Cell Death and Differentiation (2006) 13, 738–747

www.nature.com/cdd

Review

NF-κB and IKK as therapeutic targets in cancer

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Received 08.11.05; revised 22.12.05; accepted 09.1.06; published online 17.2.06
Edited by G Kroemer

Abstract

The transcription factor NF-κB and associated regulatory factors (including IκB kinase subunits and the IκB family member Bcl-3) are strongly implicated in a variety of hematologic and solid tumor malignancies. A role for NF-κB in cancer cells appears to involve regulation of cell proliferation, control of apoptosis, promotion of angiogenesis, and stimulation of invasion/metastasis. Consistent with a role for NF-κB in oncogenesis are observations that inhibition of NF-κB alone or in combination with cancer therapies leads to tumor cell death or growth inhibition. However, other experimental data indicate that NF-κB can play a tumor suppressor role in certain settings and that it can be important in promoting an apoptotic signal downstream of certain cancer therapy regimens. In order to appropriately move NF-κB inhibitors in the clinic, thorough approaches must be initiated to determine the molecular mechanisms that dictate the complexity of oncologic and therapeutic outcomes that are controlled by NF-κB.
Cell Death and Differentiation (2006) **13,** 738–747.
doi:10.1038/sj.cdd.4401877; published online 17 February 2006

Keywords: NF-κB; IKK; cancer; cancer therapy; NF-κB inhibitors

Abbreviations: IKK, IκB kinase; SR-IκBα, super-repressor IκBα

NF-κB Family and Control by IκB Kinase Signaling

The mammalian NF-κB family of proteins contains the RelA/p65, NF-κB1, NF-κB2, c-Rel, and RelB subunits, which can form a variety of hetero- and homodimers to differentially control gene expression downstream of signals elicited by cytokines, bacterial products, viral expression, growth factors, and stress stimuli.[1] Negative regulation of NF-κB is controlled primarily through interactions with the IκB family of proteins, which prevent DNA binding and promote cytoplasmic accumulation of the interacting dimeric complex. Contrary to the normal inhibitory roles for the classical forms of IκB, the Bcl-3 protein can function to regulate gene expression through interactions with the NF-κB1 and NF-κB2 subunits. Positive regulation of NF-κB is controlled through the activity of the IκB kinase (IKK) complex, which phosphorylates IκB proteins leading to their ubiquitination and subsequent proteasome-dependent degradation, leading to the nuclear accumulation of the released NF-κB complexes. In the nucleus, NF-κB complexes 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.[1] Cancer relevant, NF-κB-dependent genes include those encoding cytokines, chemokines, cyclin D1, matrix metalloproteinases, and antiapoptotic proteins such as Bcl-xL (Figure 1). As described below, NF-κB and IKK proteins are associated with oncogenesis and cancer therapy responses through a complex set of regulatory pathways.

Common Mechanisms Associated with Human Cancer

Human cancer is a highly diverse and complex disease based on multiple etiologies, multiple cell targets, and distinct developmental stages. Additionally, cancer cells often exhibit genomic instability, which contributes to enhanced disease complexity and therapeutic outcome. However, it has been proposed that cancer cells share common features that override normal controls on proliferation and homeostasis.[2] Most of these shared properties are intrinsic to the cancer cells themselves but others are derived from signals that originate from the surrounding tumor microenvironment. Properties that are held in common by advanced cancer cells and that drive malignant growth include strong resistance to growth inhibitory signals, self-sufficiency in growth, resistance to apoptosis, extended replication potential, enhanced angiogenic potential, and the ability to invade local tissue and to metastasize to distant sites.[2]

Autonomous cell growth can be provided by dysregulated expression of growth factors or growth factor receptors, leading to uncontrolled cell proliferation. Thus, a fairly common mechanism in cancer is the upregulation of expression of members of the epidermal growth factor receptor family such as EGF receptor or Her2/ErbB2. Furthermore, certain cancer cells produce growth factors such as PDGF and TGFα, which can promote cell proliferation in an autocrine manner.[2] Mutations in proteins that regulate cell proliferation are also relatively common in cancers. For example, mutations in Ras alleles drive cell proliferation through chronic stimulation of signal transduction pathways such as those involving ERK, PI3K/Akt, and RalGDS. Growth promotion can also be manifested through paracrine mechanisms involving

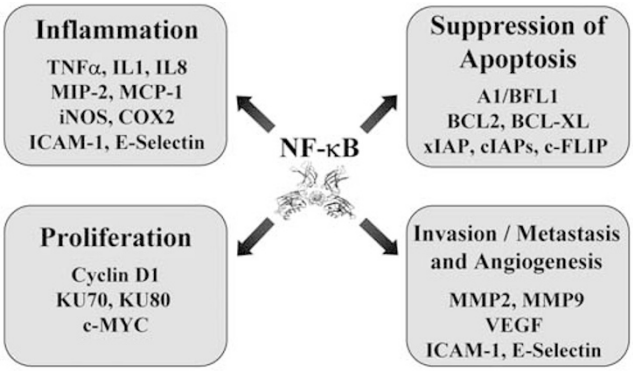

Figure 1 Representation of NF-κB-dependent genes involved in different aspects of oncogenesis. Target genes are included with the relevant oncogenic process. See text for a description

normal stromal bystanders or recruited inflammatory cells. Resistance to growth inhibitory signals can be achieved through mutations in tumor suppressor genes such as p53, Rb, Arf, and APC, or in receptors such as those for TGFβ. Additionally, upregulation of expression of cyclin D1 or c-myc, or activating mutations in transcription factors such as β-catenin can promote cell proliferation or cell growth.[2]

