183**: 1829–1840.
228 Manna SK, Aggarwal BB. IL-13 suppresses TNF-induced activation of nuclear factor-kappa B, activation protein-1, and apoptosis. *J Immunol* 1998; **161**: 2863–2872.
229 Singh S, Aggarwal BB. Protein-tyrosine phosphatase inhibitors block tumor necrosis factor-dependent activation of the nuclear transcription factor NF-kappa B. *J Biol Chem* 1995; **270**: 10631–10639.
230 Frantz B, O'Neill EA. The effect of sodium salicylate and aspirin on NF-kappa B. *Science* 1995; **270**: 2017–2019.
231 Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. *Science* 1994; **265**: 956–959.
232 Palayoor ST, Bump EA, Calderwood SK, Bartol S, Coleman CN. Combined antitumor effect of radiation and ibuprofen in human prostate carcinoma cells. *Clin Cancer Res* 1998; **4**: 763–771.
233 Katsuyama K, Shichiri M, Marumo F, Hirata Y. NO inhibits cytokine-induced iNOS expression and NF-kappaB activation by interfering with phosphorylation and degradation of IkappaB-alpha. *Arterioscler Thromb Vasc Biol* 1998; **18**: 1796–1802.
234 Matthews JR, Botting CH, Panico M, Morris HR, Hay RT. Inhibition of NF-kappaB DNA binding by nitric oxide. *Nucleic Acids Res* 1996; **24**: 2236–2242.
235 Spiecker M, Liao JK. Assessing induction of I kappa B by nitric oxide. *Methods Enzymol* 1999; **300**: 374–388.
236 Rossi A, Elia G, Santoro MG. Inhibition of nuclear factor kappa B by prostaglandin A1: an effect associated with heat shock transcription factor activation. *Proc Natl Acad Sci USA* 1997; **94**: 746–750.
237 Chaturvedi MM, Kumar A, Darnay BG, Chainy GB, Agarwal S,

Aggarwal BB. Sanguinarine (pseudochelerythrine) is a potent inhibitor of NF-kappaB activation, IkappaBalpha phosphorylation, and degradation. *J Biol Chem* 1997; **272**: 30129–30134.
238 Schesser K, Spiik AK, Dukuzumuremyi JM, Neurath MF, Pettersson S, Wolf-Watz H. The yopJ locus is required for Yersinia-mediated inhibition of NF-kappaB activation and cytokine expression: YopJ contains a eukaryotic SH2-like domain that is essential for its repressive activity. *Mol Microbiol* 1998; **28**: 1067–1079.
239 Ji C, Kozak KR, Marnett LJ. IkappaB kinase, a molecular target for inhibition by 4-hydroxy-2-nonenal. *J Biol Chem* 2001; **276**: 18223–18228.
240 May MJ, D'Acquisto F, Madge LA, Glockner J, Pober JS, Ghosh S. Selective inhibition of NF-kappaB activation by a peptide that blocks the interaction of NEMO with the IkappaB kinase complex. *Science* 2000; **289**: 1550–1554.
241 Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science* 1995; **270**: 286–290.
242 Brostjan C, Anrather J, Csizmadia V, Stroka D, Soares M, Bach FH, Winkler H. Glucocorticoid-mediated repression of NFkappaB activity in endothelial cells does not involve induction of IkappaBalpha synthesis. *J Biol Chem* 1996; **271**: 19612–19616.
243 Ehrlich LC, Hu S, Peterson PK, Chao CC. IL-10 down-regulates human microglial IL-8 by inhibition of NF-kappaB activation. *Neuroreport* 1998; **9**: 1723–1736.
244 Lentsch AB, Shanley TP, Sarma V, Ward PA. In vivo suppression of NF-kappa B and preservation of I kappa B alpha by interleukin-10 and interleukin-13. *J Clin Invest* 1997; **100**: 2443–2448.
245 Li N, Karin M. Is NF-kappaB the sensor of oxidative stress? *FASEB J* 1999; **13**: 1137–1143.
246 Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene* 1999; **18**: 6853–6866.
247 Siebenlist U, Franzoso G, Brown K. Structure, regulation and function of NF-kappa B. *Annu Rev Cell Biol* 1994; **10**: 405–455.
