

# Nuclear Factor- $\kappa$ B: A Holy Grail in Cancer Prevention and Therapy

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**Abstract:** Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is a transcription factor that is activated in response to various inflammatory stimuli such as cytokines, growth factors, hormones, mitogens, carcinogens, chemotherapeutic agents, viral products, eukaryotic parasites, endotoxin, fatty acids, metals, radiation, hypoxia, and psychological, physical, oxidative, and chemical stresses. In addition, constitutively active NF- $\kappa$ B is frequently encountered in a wide variety of tumors, including breast, ovarian, colon, pancreatic, thyroid, prostate, lung, head and neck, bladder, and skin cancers; B-cell lymphoma; Hodgkin's disease; T-cell lymphoma; adult T-cell leukemia; acute lymphoblastic leukemia; multiple myeloma; chronic lymphocytic leukemia; and acute myelogenous leukemia. Furthermore, NF- $\kappa$ B activation has been shown to regulate the expression of over 400 genes involved in cellular transformation, proliferation, inflammation, viral replication, antiapoptosis, angiogenesis, invasion and metastasis, oxidative stress, and osteoclastogenesis. Therefore, because of the critical role NF- $\kappa$ B plays in the pathogenesis of cancer, specific inhibitors of this factor are being sought. Agents that prevent cancer or inflammation have been found to suppress NF- $\kappa$ B activation. Although I $\kappa$ B $\alpha$  kinase is the major kinase, over 30 different protein kinases have been linked to the activation of NF- $\kappa$ B by different stimuli. The development of a drug that can specifically suppress NF- $\kappa$ B activation requires a full understanding of the mechanism by which NF- $\kappa$ B is activated in response to these various stimuli.

**Key Words:** NF- $\kappa$ B, IKK, antitumor, TNF, inflammation.

## 1. INTRODUCTION

Nuclear factor- $\kappa$ B (NF- $\kappa$ B), a nuclear transcription factor, was first identified in 1986 by Sen and Baltimore [1]. As its name implies, it is a nuclear factor bound to an enhancer element of the immunoglobulin kappa light chain gene in B cells [1]. First considered a B-cell transcription factor, NF- $\kappa$ B is now known to comprise a family of ubiquitous proteins. NF- $\kappa$ B proteins contain a Rel homology domain (DNA-binding domain/dimerization domain) with a nuclear localization sequence; such sequences are conserved from *Drosophila* to man. Class I proteins include p50, p52, p100, and p105. Multiple copies of ankyrin repeats are present in p100 and p105; proteolytic cleavage of p100 forms p52 and that of p105 forms p50. These protein, in turn, form dimers with class II proteins (c-Rel, RelB, and RelA/p65), which exclusively contain C-terminal activation domains. Whereas RelB forms only heterodimers, all the other proteins can form both homo- and heterodimers. NF- $\kappa$ B is the most common heterodimer formed between Rel A and p50. Dimeric NF- $\kappa$ B transcription factors bind to the 10-base-pair consensus site GGGPuNNPyPyCC, where Pu is purine, Py is pyrimidine, and N is any base. The individual dimers have distinct DNA-binding specificities for a collection of related  $\kappa$ B sites [2, 3].

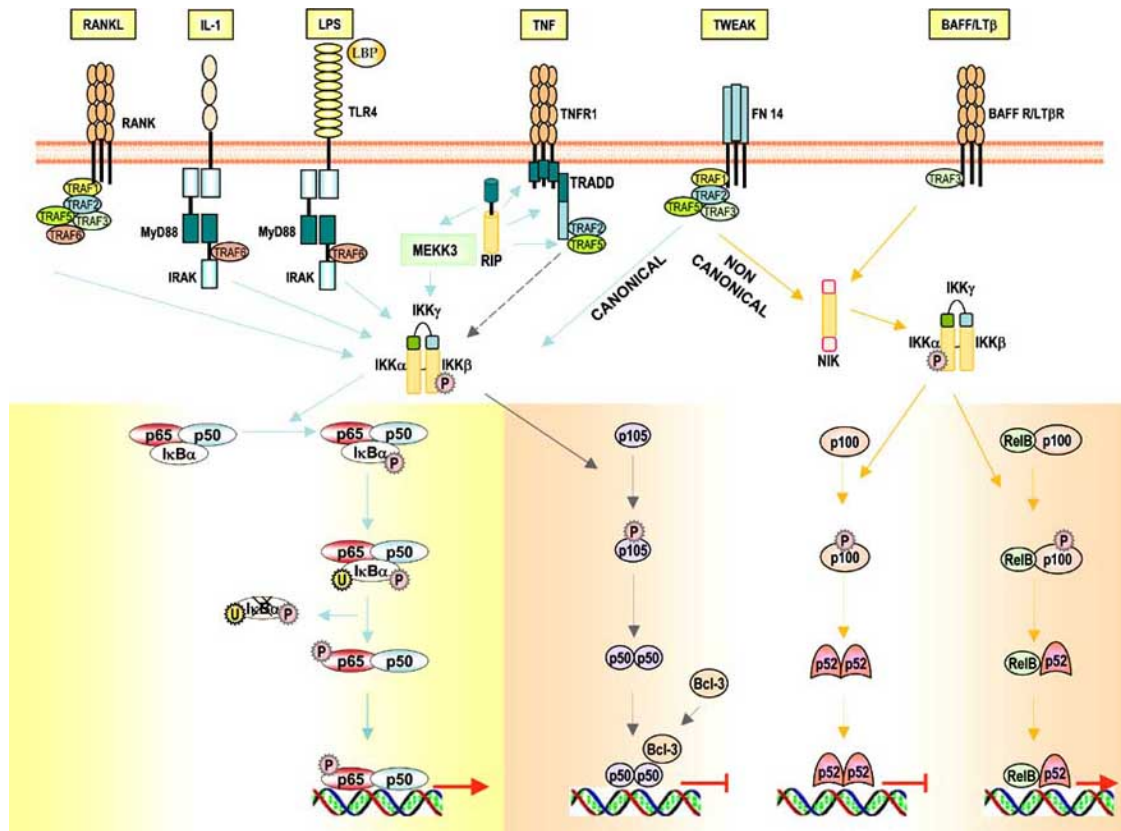
The various inhibitors of NF- $\kappa$ B include I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$ , I $\kappa$ B $\gamma$  (derived from the C-terminal of p100), I $\kappa$ B $\epsilon$ , Bcl-3, pp40 (a chicken homologue), cactus (a *Drosophila* homologue), and avian swine fever virus protein p28.2. p105 and p100 can also function to retain NF- $\kappa$ B subunits in the

cytoplasm. All of these proteins are characterized by the presence of multiple ankyrin repeats. Perhaps the most common and best-understood form of NF- $\kappa$ B consists of p50, p65, and I $\kappa$ B $\alpha$ . I $\kappa$ B $\alpha$  mediates transient gene expression, whereas I $\kappa$ B $\beta$  mediates persistent response.

The I $\kappa$ B proteins are expressed in a tissue-specific manner and have distinct affinities for individual Rel/NF- $\kappa$ B complexes. I $\kappa$ Bs contain six or more ankyrin repeats, an N-terminal regulatory domain, and a C-terminal domain that contains a proline-glutamic acid-serine-threonine motif. I $\kappa$ Bs bind to NF- $\kappa$ B dimers and sterically block the function of their nuclear localization sequences, thereby causing their cytoplasmic retention. Most agents that activate NF- $\kappa$ B mediate the phosphorylation-induced degradation of I $\kappa$ B. On receipt of a signal, phosphorylation of I $\kappa$ B $\alpha$  takes place on two conserved serine residues (S32 and S36) in the N-terminal regulatory domain. However, another member of the I $\kappa$ B family, Bcl-3, stimulates transcription after interacting with p50 and p52 subunits of NF- $\kappa$ B.

Several of the I $\kappa$ B kinases (IKKs) have been characterized, namely, IKK $\alpha$ , IKK $\beta$ , and IKK $\gamma$ . Mutation analysis revealed that IKK $\alpha$  and not IKK $\beta$  mediates proinflammatory signals. Once phosphorylated, the I $\kappa$ Bs, which are still bound to NF- $\kappa$ B, almost immediately undergo a second posttranslational modification known as polyubiquitination. The major ubiquitin acceptor sites in human I $\kappa$ B $\alpha$  are lysines 21 and 22. Protein ubiquitination occurs through the E1 ubiquitin-activating enzyme, the E2 ubiquitin-conjugating enzyme, and the E3 ubiquitin protein ligases. After ubiquitination, I $\kappa$ Bs are degraded in 26S proteasomes, leading to the release of NF- $\kappa$ B dimers, which translocate into the nucleus [3, 4]. In contrast, the activation of NF- $\kappa$ B in response to ultraviolet (UV) radiation is accompanied by I $\kappa$ B $\alpha$  degradation but not phosphorylation on the N-terminus of I $\kappa$ B $\alpha$  [5]. Hypoxia or

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**Fig. (1).** Signaling pathway of NF- $\kappa$ B.

pervanadate treatment stimulates the phosphorylation of I $\kappa$ B $\alpha$  at tyrosine 42, but other I $\kappa$ Bs do not have a tyrosine at this position [6]. Phosphorylation on Ser-276 by the catalytic subunit of protein kinase A contributes to the intrinsic transcriptional capacity of the p65 subunit of NF- $\kappa$ B. The catalytic subunit of protein kinase A was also found to be associated with NF- $\kappa$ B and I $\kappa$ B in the cytoplasm and was able to phosphorylate p65 only after I $\kappa$ B degradation [7]. In addition, a site-directed mutant of p65 (Ser-276 to Ala) is phosphorylated at Ser 529 in response to tumor necrosis factor (TNF), suggesting that multiple physiologic stimuli modulate p65 through distinct phosphorylation sites to control transcriptional activity. RelA (C-terminus) has been shown to interact with basal transcriptional apparatus proteins such as TATA-binding protein (TBP), transcription factor (TF) IIB and TBP-associated factor (TAF) 105 and with coactivators such as cAMP responsive element binding protein (CBP) and p300, although the actual role of these interactions is not clear [7]. This pathway is well conserved, both in structure and function, from *Drosophila* to humans.

NF- $\kappa$ B is activated by many divergent stimuli, including proinflammatory cytokines (e.g., TNF- $\alpha$ , interleukin-1 [IL-1]), T- and B-cell mitogens, bacteria, lipopolysaccharide (LPS), viruses, viral proteins, double-stranded RNA, and physical and chemical stresses [8] (Table 1). Cellular stresses, including ionizing radiation and chemotherapeutic agents, also activate NF- $\kappa$ B [9].

Although much has been learned since the discovery of NF- $\kappa$ B, the precise mechanism of its activation is still not fully understood. Depending on the stimulus, this mechanism involves overlapping and nonoverlapping steps. For example, TNF, one of the most potent activators of NF- $\kappa$ B, interacts with the TNF receptor (TNFR) and then recruits a protein called TNFR-associated death domain. This protein binds to TNFR-associated factor (TRAF) 2, which recruits NF- $\kappa$ B-inducing kinase (NIK), which in turn activates IKK. IKK phosphorylates I $\kappa$ B $\alpha$  at serines 32 and 36, which leads to ubiquitination at lysines 21 and 22, and this leads to the degradation of I $\kappa$ B $\alpha$  by the 26S proteasome. This degradation results in translocation of NF- $\kappa$ B to the nucleus, where it binds to its consensus sequence (5'-GGGACTTTC-3') and activates gene expression. Thus, NF- $\kappa$ B can be monitored by the I $\kappa$ B $\alpha$  degradation seen on Western blotting, by the NF- $\kappa$ B binding to DNA seen on electrophoretic mobility shift assay, or by the NF- $\kappa$ B-dependent reporter gene expression seen on transient transfection.

Besides the previously described canonical NF- $\kappa$ B activation pathway, a noncanonical NF- $\kappa$ B activation pathway is activated by CD40L, lymphotoxin (LT)- $\beta$ , receptor activator of NF- $\kappa$ B ligand (RANKL), and B-cell-activating factor of the TNF family (BAFF), all members of the TNF family (Table 1) [10, 11]. This pathway does not involve I $\kappa$ B $\alpha$  but instead involves direct phosphorylation and ubiquitin-dependent degradation of p100. Current research

**Table 1. A List of Inducers of NF- $\kappa$ B Activation**

<b>Cytokines</b>	All-trans retinoic acid	<i>Borrelia burgdorferi</i>	Pneumocystis
TNF $\alpha$ <sup>10,11</sup>	RET/PTC3 fusion oncoprotein	<i>Chlamydia pneumoniae</i>	<i>Theileria parva</i>
TNF $\beta$	S100B	<i>Cryptococcus neoformans</i>	<i>Trypanoplasma borreli</i>
RANKL <sup>10,11,154</sup>	Serum	<i>Ehrlichia chaffeensis</i>	
IL-1	Sulphatide	Enteropathogenic <i>Escherichia coli</i>	<b>Physiologic (stress) conditions</b>
TRAIL	TGF- $\alpha$	<i>Fusobacterium nucleatum</i>	Asthma
CD40 ligand <sup>10,11</sup>	TGF- $\beta$	<i>Gardnerella vaginalis</i>	Rheumatoid arthritis
CD30 ligand <sup>10,11</sup>		<i>Helicobacter pylori</i>	Crohn's disease/ulcerative colitis
TWEAK <sup>10,11</sup>	<b>Viruses</b>	Lactobacilli	Corneal epidemic
THANK	Adenovirus	<i>Listeria monocytogenes</i>	keratoconjunctivitis
BAFF <sup>10,11</sup>	Cytomegalovirus	<i>Mycoplasmata fermentans</i>	Coronary artery bypass
LT $\beta$ <sup>10,11</sup>	EBV	<i>Mycobacteria tuberculosis</i>	Hypercholesterolemia
IL-2	Hammarskjöld and hepatitis B virus	<i>Neisseria gonorrhoeae</i>	Hyperglycemia
IL-4	HVS	<i>Neisseria meningitidis</i>	Hyperhomocysteinemia
IL-8	Human herpes virus 6	<i>Porphyromonas gingivalis</i>	Hyperosmotic shock
IL-12	HIV-1	<i>Prevotella intermedia</i>	Hypoxia <sup>90</sup>
IL-15	Herpes simplex virus-1	<i>Pseudomonas aerogenosa</i>	Acute lung injury
IL-17	HTLV-1	<i>Rhodococcus equi</i>	Acute respiratory distress syndrome
IL-18	Influenza virus	<i>Rickettsia rickettsii</i>	Adhesion
CD11b/CD18 ligand	Measles virus	<i>Salmonella dublin</i>	Angina pectoris
CD28 ligand	Moloney murine leukemia virus	<i>Salmonella typhimurium</i>	Antiphospholipid antibodies
CD2 ligand	Newcastle disease virus	<i>Shigella flexneri</i>	Appendicitis
CD35 ligand	Respiratory syncytial virus	<i>Staphylococcus aureus</i>	Depolarization
CD3 ligand	Rhinovirus	Streptococcus	Hemorrhage
CD43 ligand	SARS coronavirus	<i>Streptomyces californicus</i>	Ischemia (transient, focal)
CD4 ligand	Sendai paramyxovirus	<i>Trichomonas vaginalis</i>	Ischemic preconditioning
CD66a ligand	Sindbis virus	<i>urealyticum</i>	Liver regeneration
LIF	Vaccinia virus akara	<i>Yersinia enterocolitica</i>	Human labor
S100B	West Nile flavavirus		Biotin deficiency
Agonist antibodies to AT1 receptor		<b>Bacterial or fungal products</b>	Butter and walnut diet
IgM ligand	<b>Viral products</b>	LPS	Cecal ligatin and puncture
$\beta$ -D-glucan ligand	Adenovirus 5: E1A	Wogonin	Mechanical ventilation
IgG2a	Adenovirus: E3/19K	Enterotoxin	Memory retrieval
Flt-1 ligand	African swine fever virus: IAP	Glycosylphosphatidylinositols	Middle cerebral artery occlusion
Ly6A/E ligand	Antibody to dengue virus	Fumonisin B1	Muscle disuse
	nonstructural protein 1	Staphylococcus enterotoxins A and B	Muscular dystrophy
<b>Growth factors and hormones</b>	CMV: ie1	Apicularen A	Neuronal firing
GMCSF	EBNA-2	CpG	Overventilation pancreatitis
M-CSF	EBV: LMP1	Cytolysin	Proteinuria
NGF	HBV: HBx	Diphosphoryl lipid A	Reoxygenation
EGF	HBV: LHBs	Exotoxin B Lipoprotein LpK	Senescence
Insulin-like growth factor 1	HBV: MHBst	Lipoprotein LpK	Shear stress
Insulin	HCV: Core protein	Lipoteichoic acid	Neuroonal trimethyltin injury
Hepatocyte growth factor	HVS13	Membrane lipoproteins	Uniaxial cyclic cell stretching
Gastrin	HVS: StpC	( <i>Mycoplasmata fermentans</i> ,	T-cell selection
PEDF	HIV-1: gp160	<i>Mycoplasmata penetrans</i> )	
Platelet-derived growth factor	HIV-1: Nef	Mannoproteins	<b>Physical stress</b>
Bone morphogenic protein 2	HIV-1: p9 (9 aa peptide)	Muramyl peptides	UV A, B, and C radiation <sup>106</sup>
Bone morphogenic protein 4	HIV-1: Tat	<i>Mycobacterium</i> species	Bile duct ligation
Cortical releasing hormone EGF	HTLV-I: Tax	lipoarabinomannan	Cyclic mechanical muscle strain
Follicle-stimulating hormone	HTLV-II: Tax	PlcA	Exercise $\gamma$ radiation
Mullerian inhibiting substance	Influenza virus: hemagglutinin	PlcB	Heavy ion irradiation
PAF	KSHV: K1	Porins	High-fat diet
Plant steroids (diosgenin, hecogenin, tigogenin)	Parvovirus B19: NSI	Porin 1B	Laminar shear stress
Prostratin	Respiratory syncytial virus: M2-1 SV40	Toxic shock syndrome toxin 1	Dietary lipid hydroperoxide
			PPME
<b>Oncogenes</b>	<b>Bacteria and fungi</b>	<b>Eukaryotic parasites</b>	photosensitization
Ras <sup>115</sup>	<i>Anaplasma phagocytophilum</i>	<i>Eimeria tenella</i> and <i>Eimeria</i>	Mechanical lung ventilation
			Obesity

(Table 1. Contd....)