A key process in the ability of tumor cells to expand locally and to metastasize is the suppression of apoptotic potential. Resistance to apoptosis can involve the activation of expression of antiapoptotic factors, such as Bcl-2 or Bcl-xL, or the loss of expression or mutation of proapoptotic factors, such as p53. Additionally, mutation in tumor suppressors such as PTEN leads to the activation of intracellular signaling pathways (in this case, the PI3 kinase/Akt pathway) that suppress apoptosis. Relative to Akt, this Ser-Thr kinase is known to target the phosphorylation of several factors associated with regulating apoptosis.[3] An additional mechanism of suppression of cancer cell apoptosis can be derived from release of cytokines from the tumor stroma. Clearly, constitutive or induced antiapoptotic factors expressed in tumor cells provide a strong mechanism to suppress cancer therapy efficacy (see below).

Extended replication potential is controlled in part through telomere maintenance, a property associated with virtually all cancer cells. This mechanism is commonly controlled in cancer through upregulation of expression of the telomerase enzyme. Cancer cells also exhibit properties associated with inducing and sustaining angiogenesis, a process that appears to be required for tumor progression. Animal models for tumorigenesis indicate that angiogenesis is a mid-stage property, occurring before the appearance of malignancy.[2] Angiogenesis is mediated through a complex interplay of regulatory factors, including vascular endothelial growth factor (VEGF). In fact, many tumors exhibit upregulation of VEGF.[2] Associated with the appearance of angiogenesis is often the ability of tumor cells to invade local tissues and subsequently to migrate systemically to preferred sites (metastasis). Local invasion is mediated by changes in expression of cell adhesion molecules and integrins, and in changes in expression of extracellular proteases such as MMP-2 and MMP-9. In some situations, the matrix-degrading proteases are produced by the tumor-associated stromal and inflammatory cells.

An important mechanism in the progression of many cancers is the epithelial-to-mesenchymal transition (EMT). 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. The occurrence of EMT in cancer is associated with the downregulation of expression of E-cadherin, a member of the classic cadherin family that controls cell polarity and tight junctions, and with progression to a metastatic phenotype.

Cancer Progression is Promoted by Inflammation

Chronic inflammation has long been speculated to be intimately associated with the promotion of cancer. In this regard, evidence for the involvement of inflammation with cancer was provided from clinical studies correlating tumor infiltration of immune cells with poor clinical outcome.[4] A causal role for inflammation and cancer is suggested from studies reporting reduced cancer incidence in patients undergoing treatment with anti-inflammatory drugs. As many anti-inflammatory compounds inhibit cyclooxygenase-2 activity, it has been suspected that this enzyme is the primary target for prevention. Causative roles for proteolytic enzymes, the transcription factor NF-κB, and cytokines such as TNF are indicated as functionally important molecules that potentiate inflammation-based cancer.[4–7]

There are a number of examples whereby inflammation is suspected in cancer progression (pancreatitis with pancreatic cancer, and gastritis with gastric cancer, etc.). However, probably the best-established link is between inflammatory bowel disease and colorectal cancer. Colitis is associated with an approximate 10-fold risk in developing colorectal cancer, and the risk increases significantly with the duration and extent of disease.[7] Recent evidence suggests that the development of colitis-associated cancer is manifested by inflammation of the intestinal submucosa directed by contact with the intestinal microflora, which promotes tumor outgrowth in the overlying epithelium. [7]

The role of inflammation in cancer has been studied for several years. Tumor development/progression is controlled by reciprocal interactions between neoplastically initiated cells, vascular cells, fibroblasts, and immune cells. In this regard, recent evidence indicates a significant role for innate immune cell involvement in cancer development.[8] Obviously, the local production of cytokines and growth factors would favor cancer cell survival and local invasive properties, but these effects would not, on their own, be expected to generate oncogenic events. However, it is known that the local production of reactive oxygen species and metabolites from inflammation pose a mutagenic threat to DNA.

The chemokine IL-8 is a well-established factor in the initiation and progression of inflammation. Relevant to oncogenesis, Sparmann and Bar-Sagi[9] demonstrated that oncogenic Ras expression leads to the upregulation of IL-8, which then functions to promote tumor-associated inflamma-

tion, angiogenesis, and tumor growth. The authors showed that inhibition of IL-8 with a neutralizing antibody inhibited the growth of tumor cells that express IL-8. Furthermore, the antibody reduced recruitment of neutrophils and macrophages in the derived tumors and reduced recruitment of endothelial cells leading to decreased angiogenesis. These studies[9] provide evidence for a regulatory cascade initiating with oncoprotein expression and leading to upregulation of a pro-inflammatory cytokine that recruits key inflammatory cells that promote tumorigenesis.

