Nuclear Factor-κB in Development, Prevention, and Therapy of Cancer

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Abstract Nuclear factor-κB (NF-κB) is a signal transcription factor that has emerged as an important modulator of altered gene programs and malignant phenotype in development of cancer. Major carcinogens and oncogenic viruses induce NF-κB activation, and a variety of subsequent oncogenic events contribute to a progressive increase in constitutive NF-κB activation as an important common pathway in most forms of cancer. NF-κB target genes promote tumor cell proliferation, survival, migration, inflammation, and angiogenesis. Inhibition of NF-κB has been found to be an important mechanism of action of steroids, nonsteroidal anti-inflammatory drugs, and natural and synthetic compounds that show therapeutic and preventive activity. Newer agents targeting the proteasome, inhibitor-κB kinase, and other upstream kinases involved in NF-κB activation have shown anticancer activity in clinical or preclinical studies.

Nuclear factor-κB (NF-κB) was originally identified as a bacterial lipopolysaccharide-induced nuclear transcription factor that regulates immunoglobulin κ light chain expression in B lymphocytes (1). Subsequently, NF-κB family members have been shown to be expressed in all cells, where they regulate expression of multiple genes important in cell survival, host responses to injury and infection, and contribute to pathogenesis of various diseases, including cancer (1–4). Five NF-κB family members have been identified in mammalian cells, including NF-κB1 (p105/p50), NF-κB2 (p100/p52), REL A (p65), cREL, and RELB, which associate to form various heterodimeric and homodimeric combinations. These are initially expressed in inactive form in the cytoplasm, by virtue of self-folding with contiguous ankyrin repeat domains, or association with separate ankyrin-containing inhibitor-κBs (IκB; Fig. 1). Signal-induced phosphorylation and ubiquitination of the 105 and p100 precursors and IκBs results in proteasome-mediated processing of NF-κB1 (p50), NF-κB2 (p52), and IκBs. Ankyrin/IκB degradation results in exposure of the nuclear localization sequence and DNA-binding sites for nuclear translocation and DNA binding of NF-κBs (Fig. 1).

Signal regulation of IκB degradation and NF-κB transactivation involves a family of IκB kinases (IKK), other kinases, and the ubiquitin E3 ligase SCF/βTrCP, which play important roles in integrating a diversity of upstream signals (Fig. 1; reviewed in refs. 1–4). A current paradigm for signaling by "classic" (canonical) and "alternative" (noncanonical) IKK/NF-κB pathways has evolved, based largely on studies of the functional effects of knockout of specific IKK and NF-κB/REL subunits on development, immunity, and the cancer phenotype (Fig. 1; reviewed in refs. 1, 2). The classic pathway, activated by tumor necrosis factor (TNF) and many other signals (Fig. 1A and B) through an IKKα/IKKβ/IKKγ complex, has been reported to mediate IKKβ-induced phosphorylation- and proteasome-dependent degradation of IκBs and activation of NF-κB1/REL A (p50/p65) and/or p50/cREL. Injury- or pathogen-induced activation of this pathway in normal cells has been shown to be important for cell survival and innate and adaptive immunity. An alternative pathway, activated by other TNF family members via the NF-κB inducing kinase, involves IKKα/IKKα homodimers, which activate p100/RelB for processing into NF-κB2 p52/RelB heterodimers (Fig. 1C). Knockout studies indicate that the alternative pathway components regulate survival of premature B lymphocytes and development of peripheral lymphoid tissues. Additional positive signal modifications of IKK and NF-κB components and interacting cofactors by CK2 or Akt, or interactions with negative regulators, such as the p14ARF/p53 pathway, have been shown to determine whether DNA-bound NF-κB results in gene activation or repression (Fig. 1) and will be reviewed below.

