# Nuclear factor-κB and inhibitor of κB kinase pathways in oncogenic initiation and progression

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**Abundant data support a key role for the transcription factor nuclear factor-κB (NF-κB) signaling pathway in controlling the initiation and progression of human cancer. NF-κB and associated regulatory proteins such as IκB kinase (IKK) are activated downstream of many oncoproteins and there is much evidence for the activation of NF-κB-dependent target genes in a variety of solid tumors and hematologic malignancies. This review focuses on the mechanisms by which the NF-κB pathway is activated in cancer and on the oncogenic functions controlled by activated NF-κB. Additionally, the effects of NF-κB activation in tumors relative to cancer therapy are also discussed.**

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## Regulation of NF-κB

NF-κB is a dimeric transcription factor formed by members of a family of proteins that share a conserved N-terminal dimerization/DNA-binding region designated the Rel homology domain. In mammalian cells, the NF-κB family is composed of five members: RelA(p65), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2) (Gilmore, 2006). The ability of these five members to form a variety of homo- and heterodimers and, ultimately, to differentially control gene expression is regulated by signals elicited by bacterial products, cytokines, viral expression, growth factors and stress stimuli (reviewed by Hayden and Ghosh, 2004).

Based on their overall structures and processing, the NF-κB family can be further subdivided into the 'NF-κB' proteins (p50/p105, p52/p100) and the 'Rel' proteins (RelA, RelB, c-Rel). The NF-κB proteins, p50 and p52, are produced by proteolytic removal of C-terminal sequences from larger precursor proteins (p105 and p100, respectively). In contrast, RelA, RelB and c-Rel contain C-terminal transactivation domains, which are not present in p50 and p52. Nonetheless, the p50 and p52 subunits provide DNA-binding specificity by forming heterodimers with other NF-κB subunits and are known to contribute to gene expression. Specifically, p50/p50 or p52/p52 homodimers can positively and negatively regulate expression of NF-κB target genes by recruiting co-activators or co-repressors. For instance, p50 appears to both positively and negatively regulate the expression of the KAI1 metastasis suppressor gene (Kim *et al*., 2005a). In this case, the oncoprotein β-catenin simultaneously displaces the Tip60 co-activator from the p50 complex in the KalI promoter and induces recruitment of transcriptional co-repressors, thereby converting the p50 transcriptionally active complex into a repressive complex (Kim *et al*., 2005a).

Under most basal conditions, NF-κB complexes are maintained in an inactive form primarily through interactions with the inhibitor of κB (IκB) family of proteins. The IκB family has several known members, including IκBα, IκBβ, IκBε, Bcl-3, p100 and p105, all of which contain ankyrin-like repeats that mediate binding to and inhibition of NF-κB. Generally, the IκB proteins sequester the interacting NF-κB dimeric complexes in the cytoplasm, and therefore the ability of NF-κB to bind DNA and regulate gene expression is blocked. It should be noted that Bcl-3 is unusual in that it does not induce cytoplasmic retention, but rather positively regulates gene expression by acting as a transcriptional co-activator for NF-κB p50 and p52 homodimers in the nucleus on DNA (Fujita *et al*., 1993; Westerheide *et al*., 2001).

Most stimuli activate NF-κB by modulating the activity of the IκB kinase (IKK) complex, which is comprised of three subunits: IKKα, IKKβ and NF-κB essential modulator (NEMO) (IKKγ). IKKα and IKKβ are both catalytic kinases, whereas NEMO acts as a regulatory scaffold component for the IKK complex. Upon stimulation, the IKK complex phosphorylates the IκB proteins, which then undergo rapid ubiquitination and proteasome-mediated degradation, which culminates in the release of the NF-κB complexes from their inhibitory interaction (see Scheidereit, 2006). The released complexes then accumulate in the nucleus, where they bind to target DNA sequences and regulate the expression of genes involved in the immune response, cell growth control and the regulation of cell survival (Hayden and Ghosh, 2004).

There are, however, two distinct pathways that lead to the activation of NF-κB – the canonical and the

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non-canonical pathways. These pathways are activated by often distinct stimuli, require different IKK complexes, and induce different NF-κB complexes and different target genes (see Scheidereit, 2006). In the canonical pathway, complexes such as p50/RelA-IκBα are induced by an IKK complex that requires IKKα, IKKβ and NEMO, whereas the non-canonical pathway requires only IKKα and induces the partial degradation of p100/RelB to p52/RelB.

It should be noted that in addition to the well-characterized mechanisms of negative and positive regulation of NF-κB described above, other pathways involving different kinases, such as NF-κB-inducing kinase (NIK) and IKKε, can also lead to NF-κB activation through alternative pathways (Hayden and Ghosh, 2004; Adli and Baldwin, 2006). In addition, a variety of post-translational modifications of the NF-κB subunits or signaling components can also modulate the strength and duration of the NF-κB signal (Perkins, 2006). As described in this review, NF-κB and IKK proteins are also associated with oncogenesis and cancer therapy responses through a complex and dynamic set of regulatory pathways.

## Linking NF-κB with oncogenesis

The initial cloning of the NF-κB p50/p105 subunit cDNA revealed homology to the cellular homolog (c-Rel) of the oncoprotein (v-Rel) of the avian reticuloendotheliosis virus, suggesting a link between NF-κB and oncogenesis. Subsequently, it was shown that the NF-κB p52/p100 subunit encoded by the *NFKB2* gene undergoes structural alterations in certain T-cell lymphomas, chronic lymphocytic leukemias, myelomas and B-cell lymphomas (see Courtois and Gilmore, 2006). Similarly, the gene for the IκB family member Bcl-3 is found to be translocated and overexpressed in some B-cell leukemias. In addition, c-*rel* gene amplification is detected in several types of B-cell lymphoma, whereas a subset of Hodgkin's lymphoma cases contain IκBα gene mutations or deletions (Orlowski and Baldwin, 2002; Courtois and Gilmore, 2006).

In addition to these genetic observations, gene expression studies have identified NF-κB transcription targets that are likely to promote the oncogenic phenotype (see Figure 1). For example, gene profiling has identified a subset of diffuse large B-cell lymphomas (the activated B-cell-like tumors) that requires NF-κB for growth and survival; in this case, the expression of genes associated with this type of lymphoma is blocked when NF-κB activity is inhibited (Lam *et al.*, 2005). Similarly, in murine fibroblasts, oncogenic Ras expression regulates a set of approximately 25 genes in a manner dependent on NF-κB activity (Hanson *et al.*, 2004). Interestingly, these studies also indicate that the NF-κB-controlled gene sets in distinct oncogenic settings are significantly different. Nonetheless, cancer relevant, NF-κB-dependent genes include those encoding cytokines, chemokines, cyclin D1, matrix metalloproteinases and antiapoptotic proteins (Figure 1).

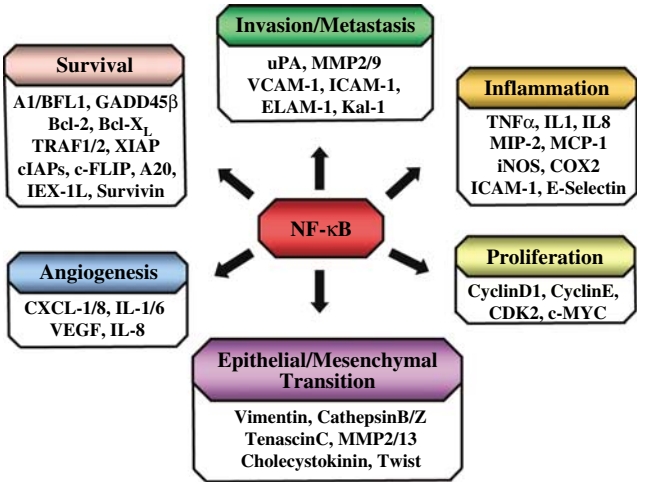


**Figure 1** Representation of NF-κB-dependent targets involved in different aspects of oncogenesis. See text for further information.