NF-κB and IKK Activation are Associated with Oncogenesis

A potential link between NF-κB and cancer was suggested by the cloning of the NF-κB p50/p105 subunit, which revealed homology to the cellular homologue (c-rel) of the oncogene (v-Rel) for the avian reticuloendotheliosis virus. Later, the p52 NF-κB subunit was shown to be encoded by a gene that undergoes translocations in certain B-cell lymphomas (the lyt-10 translocation).[10,11] Extensive evidence has now emerged indicating a critical role for NF-κB in promoting oncogenic conversion and in facilitating later stage tumor properties such as metastasis.[10,11]

A role for NF-κB in cancer is supported from numerous reports showing that NF-κB is activated (i.e., nuclear) in a number of tumors. Thus, NF-κB (RelA) activation has been detected in a variety of solid tumors, including prostate tumors, breast tumors, melanoma, pancreatic cancer, and lung adenocarcinoma.[10–12] Whether other NF-κB subunits could contribute to oncogenesis is not well established. Relative to this point, the p52 subunit has been shown to be nuclear in a number of breast tumors.[10] Additionally, the p50 subunit of NF-κB appears to control both the positive and negative regulation of the expression of the KAI1 tumor suppressor gene. Expression of the oncoprotein β-catenin converts the p50 transcriptional complex to a repressive complex through loss of Tip60 coactivator association and the induction of recruitment of transcription co-repressors, leading to loss of expression of the metastasis suppressor gene.[13] Thus, expression of p50 in a tumor should not be considered as an indication of lack of NF-κB functional activity.

NF-κB activation has been reported in a number of hematological malignancies. For example, NF-κB is activated, as measured by EMSA, in blasts and stem cells associated with acute myelogenous leukemia (AML) and this is associated with increased IKK activity.[14] Importantly, NF-κB activation can be detected in the bone marrow of patients with myelodysplastic syndrome,[15] which is considered a precursor disease of AML. RelA/p65 activation was limited to those cells with cytogenetic alterations. NF-κB was also found to be activated in childhood acute lymphoblastic leukemia.[16] High levels of NF-κB activation are found in Hodgkin–Reed–Sternberg cells, in HTVL-1-positive leukemias, B-cell lymphomas of mucosa-associated lymphoid tissue, and in primary blasts of chronic myelogenous leukemia. NF-κB activation is also associated with multiple myeloma,[17] which is currently treated with compounds that can block NF-κB activation (see below).

A number of oncoproteins have been shown to induce NF-κB activation (see Figure 2) as measured either through reporter assays or through analysis of nuclear levels of NF-κB.[10,11] For example, oncogenic Ras and Her-2/Neu (ErbB2)[10,11] have been demonstrated to activate NF-κB. In murine fibroblasts, p65/RelA and c-Rel are required for efficient cellular transformation induced by oncogenic Ras.[18] Bcr-Abl, the fusion protein associated with chronic myelogenous leukemia, was shown to activate an NF-κB-dependent reporter, and inhibition of NF-κB with super-repressor IκBα (SR-IκBα) expression blocked Bcr-Abl-induced tumor growth.[19] Activation of NF-κB by Bcr-Abl has been reported to involve the activation of the kinase MEKK1[20] (see Figure 2). Consistent with these findings, NF-κB is found activated in Bcr-Abl-positive leukemia,[21] although the activation was not associated with IKK activity. The mechanisms of stimulation of NF-κB functional activity by oncoproteins often lags behind the significantly more clear understanding of pathways associated with cytokine and LPS -induction of NF-κB. One of the better understood mechanisms for the activation of NF-κB by an oncoprotein is the mechanism whereby the HTVL-I Tax protein activates NF-κB. This protein directly binds and activates the IKK complex.[22] The MALT1/c-IAP2 fusion found in certain lymphomas leads to ubiquitination of NEMO/IKKγ and subsequent NF-κB activation.[23] Several studies indicate that PI3K/Akt-dependent signaling activates NF-κB activity and that this occurs in a manner dependent on the relative levels of the IKKα subunit.[24] Consistent with this, Akt activation in cancer is proposed to mediate NF-κB activation. In one study, it was shown that Akt is activated in primary acute myeloid leukemia and that this is associated with cell survival and NF-κB activation.[25] Another example is the activation of Akt in human melanoma, which leads to NF-κB activation and tumor progression.[26] Interestingly, this same group showed that NF-κB-inducing kinase, a regulator of the non-classical pathway, is involved in the activation of NF-κB in melanoma. The activation of NF-κB downstream of Her-2/ErbB2 was shown to involve PI3K/Akt-dependent signaling.[27] Relative to other signaling pathways, phosphorylation of FADD was detected in a significant number of lung adenocarcinomas. This was associated with poor clinical outcome and was reported to induce NF-κB activation. Interestingly, high levels of expression of the E3-ubiquitin ligase receptor βTRCP1 are proposed to activate NF-κB in pancreatic cancer cells. A summary of the regulatory pathways initiated by oncoproteins and growth factors that target NF-κB is shown in Figure 2.