Myc <sup>117</sup>	<i>Angiostrongylus cantonensis</i>	<i>necatrix</i>	Wounding combined with
bcr-ab1 <sup>116</sup>	<i>Bacteroides forsythus</i>	Leishmania	TUDCA
HeNe irradiation	<i>Bartonella henselae</i>	Phospholipomannan	Tuberous sclerosis complex
Wounding combined with thermal irradiation	<i>Bordetella pertussis</i>		Uremic toxins VLDL
	Cycloprodigiosin	<b>Physiological Mediators</b>	
<b>Oxidative stress</b>	Dacarbazine	Alloxan	<b>Chemical Agents</b>
Glutathione	Orengedeokuto	Angiotensin II	L-NMA
Hydrogen peroxide <sup>224</sup>	Diazoxide	Bradykinin	Ethanol
Catalase	5,6-dimethylxanthenone-4-acetic acid	Fibrinogen	Monensin
Nitric oxide	Flavone-8-acetic acid	Hemoglobin	Nicotine
Ozone	Haloperidol	Hyaluronan	Adriamycin
Peroxyinitrite	Kunbi-Boshin-Hangam-Tang	Thioredoxin	2-Deoxyglucose
Butyl peroxide	Methamphetamine	Kainic acid	Anisomycin
Cerulein	Norepinephrine	L-Glutamate	Benzyl isothiocyanate
Pervanadate	Oltipraz	Melanin	Bisperoxovanadium phosphotyrosyl
Reoxygenation	Phenobarbital	Violacein	Brefeldin A
	Protocatechuic acid	Vitamin D <sub>3</sub>	Cadmium
<b>Environmental hazards</b>		Adenosine	Colchicine
Cigarette smoke <sup>87-88</sup>	<b>Fatty acids</b>	Activin A	Calcium Ionophore plus phorbol ester
PH <sup>91</sup>	Palmitate	Albumin	Calyculin A
Arsenic	Leukotriene B4	Allergin	Ceramide-β-galactose
Polychlorinated biophenyl-77	Free fatty acids	Acrp30/adiponectin	2-chloroethyl ethyl sulfide
Benzo[a]pyrene diol epoxide <sup>87</sup>	Linoleic acid	Adrenomedullin	Con A
Crocidolite asbestos fibers	Oleic acid	<i>Aeginetia indica</i> L (AIL)b-A	Cycloheximide
Dicamba	Saturated fatty acids	Bronchoalveolar lavage fluid	Cyclopiazonic acid
Diesel exhaust particles	Lysophosphatidic acid	Brusatol	Diquat
Gliadin		Homocysteine	2,4-Dinitrofluorobenzene
House dust mite	<b>Modified Proteins</b>	Cryptdins	Ferrocene
Dust particles	LDL	Deoxycholic acid	Forskolin
Fear-potentiated startle response	AGEs	12(R)-hydroxyeicosatrienoic acid	Gadolinium chloride
Lead	Amyloid protein fragment	6-Hydroxydopamine	Glass fibers
Lead chromate	Glycosylated oxyhemoglobin	Human peptide transporter 1	Sodium butyrate trichostatin A
Noise	Maleylated bovine serum Albumin	LTP	Malondialdehyde
Oily fly ash	Neurotrophin receptor proteolytic fragments	LysoPC	MDMA
Silica particles	Nonamyloid beta component of Alzheimer's disease	Neuormelanin	MEN 17055 <sup>113</sup>
Wood smoke	ATM <sup>108</sup>	NS-398	N-methyl-D-aspartate
Zymosan		Osteopontin	Mycophenolic acid
		PAF	Nafenopin
<b>Chemotherapeutic drugs</b>	<b>Heat shock protein</b>	PCSC	N-Nitrosomorphine
Cisplatin	HSP60	Prolactin	Nocodazol
Paclitaxel <sup>103</sup>	HSPb1	PIF	Okadaic acid <sup>89</sup>
Doxorubicin <sup>100</sup>		12-Lipoxygenase	Peplomycin
Tamoxifen		Thrombin	Phytohemagglutinin
Vinblastine	<b>Apoptotic Mediators</b>	Streptozotocin	Phorbol ester <sup>87</sup>
Vincristine <sup>222</sup>	Anti-Fas/Apo-1	Substance P	Podophyllotoxin
Etoposide	PARP	Potassium	Polychlorinated biophenyl-77
ara-C		Burn patient serum	Prostratin
Bryostatin-1	<b>Metals</b>	Anaphylatoxin C3a and C5a	Pyrogallol
Camptothecin <sup>101</sup>	Lithium	Angiotensin II type 1 receptor-activating antibodies	Staurosporine
Daunomycin	Titanium and copper implants	Antiphospholipid antibodies	Transglutaminase 2
Daunorubicin	Nickel sulfate	Basic calcium phosphate crystals	Tunicamycin
Mitoxantrone	Iron	C2-ceramide cerulein	WF10
Gemcitabine <sup>102</sup>	Manganese	Chelidonium majus extract	WY-14 643
	Nickel	Collagen lattice	CFTR
<b>Therapeutically used drugs</b>	Chromium	Collagen type I	GPR56
Celecoxib	Cobalt	Cysteinyl leukotrienes	Hematopoietic progenitor kinase 1
AZT	Aluminum	Des-Arg10-kallidin	Ig heavy chain
Bleomycin		Mixed meal ingestion Monosodium	MHC Class I

(Table 1. Contd...)

WR1065	<b>Natural products</b>	urate crystals	
ABR-25757 SN38	Areca nut extract	Neutrophil elastase	
SA 981	Baicalin	Phellinus linteus proteoglycan	
AdAMP	$\beta$ -carotene	Platelet type arachidonate	
Anthrakinone	Quercetin	Polysaccharides of <i>Poria cocos</i>	
Ciprofibrate	Quinolinic acid	Regulatory RNA	
	Safflower polysaccharides	S100B	
	Sanglifehrin A	Sleep deprivation	

TNF, tumor necrosis factor; RANKL, receptor activator of nuclear factor- $\kappa$ B ligand; IL, interleukin; TRAIL, TNF-related apoptosis-inducing ligand; TWEAK, TNF-like weak inducer of apoptosis; THANK, TNF homologue that activates apoptosis, nuclear factor- $\kappa$ B, and c-Jun NH2 terminal kinase; BAFF, B cell-activating factor; LT, lymphotoxin; LIF, leukemia inhibitory factor; AT, ataxia telangiectasia; Ig, immunoglobulin; GM-CSF, granulocyte macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; NGF, nerve growth factor; EGF, epidermal growth factor; PEDF, pigment epithelium-derived growth factor; PAF, platelet-activating factor; TGF, transforming growth factor; EBV, Epstein-Barr virus; HVS, herpesvirus saimiri; HTLV-1, human T-cell leukemia virus type 1; SARS, severe acute respiratory syndrome; IAP, inhibitor of apoptosis protein; CMV, cytomegalovirus; EBNA, EBV nuclear antigen; LMP1, latent membrane protein 1; HBx, hepatitis B virus X; LHBs, large hepatitis B virus surface protein; MHBst, C-terminally truncated middle surface protein; HCV, hepatitis C virus; KSHV, Kaposi sarcoma herpesvirus; L-NMA, Nomega-methyl-L-arginine; NSI, non-specific lymphadenitis; SV40, simian virus 40; LPS, lipopolysaccharide; PPME, Pyropheophorbide-a methyl ester; LpK, L pyruvate kinase; P1c, poly(rI:rC); UV, ultraviolet; ara-C, 1- $\beta$ -D-arabinofuranosylcytosine; AZT, azidothymidine; AdAMP, 2-1-adamantylamino)-6-methylpyridine; LDL, low-density lipoprotein; AGEs, advanced glycosylated end products; HSP, heat shock protein; PARP, poly(ADP)ribose polymerase; Acip30, adipocyte complement-related protein of 30 kDa; LTP, long-term potentiation, LysoPC, lysophosphatidylcholine; PCSC, polysaccharide from *Poria cocos*; PIF, proteolysis-inducing factor; TUDCA, taurodeoxycholic acid; VLDL, very LDL; MDMA, 3,4-methylenedioxymethamphetamine.

Source: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>, [http://people.bu.edu/gilmore/nf- \$\kappa\$ b/](http://people.bu.edu/gilmore/nf-<math>\kappa</math>b/).

indicates that NF- $\kappa$ B activation is highly complex and may involve dozens of different protein kinases [12-53] (Table 2). Besides NIK, IKK- $\alpha$ , and IKK- $\beta$ , NF- $\kappa$ B activation may also require the involvement of other kinases, such as atypical protein kinase C, protein kinase C-z, pp90rsk, double-stranded RNA-dependent protein kinase, cot kinase (also called TPL2), mitogen-activated protein kinase kinase kinase 1, 2, and 3), phosphatidylinositol 3 protein kinase, Akt, mixed lineage kinase 3, hematopoietic progenitor kinase-1, transforming growth factor  $\beta$ -activated kinase 1, and c-raf kinase. These kinases may form a cascade, and different cascades may form depending on the NF- $\kappa$ B activator. For instance, IKK can be phosphorylated by NIK, mitogen-activated protein kinase kinase, or Akt. Although IKK is required for NF- $\kappa$ B activation by most agents, a few (such as human epithelial receptor type 2, H<sub>2</sub>O<sub>2</sub>, pervanadate, x-rays, and  $\gamma$ -radiation) activate NF- $\kappa$ B through IKK-independent pathways [54]. Although several signaling proteins and protein kinases have been identified recently that mediate NF- $\kappa$ B activation, more kinases and protein phosphatases remain to be identified. Besides the ubiquitin-dependent 26S proteasome, which has a role in I $\kappa$ B $\alpha$  degradation [55], other proteases have also been implicated in NF- $\kappa$ B activation [56].

The genetic deletions of different NF- $\kappa$ B proteins produce numerous phenotypic changes [57]. For instance, deletion of the *rel* a gene induced embryonic lethality in mice, probably due to massive apoptosis in the liver [58]. In addition, the mouse embryo fibroblasts (MEFs) from *rel* a-deletion mice were found to be hypersensitive to TNF-induced apoptosis. These results indicate a negative role for NF- $\kappa$ B in TNF-induced apoptosis. Furthermore, mice lacking the RelA subunit were brought to term only in a TNFR1-deficient background [59]. These mice lacked lymph nodes, Peyer's patches, and an organized splenic microarchitecture, and they had a profound defect in their T-cell-dependent antigen responses. Analyses of TNFR1/RelA-deficient embryonic tissues and of radiation chimeras suggest that the dependence on RelA is manifested not in hematopoietic cells

but rather in radioresistant stromal cells, which are needed for the development of secondary lymphoid organs. In contrast to the deletion of Rel A, the deletion of the I $\kappa$ B $\alpha$  gene leads to early neonatal lethality caused by inflammatory dermatitis and granulocytosis [60] that are most likely induced by constitutive activation of NF- $\kappa$ B, leading to expression of the granulocyte colony-stimulating factor.

NF- $\kappa$ B activation has been implicated in a wide variety of diseases, including cancers, diabetes mellitus, cardiovascular diseases, autoimmune diseases, viral replication, septic shock, neurodegenerative disorders, ataxia telangiectasia (AT), arthritis, asthma, inflammatory bowel disease, and several other inflammatory conditions (Table 3). For example, activation of NF- $\kappa$ B by LPS may contribute to the development of septic shock because NF- $\kappa$ B activates transcription of the inducible nitric oxide synthase (iNOS) genes known to be involved in septic shock [61]. Similarly, autoimmune diseases such as systemic lupus erythematosus may also involve activation of NF- $\kappa$ B. Additionally, in chronic Alzheimer's disease, the amyloid  $\beta$  peptide causes production of reactive oxygen intermediates and indirectly activates gene expression through  $\kappa$ B sites [62]. The influenza virus protein hemagglutinin also activates NF- $\kappa$ B, and this activation may contribute to viral induction of cytokines and to some of the symptoms associated with influenza [63]. Furthermore, the oxidized lipids from the low density lipoproteins associated with atherosclerosis activate NF- $\kappa$ B, which then activates other genes [64], and mice that are susceptible to atherosclerosis exhibit NF- $\kappa$ B activation when fed an atherogenic diet [65]. Another important contributor to atherosclerosis is thrombin, which stimulates the proliferation of vascular smooth muscle cells through the activation of NF- $\kappa$ B [66]. Finally, a truncated form of I $\kappa$ B $\alpha$  was shown to protect AT cells, which express constitutive levels of an NF- $\kappa$ B-like activity, from ionizing radiation [67]. In light of all these findings, the abnormal activation or expression of NF- $\kappa$ B is clearly associated with a wide variety of pathologic conditions. How NF- $\kappa$ B activation mediates tumorigenesis is the focus of the current review.

**Table 2. A List of Protein Kinases Implicated in NF- $\kappa$ B Activation**

Kinase	Ligand
IKK $\alpha$	Proinflammatory cytokines (eg TNF, IL-1, IL6), LPS <sup>20-22</sup>
IKK $\beta$	Proinflammatory cytokines (eg TNF, IL-1, IL6), LPS <sup>20-22</sup>
IKK $\gamma$	Proinflammatory cytokines (eg TNF, IL-1, IL6), LPS <sup>14,15</sup>
IKKi/IKK $\epsilon$	TNF, PMA <sup>16-18</sup>
NIK	TNF, CD95, and IL-1 <sup>19</sup>
NAK	Phorbol esters, growth factors <sup>38-40</sup>
PI3K	IL-1 <sup>41</sup>
Akt1	TNF, TCR/CD28 <sup>31,42</sup>
HPK-1	Unknown <sup>44</sup>
PKC- $\alpha$	TPA, TNF, Bimp1, Bcl 10/MALT1 <sup>26-28</sup>
PKC- $\beta$	IgM receptor, Bimp1, Bcl 10/MALT1 <sup>27,29</sup>
PKC- $\tau$	CD3-CD28 (TCR/CD28), Bimp 1, Bcl 10/MALT1 <sup>27,30,31</sup>
PKC- $\delta$	TNF $\alpha$ <sup>32</sup>
PKC- $\epsilon$	Phorbol ester, Bimp1, Bcl 10/ MALT1 <sup>27,33</sup>
aPKC	TNF, ras p21 <sup>32,23</sup>
MEKK1/2	Proinflammatory cytokines <sup>22-36</sup>
MEKK3	TNF, other proinflammatory cytokines <sup>22,37</sup>
TPL-2/Cot kinase	TNF <sup>34</sup>
pp90rsk	<i>Pseudomonas aeruginosa</i> <sup>54</sup>
Raf-1 kinase	Serum growth factors, phorbol ester, and PTK oncogenes <sup>45</sup>
PKR	dsRNA <sup>33</sup>
TAK1/MEKK	XIAP, TGF- $\beta$ <sup>44</sup>
MLK3	CD3/CD28 <sup>42</sup>
PAK1	p21, LPS <sup>46</sup>
BTK	B-cell antigen receptor <sup>47</sup>
JAK2	Erythropoietin <sup>12</sup>
PKA	IL-1, LPS, many others <sup>48,49</sup>
IRAK-1	IL-1 <sup>50</sup>
IRAK-2	IL-1, LPS <sup>52</sup>
IRAK-M	IL-1, LPS <sup>52</sup>
P56 lck	Ceramide <sup>53</sup>
Syk	TNF, H <sub>2</sub> O <sub>2</sub> <sup>224</sup>

IKK,  $\kappa$ B kinase; TNF, tumor necrosis factor; IL, interleukin; LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; NIK, NF- $\kappa$ B-inducing kinase; NAK, NF- $\kappa$ B-activating kinase (also called T2K-, TBK1-, or TRAF2-interacting kinase, PI3K, phosphatidylinositol 3 kinase; Akt, protein kinase B $\alpha$ ; TRC, T-cell receptor; HPK, hematopoietic protein kinase; PKC, protein kinase C; TPA, 12-O-tetradecanoylphorbol-13-acetate; MALT, mucosa-associated lymphoid tissue; IgM, immunoglobulin M; aPKC, atypical PKC; MEKK, mitogen-activated protein kinase kinase; pp90rsk, ribosomal protein S6 kinase; PKR, RNA-dependent protein kinase; dsRNA, double-stranded RNA; XIAP, X-linked inhibitor of apoptosis; TGF, transforming growth factor; MLK, mixed lineage kinase; PAK, p21 activated; BTK, Bruton's tyrosine kinase; JAK, Janus kinase; PKA, protein kinase A; IRAK, IL-1 receptor-associated kinase; Syk, spleen tyrosine kinase.