As described below, recent evidence has accumulated from a large variety of human malignancies indicating a role for NF-κB in promoting oncogenic conversion and in facilitating later stage tumor properties such as metastasis (Rayet and Gélinas, 1999; Baldwin 2001; Karin *et al.*, 2002).

## Cancer-associated mechanisms and NF-κB

Human cancer is a disease that stems from diverse etiologies and that harbors distinct affected cell targets, having therefore a very complex nature. Nevertheless, Hanahan and Weinberg (2000) have outlined a number of common features that override normal controls on proliferation and homeostasis and which appear to be associated with virtually all cancers. These shared properties are intrinsic to the cancer cells, but also can originate from signals that come from the surrounding tumor microenvironment. These common, cancer-associated mechanisms include the following: self-sufficiency in growth and loss of growth inhibitory mechanisms; suppression of apoptotic thresholds; enhanced angiogenic properties; and the ability to invade local tissue and metastasize to distant sites (reviewed by Hanahan and Weinberg, 2000). Interestingly, all these cellular processes are known to be affected in one way or another by the NF-κB pathway (see below).

### *Growth and proliferation*

The growth of cancer cells in an autonomous manner is often controlled by altered expression of growth factors or growth factor receptors, leading to cell proliferation. A relatively common growth mechanism in epithelial cancers is the upregulation of expression of members of the epidermal growth factor (EGF) receptor (EGF-R) family such as the EGF receptor or the Her-2/ErbB2 receptor. Additionally, growth factors such platelet-derived growth factor (PDGF) and transforming growth

factor alpha (TGFα) are often expressed in cancers and promote cell proliferation in an autocrine manner (reviewed by Hanahan and Weinberg, 2000). Nonetheless, cancer cell growth promotion can also be stimulated by cancer-associated stromal cells or recruited inflammatory cells through paracrine mechanisms.

Another common mechanism that promotes the growth of cancer cells is the presence of mutations in proteins that regulate cell proliferation. Thus, activating mutations in cellular *RAS* alleles lead to chronic stimulation of signal-transduction pathways such as extracellular signal-regulated protein kinase, phosphatidylinositol 3-kinase (PI3K)/Akt and RalGDS, which are involved in cell growth and survival. Additionally, cancer cell growth can be promoted by activating mutations in transcription factors, such as β-catenin, which controls expression of genes that stimulate proliferation, such as cyclin D1 or c-*myc*. Furthermore, resistance to growth inhibitory signals can be achieved through mutations in tumor suppressor genes such as Rb, APC, Arf or p53, or in receptors such as those for TGFβ (Hanahan and Weinberg, 2000).

Interestingly, NF-κB can promote cellular proliferation through regulation of specific target genes. For example, NF-κB can promote Rb hyperphosphorylation by binding and activating the cyclin D1 promoter (Karin, 2006; Kim *et al*., 2006). Additionally, the IκB homolog Bcl-3 in association with p52 homodimers has also been found to transactivate potently the cyclin D1 gene (Westerheide *et al*., 2001). Furthermore, IKKα has been proposed to play a role in cyclin D1 transcription through a T-cell factor site in the promoter, via its ability to control β-catenin phosphorylation (Albanese *et al*., 2003). Consistent with a role for IKKα in promoting cyclin D1 transcription, Karin *et al*. (2002) have found that during mammary gland development IKKα is required for RANK signaling and cyclin D1 expression. Other mechanisms whereby NF-κB may potentiate cancer cell growth is through its reported requirement for the upregulation of HIF-1α (Jung *et al*., 2003) and its regulation of c-*myc* transcription (see Baldwin 2001; Karin *et al*., 2002). Consistent with these reports, tumor growth in several mouse xenograft models is impaired when NF-κB is inhibited (see Reuther *et al*., 1998; Baldwin, 2001; Karin *et al*., 2002).

*Apoptosis evasion*

Resistance to apoptosis is a common feature of cancer cells and is associated with the increased expression of antiapoptotic factors, such as Bcl-2 or Bcl-xL, or the loss of expression, inactivation or mutation of proapoptotic factors, such as Foxo3a or p53. For instance, mutations in the tumor suppressor PTEN lead to the activation of intracellular signaling pathways, which suppress apoptosis via increased PI3K/Akt activity. Relative to Akt, this Ser-Thr kinase is known to target the phosphorylation of several factors associated with regulating apoptosis (Downward, 2004). An additional mechanism of suppression of cancer cell apoptosis can be derived from the release of cytokines from the tumor stroma. Importantly, expression of antiapoptotic factors in tumor cells provides a strong mechanism to suppress cancer therapy efficacy (see below).

Interestingly, NF-κB directly regulates a potent antiapoptotic pathway. Genes regulated by NF-κB that suppress apoptosis, such as Bcl-2 and Bcl-xL (see Figure 1), are often expressed in human cancers. Given the strong association between NF-κB and the regulation of apoptosis (Burstein and Duckett, 2003; Kucharczak *et al*., 2003; Dutta *et al*., 2006), it is not surprising that many studies suggest that NF-κB controls the antiapoptotic mechanisms associated with oncogenesis. Experiments revealed that induction of expression of H-RasV12 in immortalized mouse fibroblasts leads to cellular transformation but not to apoptosis, and inhibition of NF-κB in H-RasV12-transformed cells by the expression of a 'super-repressor' degradation-resistant form of IκBα (IκBα-SR) is associated with high levels of apoptosis (Mayo *et al*., 1997; Baldwin, 2001), indicating that NF-κB provides a prosurvival function in the context of Ras-induced oncogenesis. Consistent with this point, inhibition of NF-κB in certain tumor cell lines leads to apoptotic cell death (Karin *et al*., 2002; Kim *et al*., 2006). For instance, proliferation and survival of Hodgkin/Reed–Sternberg cells is blocked when NF-κB is inhibited by IκBα expression (Bargou *et al*., 1997). Moreover, inhibition of NF-κB in Hodgkin/Reed–Sternberg cells leads to the loss of expression of antiapoptotic effectors A1/Bfl-1, cellular-inhibitor of apoptosis 2 (c-IAP2), tumor necrosis factor receptor-associated factor1 and Bcl-xL (Hinz *et al*., 2001). Finally, another mechanism whereby NF-κB may block cell death is through its ability to suppress persistent c-Jun $NH_2$-terminal kinase (JNK) activation and the generation of reactive oxygen species (see Luo *et al*., 2005; Bubici *et al*., 2006).

The NF-κB upstream regulatory pathway also plays a role in preventing apoptosis. In that sense, Hu *et al*. (2004) have shown that IKKβ activation in breast cancer cells promotes cell proliferation and suppresses apoptotic potential through phosphorylation and degradation of the proapoptotic factor Foxo3a.

*Angiogenesis, invasion and metastasis*

Tumor progression is determined by different mechanisms. Most tumors exhibit properties associated with inducing and sustaining angiogenesis, a process that appears to be required for tumor maintenance and progression. Often associated with the appearance of angiogenesis is the ability of tumor cells to invade local tissues and subsequently to migrate systemically to preferred sites (metastasis). The ability of tumor cells to invade locally and to metastasize is also dependent on suppression of apoptotic potential, on changes in the expression of cell adhesion molecules and integrins, and on changes in the expression of extracellular proteases such as matrix metalloproteinase (MMP)-2 and MMP-9.

An additional key mechanism in the progression of many cancers is the epithelial-to-mesenchymal transition. While the differentiated epithelial phenotype is typically characterized by the polarization of the cell surface into apical and basolateral domains and by a junctional complex that controls intercellular adhesion, many cancers exhibit an alteration in this phenotype, with progression to a metastatic phenotype (Thiery, 2002).