Given the important role that growth factors play in promoting oncogenesis, it is not surprising that certain growth factors or expression of growth factor receptors has been shown to activate NF-κB (Figure 2). For example, Biswas *et al*.[28] have shown that EGF can activate NF-κB in certain cell types. We have provided evidence that EGF can induce recruitment of p65/RelA to the EAAT2/glutamate transporter promoter through a mechanism independent of IκB degradation. As described previously, the EGF receptor family member Her-2/ErbB2 has been shown to activate NF-κB,[27] and evidence indicates that a specific set of genes controlled downstream of Her-2/ErbB2 by NF-κB (E Merkhofer and A Baldwin, unpublished). We and others showed that PDGF can

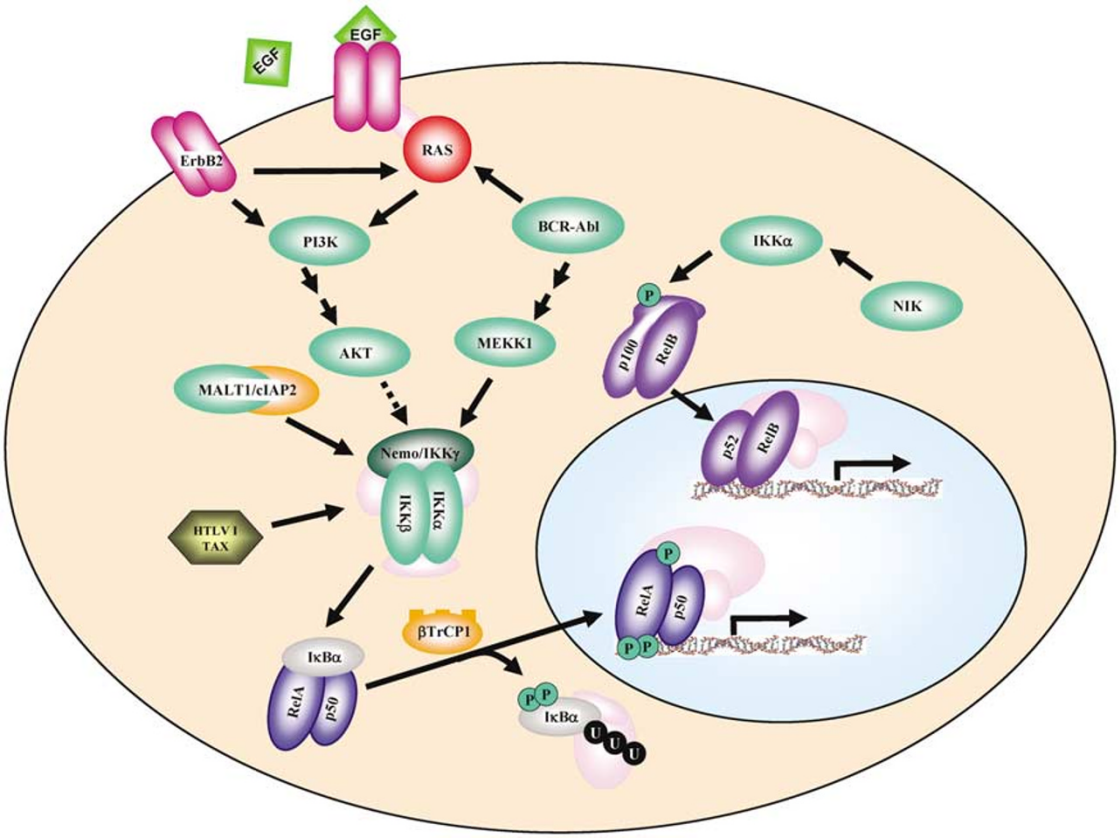

Figure 2 Pathways of activation of NF-κB by oncoproteins and growth factors. Most of the pathways ultimately target IKK for NF-κB activation. Steps linking certain regulators to IKK may not be direct (e.g., Akt). See text for a description

induce NF-κB activation and promote c-myc transcription.[10] Also, it has been shown that activated c-kit receptors lead to NF-κB activation.

In addition to the known responses of oncoproteins in activating NF-κB, evidence has been presented that certain tumor suppressors can block NF-κB activation. For example, the tumor suppressor Arf has been shown to inhibit NF-κB through a mechanism involving phosphorylation of Thr505 on p65/RelA induced by ATR and Chk1.[29] Additionally, the tumor suppressor CYLD has been demonstrated to block NF-κB activation through its deubiquitinating activity. Regarding the tumor suppressor p53, it was reported that p53 generally inhibits NF-κB function and *vice versa*;[30] however, more complex relationships between p53 and NF-κB have emerged (see below).

Although there is strong evidence that NF-κB functions to promote oncogenesis in a variety of tumors, evidence has been presented that inhibition of NF-κB in skin leads to oncogenic potential and potentiates Ras-induced transformation.[31] One mechanism to explain this concept is that inhibition of NF-κB in the skin leads to JNK activation,[32] 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. Vousden and co-workers[33] have presented evidence that NF-κB activation is required downstream of p53 in order for this tumor suppressor to induce apoptosis, whereas other experimentation indicates that NF-κB/IKK activation can destabilize p53.[34] Similar to the findings of Verma and co-workers,[34] we have shown that Bcl-3 activation suppresses p53 activation through upregulated Hdm2 gene expression.[35] Hung and co-workers[36] have reported that the oncoprotein β-catenin can block NF-κB activation. Note that evidence described above indicates that β-catenin can interact with the p50 NF-κB transcriptional complex and convert it to a transcriptional repressor.[13] Overall, these findings suggest that NF-κB can function, under certain conditions, as a tumor suppressor.