## 2. NF- $\kappa$ B MEDIATES CARCINOGENESIS

NF- $\kappa$ B has been implicated in carcinogenesis because it plays a critical role in cell survival, cell adhesion, inflammation, differentiation, and cell growth. Cancer is a hyperproliferative disorder that results from tumor initiation and tumor promotion and ultimately produces tumor metastasis. Notably, several genes involved in cellular transformation, proliferation, invasion, and angiogenesis are regulated by NF- $\kappa$ B.

### 2.1. NF- $\kappa$ B Genes are Proto-Oncogenes

NF- $\kappa$ B genes are members of a proto-oncogene family, and many functions of their encoded proteins have obvious implications for the development of cancer and its therapy. Retroviruses encoding *v-rel* were shown to be oncogenic in avian species, and *rel* genes to be prone to rearrangements and translocations [68]. For example, tumors with *rel* amplification had an increased frequency of chromosomal aberrations previously associated with tumor progression,

**Table 3. Association of NF- $\kappa$ B Activation with Major Diseases**

Cancer	Inflammatory diseases
B-cell lymphoma	Alzheimer's disease
Breast cancer	Arthritis
Hodgkin's disease	Asthma
Liver cancer	Bone resorption
Mantle cell lymphoma	Chronic obstructive pulmonary disease
Multiple myeloma	Crohn's disease
Non-Hodgkin's lymphoma	Huntington's disease
Ovarian cancer	Inflammatory bowel disease
Prostate cancer	Inflammatory response syndrome
T-cell lymphoma	Multiple sclerosis
Thyroid cancer	Ocular allergy
Bladder cancer	Parkinson's disease
Colon cancer	
Esophageal cancer	<b>Other diseases</b>
Head and neck squamous cell carcinoma	Aging
Laryngeal cancer	AIDS
Lung cancer	Catabolic disorders
Pancreatic cancer	Ectodermal dysplasia
Pharyngeal cancer	Gut diseases
Renal carcinoma	Headache
Acute lymphoblastic leukemia	<i>Helicobacter pylori</i> -associated gastritis
Adult T-cell leukemia	Incontinentia pigmenti
Cervical cancer	Ischemia/reperfusion injury
Nasopharyngeal carcinoma	Systemic lupus erythematosus
Melanoma	Muscular dystrophy
	Neuropathologic diseases
<b>Cardiovascular diseases</b>	Psychosocial stress diseases
Atherosclerosis	Renal diseases
Cardiac hypertrophy	Sepsis
Heart failure	Skin diseases
Hypercholesterolemia	Sleep apnea
	Viral infection
<b>Diabetes mellitus</b>	
Type I	
Type II	

Source: <http://www.ncbi.nih.gov/entrez/query.fcgi>, <http://people.bu.edu/gilmore/nf-kb/>.

thus suggesting an oncogenic effect of amplified *rel* in B-lymphoid cells already containing a transforming genetic lesion. According to one study's findings, *rel* amplification is a frequent event in diffuse lymphoma with a large-cell component and probably constitutes a progression-associated marker of primary extranodal lymphomas [69]. The *rel* proto-oncogene has been mapped to chromosome region 2p11.2-14, a site associated with nonrandom rearrangements in non-Hodgkin's lymphoma. Lu *et al.* [28] characterized an

abnormal *rel* mRNA from a cell line derived from a diffuse large cell lymphoma in which the evolutionarily conserved N-terminal half of the *rel* coding region was fused with the C-terminal coding region of an unrelated gene. In addition, the rearrangement or amplification of the *rel* locus was found in the lymphomatous tissue of two follicular and one diffuse large cell lymphoma [28]. These findings suggest the involvement of *rel* in the pathogenesis of large-cell lymphoma [70].

Recent studies have also implicated the p50 subunit of the NF- $\kappa$ B transcription factor complex in tumorigenesis. Mukhopadhyay *et al.* [29] investigated the expression of this complex in paired normal and non-small cell lung carcinoma (NSCLC) tissues and found that 81.8% of fresh NSCLC tissues expressed from two- to 20-fold the levels of the p50 subunit compared with normal lung tissue. Thirteen NSCLC cell lines also exhibited high levels of p50. Such alterations in the normal NF- $\kappa$ B/*rel* pathway of regulation may therefore play a role in the genesis of NSCLC [71]. Alterations in the Rel A protein were also implicated in squamous head and neck carcinoma [72], adenocarcinomas of the breast and stomach [73], thyroid carcinoma [74], and multiple myeloma [75]. In addition, the overexpression of the p50 subunit of NF- $\kappa$ B has been detected in cell lines derived from cancers of the lung, prostate, breast, bone, and brain [71].

In certain lymphomas, the *bcl-3* and *nf- $\kappa$ b2* genes are known to be translocated [76]. Furthermore, mutations in the *ikb $\alpha$*  gene were observed in Hodgkin's lymphoma [77], and blocking of *ikb $\alpha$*  by antisense mechanisms appears to induce oncogenic transformation [78]. Additionally, antisense to *relA* blocks tumorigenesis induced by Tax, a protein derived from human T-cell leukemia virus type 1 [79]. A recent study similarly showed that human breast tumors accumulate activated NF- $\kappa$ B complexes consisting of p50, p52, and Bcl-3 rather than p65 [7].

In 1992, Liptay *et al.* [38] discovered that the NF- $\kappa$ B subunits p49/p100 and p105 map to regions associated with certain types of acute lymphoblastic leukemia. During molecular characterization of translocations associated with some human T-cell acute lymphocytic leukemias, the LYL1 gene was first identified. In adult tissues, LYL1 expression was restricted to hematopoietic cells, with the notable exception of cells of the T-cell lineage. LYL1 encodes a basic helix-loop-helix protein that is highly related to TAL-1, whose activation is also associated with a high proportion of human T-cell acute lymphocytic leukemias. Liptay *et al.* found that p105, the precursor of NF- $\kappa$ B1 p50, was the major LYL1-interacting protein in this system. Ectopic expression of LYL1 in a human T-cell line caused a significant decrease in NF- $\kappa$ B-dependent transcription and a reduced level of NF- $\kappa$ B1 proteins [80]. It was also reported that p105/p50 is altered in cancers of the bone, colon, prostate, breast, and brain [81].

Alterations in p100/p52 implicated in chronic lymphocytic leukemia, multiple myeloma, and cutaneous T-cell lymphomas [82]. In addition, NF- $\kappa$ B2/p52 (lyt-10) was shown to be involved in the breakpoint of at (10;14)(q24;q32) chromosomal translocation in a case of B-cell lymphoma. Fracchiolla *et al.* [42] have demonstrated that lyt-10 gene

rearrangements are recurrent lesions that may be involved in the pathogenesis of both B-cell and T-cell malignancies and suggested that the truncation of the ankyrin domain is a common mechanism leading to abnormal I $\kappa$ B activation in lymphoid neoplasia. These authors also showed in an MDA-MB-435 breast cancer model that most p65 protein is complexed with p100 in these cells but predominantly with I $\kappa$ B $\alpha$  in cell lines expressing less p100. Based on these observations, Dejardin *et al.* [43] hypothesized that NF- $\kappa$ B is involved in carcinogenesis and suggested that the p100/p52 NF- $\kappa$ B subunit plays a role in the development of human breast cancers, possibly by sequestering other NF- $\kappa$ B-related proteins in the cytoplasm.

## 2.2. Constitutively Active NF- $\kappa$ B is Common in Tumor Cells

Constitutive expression of NF- $\kappa$ B has been shown in cell lines derived from breast, ovarian, colon, pancreatic, thyroid, prostate, lung, head and neck, bladder, and skin tumors [83]. This has also been seen for B-cell lymphoma, Hodgkin's disease, T-cell lymphoma, adult T-cell leukemia, acute lymphoblastic leukemia, multiple myeloma, chronic lymphocytic leukemia, and acute myelogenous leukemia.

Besides these cell lines, activated NF- $\kappa$ B has also been noted in tissue samples from patients with cancer. For instance, NF- $\kappa$ B is constitutively activated in high-grade squamous intraepithelial lesions and squamous cell carcinomas of the human uterine cervix [84]. NF- $\kappa$ B was also implicated in an aggressive phenotype of renal cell carcinoma [47]. Of 45 cases of renal cell carcinoma, 33% showed an NF- $\kappa$ B activity that was greater than 200% higher than that of normal renal tissue. In locally advanced cases, 64% showed an increased activity, and tissue from their metastases showed an even greater increase. In addition, the elevation of patients' serum C-reactive protein levels correlated with increases in the NF- $\kappa$ B activation in their tumors; therefore, NF- $\kappa$ B may be a cause of the inflammatory paraneoplastic syndrome [85]. Along similar lines, Yu *et al.* [48] reported that increased expression of NF- $\kappa$ B in colorectal tumorigenesis plays an important role in the pathogenesis of colon cancer in humans by mediating the transition from colorectal adenoma with low-grade dysplasia to adenocarcinoma.

Why tumor cells express constitutively active NF- $\kappa$ B, however, is not fully understood. The aberrant IKK activity and shorter half-life of I $\kappa$ B $\alpha$  in B-cell lymphoma, the mutated I $\kappa$ B $\alpha$  in Hodgkin's lymphoma, the IL-1 $\beta$  production in acute myelogenous leukemia, and the TNF production in cutaneous T-cell lymphoma and Burkitt's cell lymphoma are some of the reasons that have been postulated for constitutive NF- $\kappa$ B activation [57].

## 2.3. Carcinogens, Inflammatory Agents, Pro-Oxidants, and Stress and Tumor Promoters Activate NF- $\kappa$ B

During the last few years, various carcinogens have been shown to activate NF- $\kappa$ B, including 7,12-dimethylbenz(a)anthracene (DMBA) [86], benzo(a)pyrene diol epoxide, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, and nicotine (for a review, see reference[87]) (Table 1). For example, DMBA-induced NF- $\kappa$ B activation was shown to occur not

only *in vitro* but also when DMBA was administered *in vivo* to animals [49]. Furthermore, our laboratory showed that cigarette smoke condensate is a potent activator of NF- $\kappa$ B in different cell types [88]. Unlike TNF-induced NF- $\kappa$ B activation, the NF- $\kappa$ B activation induced by cigarette smoke condensate was persistent [87]. Besides tumor initiators, tumor promoters such as phorbol ester and okadaic acid have also been shown to activate NF- $\kappa$ B [89]. Additionally, NF- $\kappa$ B is activated by hypoxia [90] and an acidic pH [91], both characteristics of the tumor microenvironment.

TNF is one of the most potent activators of NF- $\kappa$ B. Although initially identified as an anticancer agent [92], TNF has since been shown to be involved in cellular transformation [93], tumor promotion [94], and induction of metastasis [95]. In agreement with these observations, mice deficient in TNF were shown to be resistant to skin carcinogenesis [96]. For several tumors, TNF has even been shown to be a growth factor [97]. Like phorbol ester, TNF mediates these effects in part by activating a protein kinase C pathway [98]. Other inflammatory cytokines have also been implicated in tumorigenesis [99].

## 2.4. Chemotherapeutic Agents and $\gamma$ -Radiation Induce NF- $\kappa$ B Activation

Besides inducing apoptosis, almost all chemotherapeutic agents also activate NF- $\kappa$ B (Table 1). These include DNA-damaging agents such as doxorubicin (DoxR) [100], camptothecin [101], gemcitabine [102], and cisplatin; microtubule depolymerizing agents such as taxol [103]; alkylating agents such as melphalan [104]; and the glutathione reductase inhibitor 1,3-bis(2-chloroethyl)-1-nitrosourea [105]. Similarly,  $\gamma$ -radiation, x-rays, and UV radiation have also been shown to activate NF- $\kappa$ B [106]. Egan *et al.* [107] found that I $\kappa$ B-kinase- $\beta$ -dependent NF- $\kappa$ B activation provides radioprotection to the intestinal epithelium, and Panta *et al.* [108] found that the ataxia telangiectasia mutated (ATM) gene/protein and the catalytic subunit of DNA-dependent protein kinase activate NF- $\kappa$ B through a common mitogen-activated protein kinase/extracellular signal-regulated kinase/p90(rsk) signaling pathway in response to distinct forms of DNA damage. Why these proapoptotic agents activate NF- $\kappa$ B, however, is not clear.

Although in most instances NF- $\kappa$ B mediates chemoresistance [109] or radioresistance [110], in other cases it may mediate apoptosis [111]. Additionally, chemotherapeutic agents activate NF- $\kappa$ B by mechanisms that appear to differ from that of TNF. The antiapoptotic activity of NF- $\kappa$ B in tumors contributes to their acquired resistance to chemotherapy.

Degradation of I $\kappa$ B is a seminal step in the activation of NF- $\kappa$ B. The I $\kappa$ B kinases, IKK $\alpha$  and IKK $\beta$ , have been implicated in both I $\kappa$ B degradation and subsequent modification of NF- $\kappa$ B. Using MEFs devoid of both IKK $\alpha$  and IKK $\beta$  genes (IKK $\alpha/\beta$ (-/-)), Tergaonkar *et al.* [112] showed that the novel I $\kappa$ B degradation mechanism induced by the chemotherapeutic agent DoxR does not require the classic serine 32 and 36 phosphorylation or the proline-glutamic acid-serine-threonine domain of I $\kappa$ B $\alpha$ . The degradation of I $\kappa$ B $\alpha$  was also shown to be partially blocked by the phosphatidylinositol 3-kinase inhibitor LY294002 and to be



mediated by the proteasome. The free NF- $\kappa$ B generated by the DoxR-induced I $\kappa$ B degradation in IKK $\alpha$ / $\beta$ (-/-) cells activated the chromatin-based NF- $\kappa$ B reporter gene and the expression of the endogenous target gene, I $\kappa$ B $\alpha$  [112]. These results also imply that modification of NF- $\kappa$ B by IKK $\alpha$  or IKK $\beta$ , either prior or subsequent to its release from I $\kappa$ B, is not essential for NF- $\kappa$ B-mediated gene expression, at least in response to DNA damage. In addition, DoxR-induced cell death in IKK $\alpha$ / $\beta$ (-/-) MEFs was enhanced by the simultaneous inhibition of NF- $\kappa$ B activation by the blockage of proteasomal activity [112]. These findings show an additional pathway for activating NF- $\kappa$ B during anticancer therapy and provide a mechanistic basis for the observation that proteasome inhibitors can be used as adjuvant agents in chemotherapy. Thus, DoxR-induced NF- $\kappa$ B activation is an IKK-independent I $\kappa$ B $\alpha$  degradation pathway.

The new disaccharide anthracycline MEN 10755 induces activation of both NF- $\kappa$ B and p53 transcription factors in A2780 cells [113]. However, the pharmacologic inhibition of NF- $\kappa$ B activation does not modify the sensitivity of A2780 cells to MEN 10755 treatment. To better characterize the role of NF- $\kappa$ B in MEN 10755-induced cytotoxicity, Camarda *et al.* [113] analyzed the expression of a number of genes known to be regulated by NF- $\kappa$ B and found that none of them was modified by MEN 10755. On the contrary, as our results also suggest, the p53 DNA damage-responsive pathway is fully activated in A2780 cells, with several genes controlled by p53 being either up- or downregulated, according to the described action of p53 on their promoters [113]. Thus, in the A2780 cell line, the role of p53 in transducing the DNA-damage signal appears to be relevant, whereas NF- $\kappa$ B, although activated, appears to be non-functional. Other human carcinoma cell lines also activate NF- $\kappa$ B DNA binding in response to MEN 10755, but again this binding does not always lead to target gene activation. These results suggest that other tumor type-specific factors different from those that merely activate influence NF- $\kappa$ B transcriptional activity. Therefore, care should be taken when considering the pharmacologic inhibition of NF- $\kappa$ B as a means to improve anticancer therapy.