NF-$\kappa$B appears to play a role in each of these processes. In this regard, NF-$\kappa$B has been reported to promote both angiogenesis and metastasis in certain tumor models, potentially through the regulation of vascular endothelial growth factor (VEGF) and MMPs (Figure 1) (see Baldwin, 2001; Karin *et al*., 2002). In addition, expression of the super-repressor form of I$\kappa$B$\alpha$ in human melanoma and ovarian cancer cells correlates with reduced VEGF and interleukin (IL)-8 expression and blocks growth, angiogenesis and metastasis of tumor xenografts (Huang *et al*., 2000b). Moreover, NF-$\kappa$B inhibition by overexpression of I$\kappa$B$\beta$ in lung cancer cells suppressed their ability to form metastasis (Jiang *et al*., 2001). It should be noted that these studies often demonstrate that inhibition of NF-$\kappa$B also causes reduction of tumor growth, so that it is difficult to demonstrate absolutely that NF-$\kappa$B directly controls angiogenesis and metastasis. Finally, NF-$\kappa$B has been shown to mediate the epithelial–mesenchymal transition (Huber *et al*., 2004a, b), although the exact mechanism is unclear.

## NF-$\kappa$B in the tumor micro-environment

As described above, NF-$\kappa$B- and IKK-controlled pathways appear to play key roles in cancer cell autonomous phenotypes, but there is also evidence that NF-$\kappa$B functions in tumor-associated cells to affect oncogenic mechanisms. Using IKK$\beta$ ablation in myeloid cells in a carcinogen/inflammation model for colorectal cancer, Greten *et al*. (2004) showed that NF-$\kappa$B controls the ability of invading myeloid cells to produce cytokines, which are involved in promoting tumor growth. In another interesting study, NF-$\kappa$B appears to mediate an IL-1/nitric oxide paracrine growth loop involving stromal fibroblasts and pancreatic neoplastic cells (Muerkoster *et al*., 2004).

## Mechanisms of NF-$\kappa$B activation in cancers

An outline of the regulatory pathways that target NF-$\kappa$B in oncogenesis is presented in Figure 2.

*Activation by oncoproteins*

Many oncoproteins can activate NF-$\kappa$B (see Figure 2) as measured through reporter assays or through analysis of nuclear levels of NF-$\kappa$B DNA-binding activity (Baldwin, 2001; Karin *et al*., 2002). For instance, NF-$\kappa$B is activated by both Her-2/Neu (ErbB2) and oncogenic H-Ras expression (Finco *et al*., 1997; Pianetti *et al*., 2001). Interestingly, in immortalized murine fibroblasts, oncogenic H-Ras requires the NF-$\kappa$B subunits RelA and c-Rel for efficient cellular transformation (Hanson *et al*., 2004). In addition, activation of NF-$\kappa$B by oncogenic Ras was reported to involve the IKK complex (Arsura *et al*., 2000; Figure 2). Another oncoprotein shown to activate an NF-$\kappa$B-dependent reporter is Bcr-Abl, the fusion protein associated with chronic myelogenous leukemia (CML) (Reuther *et al*., 1998). Interestingly, Bcr-Abl-induced tumor growth is

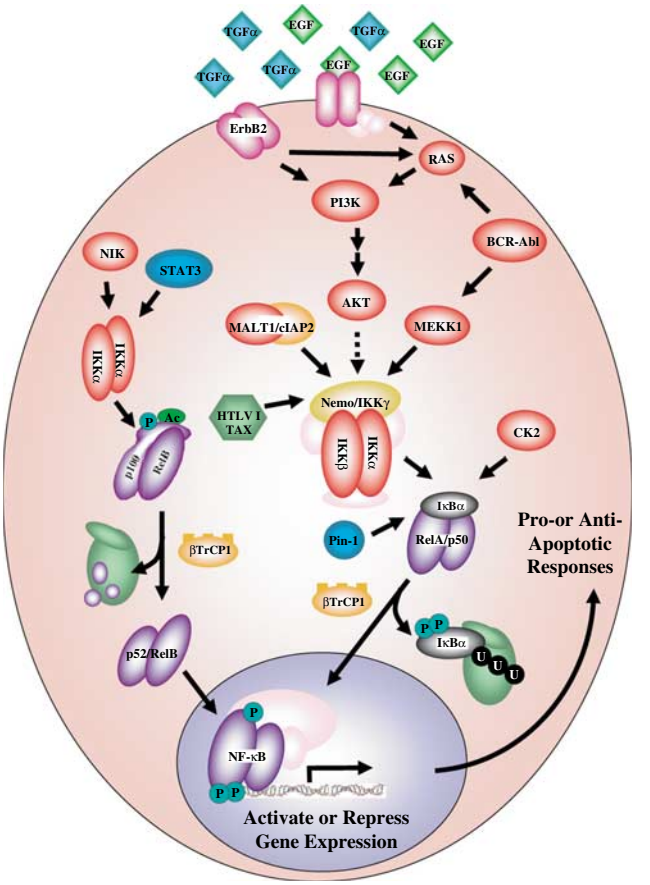


**Figure 2** Pathways of activation of NF-$\kappa$B by oncoproteins and growth factors. Overexpression of EGF-R family members (EGFr or ErbB2) by or ligand overexpression (EGF, TGF$\alpha$, etc.), as well expression of oncoproteins, such as Ras, Bcr-Abl, MALT1/c-IAP2 and Tax ultimately activate NF-$\kappa$B through the canonical pathway by targeting the IKK complex and leading to nuclear translocation of RelA/p50 heterodimers. Nonetheless, whereas MALT1/c-IAP2 and Tax can directly activate IKK, EGF-R family receptors, Ras and Bcr-Abl activate IKK indirectly through stimulation of cancer-associated pathways, such as PI3K/Akt and MEKK1. Interestingly, Ras can also be activated downstream of EGF-R family receptors and Bcr-Abl. Both CK2 and Pin-1 can activate NF-$\kappa$B downstream of IKK. Whereas Pin-1 binds to RelA, releasing its interaction with I$\kappa$B$\alpha$, CK2 promotes I$\kappa$B$\alpha$ degradation. NF-$\kappa$B can also be activated by the non-canonical pathway in cancer. For instance, NIK and STAT3 promote p100 processing by activating IKK$\alpha$, resulting in nuclear translocation of p52/RelB heterodimers. Interestingly, the E3-ubiquitin ligase receptor $\beta$TRCP can activate NF-$\kappa$B both through the canonical and non-canonical pathways; it is able to promote ubiquitination of both p100 and I$\kappa$B$\alpha$, ultimately leading to p100 processing or I$\kappa$B$\alpha$ degradation.

blocked by IκBα-SR-mediated NF-κB inhibition (Reuther *et al*., 1998). In addition, Bcr-Abl-induced NF-κB activation seems to involve the activation of the kinase MEK kinase 1 (MEKK1) (Nawata *et al*., 2003) (see Figure 2). Consistent with a role for NF-κB in Bcr-Abl-associated malignancies, NF-κB was found to be chronically active in CML and in Bcr-Abl-positive acute lymphocytic leukemias (Kirchner *et al*., 2003; Munzert *et al*., 2004; Cilloni *et al*., 2006). Another example of activation of NF-κB by an oncoprotein is the mechanism whereby the HTVL-1 Tax protein activates NF-κB; that is, Tax can directly bind and activate the IKK complex (Hiscott *et al*., 2006). In certain lymphomas, NF-κB activation can also be triggered by the fusion protein mucosa-associated lymphoid tissue (MALT)1/c-IAP2; this protein activates NF-κB through ubiquitination of NEMO (Zhou *et al*., 2005a). A number of other transforming proteins have been shown to activate NF-κB, such as Vav (Palmby *et al*., 2004), Pim-2 (Hammerman *et al*., 2004) and B-Raf (Ikenoue *et al*., 2003).