248 Schreck R, Meier B, Mannel DN, Droge W, Baeuerle PA. Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. *J Exp Med* 1992; **175**: 1181–1194.
249 Singh S, Aggarwal BB. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) *J Biol Chem* 1995; **270**: 24995–25000.
250 Cho S, Urata Y, Iida T, Goto S, Yamaguchi M, Sumikawa K, Kondo T. Glutathione downregulates the phosphorylation of I kappa B: autoloop regulation of the NF-kappa B-mediated expression of NF-kappa B subunits by TNF-alpha in mouse vascular endothelial cells. *Biochem Biophys Res Commun* 1998; **253**: 104–108.
251 Staal FJ, Roederer M, Raju PA, Anderson MT, Ela SW, Herzenberg LA, Herzenberg LA. Antioxidants inhibit stimulation of HIV transcription. *AIDS Res Hum Retroviruses* 1993; **9**: 299–306.
252 Lin YZ, Yao SY, Veach RA, Torgerson TR, Hawiger J. Inhibition of nuclear translocation of transcription factor NF-kappa B by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. *J Biol Chem* 1995; **270**: 14255–14258.
253 Pieper GM, Riaz-ul-Haq. Activation of nuclear factor-kappaB in cultured endothelial cells by increased glucose concentration: prevention by calphostin C. *J Cardiovasc Pharmacol* 1997; **30**: 528–532.
254 Shoji S, Furuishi K, Ogata A, Yamataka K, Tachibana K, Mukai R, Uda A, Harano K, Matsushita S, Misumi S. An allosteric drug, o,o′-bismyristoyl thiamine disulfide, suppresses HIV-1 replication through prevention of nuclear translocation of both HIV-1 Tat and NF-kappa B. *Biochem Biophys Res Commun* 1998; **249**: 745–753.
255 Bentires-Alj M, Hellin AC, Ameyar M, Chouaib S, Merville MP, Bours V. Stable inhibition of nuclear factor kappaB in cancer cells does not increase sensitivity to cytotoxic drugs. *Cancer Res* 1999; **59**: 811–815.
256 Jobin C, Panja A, Hellerbrand C, Iimuro Y, Didonato J, Brenner DA, Sartor RB. Inhibition of proinflammatory molecule production by adenovirus-mediated expression of a nuclear factor kappaB super-repressor in human intestinal epithelial cells. *J Immunol* 1998; **160**: 410–418.
257 Van Antwerp DJ, Verma IM. Signal-induced degradation of I(kappa)B(alpha): association with NF-kappaB and the PEST sequence in I(kappa)B(alpha) are not required. *Mol Cell Biol* 1996; **16**: 6037–6045.
258 Abu-Amer Y, Dowdy SF, Ross FP, Clohisy JC, Teitelbaum SL. TAT fusion proteins containing tyrosine 42-deleted IkappaBalpha arrest osteoclastogenesis. *J Biol Chem* 2001; **276**: 30499–30503.
259 Chen LF, Fischle W, Verdin E, Greene WC. Duration of nuclear NF-kappaB action regulated by reversible acetylation. *Science* 2001; **293**: 1653–1657.
260 Chapman NR, Perkins ND. Inhibition of the RelA(p65) NF-kappaB subunit by Egr-1. *J Biol Chem* 2000; **275**: 4719–4725.
261 Cogswell PC, Mayo MW, Baldwin AS Jr. Involvement of Egr-1/RelA synergy in distinguishing T cell activation from tumor necrosis factor-alpha-induced NF-kappa B1 transcription. *J Exp Med* 1997; **185**: 491–497.
262 Yang JP, Hori M, Sanda T, Okamoto T. Identification of a novel inhibitor of nuclear factor-kappaB, RelA-associated inhibitor. *J Biol Chem* 1999; **274**: 15662–15670.
263 Diaz-Meco MT, Lallena MJ, Monjas A, Frutos S, Moscat J. Inactivation of the inhibitory kappaB protein kinase/nuclear factor kappaB pathway by Par-4 expression potentiates tumor necrosis factor alpha-induced apoptosis. *J Biol Chem* 1999; **274**: 19606–19612.