Chemotherapeutic agents simultaneously induce transcription factors p53 and NF- $\kappa$ B. p53 induction can activate an apoptotic program, and resistance to chemotherapy correlates with the loss of a functional p53 pathway. By contrast, NF- $\kappa$ B prevents apoptosis in response to chemotherapeutic agents. Tergaonkar *et al.* [114] analyzed the p53 response in IKK $\alpha$ / $\beta$ (-/-) MEFs, which lack detectable NF- $\kappa$ B activity. Compared with wild-type fibroblasts, IKK $\alpha$ / $\beta$ (-/-) fibroblasts showed increased cell death and p53 induction in response to the chemotherapeutic agent DoxR [114]. Reconstitution of IKK $\beta$ , but not IKK $\alpha$ , increased murine double minute 2 (Mdm2) levels and decreased DoxR-induced p53 stabilization and cell death. IKK $\beta$ -mediated effects required its kinase function and were abrogated by coexpression of the dominant negative mutant I $\kappa$ B $\alpha$  (I $\kappa$ B $\alpha$ M), thus suggesting a role for NF- $\kappa$ B in the blocking of chemotherapy-induced p53 and cell death.

### 2.5. Oncogenes Activate NF- $\kappa$ B

Several oncogene products that can activate NF- $\kappa$ B have been identified, including *ras* [115], *bcr-abl* [116], and *myc*

[117]. Oncogenic Ha-Ras-induced signaling also activates NF- $\kappa$ B transcriptional activity, which is required for cellular transformation [118]. How these genes induce NF- $\kappa$ B activation, however, is poorly understood. Oncogenic Ras enhances NF- $\kappa$ B transcriptional activity through Raf-dependent and Raf-independent mitogen-activated protein kinase signaling pathways [119]. NF- $\kappa$ B activation was also found to be required for apoptosis induced by oncogenic Ras [120]. As many as 50% of all cancers (mostly solid tumors) express activated ras [121], which can lead to NF- $\kappa$ B activation. Bcr-abl, which has been shown to cause chronic myelogenous leukemia, can elicit survival signals through induction of NF- $\kappa$ B [122]. NF- $\kappa$ B activation is also required in Bcr-abl-mediated transformation. Finally, the oncogene *c-myc*, which can mediate tumor cell survival, has also been shown to be regulated by NF- $\kappa$ B [117].

### 2.6. NF- $\kappa$ B Activation is Required for Cell Proliferation

Several genes that mediate cell proliferation are also regulated by NF- $\kappa$ B, including the growth factors TNF, IL-1 $\beta$ , and IL-6 [123]. For instance, TNF was shown to be a growth factor for glioblastoma cells [124] and cutaneous T-cell lymphomas [97], IL-1 $\beta$  for acute myelogenous leukemia [125], and IL-6 for multiple myeloma [126] and head and neck squamous cell carcinoma [127]. Suppression of NF- $\kappa$ B in these tumors downregulates the cytokine expression and inhibits tumor cell proliferation. Besides growth factors, certain cell cycle regulatory proteins such as cyclin D1, which is required for transition of cells from the G1 to S phase, are also regulated by NF- $\kappa$ B [128]. Additionally, in some cells, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) was shown to induce proliferation of tumor cells, and the synthesis of cyclooxygenase-2, which controls PGE<sub>2</sub> production, was also shown to be regulated by NF- $\kappa$ B activation [129].

The constitutive activation of NF- $\kappa$ B also appears to have a role in cell proliferation. Bargou *et al.* [130] showed that proliferation of Hodgkin/Reed-Sternberg cells depended on activation of NF- $\kappa$ B. Furthermore, constitutive NF- $\kappa$ B prevented Hodgkin's lymphoma cells from undergoing apoptosis under stress conditions [130].

It was further shown that growth factors such as epithelial growth factor (EGF) and platelet-derived growth factor induce proliferation of tumor cells through activation of NF- $\kappa$ B [131]. The EGF receptor was found to engage a receptor-interacting protein and NIK to activate NF- $\kappa$ B, thus identifying a novel receptor-tyrosine kinase signalosome [132]. NF- $\kappa$ B signaling was also shown to promote both cell survival and the formation of neurite processes in nerve growth factor-stimulated PC12 cells [133].

### 2.7. Activation of NF- $\kappa$ B Inhibits Apoptosis

Both the prosurvival and antiapoptotic functions of NF- $\kappa$ B were recently reviewed [134]. Several gene products that negatively regulate apoptosis in tumor cells, including inhibitor of apoptosis proteins (IAPs) 1 and 2, X-linked IAP, cellular Fas-associated death domain-like interleukin-1 $\beta$ -converting enzyme (FLICE)-like inhibitory protein (cFLIP), were shown to be controlled by NF- $\kappa$ B activation (Table 4) [134]. For example, Bcl-x<sub>L</sub> suppressed cytochrome C release from the mitochondria, the IAPs inhibited caspase-3 and caspase-9 [135], and FLIP inhibited caspase-8 [136]. A n

**Table 4. A List of Genes Regulated by NF- $\kappa$ B**

Cytokines	Receptors	TIEG	Cell proliferation	P450	Miscellaneous
TNF $\alpha$ <sup>123</sup>	$\beta$ 2 microglobulin		COX-2 <sup>129</sup>	CYP2C11	$\alpha$ -1 Acid glycoprotein
TNF $\beta$	$\mu$ -opioid receptor	<b>Transcription factors</b>	c-myc	CYP2E1	a1-Antitrypsin,
IL-1 $\alpha$ <sup>123,125</sup>	2A	NF- $\kappa$ B1			a2(1)-Collagen
IFN- $\alpha$	A1 Adenosine receptor	NF- $\kappa$ B2	<b>Cell cycle</b>	<b>Oxidative stress</b>	$\alpha$ -Fetoprotein
IFN- $\gamma$	Amiloride-sensitive sodium channel	c-Rel	Cyclin D1 <sup>128</sup>	XOD	AMH
IL-1 $\beta$ <sup>123,126</sup>	Androgen receptor	p53	p21	GST	Apolipoprotein C III
TRAIL	B 7.1	RelB	Cyclin D2	Mn SOD	$\beta$ -Amyloid
M-CSF	Bradykinin B-1 receptor	I $\kappa$ B $\alpha$	Cyclin D3	iNOS	Biglycan
RANTES	BRL-1	c-fos	GADD 45 $\beta$		Caveolin-1
FAS ligand	CCR 5	c-myb		<b>Enzymes</b>	Claudin-2
Lymphotoxin $\alpha$	CCR7	JunB	<b>Viruses</b>	$\gamma$ -GCS	Clone 156
Lymphotoxin $\beta$	CD 137	E2F3a	HIV-1	11bGSD2	Clone 330
G-CSF	CD 154	Elf 3	Epstein-Barr virus (Wp promoter)	12-LOX	Clone 68
GM-CSF	CD 23	ELYS	Hepatitis B virus (pregenomic promoter)	5-LOX	Connexin 32
IL-2	CD 40	ETR 1001	HSV	ABC transporters	Epsilon-globin
IL-6 <sup>86</sup>	CD 48	IRF-1	SV-40	ADH	Factor VIII
IL-8 <sup>111</sup>	CD 69	IRF-2	Adenovirus (E3 region)	ARFRP 1	Gadd45 $\beta$
IL-9	CD 83	IRF-4	Avian leukosis virus	Aromatase	Galectin 3
IL-10	CD 95	IRF-7	Bovine leukemia virus	BACE	GIF
IL-11	EGFR <sup>132</sup>	Mail	Cytomegalovirus	Catepsin B and ceramide glycosyl transferase	GS 3686
IL-12	Gal 1 receptor	Nurr1	JC virus	Collagenase 1	HMG-14
IL-13	GFBP-2	Stat5a	Human papillomavirus type 16	CRAD1 and 2	K15 keratin
IL-15	Glucocorticoid receptor	WT1	SIV	Dihydrodiol dehydrogenase	K3 keratin
NK4	IGFBP-1	<b>Metastasis</b>		DT-diaphorase	K3 keratin
GCP-2 (CXCL6)	IL-1 receptor antagonist	ICAM-1	<b>Acute phase response proteins</b>	Gelatinase B	K6 keratin
Gro $\alpha$	IL-2 receptor $\alpha$ -chain	VCAM-1	Angiotensinogen	GSTP1-1	Laminin B2 chain
Gro $\gamma$	Immunoglobulin $\gamma$ 4	ELAM-1	$\beta$ -defensin-2	Guanylyl cyclase $\alpha$	mCGM3
Gro-1	Immunoglobulin C $\gamma$ 1	E-selectin	C4b binding protein	H <sup>+</sup> -K <sup>+</sup> -ATPase a2	Mts1
ICOS	Immunoglobulin E heavy chain	Endoglin	Complement factor B	Heparanase	MUC-2
IL-1 receptor antagonist	Immunoglobulin $\kappa$ light chain	Fibronectin	Complement factor C4	HO-1	Mx 1
IP-10	Invariant chain II	MadCAM-1	C-reactive protein	Hyaluronan synthase	Neutrophil gelatinase-associated lipocalin
KC	Lox-1	P-selectin	LPS binding protein	Iodothyronine deiodinase	NLF 1
BMP-2	Mdr 1	Tenascin-C	Pentraxin PTX3	Lysozyme	P11
CCL15	MHC class (HLA-B7)	DC-SIGN	SAA1 and SAA2	MKP-1	PAI-1
CCL22	MHC class I (H-2Kb)	MMP-3	Tissue factor-1	N-	Pax 8
CCL28	Neuropeptide YY1-receptor	MMP-9 <sup>139</sup>	Urokinase-type plasminogen activator	Acetylglucosaminyltransferase 1	PCBD
CCL5	NMDA receptor subunit NR-1	CXCR4 <sup>151</sup>		NGAL	Perforin
CD40 ligand	NMDA receptor subunit	KAI1/CD82 <sup>137</sup>		NQO1	PGK
CINC-1		uPA <sup>142</sup>		PDE 7A1	PSA
CXCL 11		<b>Antiapoptosis</b>	<b>Proteins involved in antigen presentation</b>	PGES/L-PGDS	RICK
ENA-78 (CXCL5)		Bcl-2 <sup>134</sup>	Tapasin	Phospholipase A2	S100A6 (calcyclin)
Eotaxin		Bcl-x <sub>L</sub> <sup>134</sup>			Spergen-1
Erythropoietin		Bfl1/A1 <sup>134</sup>			Syndecan-4
MCP-1/JE					TFPI-2

(Table 4. Contd....)

MIP-1 $\alpha$ , $\beta$	Nod 2	TRAF-1 <sup>134</sup>	LMP 2	Phospholipase C $\delta$ 1	Transferrin
MIP-2	Polymeric Ig receptor	TRAF-2 <sup>134</sup>	TAP 1	PIM-1	Urokin 16
MIP-3 $\alpha$	RAF receptor 1	TRAF6	Complement B	PTGIS	UBE 2M
Mob-1	RAGE	c-FLIP <sup>134</sup>	Complement receptor 2	RACK 1	UCP-2
Neutrophil-activating peptide 78	T-cell receptor $\beta$ chain	IAPs <sup>134</sup>	Complement component 3	REV 3	Vimentin
NK-1R	T-cell receptor/ CD3 $\gamma$	Survivin <sup>135</sup>		Seprin 2A	Wilms' tumor suppressor gene
Stem cell factor	TNF-Receptor, p75/80	A20		SNARK	
Angiotensinogen	DR4 <sup>167</sup>	Bax	<b>Kinases</b>	TERT	
TCA3	DR5 <sup>167</sup>	Caspase-11	PI3K <sup>143</sup>	Transglutaminase	
TFF3		FAP-1	PIK3		
TSP-1	<b>Early response genes</b>	Fas-ligand	PKC $\delta$		
TSP-2	p62	IEX-1L	MAP 4K1		
	p22/RPG1	Nr13			
	B 94				
	Egr-1	<b>Angiogenesis</b>			
		VEGF <sup>144,145</sup>			

TNF, tumor necrosis factor; IL, interleukin, IFN, interferon; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; M-CSF, macrophage colony-stimulating factor; RANTES, regulated upon activation, normal T-cell expressed and secreted; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; NK, neurokinin; GCP, granulocyte chemotactic protein; CXCL, CXC chemokine ligand; Gro, growth-related oncogene; ICOS, inducible costimulator; IP-10, IFN- $\gamma$ -inducible protein 10; KC, Kupffer cells; BMP, bone morphogenic protein; CCL, chemokine ligand; CINC, cytokine-induced neutrophil chemoattractant; CXCL, CXC ligand, ENA-78, epithelial cell-derived neutrophil-activating protein 78; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; TCA, T-cell activation; TFF3, trefoil factor 3; Tsp, thrombospondin; BRL, ; EGFR, epidermal growth factor receptor; GFBP, growth factor binding protein; IGFBP, insulin-like GFBP; Lox-1, lectin-like oxidized low-density lipoprotein receptor; Mdr, Mdr1, multidrug resistance mediator 1; MHC, major histocompatibility complex; NMDA, N-methyl-D-aspartate; Ig, immunoglobulin; RAGE, receptor of advanced glycation end products; DR, death receptor; Egr-1, early growth response 1; TIEG, TGF $\beta$ -inducible early gene; NF- $\kappa$ B, nuclear factor  $\kappa$ B; IRF, interferon regulatory factor; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule 1; ELAM, endothelial leukocyte adhesion molecule; MadCAM-1, mucosal addressin cell adhesion molecule; DC-SIGN, dendritic cell surface C-type lectin; MMP, matrix metalloproteinase; CXCR, CXC chemokine receptor; TRAF, TNF-receptor associated factor; c-FLIP, c-FLICE inhibitory protein; IAPs, inhibitors of apoptosis; IEX-1L, immediate early response factor 1; VEGF, vascular endothelial growth factor; COX-2, cyclooxygenase-2; GADD, growth arrest and DNA damage-inducible; HSV, herpes simplex virus; SV-40, simian virus 40; SIV, simian immunodeficiency virus; SAA, serum amyloid A proteins; LMP, latent membrane protein; PI3K, phosphatidylinositol 3 kinase; PIK3, polo-like kinase 3; MAP, mitogen-activated protein; CYP, cytochrome p450; XOD, xanthine oxidase; GST, glutathione S-transferase; Mn SOD, superoxide dismutase; iNOS, inducible nitric oxide synthase; GCS, glutamylcysteine synthetase; LOX, lipoxigenase; ADH, alcohol dehydrogenase; ARFRP 1, ARF-related protein 1; CRAD, conditionally replicating adenoviruses; NQO1, NAD(P)H quinone oxidoreductase 1; TERT, telomerase catalytic subunit; AMH, anti-mullerian hormone; HMG-14, high mobility group 14; Nod 2, Nucleotide oligomerization domain 2; TAP1, Human transporter associated with antigen processing 1; BACE,  $\beta$ -secretase, GSTP1, glutathione S-transferase P1; MKP-1, MAP kinase phosphatase-1; NGAL, Neutrophil gelatinase-associated lipocalin; NQO, NAD(P)H:quinone oxidoreductase; PGE, PGE synthase, L-PGDS, lipocalin-type PGD synthase, PIM, phosphatidylinositol; PTGIS, prostacyclin synthase; Rack1, Receptor for activated C kinase 1; SNARK, SNF1/AMP kinase-related kinase; GIF, growth inhibitory factor; PSA, prostate-specific antigen; RICK, Rip-like interacting caspase-like apoptosis-regulatory protein kinase; TFPI, tissue factor pathway inhibitor; UCP, uncoupling protein. Source: <http://www.ncbi.nih.gov/entrez/query.fcgi>, <http://people.bu.edu/gilmore/nf-kb/>.

antiapoptotic role of NF- $\kappa$ B has been alleged in T-cell lymphoma, osteoclasts, melanoma, pancreatic cancer, bladder cancer, and breast cancer. Cell types that display an antiapoptotic role for NF- $\kappa$ B include B cells, T cells, granulocytes, macrophages, neuronal cells, and smooth muscle cells [57].