*Activation of NF-κB by oncogenic-associated pathways*

Different signaling pathways known to play a role in oncogenesis activate NF-κB. For example, PI3K/Akt-dependent signaling activates NF-κB (Figure 2); this occurs in a manner dependent on the relative levels of the IKKα subunit (Gustin *et al*., 2004). In addition, Akt is activated in primary acute myeloid leukemia and this is associated with cell survival and NF-κB activation (Grandage *et al*., 2005). Another example is the activation of Akt in human melanoma, which leads to NF-κB activation and tumor progression (Dhawan *et al*., 2002). Moreover, PI3K/Akt-dependent signaling is involved in the activation of NF-κB downstream of Her-2/ErbB2 (Pianetti *et al*., 2001). Relative to other signaling pathways, in a significant number of lung adenocarcinomas, Fas-associated death domain phosphorylation is associated with poor clinical outcome and induces IKK-mediated NF-κB activation (Chen *et al*., 2005). Interestingly, in pancreatic cancer cells, high levels of expression of the E3-ubiquitin ligase receptor beta-transducin repeat-containing protein 1 (βTRCP1) is proposed to activate NF-κB (Muerkoster *et al*., 2005; Figure 2). Finally, in breast cancer, CK2 was proposed to induce NF-κB (Figure 2) and this effect was recently proposed to involve the upregulation of the IKKε subunit (Eddy *et al*., 2005).

*Activation downstream of growth factors and growth factor receptors*

Growth factors play an important role in promoting oncogenesis; thus, it is not surprising that certain growth factors or expression of growth factor receptors can activate NF-κB (Figure 2). For example, in certain cell types, NF-κB can be activated by EGF (Biswas *et al*., 2000), although the mechanism associated with this activation is poorly characterized. Sitcheran *et al*. (2005) have shown that EGF can induce recruitment of RelA to the excitatory amino-acid transporter (EAAT)2/glutamate transporter promoter through a mechanism independent of IκB degradation. Moreover, NF-κB is activated by the EGF-R family member Her-2/ErbB2 (Pianetti *et al*., 2001), and there appears to be a specific set of genes controlled by NF-κB downstream of Her-2/ErbB2 (Merkhofer E and Baldwin AS, unpublished). NF-κB activation can also be induced by the growth factor PDGF, leading simultaneously to cell proliferation by induction of c-*myc* transcription and to increased survival by suppression of apoptosis (Romashkova and Makarov, 1999). Finally, in human mast cells, the NF-κB pathway can also be induced by constitutively activated c-Kit receptors, resulting in the upregulation of cyclin D3 expression and cell cycle progression (Tanaka *et al*., 2005).

*Other mechanisms for the activation of NF-κB in cancer*

The proinflammatory cytokine IL-6 can act as a survival factor for different cancer cells and induces signaling through the signal transducers and activators of transcription (STAT) proteins. Interestingly, constitutively active STAT3 is found in IL-6-dependent multiple myeloma cell lines that are resistant to apoptosis (Catlett-Falcone *et al*., 1999). In addition, STAT3 levels are elevated in a number of human cancers, including breast cancer, prostate cancer, head and neck cancers, and hematologic malignancies (Bowman *et al*., 2000). Furthermore, STAT3 induces cellular transformation *in vitro* and tumor formation in nude mice (Bromberg *et al*., 1999). When acetylated, STAT3 promotes p100 processing to p52 and thereby activates NF-κB (Nadiminty *et al*., 2006, Figure 2). Therefore, the regulation of NF-κB downstream of STAT3 activation in IL-6-producing cancers should be considered as a relevant oncogenic event.

Another unexpected mechanism to activate NF-κB in cancer has been proposed to involve the prolyl isomerase Pin1. Ryo *et al*. (2003) showed that Pin1 expression in human breast cancer samples correlates with nuclear levels of RelA. This study suggests that Pin1 binds to RelA, inhibiting interaction with IκB and resulting in increased nuclear accumulation of this NF-κB subunit (Figure 2). Consistently, downregulation of Pin1 with small interfering RNA in two breast cancer cell lines suppressed NF-κB activity.

## Inhibition of NF-κB by tumor suppressors

NF-κB activity is clearly modulated by oncoproteins, but evidence has also been presented that NF-κB activation can be blocked by certain tumor suppressors. One example is the tumor suppressor Arf, which has been shown to inhibit NF-κB through a mechanism involving ATR- and Chk1-induced phosphorylation of RelA (Rocha *et al*., 2005). Additionally, the tumor suppressor CYLD blocks NF-κB and Bcl-3 activation through its deubiquitinating activity (Kovalenko *et al*., 2003; Trompouki *et al*., 2003; Massoumi *et al*., 2006). Moreover, the tumor suppressor menin, which is mutated/deleted in parathyroid tumors, was reported

to bind and suppress NF-κB activation (Heppner *et al*., 2001). Regarding the tumor suppressor p53, it was reported that p53 generally inhibits NF-κB function, and *vice versa* (Webster and Perkins, 1999); however, more complex relationships between p53 and NF-κB have emerged (see below).

## NF-κB in human cancers

NF-κB activation as measured by nuclear accumulation or by increased DNA-binding activity has been observed in a wide variety of solid tumors and hematologic malignancies (see Figure 3). Below we discuss a few examples of NF-κB activation and its involvement in different cancers.

### *Breast cancer*

Breast cancers are phenotypically heterogeneous, presently classified into six subtypes based on gene expression profiling (Ramaswamy and Perou, 2003). Nonetheless, different studies indicate that NF-κB is activated in breast cancer cells, where it affects cell proliferation, suppresses apoptosis as well as promotes anchorage-independent growth. Indeed, a variety of breast cancer cell lines displays increased NF-κB DNA-binding activity (Nakshatri *et al*., 1997). However, the classic NF-κB complex composed of p50/RelA heterodimers is detected only in a minority of breast cancers (Cogswell *et al*., 2000). Consistent with this, the RelA subunit was detected as nuclear in approximately 16% of primary breast cancers. On the other hand, Biswas *et al*. (2004) reported that the p50/RelA heterodimer is activated in the majority (86%) of estrogen receptor (ER)-negative, ErbB2/Her2-positive breast tumors. However, since elevated ErbB2 expression occurs in approximately 20–25% of breast tumors, the above results suggest that the activation of RelA is not widely prevalent in primary breast tumors but may be associated more with distinct subtypes (i.e., Her2 positive/ER negative). In addition, these results also suggest that it is the loss of ER and the expression of ErbB2 that strongly activates the binding potential of the p50/RelA heterodimer. Indeed, whereas Her2/ErbB2 activates NF-κB by a mechanism that involves the PI3K/Akt pathway and utilizes calpain to degrade IκBα (Pianetti *et al*., 2001), ER has been shown to regulate NF-κB negatively by a variety of mechanisms (Kalaitzidis and Gilmore, 2005).

Interestingly, other NF-κB family members have been implicated in breast cancer. For example, transgenic mice with mammary cell-specific c-Rel expression develop mammary tumors, albeit with a very long latency (Romieu-Mourez *et al*., 2002). Moreover, Cogswell *et al*. (2000) noted, in approximately 40% of breast tumors, an increased binding potential of p50- and p52-containing complexes with an increased expression of Bcl-3. Additionally, it was reported that enhanced DNA-binding activity associated with the p50 NF-κB subunit is associated with ER-positive breast cancers destined for early relapse despite adjuvant endocrine therapy with tamoxifen, suggesting an involvement of this aspect of the NF-κB pathway in endocrine-resistant breast cancer (Zhou *et al*., 2005b). Recently, Eddy *et al*. (2005) provided evidence that NF-κB activation in breast cancer cell lines is associated with aberrant expression of the IKKε subunit, and