Modes of Action of NF-κB and IKK in Cancer

Clearly, NF-κB functions as a transcriptional regulator in a variety of cancer cells as evidenced through the identification of cancer-specific, cancer-relevant gene targets controlled by NF-κB (see Figures 1 and 3). For example, gene profiling has identified a subset of diffuse large B-cell lymphoma that requires NF-κB for growth and survival. Inhibition of NF-κB activation blocks the expression of genes associated with this type of lymphoma.[37] We have identified a set of approximately 25 genes that are regulated by NF-κB in a manner dependent on oncogenic Ras expression in murine

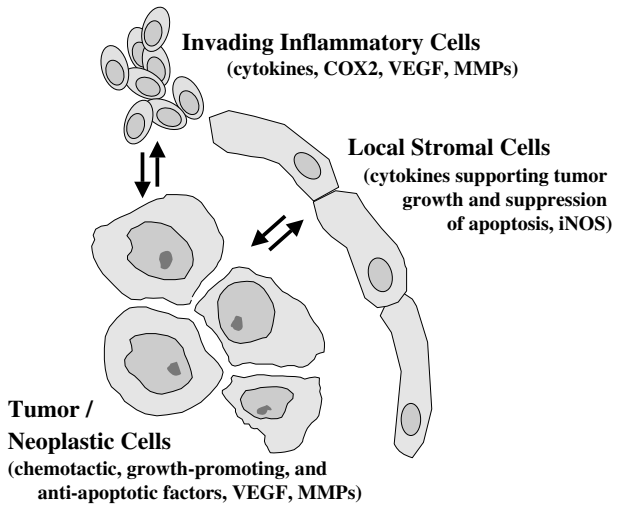

Figure 3 Roles for NF-κB in tumor and tumor-associated cells. NF-κB activation is relevant in tumor/neoplastic cells, in invading inflammatory cells, and in tumor-associated stromal compartment. See text for a description

fibroblasts.[18] Interestingly, these studies indicate that the NF-κB-controlled gene sets in distinct oncogenic settings are significantly different. As described above,[13] the p50 NF-κB subunit can positively or negatively regulate KAI-1 tumor suppressor gene expression in a manner dependent on the activation levels of β-catenin.

One of the key properties associated with transformed cells is their ability to resist apoptosis. Experiments revealed that induction of RasV12 in immortalized Rat1 fibroblasts leads to cellular transformation but not to apoptosis. If NF-κB was inhibited in these cells by expression of SR-IκBα, then the induction of RasV12 expression was associated with high levels of apoptosis.[10] Consistent with this point, inhibition of NF-κB in certain tumor cell lines leads to apoptotic cell death.[10,11] Hodgkin's lymphoma has proven to be a cancer that is strongly controlled by NF-κB activation. Proliferation and survival of Hodgkin/Reed–Sternberg cells is blocked when NF-κB is inhibited by IκBα expression.[38] Genes regulated by NF-κB that suppress apoptosis, such as Bcl-2 and Bcl-xL, are often expressed in human cancers (see Figures 1 and 3). Consistent with this, inhibition of NF-κB in Hodgkin/Reed–Sternberg cells led to the loss of expression of antiapoptotic effectors A1/Bfl-1, c-IAP2, TRAF1, and Bcl-xL.[39] Another mechanism whereby NF-κB may block cell death is through its ability to suppress persistent JNK activation and the generation of reactive oxygen species.[40] Relative to a role for the NF-κB upstream pathway in preventing apoptosis, Hu *et al*.[41] have shown that IKKβ activation in breast cancer cells leads to the direct phosphorylation and degradation of the proapoptotic factor Foxo3a, suppressing apoptotic potential in certain breast cancer cells, and promoting cell proliferation.

NF-κB activation also appears to promote cellular proliferation, consistent with a role in promoting growth sufficiency of cancer cells. Evidence has been presented that NF-κB can bind and activate the cyclin D1 promoter, promoting Rb hyperphosphorylation.[10,11] Additionally, the IκB homologue Bcl-3 in association with p52 homodimers has also been found to potently activate transcription of the cyclin D1 gene. Interestingly, IKKα has been proposed to play a role in cyclin D1 transcription through the Tcf site, via its ability to control β-catenin phosphorylation.[42] Consistent with a role for IKKα in promoting cyclin D1 transcription, Karin *et al*.[11] colleagues found that IKKα is required for RANK signaling and cyclin D1 expression in mammary gland development. Other mechanisms whereby NF-κB may potentiate oncogenic conversion and maintenance is through its reported requirement for the upregulation of HIF-1α[43] and its regulation of c-myc transcription.[10,11] Consistent with these reports, inhibition of NF-κB in several tumor xenografts inhibits growth of those tumors.[10,11,19]