Although rare, there are systems in which NF- $\kappa$ B plays a proapoptotic role in addition to its more common antiapoptotic role. This is seen, for example, in B-cells, T-cells, neuronal cells, and endothelial cells. These opposing effects of NF- $\kappa$ B are thought to be cell-type specific and/or dependent on the inducing signal (e.g., IL-1, TNF, UV radiation). These outwardly paradoxical effects of NF- $\kappa$ B on apoptosis may result from the different activation pathways of NF- $\kappa$ B that cause the expression of proteins that promote (e.g., Fas, c-myc, p53, and IB) or inhibit (e.g., TRAF2, IAP proteins, and Bcl-2-like proteins) apoptosis. In addition, NF- $\kappa$ B activation variably regulates cell cycle proteins (e.g.,

cyclin D1 and CDK2 kinase) and their interaction with various cellular components (e.g., p300 and p53) that promote or induce apoptosis [134]. Along similar lines, Shinohara *et al.* [137] showed that NF- $\kappa$ B activation induces the expression of the metastasis suppressor gene KAI1/CD82 in lung cancer cell lines expressing mutant p53. Mucosa-associated lymphoid tissue (MALT) lymphoma, the most common extranodal lymphoid cell neoplasia that frequently follows chronic bacteria-induced inflammation in various tissues, is also related to NF- $\kappa$ B inhibition of apoptosis. MALT lymphomas are characterized genetically by the t(11;18)(q21;q21) translocation, which yields chimeric transcripts encoding structurally distinct API2/MALT1 fusion proteins. Stoffel *et al.* [138] provided functional evidence for the contribution of API2/MALT1 fusion proteins to the transformation of cells in culture by activating the NF- $\kappa$ B pathway through a RelB/p50 dimer. They also provided evidence of the emerging role of the NF- $\kappa$ B signaling pathway in the inhibition of apoptosis. Thus,

activation of NF- $\kappa$ B and inhibition of p53-mediated apoptosis by API2/MALT 1 fusions promote oncogenesis.

### 2.8. NF- $\kappa$ B Mediates the Invasion of Tumor Cells

When cancer treatment is ineffective, the tumor cells remaining after treatment inevitably infiltrate the surrounding normal tissue, which leads to tumor recurrence. According to recent studies, the ability of tumor cells to digest the extracellular matrix (ECM) by secreting proteolytic enzymes correlates well with their tissue invasiveness. [139-141] For most primary human tumors, invasion is thought to be accomplished, at least in part, by proteases — serine, cysteine, and metalloproteinases — that penetrate connective tissue barriers, induce vascular remodeling, and destroy normal tissue. Several proteases (e.g., matrix metalloproteinases [MMPs] and the serine protease urokinase-type plasminogen activator [uPA]) that influence the invasive characteristics of tumors are regulated by NF- $\kappa$ B [139-141].

MMPs are a pivotal family of zinc enzymes responsible for the degradation of ECM components, including basement membrane collagen, interstitial collagen, fibronectin, and various proteoglycans. MMPs promote the growth of cancer cells through the interaction of ECM molecules and integrins, thereby cleaving insulin-like growth factors and shedding transmembrane precursors of growth factors, including TGF- $\beta$ . MMPs promote angiogenesis by increasing the bioavailability of proangiogenic growth factors. MMPs also regulate invasion and migration by degrading structural ECM components — in particular, by cleaving laminin-5. Notably, it has been shown that MMP-9 expression is regulated transcriptionally through NF- $\kappa$ B elements within the MMP-9 gene [139]. Bond *et al.* [141], showed by using an adenovirus that overexpressed the inhibitory subunit I $\kappa$ B $\alpha$  that NF- $\kappa$ B activation was an absolute requirement in the upregulation of MMP-9.

uPA is another critical protease involved in tumor invasion and metastasis. Novak *et al.* [140] reported that the transcriptional activation of the uPA gene by phorbol myristate, IL-1, and TNF $\alpha$  requires the induction of NF- $\kappa$ B activity and the decay of its short-lived repressor protein I $\kappa$ B $\alpha$ . Wang *et al.* [142] also reported that uPA was overexpressed in pancreatic tumor cells and that its overexpression was induced by constitutive RelA activity. The uPA promoter contains an NF- $\kappa$ B binding site that directly mediates the induction of uPA expression by RelA. Treating pancreatic tumor cell lines with the NF- $\kappa$ B inhibitors dexamethasone and N-tosyl-L-phenylalanine chloromethyl ketone abolished constitutive RelA activity and uPA overexpression [142]. These results showed that uPA is one of the downstream target genes induced by constitutively activated RelA in human pancreatic tumor cells and suggested that constitutive RelA activity plays a critical role in tumor invasion and metastasis.

It was further shown that constitutively active phosphatidylinositol 3'-kinase (PI3k) controls cell motility by regulating the expression of uPA through the activation of NF- $\kappa$ B [143]. Mahabeleshwar *et al.* [107] demonstrated that the activation of Syk, a protein-tyrosine kinase, suppressed cell motility and NF- $\kappa$ B-mediated secretion of uPA by inhibiting PI3k activity in breast cancer cells. Thus, one of

the ways to block the invasion of tumor cells is to target NF- $\kappa$ B and thus prevent its activation of the genes involved in cancer progression.

### 2.9. NF- $\kappa$ B Activation is Needed for Angiogenesis

It is now well recognized that the induction of the tumor vasculature (i.e., angiogenesis) is critical for the progression of tumors. Tumor vascularization has been shown to be dependent on chemokines (e.g., monocyte chemoattractant protein-1, IL-8), and growth factors (e.g., TNF, vascular EGF [VEGF]) produced by macrophages, neutrophils, and other inflammatory cells [144]. The production of these angiogenic factors has been shown to be regulated by NF- $\kappa$ B activation [145].

NF- $\kappa$ B has been shown to mediate the upregulation of IL-8 and VEGF expression in bombesin-stimulated PC-3 cells [146]. Yu *et al.* [147] demonstrated that NF- $\kappa$ B expression was associated with VEGF expression and microvessel density in human colorectal cancer. Specifically, they detected the immunohistochemical expression of NF- $\kappa$ B, VEGF, and CD34 in 10 paraffin-embedded specimens of normal colorectal mucosa and 52 paraffin-embedded colorectal adenocarcinomas obtained by surgery or endoscopy; NF- $\kappa$ B and VEGF were significantly overexpressed and associated with increased microvessel density in the colorectal cancer specimens. These findings suggest that increased expression of NF- $\kappa$ B contributes to tumor angiogenesis in colorectal cancer and that VEGF plays an important role in mediating the NF- $\kappa$ B angiogenic pathway.

Highly metastatic melanoma cells were found to express high levels of constitutive NF- $\kappa$ B activity that was suppressed by transfection with I $\kappa$ B $\alpha$ M, and suppression of constitutive NF- $\kappa$ B activity inhibited tumor growth, prevented lung metastasis, and decreased microvessel density (angiogenesis), which correlated with a decrease in the level of IL-8 expression [148]. In another study, Pollet *et al.* (112) demonstrated that LPS directly stimulated endothelial sprouting *in vitro*, which is mediated through TRAF6. Inhibition of NF- $\kappa$ B activity, downstream of TRAF6, was sufficient to inhibit LPS-induced endothelial sprouting [149]. Also, inhibition of NF- $\kappa$ B activity blocked basic fibroblast growth factor-induced angiogenesis. These findings further underscore the role of NF- $\kappa$ B activation in mediating angiogenesis.

### 2.10. NF- $\kappa$ B is Involved in Metastasis of Tumor Cells

The metastasis of cancer requires the migration of cancerous cells both into and out of the vessel walls and their transport to other parts of the body. The ability of tumor cells to penetrate vessel walls is mediated by specific molecules that are expressed on the endothelial cells of the blood vessels in response to a number of signals from inflammatory cells, tumor cells, and so on. Among those special molecules are intercellular adhesion molecule-1, endothelial leukocyte adhesion molecule-1, and vascular cell adhesion molecule-1, all of which are expressed in response to NF- $\kappa$ B activation [150] (Table 4).

Helbig *et al.* [151] demonstrated that NF- $\kappa$ B regulates the motility of breast cancer cells by directly upregulating the expression of CXCR4. They further showed that the NF-

$\kappa$ B subunits p65 and p50 bind directly to sequences within the -66 to +7 region of the CXCR4 promoter and activate transcription. They also showed that the cell surface expression of CXCR4 and that stromal derived factor-1 $\alpha$ -mediated migration are enhanced in breast cancer cells isolated from mammary fat pad xenografts compared with parental cells grown in culture. A further increase in CXCR4 cell surface expression and stromal-1 $\alpha$ -mediated migration was observed for cancer cells that metastasized to the lungs. These results implicate NF- $\kappa$ B in the migration and organ-specific homing of metastatic breast cancer cells. In addition, Fujioka *et al.* [152] showed that inhibiting constitutive NF- $\kappa$ B activity by expressing I $\kappa$ B $\alpha$ M suppressed liver metastasis, but not tumorigenesis, in the metastatic human pancreatic tumor cell line AsPc-1 in an orthotopic nude mouse model. These findings demonstrate the importance of the suppression of NF- $\kappa$ B activation in reducing the metastasis of cancer cells to other sites. Recently, Loercher *et al.* [153] further showed that NF- $\kappa$ B is an important modulator of the altered gene expression profile and malignant phenotype in squamous cell carcinoma.

### 2.11. NF- $\kappa$ B Activation Mediates Bone Loss

The adult skeleton is in a dynamic state, undergoing turnover by the coordinated actions of osteoclasts and osteoblasts. The focal net loss of bone at sites of inflammation in conditions such as arthritis and osteoporosis is due to an imbalance in favor of bone resorption. The loss of bone is also common to certain type of cancers, such as breast cancer, melanoma, and prostate cancer (Table 3). Binding of RANKL, a member of the TNF superfamily, to its receptor RANK leads to the activation of NF- $\kappa$ B [154]. Furthermore, osteoclast differentiation and activation are mediated by RANKL, which is induced by osteoblasts or bone-lining cells in response to stimuli by the parathyroid hormone, IL-1, or PGE<sub>2</sub>. Double-knockout mice for both NF- $\kappa$ B1 and NF- $\kappa$ B2 developed osteopetrosis because of a defect in osteoclast differentiation [155] and could not generate mature osteoclasts and B cells [156]. These findings establish the criticality of NF- $\kappa$ B in bone loss and development.

NF- $\kappa$ B proteins transmit growth factor signals between the ectoderm and the underlying mesenchyme during embryonic limb formation. In one study, the interruption of NF- $\kappa$ B activity through the virus-mediated delivery of an inhibitor resulted in a highly dysmorphic apical ectodermal ridge, a reduction in overall limb size, a loss of distal elements, and a reversal in the direction of limb outgrowth [157]. Yao *et al.* [158] showed that ethyl alcohol induced NF- $\kappa$ B nuclear translocation through p56lck in human osteoblast-like cells. This activation of NF- $\kappa$ B thus may contribute to bone loss through activation of signal transduction that results in production of the osteoclastogenic cytokine IL-6 in osteoblasts [158].

Inhibition of NF- $\kappa$ B signaling activity in Saos-2 cells resulted in a marked decrease in cellular proliferation accompanied by the induction of bone morphogenic proteins 4 and 7 and of the osteoblast-specific transcription factor Cbfa1, which heralds osteoblast differentiation, the induction of alkaline phosphatase, osteopontin and osteocalcin

production, and an attendant increase in matrix deposition and mineralization *in vitro*. These results point to the negative regulation of osteoblast differentiation by NF- $\kappa$ B, with implications for the pathogenesis and progression of osteosarcomas [159]. They also suggest the critical involvement of NF- $\kappa$ B proteins in regulating the dynamics of bone development, thus raising new possibilities for the treatment of bone disorders.

### 3. SUPPRESSION OF NF- $\kappa$ B INHIBITS TUMORIGENESIS

The evidence presented in the previous sections suggests that activation of NF- $\kappa$ B can lead to tumor cell proliferation, invasion, angiogenesis, and metastasis. Therefore, the suppression of NF- $\kappa$ B in cancer cells may provide a target for treatment that prevents cancer.

#### 3.1. Tumor-Suppressor Genes Inhibit NF- $\kappa$ B Activation

Whereas oncogenes can stimulate NF- $\kappa$ B activation, several tumor-suppressor genes, including the phosphatase and tensin gene (PTEN) [160], CYLD [161-163], and p53 [164], inactivate NF- $\kappa$ B. (Table 5). The mutated in multiple advanced cancers (MMAC)/PTEN homologue is a natural antagonist of PI3k activity. PTEN is a lipid phosphatase responsible for downregulating the PI3k product phosphatidylinositol 3,4,5-triphosphate. In a glioma cell line that was stably transfected with MMAC/PTEN, the IL-1-induced DNA binding and transcriptional activity of NF- $\kappa$ B were both inhibited [160]. The ability of IL-1 to induce I $\kappa$ B $\alpha$  degradation or the nuclear translocation of NF- $\kappa$ B was, however, unaffected by MMAC/PTEN expression. Moreover, the IL-1-induced phosphorylation of p50 NF- $\kappa$ B was potently inhibited in MMAC/PTEN-expressing cells. However, the IL-1-induced interaction between the PI 3-k target Akt kinase and the IKK complex was antagonized by MMAC/PTEN.

Mayo *et al.* [128] found that PTEN inhibits TNF-stimulated NF- $\kappa$ B transcriptional activity. Specifically, PTEN did not block TNF-induced IKK activation, I $\kappa$ B $\alpha$  degradation, p105 processing, p65 (RelA) nuclear translocation, or DNA binding of NF- $\kappa$ B [165]. However, PTEN did inhibit NF- $\kappa$ B-dependent transcription by blocking the ability of TNF to stimulate the transactivation domain of the p65 subunit. Thus, maintenance of the PTEN tumor-suppressor protein is required to modulate Akt activity and to concomitantly control the transcriptional activity of NF- $\kappa$ B as an antiapoptotic transcription factor.

Familial cylindromatosis is caused by mutations in a gene encoding CYLD, a tumor-suppressor gene with deubiquitinating enzymatic activity that negatively regulates the activation of NF- $\kappa$ B. A loss of the deubiquitinating activity of CYLD correlates with tumorigenesis. CYLD inhibits activation of NF- $\kappa$ B by the TNFR family members CD40, X-linked ectodermal dysplasia receptor (XEDAR), and ectodysplasin-A receptor (EDAR) in a manner that depends on the deubiquitinating activity of CYLD. Downregulation of CYLD by RNA-mediated interference augments both basal and CD40-mediated activation of NF- $\kappa$ B. The inhibition of NF- $\kappa$ B activation by CYLD is mediated, at least in part, by the deubiquitination and