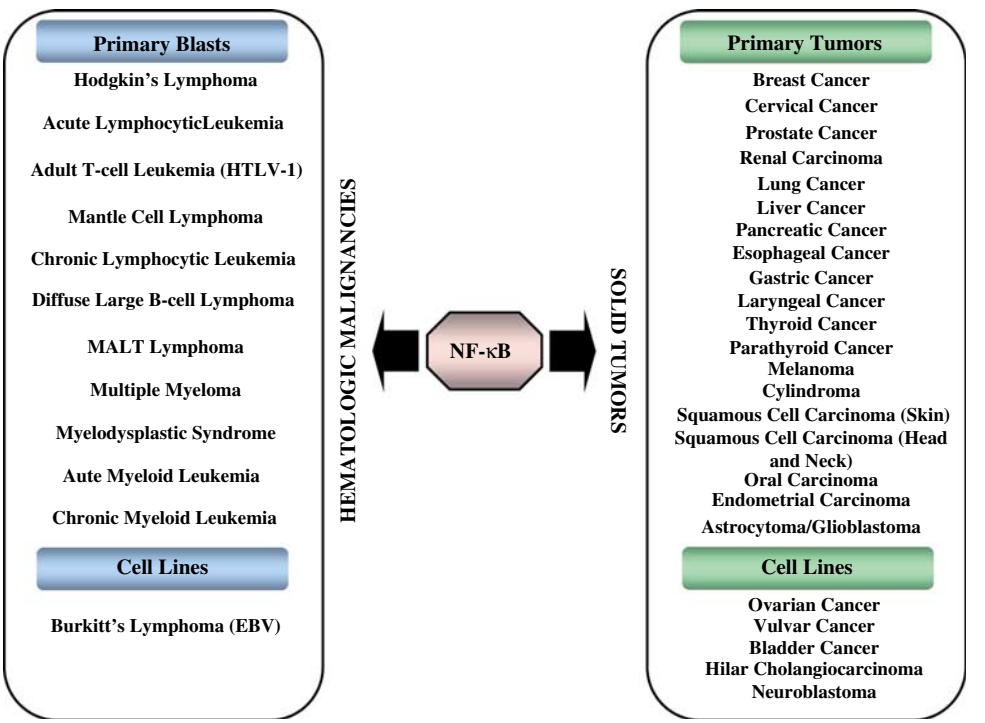


**Figure 3** Constitutive NF-κB activation in human cancers.

elevated expression of IKKε was detected in 4/6 primary tumor samples. Interestingly, we have recently found that, in ceratin cancer cells, IKKε can induce the phosphorylation of RelA at Ser-536 (Adli and Baldwin, 2006). These results raise the possibility that breast tumor subtypes exhibit different forms of NF-κB activation, some associated with enhanced p50/RelA DNA binding, some with enhanced p50 or p52 DNA-binding activity, some with enhanced c-Rel expression, and some with increased Ser-536 phosphorylation of RelA.

*Melanoma and NF-κB*

Melanoma is a skin cancer that originates in melanocytes and which progresses from pigmented lesions called benign nevi to dysplastic nevi. The disease can then progress to *in situ* melanoma followed by the potential for invasive melanoma. Invasive melanoma is highly resistant to standard forms of cancer therapy (for a review, see Ueda and Richmond, 2006). Based on the fact that several NF-κB-regulated chemokines are constitutively expressed in human melanoma (Mrowietz *et al*., 1999; Singh *et al*., 1999; Ueda and Richmond, 2006), it has been proposed that NF-κB plays an important role in melanoma. The role for these chemokines is thought to be at the level of enhancement of growth and survival through autocrine and paracrine mechanisms. Consistent with this, inhibition of IL-8 (an NF-κB target) through the use of neutralizing antibodies inhibits tumor angiogenesis in xenograft models (Huang *et al*., 2002). In addition, suppression of NF-κB activation by expression of the IκBα super-repressor can block melanoma xenograft growth, angiogenesis and metastasis (Huang *et al*., 2000a). Interestingly, the activation of NF-κB in melanoma can be mediated by NIK, a regulator of the non-canonical pathway of NF-κB activation (Dhawan and Richmond, 2002). Other mechanisms associated with NF-κB activation in melanoma have been proposed to involve loss of p16, signaling downstream of N-Ras and B-raf, Akt activation associated with PTEN loss, and autocrine/paracrine mechanisms associated with chemokines and G-coupled protein receptors or with growth factors such as EGF (Ueda and Richmond, 2006).

*NF-κB and pancreatic cancer*

Pancreatic ductal adenocarcinoma is a fatal disease, affecting about 30 000 people per year in the United States. Pancreatic cancer is associated with inflammation either through pancreatitis or through *Helicobacter pylori* positivity (Garcea *et al*., 2005). In a variety of pancreatic cancer cell lines, but not in immortalized pancreatic epithelial cells, NF-κB was found to be constitutively activated. Activation of NF-κB also has been observed in animal models for pancreatic cancer and in human tissue from pancreatic tumors (Wang *et al*., 1999c; Liptay *et al*., 2003; Garcea *et al*., 2005). Using cell lines and xenografts, it appears that NF-κB promotes proliferation and suppresses apoptosis in these cancer cells (Liptay *et al*., 2003). Inhibition of NF-κB via super-repressor IκBα expression blocks angiogenesis and metastasis in human cancer-derived xenograft tumors (Fujioka *et al*., 2003). Mechanisms of NF-κB activation in pancreatic cancer appear to involve K-Ras and Akt activation, as well as Notch-1 signaling, nuclear glycogen synthase kinase-3β, and Vav1 (Wang *et al*., 1999c, 2006; Asano *et al*., 2004; Fernandez-Zapico *et al*., 2005; Ougolkov *et al*., 2005). Additionally, it has been reported that autocrine production of IL-1α regulates NF-κB activation in pancreatic cancer cells (Niu *et al*., 2004). NF-κB-regulated gene targets that encode proteins potentially relevant to the maintenance and invasion of pancreatic cancer cells are proposed to be GADD45α and urokinase-type plasminogen activator (Wang *et al*., 1999b; Schneider *et al*., 2006).

*NF-κB in hematologic malignancies*

In addition to the observed NF-κB activity in solid tumors, there is widespread observation of NF-κB activation in hematological malignancies (see Figure 3). For example, NF-κB activation and increased IKK activity are found in blasts and stem cells associated with acute myelogenous leukemia (AML) (Baumgartner *et al*., 2002). Consistent with this, NF-κB activation is detected in the bone marrow of patients with myelodysplastic syndrome (Braun *et al*., 2006), which is considered a precursor disease of AML. NF-κB is also activated in childhood acute lymphoblastic leukemia (Kordes *et al*., 2000), Hodgkin–Reed–Sternberg cells (Bargou *et al*., 1997), HTLV-1-positive leukemias (Hiscott *et al*., 2006), B-cell chronic lymphocytic leukemia (Furman *et al*., 2000; Zaninoni *et al*., 2003) and in primary blasts of CML (Kirchner *et al*., 2003). In addition, NF-κB-dependent gene transcription is a hallmark of the activated B-cell-like subset of diffuse large B-cell leukemia (Lam *et al*., 2005; Ngo *et al*., 2006). Finally, NF-κB activation is also associated with multiple myeloma (Ni *et al*., 2001), where it seems to play a crucial role. The importance of NF-κB in multiple myeloma is suggested from its involvement downstream of CD40, the tumor necrosis factor (TNF) receptor family member that is expressed in a variety of B-cell malignancies and which is associated with multiple myeloma homing. Consistent with this, monoclonal antibodies to CD40 block CD40L-induced NF-κB activation as well as IL-6 and VEGF secretion in co-cultures of multiple myeloma cells and bone marrow-derived stromal cells (Tai *et al*., 2005). Interestingly, multiple myeloma is currently treated with compounds that can block NF-κB activation (Mitsiades *et al*., 2002a) (see below).