Many pathogens, growth factors, cytokines, and carcinogens that induce activation of the classic pathway and heterodimer NF-κB1/REL A (p50/p65) have been implicated in promotion and pathogenesis of cancer. Induced activation by cytotoxic agents, and constitutive activation of the classic pathway by oncogenic activation of upstream receptor and non-receptor tyrosine kinases, has been shown to promote therapeutic resistance and cancer cell survival (Fig. 1A and B; refs. 2, 3). These findings have resulted in interest in development of proteasome and βTrCP inhibitors for therapy, and more specific IKKβ-specific inhibitors, to target the classic pathway for prevention and therapy of cancer. However, recent evidence indicating that signal activation of both IKKα and IKKβ, and NF-κB1 and NF-κB2, may promote survival in different cancers,

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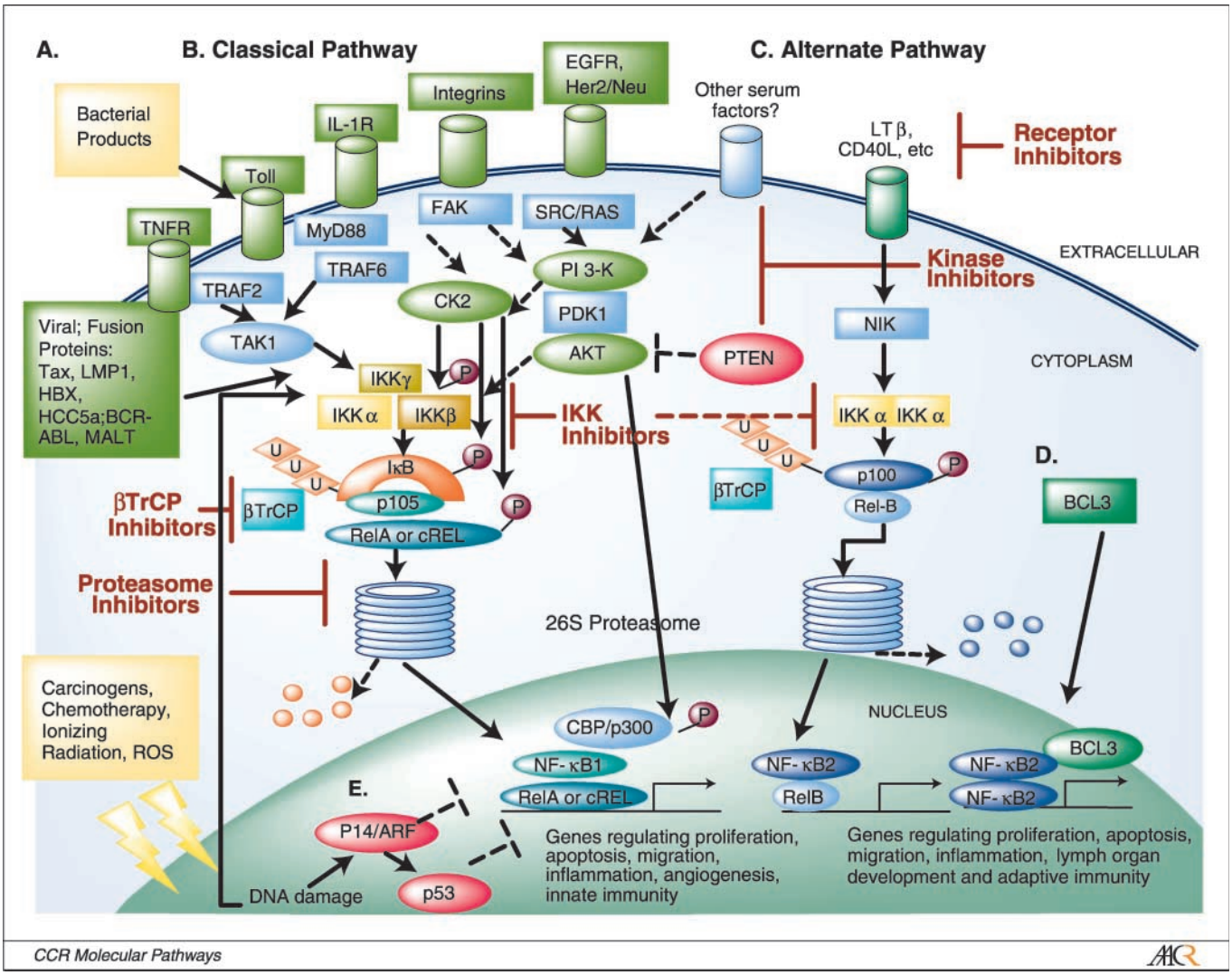