**Table 5. A List of Agents that Inhibit NF- $\kappa$ B Activation**

<p><b>Anti-inflammatory agents</b></p> <p>Aspirin<sup>57</sup>  Indomethacin<sup>57</sup>  Diclofenac<sup>57</sup>  Naproxen<sup>57</sup>  Dexamethasone<sup>57</sup>  Phenylbutazone<sup>57</sup>  Tamoxifen<sup>57</sup>  Sulfasalazine  Sulindac  Ibuprofen<sup>57</sup>  Leflunomide<sup>173</sup>  Trichodion  Acetaminophen  Gabexate mesilate  Glucosamine sulfate  Petrosaspongiolide M  Dexanabinol  BSASM  Cloricromene  Rolipram  Pranlukast</p> <p><b>Immunosuppressive agents</b></p> <p>Cyclosporin  LF15-0195  15-Deoxyspergualin  6(5H)-Phenanthridinone and benzamide</p> <p><b>Chemopreventive agents</b></p> <p>Carboplatin  Vesnarinone<sup>232</sup>  Cycloprodigiosin  (-)-Cycloepoxydon  Camphothecin  Gemcitabine  Levamisole  Epoxomicin</p> <p><b>Angiogenesis</b></p> <p>2-Methoxyestradiol  TNP-470</p> <p><b>Cell proliferation</b></p> <p>6-Aminoquinazoline derivatives  Raxofelast</p> <p><b>Virus</b></p> <p>K1L  HIV-1 Vpu protein  Canine distemper virus  Hypochlorite  Inhaled isobutyl nitrite  Jesterone dimer  KT-90 APC  Losartin  Calcitriol</p>	<p><b>Antioxidants</b></p> <p>ADT  Apocynin  Benidipine  BHA  Carvedilol  Catechol derivatives  DHEAS  DMDTC  DMSO  Disulfiram  Ebselen  EPC-K1 21  Ethyl pyruvate  Ethylene glycol tetraacetic acid  Iron tetrakis  Mn-SOD  Melatonin  NAL  NDGA  Orthophenanthroline  Hydroquinone and tert-butyl hydroquinone  Ref-1  Tempol  Tepoxaline  2-Amino-3-cyano-4-aryl-6-(2-hydroxy-phenyl)  N-Acetylcysteine</p> <p><b>Reactive oxygen species</b></p> <p>Nitric oxide</p> <p><b>Antibacterial agents</b></p> <p>Fosfomicin  5-Aminosalicylic acid  Clarithromycin  Rifampicin  Roxithromycin</p> <p><b>Antidiabetes agents</b></p> <p>Adiponectin  Glimepiride</p> <p><b>Tyrosine phosphatase inhibitors</b></p> <p>Diamides<sup>242</sup>  Pervanadate<sup>242</sup>  Phenylarsine oxide<sup>242</sup></p> <p><b>Serine protease inhibitor</b></p> <p>Pefabloc</p> <p><b>Phosphatidylcholine-phospholipase</b></p> <p><b>C inhibitor</b></p> <p>D609  Hyperosmolarity  Hypothermia  IMD-0354</p>	<p><b>HIV-1 protease inhibitors</b></p> <p>Nelfinavir  Ritonavir  Saquinavir</p> <p><b>ACE inhibitor</b></p> <p>Quinadril</p> <p><b>COX-2 inhibitor</b></p> <p>Celecoxib<sup>216,218</sup></p> <p><b>Interferon inducible protein</b></p> <p>p202a</p> <p><b>Methanesulfonilide antiarthritis inhibitor</b></p> <p>T-614</p> <p><b>PTK inhibitor</b></p> <p>Herbimycin A<sup>244</sup></p> <p><b>Farnesyl protein transferase inhibitor</b></p> <p>SCH66336<sup>220</sup></p> <p><b>Protease inhibitor</b></p> <p>Nafamostat mesilate</p> <p><b>PPAR<math>\gamma</math> ligand</b></p> <p>Pioglitazone</p> <p><b>Heat shock proteins</b></p> <p>HSP70  HSP72</p> <p><b>Statins</b></p> <p>Atorvastatin  Erbstatin  Fluvastatin  Lovastatin</p> <p><b>Metals</b></p> <p>Chromium  Cadmium  Gold  Lead  Mercury  Zinc  Arsenic  Titanium</p> <p><b>I<math>\kappa</math>B-like protein</b></p> <p>Encoded by ASFV</p> <p><b>Acetylation inhibitor</b></p> <p>HDAC<sup>187</sup>  Nucling  ox-LDL and HNE  OXPAPC</p>	<p><b>Others</b></p> <p><math>\beta</math>-Amyloid protein  <math>\beta</math>-Catenin  <math>\gamma</math>-Glutamylcysteine synthetase<sup>234</sup>  1,2,3,4,6-Penta-O-galloyl-<math>\beta</math>-D-glucose  1,2,4-Thiadiazolidine derivatives  15-Deoxy-prostaglandin J(2)  17-Allylamino-17-demethoxygeldanamycin  2-Acetylaminofluorene  3,4,5-Trimethoxy-4'-fluorochalcone  4-Hydroxy-2-nonenal<sup>183</sup>  5'-Methylthioadenosine  Acrolein  Adenosine<sup>226</sup>  AMP-activated protein kinase  Amrinone  Anandamide  Angiotensin-1  ANP  Antithrombin III  APC0576  Arsenite<sup>243</sup>  Auranofin  AvrA protein  AZT  Bcl-Abl<sup>228</sup>  Benfotiamine  Biliverdin  Biotinylated isopanepoxydone  Bisphenol A  BMT  Bovine serum albumin  BZLF1  C5a  Carbon monoxide  Cyclopentones  CYL-19s and CYL-26z  CYLD<sup>161-163</sup>  Desloratadine  Disulfiram  Dobutamine  DQ 65-79  DTD  E3330  E-73  Ecabet sodium  Hydroquinone  RKIP  Rocaglamide  Saline  Selenomethionine  Siah2</p>
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(Table 5. Contd....)

CaMKK	Intravenous immunoglobulin	p8	SiRNA <sup>191</sup>
Camptothecin	Jesterone	p53 <sup>164</sup>	SLPI
Caprofin	JSH-21	PACAP	SOCS1
Decoy oligonucleotides	JSH-23	PAN1	Sodium salicylate
Dehydroxymethylepoxyquinomicin	Kaposi's sarcoma-associated	Panepoxidone	SUN C8079
Diethyldithiocarbamic acid	herpesvirus K1 protein	PBN	Surfactant protein A
Diethylmaleate	Ketamine	PDTC	Survanta
Diltiazem	KL-1156	PEDF	TAT-SR-I $\kappa$ B $\alpha$
DMF	LMB	Pentoxifylline	Tetrathiomolybdate
Epoxyquinol	Macrolide antibiotics	Photo oxidative stress <sup>236</sup>	Thalidomide <sup>229</sup>
Erythromycin	MAST205	PIAS	THI 52
Estrogen (E2)	MEB	Pirfenidone	Thiopental
Ethacrynic acid	Melatonin	PPM-18	TNAP
Ethyl 2-[(3-methyl-2,5-dioxo(3-pyrrolinyl)]	Mercaptopyrazine	Probiotics	Tom1
Ethyl pyruvate	Mesalamine	Probiotics	Tranilast
Evans blue	Methotrexate <sup>230</sup>	Prostaglandin 15-deoxy-Delta(12,14)-PGJ(2)	Triflusal
Fibrates	Monochloramine	15-deoxy-delta 12,14-prostaglandin J2	Triglyceride-rich lipoproteins
Fish oil feeding	Monomethylfumarate	Prostaglandin A1	Troglitazone
Flunixin meglumine	MTA	Prostaglandin E2	Tyrphostin
Flurbiprofen	Murr1	Prostaglandin E2	Ursodeoxycholic acid
Fox1j	MX781	PSK	Uteroglobin
G-120 3032	Myxoma Virus MNF	Psychosine	Vasoactive intestinal peptide
Gangliosides	N-(p-coumaroyl) serotonin	PTEN <sup>160</sup>	vIRF3
Gemfibrozil	NEM	PTX-B	YopJ
Ghrelin	Neurofibromatosis-2 protein	Pyrrhione	ZAS3 protein
GILZ	nicotinamide, 3-aminobenzamide	Pyrrolidinedithiocarbamic -acid	ZUD protein
Glucocorticoids	Nilvadipine	Quinazolines	
Hydroquinone	Nitrosoglutathione	Rapamycin	
HSCO	Nitrosylcobalamin mild hypothermia	Rebamipide	
Human breast milk	N-Octylcafeate	RelA peptides	
Hydrochloride	N-Oleoyldopamine	Retinoic acid receptor-related orphan receptor-alpha	
	NPM-ALK oncoprotein	Ribavirin	
	NRF	Rifamides	
		Ritonavir	

ADT, anethole dithiolthione; BHA, butylated hydroxyanisole; DHEAS, dehydroepiandrosterone sulfate; DMDTC, dimethyldithiocarbamates; DMSO, dimethylsulfoxide; EPC-K1, phosphodiester compound of vitamin E and vitamin C; EGTA, ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid; Mn-SOD, manganese superoxide dismutase; NAC, N-acetylcysteine; NAL, nacyselyn; NDGA, nordihydroguaiaritic acid phenolic antioxidants; Ref-1, redox factor 1; ACE, angiotensin-converting enzyme; COX-2, cyclooxygenase-2; PTK, protein tyrosine kinase; PPAR, peroxisome proliferator-activated receptor; HSP, heat shock protein; ASFV, African swine fever virus; HDAC, histone deacetylase; ANP, atrial natriuretic peptide; APC, activated protein C; BMT, o,o'-bismyristoyl thiamine disulfide; ALLnL, N-acetyl-leuciny-leucynil-norleucynal; APNE, N-acetyl-DL-phenylalanine-b-naphthylester; BTEE, N-benzoyl L-tyrosine-ethylester; DCIC, 3,4-dichloroisocoumarin; LLM, N-acetyl-leuciny-leucynil-methional; TLCK, N-a-tosyl-L-lysine chloromethyl ketone; TPCK, N-a-tosyl-L-phenylalanine chloromethyl ketone; Z-LLL, carbobenzoxy-leuciny-leucynil-leucynal; Z-LlnV, carbobenzoxy-leuciny-leucynil-norvalinal; a-MSH, alpha-melanocyte-stimulating hormone; ox-LDL, oxidized low density lipoprotein; HNE, Hydroxynonenal; HB-EGF, Heparin-binding epidermal growth factor-like growth factor; PACAP, Pituitary adenylate cyclase-activating polypeptide; THI 52; 1-naphthylethyl-6,7-dihydroxy-1,2,3,4- tetrahydroisoquinoline; PDTC, Pyrrolidinedithiocarbamate; DMF, Dimethylfumarate; DTD, 4,10-dichloropyrido[5,6:4,5]thieno[3,2-d':3,2- d]-1, 2, 3-ditriazine; Tranilast; N-(3,4-dimethoxycinnamoyl)anthranilic acid, MEB; 2-(4-morpholynl) ethyl butyrate hydrochloride; FLIP, FLICE-Like Inhibitory Protein; PTX-B, pertussis toxin binding protein; RKIP; Raf Kinase Inhibitor Protein; GILZ, Glucorticoid-induced leucine zipper protein; LMB, Leptomycin B; MTA, Mevinolin; 5'-methylthioadenosine; NEM, N-ethyl-maleimide; NRF, NF- $\kappa$ B repression factor; OXPAPC, oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine; PEDF, pigment epithelium derived factor; PTX, Pentoxifylline; PBN, Phenyl-N-tert-butylitron; PIAS1, protein inhibitor of activated STAT1; PSK, Protein-bound polysaccharide; VEGF; Vascular endothelial growth factor; Avr, avirulence; AZT, 3'-azido-3'-deoxythymidine; CaMKK, Calcium/calmodulin-dependent protein kinase kinase; DMF, dimethylfumarate; HSCO, Hepatoma Subtracted-cDNA library Clone One; MEB, 2-(4-morpholynl) ethyl butyrate hydrochloride; NPM, nucleophosmin; ALK, anaplastic lymphoma kinase; PAN, PYRIN and NACHT domain; Ppm-18, a-benzoylamino-1,4-naphthoquinone; Siah, seven in absentia homolog; SLPI, secretory leukocyte protease inhibitor; SOCS, Suppressor of cytokine signalin; THI52, 1-naphthylethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline; TNAP, TRAFs and NIK-associated protein; vIRF, human herpesvirus 8-encoded interferon regulatory factor; YopJ, Yersinia outer protein; ZUD, ZU 5 and death domain-containing protein.

Source: <http://www.ncbi.nih.gov/entrez/query.fcgi>, <http://people.bu.edu/gilmore/nf-kb/>.

inactivation of TRAF2. CYLD also binds to the NF- $\kappa$ B essential modulator (also known as IKK $\gamma$ ) component of the IKK complex and appears to regulate its activity through deubiquitination of TRAF2, since TRAF2 ubiquitination can be modulated by CYLD [161-163]. These results indicate that CYLD is a negative regulator of the cytokine-mediated activation of NF- $\kappa$ B that is required for appropriate cellular

homeostasis of skin appendages. In addition, the inhibition of CYLD increases resistance to apoptosis, suggesting a mechanism through which the loss of CYLD contributes to oncogenesis. This effect can be reversed by aspirin derivatives that inhibit NF- $\kappa$ B activity, which suggests a therapeutic intervention strategy to restore growth control in patients with familial cylindromatosis.

Whereas NF- $\kappa$ B provides a proliferative signal, p53 is a mediator of antiproliferation. A mechanism by which p53 regulates NF- $\kappa$ B function and cell cycle progression has been proposed: p53 was shown to downregulate the expression of cyclin D1 by inhibiting the expression of the Bcl-3 protein [166], a member of the I $\kappa$ B family that functions as a transcriptional coactivator for p52 NF- $\kappa$ B; p53 also reduced p52/Bcl-3 complex levels. At the same time, p53 induced a significant increase in the association of p52 and histone deacetylase 1 (HDAC1). Importantly, p53-mediated suppression of the cyclin D1 promoter was reversed by coexpression of Bcl-3 and inhibition of p52 or deacetylase activity. p53 therefore induces a transcriptional switch by which p52/Bcl-3 activator complexes are replaced by p52/HDAC1 repressor complexes, resulting in active repression of cyclin D1 transcription.

The alternative reading frame (ARF) tumor suppressor is a central component of the cellular defense against oncogene activation. In addition to activating p53 by binding to Mdm2, ARF can repress the transcriptional activity of the antiapoptotic RelA(p65) NF- $\kappa$ B subunit. Rocha *et al.* [166] demonstrated that ARF induces the ataxia-telangiectasia mutated and Rad3-related (ATR) and checkpoint kinase 1 (Chk1)-dependent phosphorylation of the RelA transactivation domain at threonine 505, a site required for ARF-dependent repression of RelA transcriptional activity [166]. Consistent with this finding, ATR and Chk1 are required for ARF-induced sensitivity to TNF-induced cell death. Significantly, ATR activity was also required for ARF-induced p53 activity and inhibition of proliferation; ARF achieved these effects by activating ATR and Chk1. These results reveal novel functions for ARF, ATR, and Chk1, together with a new pathway regulating the RelA NF- $\kappa$ B function. Moreover, this pathway provides a mechanism through which ARF can remodel the cellular response to an oncogenic challenge and execute its function as a tumor suppressor.

### 3.2. Inhibition of NF- $\kappa$ B Inhibits Tumorigenesis

That NF- $\kappa$ B is critical for tumorigenesis is also indicated by studies showing that suppression of NF- $\kappa$ B blocks tumorigenesis. For instance, fibrosarcoma and colore tumors grown in nude mice were induced to undergo apoptosis after infection with an adenovirus expressing a modified form of I $\kappa$ B $\alpha$  and the systemic delivery of camptothecin-11 chemotherapy [7]. In addition, HT1080 fibrosarcoma cells exposed to ionizing radiation, TNF- $\alpha$ , or daunorubicin exhibited enhanced activation of NF- $\kappa$ B, and inhibition of NF- $\kappa$ B dramatically enhanced apoptosis in response to radiation or daunorubicin [9]. There are, however, studies suggesting that suppression of NF- $\kappa$ B has no effect on apoptosis. For example, the stable transfection of *ikb $\alpha$*  did not result in enhanced cytotoxicity in response to chemotherapeutic agents, despite the ability of these agents to activate NF- $\kappa$ B [167].

### 3.3. Chemopreventive Agents Inhibit NF- $\kappa$ B Activation

Because of the critical role of NF- $\kappa$ B in proliferation, invasion, angiogenesis, and metastasis of tumors, there has been great interest in modulators of the NF- $\kappa$ B signaling pathway. Several agents that have been described as natural

chemopreventive agents have also been found to be potent inhibitors of NF- $\kappa$ B activation (Table 6). How these agents suppress NF- $\kappa$ B activation is becoming increasingly apparent. For example, these inhibitors may block any one or more steps in the NF- $\kappa$ B signaling pathway, such as the incoming signals that activate the NF- $\kappa$ B signaling cascade, the translocation of NF- $\kappa$ B into the nucleus, DNA binding of the dimers, and interactions with the basal transcriptional machinery. For instance, whereas curcumin blocks IKK activation [126], resveratrol suppresses p65 translocation to the nucleus [86] and caffeic acid phenethyl ester (CAPE) suppresses the binding of the p50-p65 complex directly to the DNA [168].

Other chemopreventive agents that have been shown to suppress NF- $\kappa$ B activation include betulinic acid, ursolic acid, piceatannol, green tea polyphenols, oleandrin, and anethole [89, 169-172]. Similarly leflunomide, a drug approved for the treatment of rheumatoid arthritis, was also shown to suppress NF- $\kappa$ B activation [173].

### 3.4. Some Cytokines Block NF- $\kappa$ B Activation

Several cytokines have also been found to suppress NF- $\kappa$ B activation. These include cytokines produced by Th2 cells, such as IL-4, IL-11, IL-13, and IL-10 [174], and hormones of the endocrine system, such as human chorionic gonadotropin [175], melanocyte-stimulating hormone (MSH) [176], and growth hormone [177] (Table 7). Besides these cytokines, IFN- $\alpha$  was also found to be a potent suppressor of NF- $\kappa$ B activation [178]. How these agents suppress NF- $\kappa$ B activation varies. For instance, the nuclear translocation of the RelA subunit and the degradation of I $\kappa$ B $\alpha$  induced by TNF were prevented by IL-13 and human chorionic gonadotropin in one study [175]. Both IL-10 and IL-13 suppressed nuclear localization of NF- $\kappa$ B and increased I $\kappa$ B $\alpha$  mRNA expression in another study [179].

## 4. NF- $\kappa$ B IS A POTENTIAL TARGET FOR DRUG DEVELOPMENT

NF- $\kappa$ B is an ideal target for anticancer drugs. Given the hyperproliferative nature of cancer, which involves transformation, initiation, promotion, angiogenesis, invasion, and metastasis, and the diversity of its clinical presentation, aggressiveness, and current treatment strategies, the implication is that an equally diverse number of potential targets exist in the molecular pathways leading to its formation. In this regard, several strategies have been used to block the activation of NF- $\kappa$ B, and a wide range of compounds, such as IKK inhibitors, inhibitory peptides, antisense RNA, and proteasome inhibitors, have been found to block various steps leading to NF- $\kappa$ B activation (Table 8).