## Localized inflammation promotes oncogenesis

Inflammation is the first host response to infection or tissue injury and is mediated by the innate immune system. It involves activation and directed migration of leukocytes (neutrophils, monocytes and eosinophils) from the venous system to sites of damage. Recruitment

of these inflammatory cells to sites of tissue injury is mediated by a family of chemotactic cytokines, named chemokines, which possess a relatively high degree of specificity for chemoattraction of specific leukocyte populations. These inflammatory cells then secrete growth factors and cytokines and thereby promote cell proliferation and local invasive properties at the inflammatory site. Nonetheless, these effects would not, on their own, be expected to generate oncogenic events because normal inflammation is usually self-limiting; however, persistence of the initiating factors or a failure of mechanisms required for resolving the inflammatory response can lead to chronic inflammation, which can then promote oncogenesis (Coussens and Werb, 2002).

Indeed, chronic inflammation is suspected to be an important player in the development of a number of cancers (e.g., pancreatitis with pancreatic cancer, hepatitis with liver cancer, etc.) and a link has been established between inflammatory bowel disease and colorectal cancer. In fact, there is an approximately 10-fold increased risk for developing colorectal cancer in patients who have colitis, and the risk increases significantly with the duration and extent of colitis (reviewed by Clevers, 2004). In addition, recent evidence indicates a significant role for innate immune cell involvement in colon cancer development (Balkwill *et al*., 2005). Additional evidence for the involvement of inflammation in promoting cancer is provided by clinical studies that correlated poor clinical outcome with tumor infiltration by immune and inflammatory cells (Coussens and Werb, 2002) and from studies showing reduced cancer incidence in patients undergoing treatment with anti-inflammatory drugs.

Although chronic inflammatory diseases are able to promote oncogenesis, tumor cells themselves produce various cytokines and chemokines that attract leukocytes leading to an inflammatory response. This inflammatory component of developing neoplasms can then function to promote oncogenic progression. In that sense, Sparmann and Bar-Sagi (2004) demonstrated that oncogenic Ras expression leads to the upregulation of the chemokine IL-8, which then functions to promote tumor-associated inflammation, angiogenesis and tumor growth. In this study, inhibition of IL-8 with a neutralizing antibody blocked tumor cell growth. Additionally, the IL-8 antibody reduced the recruitment of neutrophils and macrophages into the derived tumors and reduced the recruitment of endothelial cells leading to decreased angiogenesis. This study demonstrates the existence of a regulatory cascade initiating with oncoprotein expression, leading to the upregulation of a proinflammatory cytokine, which recruits key inflammatory cells to promote tumorigenesis.

Since IL-8 is a target of NF-$\kappa$B, it is likely that the ability of Ras to activate IL-8 gene expression and subsequent neutrophil recruitment is mediated through NF-$\kappa$B (see Figures 1 and 2). In addition, other cytokines involved in the potentiation of inflammation-based cancers, such as cyclooxygenase (Cox)-2, TNF$\alpha$ and IL-1 (Coussens and Werb, 2002; Clevers, 2004), can also be regulated by NF-$\kappa$B (Figure 1). Indeed, others have suggested that NF-$\kappa$B represents a causal link between inflammation and cancer (Greten *et al*., 2004; Pikarsky *et al*., 2004, see below).

## Roles for NF-$\kappa$B in inflammation-associated cancers

Consistent with the important role played by NF-$\kappa$B in both innate and acquired immunity, recent published data directly implicate NF-$\kappa$B activation as a key component in inflammation-based cancer progression. Greten *et al*. (2004) applied the IKK$\beta$ conditional knockout model to test the role of the NF-$\kappa$B activation pathway in controlling tumorigenesis in a colitis-associated model for cancer. Deletion of IKK$\beta$ in intestinal epithelial cells did not affect tumor size in this model, but dramatically reduced tumor number. The reduction in tumor number was explained by strongly enhanced apoptosis in the DNA-damaged intestinal target cells, consistent with a role of NF-$\kappa$B in suppressing apoptotic potential. Importantly, in this model, the tumor-associated inflammation component was not reduced. These data argue that NF-$\kappa$B activation is important in the early stages of DNA-damaged induced tumorigenesis (as an antiapoptotic mediator), but not in the prominent growth phase of tumorigenesis. Nonetheless, Greten *et al*. (2004) went on to show that deletion of IKK$\beta$ in myeloid cells does not affect tumor number, but leads to a significant reduction in tumor size and to a reduction in tumor-associated proinflammatory cytokine levels that are likely to serve as tumor growth factors. This result is consistent with the importance of NF-$\kappa$B in promoting myeloid cell recruitment and inflammatory gene expression as part of the inflammatory phase of oncogenesis.

Conversely, a different study suggested that NF-$\kappa$B does not play a role in the early inflammation-associated neoplastic growth, but functions to suppress apoptosis and to allow malignancy to progress (Pikarsky *et al*., 2004). In this study, which utilized a mouse model of spontaneous hepatitis followed by hepatocellular carcinoma, inhibition of NF-$\kappa$B in hepatocytes through regulatable I$\kappa$B$\alpha$ super-repressor expression did not block hepatitis or the earliest stages of neoplasia; however, inhibition of NF-$\kappa$B at later stages (either through I$\kappa$B$\alpha$ expression or through TNF antibodies) induced apoptosis and blocked progression to hepatocellular carcinoma. In a related study, Maeda *et al*. (2005) showed that, although loss of IKK$\beta$ in hepatocytes actually promotes chemical-induced hepatocarcinogenesis through a compensatory mechanism downstream of JNK-mediated cell death, knockout of IKK$\beta$ in hemopoietic-derived Kupffer cells suppressed hepatocarcinogenesis, indicating that NF-$\kappa$B expression in these cells is crucial for the inflammation-mediated neoplastic growth.

A role for NF-$\kappa$B in inflammation-associated cancer has also been presented by less direct, but highly suggestive evidence. For example, NF-$\kappa$B activation is

suggested to promote neoplastic progression in Barrett's esophagus, a disease associated with inflammation. It is proposed that this is mediated through the ability of NF-κB to regulate Cox-2 and IL-8 gene expression. In addition, Jung *et al*. (2003) reported that IL-1β induces HIF-1α gene expression through a mechanism involving the induction of $PGE_2$ through the NF-κB-dependent upregulation of Cox-2. These studies suggest a direct mechanism whereby NF-κB-regulated cytokines such as IL-1β, IL-8 or Cox-2 promote oncogenesis.

The above-discussed evidence of a role for NF-κB in controlling inflammation-based oncogenesis suggests that inhibitors of this transcription factor are likely to serve as key components in the prevention of many cancers. Thus, studies demonstrating the ability of non-steroid anti-inflammatory compounds to suppress the development of some cancers are consistent with this hypothesis (see below).

## Approaches to blocking NF-κB for cancer therapy

Given the importance of the NF-κB pathway in oncogenesis, there are a growing number of translational/clinical studies in which NF-κB activity in cancer cells is inhibited by different compounds. These include compounds that have already been approved for use in therapy, compounds that are undergoing clinical trials and compounds that show therapeutic promise in preliminary studies (see below). In summary, extensive evidence demonstrates that compounds which block NF-κB activation can serve to block cancer cell growth (Baldwin, 2001; Yamamoto and Gaynor, 2001; Karin *et al*., 2002; Gilmore and Herscovitch, 2006).

### *Proteasome inhibitors*

The proteasome regulates the turnover of a range of different proteins within the cell by promoting degradation of ubiquitinated proteins, including IκB family members. Therefore, proteasome inhibitors, which prevent degradation of IκB proteins, have been extensively used in cancer studies to block NF-κB activity. For example, proteasome inhibition in adult T-cell leukemia primary cells correlates with stabilized IκBα and consequently inhibits NF-κB; this leads to impaired cell growth and induced apoptosis (Nasr *et al*., 2005). Probably a more clinically relevant example is the use of proteasome inhibitors for the treatment of multiple myeloma, which is considered a disease dependent on NF-κB activation (Richardson *et al*., 2004). A highly specific inhibitor of the proteasome is bortezomib/PS-341/VELCADE, which is currently approved for the treatment of multiple myeloma (Richardson *et al*., 2004). A newly developed proteasome inhibitor, NPI-0052, with properties distinct from bortezomib, also induces the death of multiple myeloma cells (Chauhan *et al*., 2005).