NF-κB activation in tumor cells, and in tumor-associated stromal and endothelial cells, likely plays a role in tumor progression and invasion. The role of NF-κB in invading myeloid cells has been addressed using IKKβ ablation in these cells in a carcinogen/inflammation model for colorectal cancer.[5] In that study, NF-κB was shown to control production of cytokines in myeloid cells, which are involved in promoting tumor growth. In another interesting study, NF-κB appears to mediate an IL-1/nitric oxide paracrine growth loop involving stromal fibroblasts and pancreatic neoplastic cells.[44] In this regard, NF-κB has been reported to promote both angiogenesis and metastasis in certain tumor models, potentially through regulation of VEGF and MMPs (Figure 1).[10,11] In a more direct study, expression of the SR-IκBα blocked growth, angiogenesis, and metastasis of human melanoma and ovarian cancer cells grown as xenografts,[45] which correlated with reduced VEGF and IL-8 expression.[45] Consistent with a role in metastasis, NF-κB has been shown to mediate the EMT.[46]

Roles for NF-κB Activation in Inflammation-associated Cancer

As outlined above, there is compelling evidence that inflammation promotes cancer progression. Given the key role that NF-κB plays in both innate and acquired immunity, it would not be surprising that NF-κB plays an essential role in providing the link between inflammation and cancer progression. In fact, recently published data directly implicate NF-κB activation as a key component in inflammation-based cancer progression. Karin and co-workers[5] utilized the IKKβ conditional knockout to test the role of the NF-κB activation pathway in controlling tumorigenesis in a colitis-associated model for cancer. Deletion of IKKβ in intestinal epithelial cells dramatically reduced tumor number in this model, but not tumor size. The reduction in tumor number was explained by strongly enhanced apoptosis in the DNA-damaged intestinal target cells, consistent with a role of NF-κB in suppressing apoptotic potential. Importantly, tumor-associated inflammation was not reduced in this component of the model. These data argue that NF-κB 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. Greten *et al*.[5] went on to show that deletion of IKKβ in myeloid cells, however, leads to a significant reduction in tumor size but not tumor number, 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-κB in promoting myeloid cell recruitment and inflammatory gene expression as part of the inflammatory phase of oncogenesis (see Figure 2). In another related study, Maeda *et al*.[47] showed that loss of IKKβ in hepatocytes actually promoted chemical-induced hepatocarcinogenesis through a mechanism involving enhanced ROS production and JNK activation with associated cell death, leading to a compensatory response in surviving hepatocytes. Knockout of IKKβ in Kupffer cells suppressed hepatocarcinogenesis, indicating an inflammatory role for these hemopoietic-derived cells in this model.

In another recently described model, Ben-Neriah and coworkers[6] utilized a mouse knockout for the Mdr2 gene, which develops spontaneous hepatitis followed by hepatocellular carcinoma. Thus this serves as a straightforward model for inflammation-based oncogenesis. Inhibition of NF-κB through regulatable SR-IκBα expression did not block hepatitis or the earliest stages of neoplasia. However, inhibition of NF-κB at later stages (either through IκBα expression or through TNF antibodies) blocked progression to hepatocellular carcinoma at least partly through the induction of apoptosis. In this model, NF-κB does not play a role in the early inflammation-associated neoplastic growth but, acting downstream of TNF, functions to suppress apoptosis and to allow cancer malignancy to progress.

Less direct, but highly suggestive evidence for a role of NF-κB in inflammation-associated cancer has been presented. For example, NF-κB 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. Jung *et al*.[43] 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 cytokines such as IL-1β promote oncogenesis. Regarding the work of Sparmann and Bar-Sagi[9] described above, it is likely that the ability of Ras to activate IL-8 gene expression and subsequent neutrophil recruitment is mediated through NF-κB (see Figure 2). The evidence for a role of 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).

Pharmacological Inhibitors of NF-κB Pathways Often, but not Always, Suppress Cancer Growth: Evidence for Specificity?

Experimentation reveals that compounds that block NF-κB activation can serve to block cancer cell growth.[10–12] Probably, the most clinically relevant example of this response is the use of proteasome inhibitors for treatment of multiple myeloma, which is considered a disease dependent on NF-κB activation.[48] Bortezomib/Velcade is a highly specific inhibitor of the proteasome, and is currently approved for treatment of multiple myeloma.[48] The importance of NF-κB in multiple myeloma is suggested from its involvement downstream of CD40, the 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 cocultures of multiple myeloma cells and bone marrow-derived stromal cells.

Thalidomide and immunomodulatory thalidomide analogues have shown activity against relapsed or refractory multiple myeloma.[49] Additionally, 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.[49] There is evidence that thalidomide and its analogues 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.[50] These results are consistent with our earlier report that thalidomide blocks NF-κB activation via suppression of IKK activity.[51]

Other hematological malignancies are susceptible to NF-κB inhibition. Proteasome inhibition inhibited cell growth and induced apoptosis in adult T-cell leukemia, an NF-κB-relevant malignancy, correlated with stabilized IκBα and inhibited NF-κB.[52] A 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.[37] This compound was shown to lead to downregulation of a set of NF-κB-dependent genes.