Fig. 1. NF-κB activation in pathogenesis and therapy of cancer. *A,* NF-κB activation in cancer development is linked with chronic exposure to bacteria, certain viral products, chemical promoters, and carcinogens and reactive oxygen species, which cause repeated DNA damage. Induction also occurs in response to cytotoxic chemotherapy and ionizing radiation. *B,* classic NF-κB pathway activation occurs in response to these bacterial, viral, chemical or physical stimuli; by aberrant cytokine, integrin, and growth factor ligand/receptor activation (e.g., TNFR, IL-1R, integrin $\alpha_6\beta_4$, EGFR, HER-2/*neu*, and other serum factors); expression of activating fusion proteins following translocations (e.g., BCR-ABL and MALT1); or aberrant activation by intermediate kinases (e.g., PI3K, CK2, and AKT). Intermediate kinases convey signals to the IκB complex formed by IKKα, IKKβ, and IKKγ, and IKKβ and CK2 phosphorylate IκB, marking it for ubiquitination by E3 ligase βTrCP (SCF) and proteasome degradation. P105/RELA or cREL is processed to NF-κB1 (p50)/RELA or cREL heterodimers, which translocate to the nucleus and bind promoters of genes regulating proliferation, apoptosis, migration inflammation, angiogenesis, and innate immunity. *C,* alternative pathway. The alternative pathway may be activated by other TNF family members via the NF-κB inducing kinase and involves IKKα/IKKα homodimers, which activate NF-κB2/p100 for processing into p52/RelB heterodimers. The RelB/p52 heterodimer then translocates into the nucleus to bind the promoter of genes whose products are important for the malignant phenotype in some cancers and B-cell development and adaptive immunity. *D,* in certain leukemias, overexpression of BCL3 can activate NF-κB2. *E,* repression of gene activation can occur in the presence of intact p14ARF and p53, which favor replacement of CBP/p300 with histone deacetylases. Red highlighted inhibitors of NF-κB activation under clinical and preclinical investigation include proteasome, βTrCP and IKK antagonists, and inhibitors of receptor and intermediate kinases involved in activation. Abbreviations: NIK, NF-κB inducing kinase; IL-1R, interleukin 1 receptor; EGFR, epidermal growth factor receptor; TNFR, TNF receptor; TRAF, TNF receptor – associated factor; TAK, transforming growth factor-β – activated kinase; FAK, focal adhesion kinase; CK2, casein kinase 2; PI3K, phosphatidylinositol 3-kinase; PDK, 3-phosphoinositide – dependent protein kinase.

and that NF-κB may inhibit or promote cancer development in different models, breaks down previously accepted paradigms, suggesting a more complex role for the IKKs and NF-κBs as targets for cancer prevention and therapy.

Links between NF-κB, Infection, Inflammation, and Carcinogens in Development of Cancer

Cumulative experimental and epidemiologic evidence indicates that pathogen-, carcinogen-, and inflammation-induced activation of NF-κB plays direct or indirect roles in cellular promotion, transformation, and progression of experimental and human cancers (refs. 2, 4, 5; Fig. 1A and B). The first evidence implicating pathogen-induced NF-κB activation in development of cancer was identification of the REV-T viral oncogene that causes avian reticuloendothelial lymphomatosis, v-Rel (4, 6), which shares a Rel transactivation domain with the mammalian homologues NF-κB1, NF-κB2, RelA (p65), cRel, and RelB. Figure 1 summarizes a number of alterations and signal pathways that have been implicated in activation of NF-κB in human cancers (2–6). EBV gene *LMP-1* and human