### 4.1. Inhibitors of Proteasomes Block NF- $\kappa$ B Activation

Proteasome inhibitors block the 26S proteasome, which is necessary to degrade the I $\kappa$ B $\alpha$  inhibitory subunit after its phosphorylation and ubiquitination in the cytoplasm, and thus its subunit release from the NF- $\kappa$ B complex [55]. Some of the well-known proteasome inhibitors are peptide aldehydes, such as ALLnL, LLM, Z-LLnV, and Z-LLL; lactacystine; PS-341; N-cbz-Leu-Leu-leucinal (MG132); MG115; and ubiquitin ligase inhibitors (for a review, see reference[57]) (Table 8).



Table 6. A List of Natural Products that Suppress NF- $\kappa$ B Activation

$\alpha$ -Lipoic acid	Cheongyeolsaseuptang	Isomallotochromanol and isomallotochromene	Tetrandine
$\alpha$ -Pinene	Chromene derivatives	Isorhapontigenin	Theaflavin
$\alpha$ -Tocopherol	Chicory root	Kahweol	Trilinolein
$\alpha$ -Torphryl acetate	Chitosan	Quinic acid	Triptolide
$\alpha$ -Torphryl succinate	Curcumin <sup>219,247-266</sup>	Kamebakaurin	Ursolic acid <sup>89</sup>
$\beta$ -Lapachone <sup>235</sup>	Cyclolinteinone	Lacidipine	Vitamin C
1'-Acetoxychavicol acetate <sup>213</sup>	DDC	L-Ascorbic acid	Wedelolactone
20(S)-Protopanaxatriol	Dehydroascorbic acid	Lazaroids	Withaferin A
23-Hydroxyursolic acid	Diarylheptanoid	Lupeol	Wogonin
7-Amino-4-methylcoumarin	Dibenzylbutyrolactone lignans	Luteolin	Wortmannin <sup>238</sup>
Acetyl-boswellic acids	Diferoxamine	Magnolol	Yakuchinone A and B
Allyl-cysteine	Digitoxin	$\gamma$ -Mangostin	Zerumbone <sup>211</sup>
Amentoflavone	Dihydroisoeugenol	Manassantins	<b>Others</b>
Andalusol	Dihydrolipoic acid	Mangiferin	1,2,4-Thiadiazolidine derivatives
Andrographolide	DIM/I3C <sup>212</sup>	Nicotine	2-Amino-3-cyano-4-aryl-6-(2-hydroxy-phenyl)
Anethole <sup>170</sup>	Ebselen	Oleandrin <sup>171</sup>	Aged garlic extract
Apigenin	EGCG	Onconase	Apple juice
Artemisia sylvatica	Emodin <sup>238</sup>	Panduratin A	<i>Artemisia capillaris</i> Thunb extract
Artemisinin	ent-Kaurane diterpenoids	Palthenolide	<i>Artemisia iwaiyomogi</i> extract
Astaxanthin	Ergothioneine	PC-SPES	Blueberry and berry mix
Astragaloside IV	Evodiamine <sup>214</sup>	Phenethylisothiocyanate	Black raspberry extracts
Aucubin	Epoxyquinone A	Phomol	Cat's claw bark
Avicin	Falcarindol	Phytic acid	Extract of the stem bark of <i>Mangifera indica</i> L.
Baicalein	Flavonoids	Phallacidin	Extracts of <i>Ochna macrocalyx</i>
Bambara groundnut	Flavopiridol <sup>223</sup>	Piceatannol <sup>227</sup>	Fomes fomentarius methanol extracts
Bee venom	Fungal gliotoxin	Piperine	Fructus benincasae recens extract
Benzyl isothiocyanate	<i>Ganoderma lucidum</i> polysaccharides	Qingkailing,	<i>Ginkgo biloba</i> extract
Betulinic acid <sup>172</sup>	Garcinol	Shuanghuanglian	<i>Harpagophytum procumbens</i> extracts
Bis-eugenol	Genistein	Quercetin	<i>Kochia scoparia</i> fruit
Bupleurum fruticosum	Geldanamycin	Resiniferatoxin	Omega 3 fatty acids
phenylpropanoids	Genipin	Resveratrol <sup>225,231</sup>	<i>Phyllanthus amarus</i> extracts
Cacospongionolide B	Ginkgolide B	Rhein	<i>Platycodi radix</i> extract
Calagualine <sup>210</sup>	Glabridin	Rocaglamide	Red wine
Cancer bush	Glitoxin	Rotenone	<i>Sophorae radix</i> extract
CAPE	<i>Glossogyne tenuifolia</i>	Sanggenon C	Stinging nettle plant extracts
Capsaicin <sup>240</sup>	Glycyrrhizin	Sanguinarine	<i>Tanacetum larvatum</i> extract
Capsiate	Guggulsterone <sup>215</sup>	Saucerneol	<i>Uncaria tomentosa</i> plant extract
Carnosol	Helenarin	Sauchinone	<i>Trichomonas vaginalis</i>
Catalposide	Hematein	Sesamin	<i>Xanthium strumarium</i> L.
Cinnamaldehyde/2-methoxycinnamaldehyde	Herbal compound 861	Sesquiterpene lactones	<i>Yucca schidigera</i> extract
Cycloepoxydon	Hydroxyethyl starch	Silymarin <sup>209</sup>	
Celastrol	Hypericin	Sinomenine	
Cepharantin	10Z-hymenialdisine	<i>Solana nigrum</i> L.	
Cytochalasin D	Hypoestoxide	Sphondin	
Conophylline	Indirubin-3'-oxime	Staurosporine	
	IRFI 042	Tansinones	

SAC, garlic compound, DDC; Diethylthiocarbamate, EGCG; Epigallocatechin-3-gallate (green tea polyphenols), Flavonoids (Crategus), Garcinol from extract of *Garcinia indica* fruit rind, IRFI 042; Vitamin E-like compound, 20(S)-Protopanaxatriol; ginsenoside metabolite, Apigenin; plant flavinoid, Calagualine; fern derivative, Capsaicin; 8-methyl-N-vanillyl-6-nonenamide, Cyclolinteinone; sponge sesterterpene, Emodin; 3-methyl-1,6,8-trihydroxyanthraquinone, *Kochia scoparia* fruit; methanol extract, KIL; Vaccinia virus protein, Panduratin A from *Kaempferia pandurata*, Zingiberaceae, Phytic acid; inositol hexakisphosphate, Rocaglamides; Aglaia derivatives, Sanguinarine; pseudochelethrine, 13-methyl-[1,3]-benzodioxolo-[5,6-c]-1,3-dioxolo-4,5 phenanthridinium, Sesquiterpene lactones; parthenolide; ergolide; guaianolides, Apigenin; 4',5,7-trihydroxyflavone, Baicalein; 5,6,7-trihydroxyflavone, Bambara groundnut; Vigna subterranean, Bee venom; melittin, Blueberry and berry mix; Optiberry, Cancer bush; Sutherlandia frutescens, Onconase; Ranpirinase, Catalposide; stem bark, Cat's claw bark; *Uncaria tomentosa*; Rubiaceae, Chicory root; guaianolide 8-deoxylactucin, Diarylheptanoid (7-(4'-hydroxy-3'-methoxyphenyl)-1-phenylhept-4-en-3-one), ent-kaurane diterpenoids; Croton tonkinensis leaves, Harpagophytum procumbens, Devil's Claw extracts, PC-SPES; 8 herb mixture, Qingkailing and Shuanghuanglian; Chinese medicinal preparations, *Solana nigrum* L. 150 kDa glycoprotein, Sphondin; furanocoumarin derivative from *Heracleum laciniatum*, Tansinones; *Salvia miltiorrhiza* Bunge, Labiate roots, Sesamin from sesame oil, Triptolide; PG490, extract of Chinese herb, Wogonin; 5,7-dihydroxy-8-methoxyflavone, Wortmannin; fungal metabolite, Aged garlic extract; allicin, DIM, 3,3'-Diindolylmethane. Source: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>, <http://people.bu.edu/gilmore/nf-kb/>.

**Table 7. A List of Proteins and Peptides that Inhibit NF- $\kappa$ B Activation**

Interleukin	Peptides
IL-4 <sup>237</sup>	TRAF6 inhibitory peptide
IL-11	NEMO binding peptide
IL-10 <sup>179</sup>	p65 binding peptide <sup>198</sup>
IL-13 <sup>179, 239</sup>	Boronic acid peptide
	NLS cell permeable peptides <sup>195</sup>
<b>Interferon</b>	Peptide nucleic acid-DNA decoys
IFN- $\alpha$ <sup>147</sup>	Peptide YY
	TIRAP inhibitor peptide
<b>Growth factors and hormones</b>	
VEGF	
Hepatocyte growth factor	
MSH <sup>176</sup>	
HB-EGF	
Chorionic gonadotropin <sup>175,221</sup>	

IL, Interleukin; IFN, interferon; VEGF, vascular epidermal growth factor; MSH, melanocyte stimulating hormone TIRAP, toll IL1 receptor domain-containing adapter protein; HB-EGF, Heparin-binding epidermal growth factor-like growth factor; TRAF, TNF receptor-associated factor; NEMO, NF- $\kappa$ B essential modulator; NLS, nuclear localization sequence. Source: <http://www.ncbi.nih.gov/entrez/query.fcgi>, <http://people.bu.edu/gilmore/nf-kb/>.

PS-341 blocks TNF- $\alpha$ -induced NF- $\kappa$ B activation in multiple myeloma cells through the inhibition of I $\kappa$ B $\alpha$  phosphorylation and the degradation of I $\kappa$ B $\alpha$  [180]. Proteasome inhibitors also inhibit the chymotrypsin-like activity of the proteasome complex. Fiedler *et al.* [181] have

demonstrated that the pretreatment of cells with the proteasome inhibitor N-cbz-Leu-Leu-leucinal (MG-132) reverses TNF $\alpha$ -induced NF- $\kappa$ B activation by inhibiting proteasome-mediated I $\kappa$ B $\alpha$  degradation and NF- $\kappa$ B-mediated gene transcription [181].

Calpain inhibitor I is a cysteine protease inhibitor that is less potent than MG132 and MG115. Molecules such as lactacystin irreversibly block proteasome activity by acylating a threonine residue in the active site of the subunit X of the mammalian proteasome. Some serine protease inhibitors also act as proteasome inhibitors and block the phosphorylation and degradation of I $\kappa$ B $\alpha$  [182]. Retaining the NF- $\kappa$ B dimers in the cytoplasm by preventing the phosphorylation and degradation of I $\kappa$ B $\alpha$  is also an effective way to inhibit NF- $\kappa$ B, and this can be achieved by several signaling molecules such as nitric oxide, estrogen, oxidized low-density lipoproteins, 4-hydroxynonenal, and prostaglandin A.

#### 4.2. Inhibitors of IKK Block NF- $\kappa$ B Activation

I $\kappa$ B $\alpha$  phosphorylation is a critical step in NF- $\kappa$ B activation, and compounds that block this phosphorylation also prevent I $\kappa$ B $\alpha$ 's ubiquitination and further degradation. Recently, 4-hydroxy-2-nonenal, a lipid peroxidation product, has been shown to block this phosphorylation by directly inhibiting of IKK [183]. Also, 5-bromo-6-methoxy- $\beta$ -carbolone, a natural product derivative, is a nonspecific IKK inhibitor that inhibits the phosphorylation of I $\kappa$ B $\alpha$  and the subsequent activation of NF- $\kappa$ B; this compound has a 50% inhibitory concentration (IC<sub>50</sub>) in the nanomolar range [184]. SC-514 is another novel inhibitor of IKK, specifically inhibiting IKK $\beta$ , which has an IC<sub>50</sub> of approximately 10  $\mu$ M; it does not inhibit other IKK isoforms or other serine-

**Table 8. Synthetic Compounds That Inhibit NF- $\kappa$ B Activation**

<b>IKK inhibitors</b>	SN-50	<b>P42 MAPK inhibitor</b>
BAY-11-7082	FK506	AG 126
BAY-11-7085	Ro106-9920	
BMS-345541 <sup>186</sup>	PS5190	<b>P38 MAPK inhibitor</b>
SPC839	TLCK, TPCK	SB203580
AS602868	Cyclosporin A	
PS1145	DCIC	<b>PI3K-kinase inhibitor</b>
MNL120	Deoxyspergualin	LY294002
SC-514 <sup>185</sup>	DFP	
<b>Polyubiquitination inhibitor</b>	LLM	<b>PKC inhibitor</b>
RO106-9920	BTEE	RO31-8220
	MG115 <sup>57</sup>	
<b>Proteasome inhibitors</b>	MG120	<b>MEK1/2 inhibitor</b>
ALLnL <sup>57</sup>	APNE	U0126
PS-341 <sup>57,218</sup>	Ubiquitin Ligase Inhibitors	
Lactacystine, $\beta$ -lactone <sup>57</sup>	Z-LLL	<b>Protein kinase inhibitor</b>
	Z-LLnV	A77 1726

IKK, I $\kappa$ B kinase; PI3K, phosphatidylinositol 3; PKC, protein kinase, protein kinase C, MEK, mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase. Source: <http://www.ncbi.nih.gov/entrez/query.fcgi>, <http://people.bu.edu/gilmore/nf-kb/>.

threonine and tyrosine kinases. SC-514 inhibits the native IKK complex and the recombinant human IKK- $\alpha$ /IKK- $\beta$  heterodimer and IKK- $\beta$  homodimer in a similar manner [185] (Table 8).

BMS-345541 (4(2'-aminoethyl) amino-1,8-dimethylimidazo(1,2- $\alpha$ )quinoxaline) was identified as a selective inhibitor of the catalytic subunits of IKK with an  $IC_{50}$  of 4  $\mu$ M for IKK $\alpha$  and 0.3  $\mu$ M for IKK $\beta$  [186] (Table 8). Burke *et al.* [186] proposed a binding model in which BMS-345541 binds to similar allosteric sites on IKK $\alpha$  and IKK $\beta$  and then affects the active sites of the subunits differently. BMS-345541 was also shown to have excellent pharmacokinetics in mice, to dose dependently inhibit the production of serum TNF $\alpha$  after intraperitoneal challenge with LPS in mice, and to represent an important tool for investigating the role of IKK in disease models [186].

#### 4.3. Acetylation Inhibitors can Block NF- $\kappa$ B Activation

Histone acetylation modulates gene expression, cellular differentiation, and survival and is regulated by the opposing activities of histone acetyltransferases and HDACs (Table 5). HDAC3 acts directly on nuclear Rel A, enabling its association with I $\kappa$ B $\alpha$  and its subsequent export from the nucleus, and is another potential target for gene transfer. In one study, the expression of HDAC3 in TNF $\alpha$ -stimulated HeLa cells repressed both NF- $\kappa$ B DNA binding and the levels of Rel A, with a corresponding increase in the level of inactive cytoplasmic I $\kappa$ B $\alpha$ -NF- $\kappa$ B complexes [187]. This repressive mechanism was shown to control the duration of NF- $\kappa$ B activation and thus may be a potential weapon against constitutive NF- $\kappa$ B activation.

#### 4.4. Gene Transfer of Inhibitory Proteins can Block NF- $\kappa$ B Activation

The transfer of genes that encode inhibitory proteins is another strategy used to block the activation of NF- $\kappa$ B. The most direct target is the I $\kappa$ B gene. This transfer entails the modification of I $\kappa$ B at its specific phosphorylation (ser 32 and 36 switched with ala) and ubiquitination (lys 21 and 22 switched with arg) sites to prevent its degradation. In several studies, the superrepressor of NF- $\kappa$ B activity was a mutated, nondegradable I $\kappa$ B $\alpha$  resistant to phosphorylation and degradation that could be delivered into intestinal epithelial cells by using an adenoviral vector (Ad5 I $\kappa$ B). There was a strong inhibition of NF- $\kappa$ B activity because the superrepressor kept the NF- $\kappa$ B complex in the cytoplasm indefinitely [167]. These studies suggest an exciting approach for *in vivo* intestinal gene therapy and illustrate a key role for NF- $\kappa$ B in transcriptional regulation of the inflammatory phenotype of intestinal epithelial cells. Along similar lines, a nonphosphorylatable form of I $\kappa$ B $\alpha$  was recently shown to inhibit osteoclastogenesis and block bone resorption when injected into bone marrow macrophages [188].