Although proteasome inhibitors have shown efficacy in the treatment of multiple myeloma, evidence for clinical efficacy for the treatment of solid tumors is missing. However, proteasome inhibitors have shown efficacy in a number of preclinical models for solids tumors and these responses are typically associated with the inhibition of NF-κB and of NF-κB-dependent gene expression. For example, a recent study showed that the proteasome inhibitor PS-341 causes growth arrest and apoptosis in human glioblastoma cell lines and tumor explants (Yin *et al*., 2005), which is correlated with the inhibition of NF-κB and downregulation of Bcl-2 and Bcl-xL. In addition, proteasome inhibitors have shown antitumor activity in models of breast, lung, colon, bladder, ovary, pancreas and prostate cancers (reviewed by Yin *et al*., 2005).

Although bortezomib/PS-341/VELCADE can block NF-κB activation, it is unclear whether the primary mode of action of this compound in multiple myeloma and other malignancies is solely (or even partially) dependent on its ability to inhibit NF-κB activation. In this regard, in addition to its effects on NF-κB, bortezomib has been reported to block JNK activation through the upregulation of MAP kinase phosphatase-1 (Small *et al*., 2004) and to modulate $Ca^{2+}$/mitochondrial function (Landowski *et al*., 2005). Additionally, it has been reported that bortezomib initiates tumor cell-selective apoptosis that is correlated with the induction of the proapoptotic gene encoding the BH3-only protein Noxa (Fernandez *et al*., 2005). In that study, proteasome inhibition did not correlate with a generalized inhibitory effect on NF-κB.

### *Thalidomide and analogs*

Thalidomide is an anti-inflammatory drug that can block NF-κB activity and has been shown to have antioncogenic properties. Thalidomide and immunomodulatory thalidomide analogs have shown activity against relapsed or refractory multiple myeloma (Rajkumar, 2003). For example, thalidomide was evaluated with dexamethasone in earlier stage disease and yielded response rates in the range of 70%. Treatment of smoldering/indolent disease with single-agent thalidomide yielded overall response rates in the 35% range (Rajkumar, 2003). There is evidence that thalidomide and its analogs have direct impact on multiple myeloma cells through the induction of apoptosis and growth arrest. Importantly, thalidomide was shown to inhibit NF-κB in these cells (Mitsiades *et al*., 2002b). These results are consistent with our earlier report that thalidomide blocks NF-κB activation via suppression of IKK activity (Keifer *et al*., 2001).

### *Non-steroid anti-inflammatory drugs*

While non-steroid anti-inflammatory drugs (NSAIDs) are described generally as inhibitors of Cox-2 activity, it is also possible that these compounds target NF-κB activity. In fact, it was recently shown *in vitro* that the Cox-2 inhibitor celecoxib inhibits NF-κB activation induced by TNF through a mechanism that involved suppression of IKK and Akt activation (Shishodia *et al*., 2004). This same group showed that NSAIDs exhibit differing strengths in their ability to suppress NF-κB

activity and NF-κB-dependent gene expression, with celecoxib as the most potent inhibitor and aspirin the weakest (Takada *et al*., 2004). Interestingly, celecoxib can induce apoptosis in a variety of leukemia cell lines in a manner that is correlated with the suppression of NF-κB activation (Subhashini *et al*., 2005). Sulfasalazine, another NSAID that blocks NF-κB activation, has been shown to inhibit growth and induce apoptosis in both glioblastoma cell lines and primary cultures to a similar level as obtained by the expression of a degradation-resistant super-repressor form of IκBα (Robe *et al*., 2004). Thus, it is possible that NSAIDs might prevent cancer progression through the inhibition of NF-κB.

*Curcumin, IKK inhibitors, arsenic and parthenolide*

A range of compounds with NF-κB inhibitory activity has also been shown to have antitumor or antiangiogenic potential as studied on tumor cell lines and derived xenografts. One of the more extensively studied compounds in this group is curcumin, a diferuloylmethane derived from turmeric, which has been shown to suppress NF-κB activation and NF-κB-dependent gene expression. Previous studies had indicated that curcumin has strong antitumor effects on AML and prostate cancer cell lines (Mukhopadhyay *et al*., 2001; Anto *et al*., 2002). Recently, curcumin has been shown to induce growth arrest and apoptosis in mantle cell lymphoma cell lines (Shishodia *et al*., 2005), where it suppressed the expression of the genes encoding cyclin D1, Bcl-2 and Bcl-xL, all known NF-κB target genes.

Another group of NF-κB inhibitory compounds are small-molecule inhibitors of the IKK kinases. The IKK inhibitors BAY 11-7082 and AS602868 have shown efficacy in leukemia models via their ability to induce increased apoptosis (Frelin *et al*., 2005; Garcia *et al*., 2005). Another small-molecule inhibitor of IKK (PS-1145) was found to be selectively toxic for subtypes of diffuse large B-cell lymphoma cells that are associated with NF-κB activation (Lam *et al*., 2005). This compound was shown to lead to the downregulation of a set of NF-κB-dependent genes.

Additionally, arsenic, which is used for the treatment of acute promyelocytic leukemia, was shown to inhibit constitutive NF-κB/IKK activity and NF-κB target genes in Hodgkin/Reed–Sternberg cell lines, and to induce apoptosis in these cells (Mathas *et al*., 2003). Introduced expression of RelA overcame the arsenic-induced cell death, suggesting the relevance of NF-κB as the target for arsenic (Mathas *et al*., 2003). Another NF-κB inhibitor, the natural product parthenolide, has also shown efficacy against human AML stem and progenitor cells (Guzman *et al*., 2005), and cholangiocarcinoma cells (Kim *et al*., 2005b). Finally, a number of well-established dietary chemopreventive compounds have been shown to inhibit NF-κB (Yamamoto and Gaynor, 2001). It should be noted that a caution with some of these studies is that the therapeutic effects of certain inhibitors may involve the regulation of non-NF-κB targets.

## NF-κB activation and cancer therapy

Evidence was presented in the mid-1990s that DNA-damaging and stress-inducing agents activate NF-κB (see Baldwin, 2001; Yamamoto and Gaynor, 2001; Karin *et al*., 2002; Nakanishi and Toi, 2005). For example, NF-κB activation is seen in response to the chemotherapeutic compound daunorubicin and to irradiation (Wang *et al*., 1996). Inhibition of NF-κB by expression of the super-repressor form of IκBα strongly enhanced the apoptotic efficacy of daunorubicin and of radiation (Wang *et al*., 1996). The topoisomerase I inhibitor CPT-11 can activate NF-κB in experimental colorectal tumors and the administration of adenovirally expressed super-repressor IκBα or the proteasome inhibitor PS-341 inhibits NF-κB activation and significantly enhances the apoptotic response of the tumor to CPT-11 (Wang *et al*., 1999a; Cusack *et al*., 2001). Thus, a model was proposed whereby activation of NF-κB in response to chemotherapies and to radiation functions to suppress the cancer therapy's apoptotic potential (Baldwin, 2001) (see Figure 4).

A number of other reports using a variety of chemotherapies and a variety of approaches to blocking NF-κB support this model (Baldwin, 2001; Yamamoto and Gaynor, 2001; Karin *et al*., 2002; Nakanishi and Toi, 2005). The recent review by Nakanishi and Toi (2005) provides a thorough outline of different chemotherapies that activate NF-κB and of compounds that can be used to block NF-κB activation to promote cancer therapy efficacy. For example, a study analysing NF-κB inhibition in association with radiation indicates that NF-κB activation in experimental colorectal tumors leads to radiation resistance (Russo *et al*., 2001). In addition, a more recent report indicates that NF-κB inhibition sensitizes cancer cells to tumor-necrosis factor-related apoptosis-inducing ligand-induced apoptosis through the sustained activation of JNK (Nakshatri *et al*., 2004). Based on these studies, clinical trials utilizing certain chemotherapies in conjunction with NF-κB inhibitors (proteasome inhibitors, thalidomide, etc.) are presently underway.