264 Chakraborty M, Qiu SG, Vasudevan KM, Rangnekar VM. Par-4 drives trafficking and activation of Fas and Fasl to induce prostate cancer cell apoptosis and tumor regression. *Cancer Res* 2001; **61**: 7255–7263.
265 Camandola S, Mattson MP. Pro-apoptotic action of PAR-4 involves inhibition of NF-kappaB activity and suppression of BCL-2 expression. *J Neurosci Res* 2000; **61**: 134–139.
266 Phillips AC, Ernst MK, Bates S, Rice NR, Vousden KH. E2F-1 potentiates cell death by blocking antiapoptotic signaling pathways. *Mol Cell* 1999; **4**: 771–781.
267 Webster GA, Perkins ND. Transcriptional cross talk between NF-kappaB and p53. *Mol Cell Biol* 1999; **19**: 3485–3495.
268 Ikeda A, Sun X, Li Y, Zhang Y, Eckner R, Doi TS, Takahashi T, Obata Y, Yoshioka K, Yamamoto K. p300/CBP-dependent and -independent transcriptional interference between NF-kappaB RelA and p53. *Biochem Biophys Res Commun* 2000; **272**: 375–379.
269 Mayo MW, Wang CY, Cogswell PC, Rogers-Graham KS, Lowe SW, Der CJ, Baldwin AS Jr. Requirement of NF-kappaB activation to suppress p53-independent apoptosis induced by oncogenic Ras. *Science* 1997; **278**: 1812–1815.
270 Chen E, Li CC. Association of Cdk2/cyclin E and NF-kappa B complexes at G1/S phase. *Biochem Biophys Res Commun* 1998; **249**: 728–734.
271 Ryan KM, Ernst MK, Rice NR, Vousden KH. Role of NF-kappaB in p53-mediated programmed cell death. *Nature* 2000; **404**: 892–897.
272 Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* 1996; **84**: 299–308.
273 Cao Z, Xiong J, Takeuchi M, Kurama T, Goeddel DV. TRAF6 is a signal transducer for interleukin-1. *Nature* 1996; **383**: 443–446.
274 Rothe M, Xiong J, Shu HB, Williamson K, Goddard A, Goeddel DV. I-TRAF is a novel TRAF-interacting protein that regulates TRAF-mediated signal transduction. *Proc Natl Acad Sci USA* 1996; **93**: 8241–8246.
275 Song HY, Regnier CH, Kirschning CJ, Goeddel DV, Rothe M. Tumor necrosis factor (TNF)-mediated kinase cascades: bifurcation of nuclear factor-kappaB and c-jun N-terminal kinase (JNK/SAPK) pathways at TNF receptor-associated factor 2. *Proc Natl Acad Sci USA* 1997; **94**: 9792–9796.
276 Jang IK, Lee ZH, Kim YJ, Kim SH, Kwon BS. Human 4–1BB (CD137) signals are mediated by TRAF2 and activate nuclear factor-kappa B. *Biochem Biophys Res Commun* 1998; **242**: 613–620.
277 Ling L, Cao Z, Goeddel DV. NF-kappaB-inducing kinase activates IKK-alpha by phosphorylation of Ser-176. *Proc Natl Acad Sci USA* 1998; **95**: 3792–3797.
278 Regnier CH, Song HY, Gao X, Goeddel DV, Cao Z, Rothe M. Identification and characterization of an IkappaB kinase. *Cell* 1997; **90**: 373–383.

279 Woronicz JD, Gao X, Cao Z, Rothe M, Goeddel DV. IkappaB kinase-beta: NF-kappaB activation and complex formation with IkappaB kinase-alpha and NIK. *Science* 1997; **278**: 866–869.
280 Baeuerle PA, Baichwal VR. NF-kappa B as a frequent target for immunosuppressive and anti-inflammatory molecules. *Adv Immunol* 1997; **65**: 111–137.
281 Maniatis T. A ubiquitin ligase complex essential for the NF-kappaB, Wnt/Wingless, and Hedgehog signaling pathways. *Genes Dev* 1999; **13**: 505–510.
282 Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D. Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. *Nature* 1995; **376**: 167–170.
283 Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science* 1995; **270**: 286–290.