#### 4.5. Antisense and Small Interfering RNA can Block NF- $\kappa$ B Activation

Antisense agents that inhibit the expression of a target gene in a sequence-specific manner may be used against NF- $\kappa$ B for therapeutic purposes. Three types of anti-mRNA

strategies can be distinguished: 1) single-stranded antisense oligonucleotides, 2) RNA cleavage triggered by catalytically active oligonucleotides, referred to as ribozymes, and 3) RNA interference induced by small interfering RNA (siRNA) molecules.

In one study, phosphorothioate antisense oligonucleotides to p65 inhibited *in vitro* growth, reduced soft-agar colony formation, and eliminated the ability of cells to adhere to an ECM in diverse transformed cell lines [189]. In addition, stable transfectants of a fibrosarcoma cell line expressing dexamethasone-inducible antisense RNA to p65 showed inhibition of *in vitro* growth and *in vivo* tumor development. Furthermore, in response to inducible expression of antisense RNA, a pronounced tumor regression was seen in nude mice. The administration of antisense but not sense p65 oligonucleotides also caused a pronounced inhibition of tumorigenicity in nude mice injected with diverse tumor-derived cell lines [189].

RNA interference is the mechanism of sequence-specific, posttranscriptional gene silencing initiated by double-stranded RNA homologous to the gene being suppressed and has the potential to be a new and powerful tool in cancer therapeutics [190]. Surabhi and Gaynor [191] showed the potential of siRNAs in decreasing the level of expression of p65 protein [191]. Also, when siRNAs were directed against IKK $\alpha$ , IKK $\beta$ , and the upstream regulatory kinase transforming growth factor  $\beta$ -activated kinase-1, both IKK $\alpha$  and IKK $\beta$  were found to be important in activating the NF- $\kappa$ B pathway [192]. Another study showed that the blocking of c-Rel in primary macrophages with siRNA selectively blocked IL-12 production and normalized the minimal, residual IL-12 levels in a lupus erythematosus-prone and diabetes mellitus-prone mouse strain with aberrant IL-12 production [193].

#### 4.6. Peptides can Cross the Cell Membrane and Block NF- $\kappa$ B Activation

Another approach to inhibiting NF- $\kappa$ B activation is to use peptides that cross the cell membrane and block the nuclear localization of the NF- $\kappa$ B complex (Table 8). For example, SN-50 and *o,o'*-bismyristoyl thiamine disulfide [194] work by mimicking the sequence of p50 responsible for transporting the NF- $\kappa$ B complex from the cytoplasm to the nucleus to block the normal import machinery. A dual nuclear localization signal (NLS) peptide has been shown to block the karyopherin-NF- $\kappa$ B interaction, which is required for the translocation of NF- $\kappa$ B into the nucleus [195]. Also, a novel peptide that selectively blocks the association of IKK $\gamma$  with the rest of the IKK complex has been shown to inhibit NF- $\kappa$ B activation in response to proinflammatory cytokines in mice and at the same time to preserve basal NF- $\kappa$ B activity [196]. P1, another novel hybrid peptide derived from cecropin-A and magainin-2, reduced osteoclast differentiation in various osteoclast culture systems by inhibiting the NF- $\kappa$ B activation induced by RANKL [197].

NF- $\kappa$ B activation is known to require p65 phosphorylation at serine residues 276, 529, and 536 before it undergoes nuclear translocation. Small protein domains, termed protein transduction domains (PTDs), that are able to penetrate cell membranes can be used to transport other

proteins across the cell membrane. Our laboratory has identified two peptides from the p65 subunit of NF- $\kappa$ B (P1 and P6 from amino acid residues 271-282 and 525-537, respectively) that, when linked with a PTD derived from the third helix sequence of antennapedia, inhibited TNF-induced NF- $\kappa$ B activation *in vivo* [198]. Linkage to the PTD was not, however, required to suppress the binding of the p50-p65 heterodimer to the DNA *in vitro*, and PTD-p65-P1 had no effect on TNF-induced AP-1 activation. On the other hand, PTD-p65-P1 did suppress NF- $\kappa$ B activation induced by LPS, IL-1, okadaic acid, phorbol 12-myristate 13-acetate, H<sub>2</sub>O<sub>2</sub>, cigarette smoke condensate, and TNF. Although PTD-p65-P1 had no effect on the phosphorylation and degradation of the TNF-induced inhibitory subunit of NF- $\kappa$ B (I $\kappa$ B $\alpha$ ) or on I $\kappa$ B $\alpha$  kinase activation, it blocked TNF-induced p65 phosphorylation and nuclear translocation. These peptides also suppressed the NF- $\kappa$ B-regulated reporter gene expression induced by TNF, TNFR1, the TNFR-associated death domain, TNFR-associated factor-2, NF- $\kappa$ B-inducing kinase, I $\kappa$ B $\alpha$  kinase, and p65. Notably, the suppression of NF- $\kappa$ B by PTD-p65-P1 enhanced the apoptosis induced by TNF and chemotherapeutic agents.

#### 4.7. Antiinflammatory Agents Block NF- $\kappa$ B Activation

Additionally, several anti-inflammatory agents have been identified that suppress NF- $\kappa$ B activation. Examples include aspirin, ibuprofen, indomethacin, tamoxifen, dexamethasone, and sulindac [57] (Table 5). However, the exact mechanism of their action is not fully understood. Kopp and Ghosh [190] demonstrated that the anti-inflammatory drugs sodium salicylate and aspirin inhibited the activation of NF- $\kappa$ B by preventing the degradation of the NF- $\kappa$ B inhibitor, I $\kappa$ B, so that NF- $\kappa$ B was retained in the cytosol.

Our laboratory has investigated the effect of almost a dozen different commonly used nonsteroidal anti-inflammatory drugs and dexamethasone on TNF-induced NF- $\kappa$ B activation and NF- $\kappa$ B-regulated gene products and cell proliferation [199]. We included dexamethasone, an anti-inflammatory steroid, in this analysis for comparison with NSAIDs. As indicated by DNA binding, none of the drugs alone activated NF- $\kappa$ B. In fact, all of the compounds inhibited TNF-induced NF- $\kappa$ B activation, but with highly variable efficacy. Specifically, the IC<sub>50</sub>s required were 5.67 (aspirin), 3.49 (ibuprofen), 3.03 (sulindac), 1.25 (phenylbutazone), 0.94 (naproxen), 0.60 (indomethacin), 0.38 (diclofenac), 0.084 (resveratrol), 0.043 (curcumin), 0.027 (dexamethasone), 0.024 (celecoxib), and 0.010 (tamoxifen) mM. All drugs inhibited I $\kappa$ B $\alpha$  kinase and suppressed I $\kappa$ B $\alpha$  degradation and NF- $\kappa$ B-regulated reporter gene expression. They also suppressed NF- $\kappa$ B-regulated cyclooxygenase-2 and cyclin D1 protein expression in a dose-dependent manner. Furthermore, all compounds inhibited the proliferation of tumor cells, with IC<sub>50</sub>s of 6.09 (aspirin), 1.12 (ibuprofen), 0.65 (sulindac), 0.49 (phenylbutazone), 1.01 (naproxen), 0.19 (indomethacin), 0.36 (diclofenac), 0.012 (resveratrol), 0.016 (curcumin), 0.047 (dexamethasone), 0.013 (celecoxib), and 0.008 (tamoxifen) mM. Overall, we found that aspirin and ibuprofen are the least potent and resveratrol, curcumin, celecoxib, and tamoxifen are the most potent anti-inflammatory and antiproliferative agents of those studied.

The inhibition of NF- $\kappa$ B with these drugs represents a possible approach to solving the more complicated issue of creating drug therapies that are effective in preventing or attenuating tumorigenesis. Indeed, aspirin has been shown to be an effective therapeutic agent against cylindromatosis, colorectal cancers, and human hepatoma. Our understanding of the precise mechanisms of action, specificity, and even toxicity of these drugs with respect to NF- $\kappa$ B is still incomplete. However, targeting NF- $\kappa$ B seems central to designing an effective therapy for cancer.

#### 4.8. NF- $\kappa$ B can be Suppressed by Chemical Modifications

Various proteins that constitute the NF- $\kappa$ B machinery contain a highly reactive cysteine residue on their surfaces (Table 9). Because modifications of this residue can also suppress NF- $\kappa$ B activation, various agents have been tested for their ability to modify these cysteine residues. Some found to be effective in this regard include N-tosyl-L-phenylalanine chloromethyl ketone, caffeic acid phenethyl ester (CAPE), herbimycin, arsenite, sesquiterpene lactone, ethyl pyruvate, ethacrynic acid, and kaurane diterpene (kamebakaurin) (Table 6).

**Table 9. Inhibition of NF- $\kappa$ B Through the Critical Cysteine Residue**

IKK $\alpha$ / $\beta$	Cys 179 <sup>242</sup>
IKK $\beta$	Cys 59 <sup>243</sup>
IKK $\gamma$	Cys 417 <sup>244</sup>
p65	Cys 38 <sup>245</sup>
p50	Cys 62 <sup>246</sup>

### 5. NF- $\kappa$ B ACTIVATION IS A DOUBLE-EDGED SWORD

Although NF- $\kappa$ B activation in most cases is harmful [200], there is evidence that NF- $\kappa$ B activation also has beneficial effects. As previously shown, NF- $\kappa$ B activation regulates the expression of both antiapoptotic and proapoptotic genes. However, the downstream target genes regulated by NF- $\kappa$ B in the initiation of proapoptotic signaling are unclear. Perfettini *et al.* [201] found that NF- $\kappa$ B and p53 are the dominant apoptosis-inducing transcription factors elicited by the HIV-1 envelope. Additionally, Ravi *et al.* [202] found that NF- $\kappa$ B induces expression of death receptor 4 (DR4) and DR5 and that, conversely, a transdominant mutant of the inhibitory protein I $\kappa$ B $\alpha$  or a transactivation-deficient mutant of c-Rel reduces expression of either DR. Whereas NF- $\kappa$ B promotes DR expression, cytokine-mediated activation of the RelA subunit of NF- $\kappa$ B also increases expression of the apoptosis inhibitor Bcl-x<sub>L</sub> and protects cells from the TNF-related apoptosis-inducing ligand (TRAIL). Inhibition of NF- $\kappa$ B by blocking the activation of the IKK complex reduces Bcl-x<sub>L</sub> expression and sensitizes tumor cells to TRAIL-induced apoptosis. The ability to induce DRs or Bcl-x<sub>L</sub> may explain the dual role of NF- $\kappa$ B as a mediator and inhibitor of cell death during immune and stress responses. Fujioka *et al.* [203] showed that NF- $\kappa$ B mediates proapoptotic effects by activating the p53-signaling pathway in response to doxycycline-induced

superoxide. Additionally, Li *et al.* [204] demonstrated that the ability of doxycycline or superoxide to induce expression of polo-like kinase 3 (Plk3) depends on NF- $\kappa$ B activity. They identified a  $\kappa$ B binding site in the promoter of Plk3 and showed that this site is directly involved in Plk3 induction by the RelA-NF- $\kappa$ B complex [204]. Specifically, Plk3 formed a complex with p53 and was involved in the phosphorylation of p53 on Ser-20 in response to superoxide. The inhibition of Plk3 expression by Plk3 siRNA suppressed the doxycycline- and superoxide-mediated apoptosis. In addition, the overexpression of wild-type Plk3 in HCT116 p53<sup>+/+</sup> cells induced rapid apoptosis. However, the overexpression of wild-type Plk3 in HCT116 p53<sup>-/-</sup> cells and of the kinase-defective mutant Plk3(K91R) in p53<sup>+/+</sup> cells induced a delayed onset of apoptosis. Furthermore, the mutagenesis of Plk3 showed that the N-terminal domain (amino acids 1–26) is essential for the induction of delayed onset of apoptosis. These findings show that Plk3 is a RelA-NF- $\kappa$ B-regulated gene that induces apoptosis in both p53-dependent and -independent signaling pathways, thus suggesting a possible mechanism for RelA-NF- $\kappa$ B-regulated proapoptotic responses.

DR5 (TRAIL-R2) is a proapoptotic protein considered a potential target for cancer therapy; its expression is mediated by NF- $\kappa$ B. Shetty *et al.* [205] found that NF- $\kappa$ B differentially regulates DR5 expression that involves HDAC1. They specifically showed that etoposide-induced DR5 expression requires the first intronic region of the DR5 gene [205]. The mutation of a putative NF- $\kappa$ B binding site in this intron eliminates the DR5 promoter activity, as do mutations in the p53 binding site in this region. The reduction in p53 expression also blocked p65 binding to the intronic region of the DR5 gene, indicating cooperation between p53 and p65 in DR5 expression. They further found that HDAC inhibitors activate NF- $\kappa$ B and p53 and upregulate DR5 expression. The blockage of DR5 activation decreased HDAC inhibitor-induced apoptosis, and a combination of HDAC inhibitors and TRAIL increased apoptosis [205]. This provides a mechanism for regulating NF- $\kappa$ B-mediated DR5 expression and could explain the differential roles NF- $\kappa$ B plays in regulating apoptosis.

It is known that inactivation of apoptotic pathways is a common event in cancer and that p53 and NF- $\kappa$ B are transcription factors that regulate apoptosis during tumorigenesis. Although NF- $\kappa$ B is generally considered a suppressor of cell death, Ryan *et al.* [206] showed that NF- $\kappa$ B can contribute to p53-induced death. Specifically, they found that whereas the loss of NF- $\kappa$ B is tumor promoting, it does not substitute for the loss of p53. They also showed that the loss of p65, a critical subunit of NF- $\kappa$ B, can cause resistance to different agents that signal death through p53. The loss of p65 also enhances the tumorigenesis induced by E1a and Ras. Unlike the loss of p53, however, the loss of p65 does not cause anchorage-independent growth or enable tumor development after the expression of a single oncogene. These findings reaffirm the role of NF- $\kappa$ B in p53-induced death and also show that its loss does not substitute for the loss of p53 in tumor development. These findings further indicate that loss of the ability to induce programmed cell death, even if this ability is central to p53's function,

does not completely inactivate p53's tumor-suppressive effects. Interestingly, Bohuslav *et al.* [207] found that p53 induces NF- $\kappa$ B activation by an I $\kappa$ B kinase-independent mechanism involving the phosphorylation of p65 by ribosomal S6 kinase 1.

The role of the NF- $\kappa$ B family of transcription factors as tumor promoters is firmly established. However, some data suggest that NF- $\kappa$ B can also inhibit tumor growth. Moreover, NF- $\kappa$ B activity is modulated by tumor suppressors such as p53 and ARF whereby NF- $\kappa$ B subunits repress, rather than activate, the expression of tumor-promoting genes. This suggests a dual function of NF- $\kappa$ B during tumor progression: in the early stages, NF- $\kappa$ B inhibits tumor growth, but as further mutations lead to a loss of tumor-suppressor expression, the oncogenic functions of NF- $\kappa$ B become unleashed, allowing it to actively contribute to tumorigenesis. Perkins reviewed the implications of NF- $\kappa$ B function and how it might influence the use of NF- $\kappa$ B-based anticancer therapies [208].

## CONCLUSIONS

Overall, the cited studies have clearly shown that NF- $\kappa$ B plays a critical role in tumorigenesis. Similarly, the suppression of NF- $\kappa$ B can inhibit various steps in the tumorigenic process. Thus, the design of NF- $\kappa$ B inhibitors that are pharmacologically safe will be critical for the treatment of cancer. Because of the multiple agents that activate NF- $\kappa$ B through diverse pathways, it is unlikely that any single inhibitor of NF- $\kappa$ B could be effective against all tumors. Our efforts, therefore, should be directed toward developing NF- $\kappa$ B inhibitors that work best against individual cancers. Bortezomib (Velcade), also called PS341, is one such inhibitor that has already been approved by the US Food and Drug Administration for treatment of multiple myeloma.

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## ABBREVIATIONS

NF- $\kappa$ B	=	Nuclear factor- $\kappa$ B
IKK	=	I $\kappa$ B kinasen
TNF	=	Tumor necrosis factor
IL-1	=	Interleukin-1
LPS	=	Lipopolysaccharide
TNFR	=	TNF receptor
TRAF	=	TNF receptor-associated factor
NIK	=	NF- $\kappa$ B inducing kinase
NSCLC	=	Non-small cell lung carcinoma

MEF	=	Mouse embryo fibroblasts
PGE <sub>2</sub>	=	Prostaglandin E <sub>2</sub>
EGF	=	Epithelial growth factor
MALT	=	Mucosa-associated lymphoid tissue
MMP	=	Matrix metalloproteinase
uPA	=	Urokinase-type plasminogen activator
ECM	=	Extracellular matrix
PI3k	=	Phosphatidylinositol 3'-kinase
IκBαM	=	Mutant IκBα
HDAC	=	Histone deacetylase
PIK3	=	Polo-like kinase 3
siRNA	=	Small interfering RNA
DR	=	Death receptor
PTD	=	Protein transduction domain

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