While the evidence that NF-κB often functions in an antiapoptotic manner downstream of its activation by chemotherapy or radiation is strong, recent data indicate that NF-κB activation by certain non-traditional cancer therapies in certain cell types can also function in a proapoptotic manner (see Figure 4). For example, apoptosis induced by the retinoid 3-Cl-AHPC was reported to require NF-κB activation (Farhana *et al*., 2005). Exposure of cells to this compound blocked the expression of X-linked inhibitor of apoptosis, Bcl-xL and c-IAP1, and enhanced the expression of proapoptotic death receptors DR4 and DR5 as well as Fas. In a similar study, the retinoid CD437 was shown to activate NF-κB in DU145 prostate cancer cells and to contribute to the induction of apoptosis through the upregulation of the death receptors DR4 and DR5 (Jin *et al*., 2005). In addition, aspirin and UV-C induce apoptosis through a mechanism that drives RelA to the nucleolus, leading to an inability to activate NF-κB-dependent genes

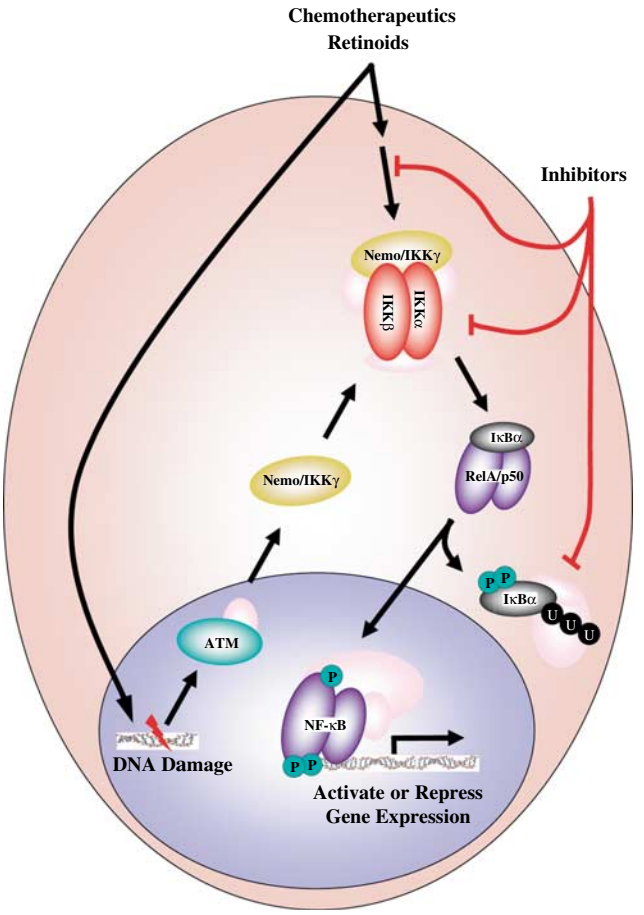


**Figure 4** Targeting the NF-κB pathway as primary or adjuvant therapy in cancer. NF-κB is activated by standard chemotherapeutic agents and by retinoids, either by the DNA damage response induced by many of these agents or by other pathways that will directly activate the IKK kinase complex. In the nucleus, NF-κB can mediate either a pro- or antiapoptotic transcriptional response, apparently depending on the cancer cell regulatory environment. Targeted inhibition of the NF-κB pathway at different levels (directed towards the upstream pathways, IKK or the proteasome-dependent activation of NF-BB has proven beneficial both as stand-alone therapy as well as in combination with other therapeutic agents.

(Stark and Dunlop, 2005). Under these conditions, NF-κB inhibition would be counterintuitive as a cancer therapy.

Nonetheless, which signaling events ultimately determine whether NF-κB activation will lead to a pro- or antiapoptotic response are presently unclear. For example, Campbell *et al.* (2004) found that NF-κB activation induced by etoposide in U-2 OS osteosarcoma cells leads to the traditional antiapoptotic response and is associated with the activation of Bcl-xL gene expression. On the other hand, NF-κB activation by doxorubicin and daunorubicin in the same cells promotes cell death and leads to the NF-κB-mediated repression of antiapoptotic genes such as Bcl-xL. Interestingly, it was found that daunorubicin induced the association of RelA with the histone deacetylases 1, 2 and 3, consistent with the role for NF-κB in repressing gene expression downstream of responses to that chemotherapy. A similar report indicated that doxorubicin-induced NF-κB is deficient in transcriptional activity, which is associated with reduced phosphorylation and acetylation of RelA (Ho *et al.*, 2005). In addition, NF-κB activation by doxorubicin in four colorectal cancer cells is required for the anticancer efficacy of the drug (Ashikawa *et al.*, 2004). However, there are other papers where doxorubicin-induced cell death is inhibited by NF-κB activation (see Baldwin, 2001; Nakanishi and Toi, 2005). The basis of these conflicting results is not known.

It is reasonable to speculate that the pro- vs antiapoptotic activity of NF-κB is determined by the phenotypic profile of the tumor in combination with the specific cancer therapy (Figure 4). In this respect, the activation of NF-κB itself by the relevant cancer therapy is not likely to be the determining outcome on gene expression. Presumably, modification of NF-κB subunits (or lack thereof) related to the regulatory status of the cancer will then determine whether NF-κB functions in the anti- or proapoptotic response (Figure 4). In this sense, this antagonic behavior of chemotherapy-induced NF-κB in different cancer settings clearly shows the importance of individualized cancer therapy, probably based on a knowledge of the molecular phenotype of a given cancer.

## NF-κB as a tumor suppressor

While there is solid evidence that NF-κB can function in a pro-oncogenic manner, other experimentation indicates that NF-κB can, under certain circumstances, function in a tumor suppressor role (see Perkins, 2004; Shishodia and Aggarwal, 2004). For example, one report demonstrated that inhibition of NF-κB in skin leads to oncogenic potential and potentiates H-Ras-induced transformation (Dajee *et al.*, 2003). One mechanism to explain this concept is that inhibition of NF-κB in the skin leads to JNK activation (Zhang *et al.*, 2004), a finding consistent with several reports that NF-κB activation suppresses the phosphorylation and activation of JNK.

Similar questions regarding the oncogenic potential of NF-κB arise with the consideration that NF-κB appears to function downstream of the tumor suppressor p53. Ryan *et al.* (2000) have presented evidence that NF-κB activation is required downstream of p53 in order for this tumor suppressor to induce apoptosis. However, other experimentation indicates that NF-κB/IKK activation can destabilize p53 (Tergaonkar *et al.*, 2002). Similar to these findings, it has been shown recently that Bcl-3/p52 activation suppresses p53 activation through upregulated Hdm2 gene expression (Kashatus *et al.*, 2006). Thus, the interplay between NF-κB/IKK signaling and p53 function is complex and needs further exploration.

Overall, these findings suggest that NF-κB can function, under certain conditions, as a tumor suppressor.

The potential role of NF-κB as a tumor suppressor has been reviewed elsewhere (Perkins, 2004; Shishodia and Aggarwal, 2004).

## Concluding remarks

An enormous amount of data strongly implicates transcription factor NF-κB in a variety of oncogenic mechanisms. Additionally, there is compelling evidence for an important role for NF-κB in modulating cancer therapy efficacy. A major challenge is to distinguish the situations where NF-κB functions pro-oncogenically/antiapoptotically from those where it may function as a tumor suppressor and a proapoptotic factor. In this regard, it is important to identify the molecular mechanisms that dictate these outcomes. A second challenge is to bring rational inhibitors of NF-κB or its upstream regulatory pathways to clinical cancer therapy in a manner consistent with an antiapoptotic/proproliferation role, either as stand-alone therapies or as adjuvants with existing or new therapies.


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