As described above, proteasome inhibitors have shown efficacy in the treatment of multiple myeloma, but evidence for clinical efficacy for treatment of solid tumors is missing. However, proteasome inhibitors have shown efficacy in a number of preclinical models for solid tumors and these responses are typically associated with the inhibition of NF-κB and of relevant gene expression. For example, proteasome inhibitors have shown antitumor activity in models of breast, lung, colon, bladder, ovary, pancreas, and prostate cancers.[53] A recent study showed that the proteasome inhibitor PS-341 caused growth arrest and apoptosis in human glioblastoma cell lines and tumor explants.[53] This response was correlated with inhibition of NF-κB and downregulation of Bcl-2 and Bcl-xL.

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 or other malignancies is dependent on its ability to inhibit NF-κB activation. In this regard, bortezomib has been reported to block JNK activation through the upregulation of MKP-1[54] and to modulate Ca^{2+}/mitochondrial function[55] in addition to its effects on NF-κB. Additionally, it has been reported that bortezomib initiates tumor cell-selective apoptosis that is correlated with the induction of the pro-apoptotic gene encoding the BH3-only protein Noxa.[56] In that study, proteasome inhibition did not correlate with a generalized inhibitory effect on NF-κB.

Although 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 suppressed IKK and Akt activation.[57] This same group showed that NSAIDs exhibit differing strengths in their ability to suppress NF-κB and to downregulate NF-κB-dependent gene expression, with celecoxib as the most potent inhibitor and aspirin the weakest.[58] Celecoxib induced apoptosis of a variety of leukemia cell lines and this was correlated with suppression of NF-κB activation.[59] Thus it is possible that an effect of NSAIDs in preventing cancer progression is through inhibition of NF-κB.

A range of compounds that have NF-κB inhibitory activity have 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 strong antitumor effects on AML and prostate cancer cell lines.[60,61] Recently, curcumin has been shown to induce growth arrest and apoptosis in mantle cell lymphoma cell lines.[62] Curcumin suppressed the expression of cyclin D1, Bcl-2, and Bcl-xL, all known NF-κB target genes. The IKK inhibitors BAY 11-7082 and AS602868 have shown efficacy in leukemia models via increased apoptosis.[63,64] Another NF-κB inhibitor, parthenolide, has shown efficacy against human AML stem and progenitor cells,[65] and cholangiocarcinoma cells.[66] Sulfasalazine, a nonsteroidal anti-inflammtory drug that blocks NF-κB activation, was shown to inhibit growth and induce apoptosis in glioblastoma cell lines and primary cultures, as did expression of SR-IκBα.[67] Finally, 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. Expression of p65/RelA overcame the arsenic-induced cell death, suggesting the relevance of NF-κB as the target for arsenic.[68] A concern with some of these studies is that the therapeutic effects of certain inhibitors may involve NF-κB-independent targets. Additionally, a number of well-established dietary chemopreventive compounds have been shown to inhibit NF-κB.[12]

The Role of NF-κB in Regulating Cancer Therapy Responses

Evidence was presented in the mid-1990s that DNA-damaging and stress-inducing agents activate NF-κB.[10–12,69] Our group demonstrated NF-κB activation in response to chemotherapy by daunorubicin and to irradiation.[70] Inhibition of NF-κB by expression of the SR-IκBα strongly enhanced the apoptotic efficacy of daunorubicin and of radiation.[70] The topoisomerase I inhibitor CPT-11 activated NF-κB in experimental colorectal tumors and administration of adenovirally expressed SR-IκBα or the proteasome inhibitor PS-341 inhibited NF-κB activation and significantly enhanced the apoptotic response of the tumor to CPT-11.[71,72] Thus, the model was that activation of NF-κB in response to chemotherapies and to radiation functioned to suppress the apoptotic potential of that cancer therapy[10] (see Figure 4). A number of other reports using a variety of chemotherapies and a variety of approaches to block NF-κB have supported this model.[10–12,69] The recent review by Nakanishi and Toi[69] provides a thorough outline of different chemotherapies that activate NF-κB and of compounds that are used to block NF-κB activation to promote cancer therapy efficacy. Furthermore, new reports indicate that NF-κB inhibition sensitizes cancer cells to TRAIL-induced apoptosis through the sustained activation of JNK.[73] Additionally, a study analyzing NF-κB inhibition in association with radiation indicated that NF-κB activation leads to radiation resistance in experimental colorectal tumors.[10] Based on these studies, clinical trials utilizing certain chemotherapies in conjunction with NF-κB inhibitors (proteasome inhibitors, thalidomide, etc.) are presently underway.

Although there is strong evidence that NF-κB often functions in an antiapoptotic manner downstream of its activation by chemotherapy or radiation , recent data indicate that NF-κB activation can also function in a proapoptotic manner after activation by certain nontraditional cancer therapies in certain cell types (see Figure 4). Under these conditions, NF-κB inhibition would presumably be counter-intuitive as a cancer therapy. Thus, apoptosis induced by the retinoid 3-Cl-AHPC was reported to require NF-κB activation.[74] Exposure of cells to this compound blocked expression of XIAP, 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 activated NF-κB in DU145 prostate cancer cells and contributed to the induction of apoptosis through the upregulation of the death receptors DR4 and DR5.[75] Another study indicated that aspirin and UV-C induce apoptosis through a mechanism that drives p65/RelA to the nucleolus, leading to an inability to activate NF-κB-dependent genes.[76]

More recently, Perkins and co-workers found that NF-κB activation by doxorubicin and daunorubicin in U-2 OS osteosarcoma cells promoted cell death. In that paper, NF-κB activation by these chemotherapeutic compounds led to the binding and repression of antiapoptotic genes such as Bcl-xL. Interestingly, the activation of etoposide in the same cells led to the traditional antiapoptotic response and was associated with the activation of Bcl-xL gene expression. Importantly, it was found that daunorubicin induced association of RelA with the histone deacetylases HDAC1, 2, and 3, consistent with the role of NF-κB in repressing gene expression downstream of responses to that chemotherapy. A similar report[78] indicated that NF-κB that is activated by doxorubicin is deficient in transcriptional activity, which is associated with reduced phosphorylation and acetylation of RelA. This paper indicated that the repressive effect was histone deacetylase independent and that RelA was not stably bound to target gene promoters. A recent paper by Aggarwal and co-workers[79] showed that NF-κB activation by doxorubicin in four colorectal cancer cells was required for the anticancer efficacy of the drug. However, there are other papers where doxorubicin-induced cell death was inhibited by NF-κB activation.[10,69] It is presently unclear which signaling events ultimately determine whether NF-κB activation will lead to a pro- or antiapoptotic response, but it is reasonable to

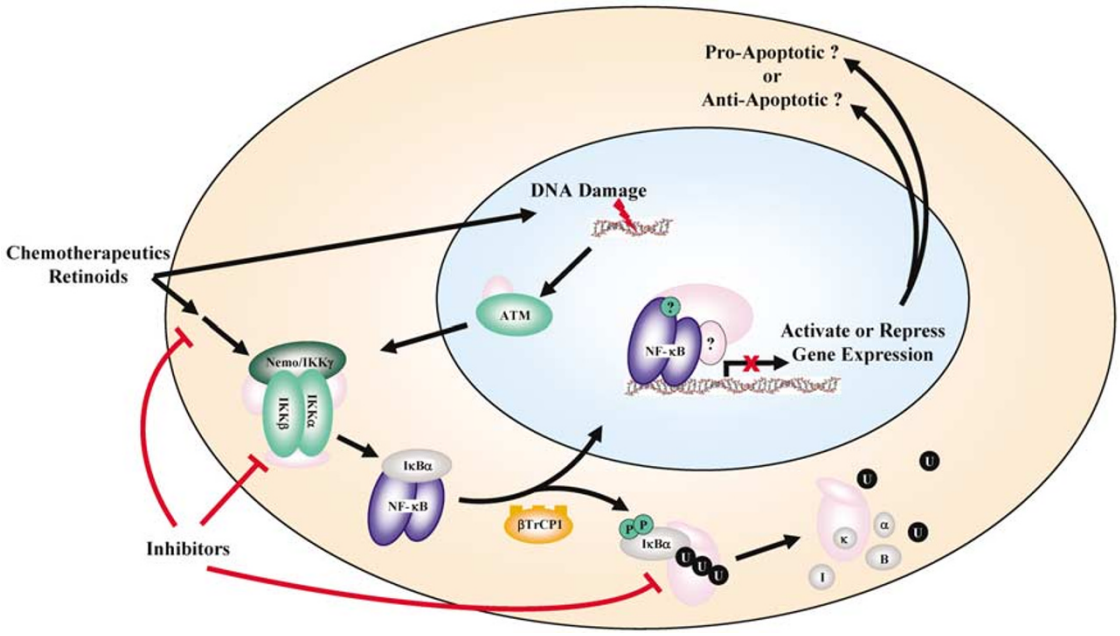

Figure 4 Activation of NF-κB by cancer therapies can lead to anti- or proapoptotic responses. NF-κB is shown activated by standard chemotherapeutics or by retinoids. Many of the chemotherapies induce a DNA damage response, as shown. In the nucleus, NF-κB can mediate a transcriptional response, apparently determined by the chemotherapy in the setting of the cancer cell regulatory environment, which either activates or inhibits apoptotic regulatory genes. Inhibitors of the NF-κB pathway can target upstream pathways, IKK, or the proteasome-dependent activation of NF-κB

speculate that the response 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 likely not 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). These studies suggest the importance of the potential of individualized cancer therapy relative to adjuvant approaches where NF-κB is inhibited.

Summary

An enormous amount of data strongly implicate the transcription factor NF-κB in a variety of oncogenic mechanisms. Additionally, compelling experimentation indicates an important role of 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 role, either as stand-alone therapies or as adjuvants with existing or new therapies.

Acknowledgements

We are very grateful for discussions with members of the Baldwin laboratory. We acknowledge scientific support from the NIH/NCI, the UNC Breast and GI SPORE programs, the Department of Defense Breast Cancer Program, and the Waxman Cancer Research Foundation to ASB. HJK is supported by a KO8 award from the NCI. Additionally, owing to editorial limitations, we regret our inability to reference all of the important published manuscripts on NF-κB and oncogenesis.

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