T lymphocyte virus gene *Tax* encode proteins that activate NF-κB and commandeer gene programs that promote cell proliferation, survival, and inflammation, contributing to the pathogenesis of lymphomas, nasopharyngeal carcinomas, and adult T-cell leukemias. Human papillomavirus genes *E6* and *E7* that inactivate *p53* and *Rb* tumor suppressor genes have also been implicated in NF-κB activation and are associated with premalignant and malignant lesions of the cervix, oropharynx, and larynx, where human papillomavirus–associated neoplasms are prevalent. Hepatitis B and C are major causes of hepatocellular carcinoma; hepatitis B X protein and hepatitis C 5A and core proteins have been shown to activate NF-κB and are implicated in hepatocellular transformation. Recently, gastric *Helicobacter pylori* and colonic bacteria in patients with ulcerative colitis have also been implicated in NF-κB activation in epithelia and inflammatory leukocytes and promotion of gastric and colon carcinomas.

Major chemical and physical carcinogens implicated in initiation and/or promotion of human cancer can also activate NF-κB. Notably, nicotine and carcinogens in tobacco and betel nut (areca), which are linked to pathogenesis of head and neck and lung malignancies, induce AKT and NF-κB and promote cell proliferation, survival, and inflammation (4, 5, 7). Nicotine has been reported to directly activate these pathways via nicotinic receptors and Akt (7), whereas chemotherapy- and radiation-induced DNA damage has been reported to induce NF-κB activation via nuclear to cytoplasmic signaling mechanism involving sumoylation of the IKK complex (8). TNF, γ radiation, and certain chemotherapeutic drugs also induce NF-κB activation and several target antiapoptotic genes (*TRAF*, *IAP*, and *BCL-2* and *Bcl-XL*) that protect cells from therapeutic injury by these agents (4).

Constitutive Activation of NF-κB in Cancer

There is now considerable evidence that sustained or constitutive activation of NF-κB is prevalent in cell lines and tumor tissue specimens and contributes to malignant progression and therapeutic resistance in most of the major forms of human cancer. NF-κB/Rels are constitutively activated in human lymphomas (9), carcinomas of the breast (10), prostate (11), lung (12), colon (13), pancreas (14), head and neck (15), esophagus (16), and cervix (17).

Although the mechanisms of activation are incompletely understood, aberrant activation of upstream tyrosine receptor and non-receptor kinases via IKKs or other kinases has been found to be the most common basis for NF-κB activation in epithelial and lymphoid malignancies (Fig. 1, highlighted in *green*). Autocrine or paracrine activation of NF-κB resulting from overexpression of epidermal growth factor (18), HER-2/*neu* (19), interleukin-1 (20), hepatocyte growth factor (21), and integrin family (22) ligands and receptors has been reported. Epidermal growth factor receptor and HER-2/*neu* signaling involving phosphatidylinositol 3-kinase (PI3K), IKK, and CK2 has been shown in breast cancer (23). Interleukin-1/interleukin-1R, transforming growth factor-α/epidermal growth factor receptor, PI3K, Akt, CK2, and IKK have been shown to mediate activation in head and neck squamous cell carcinomas (20, 24, 25). Hepatocyte growth factor/PI3K/Pak/IKK signaling is found in prostate carcinoma (21). Activation via CK2 and IKK is observed in colon carcinomas (26). PI3K/Akt and IKK seem to be important in signal activation of NF-κB and cell survival in many cancers (27–30). The BCR-ABL fusion oncogene has been implicated in NF-κB activation, cell survival, and tumorigenesis in human leukemias (ref. 31; Fig. 1A). Activation by a translocation that produces a MALT-1 fusion protein has reported in diffuse large B-cell lymphomas (ref. 32; Fig. 1A). Constitutive activation of NF-κB p52:p52 due to overexpression and association with the transactivating family member BCL-3 has been detected in breast carcinomas and lymphomas (refs. 33, 34; Fig. 1D). Direct mutation or altered expression of NF-κB molecules has only rarely been found in human cancers and in Hodgkin's lymphomas, where mutations of IκB that favor activation have been identified (35, 36).

Loss of the inhibitory effects of certain tumor suppressor genes may also be important in function of NF-κB as a prosurvival factor (Fig. 1E, highlighted in *stippled red*). Evidence indicates that activation of functional ARF, ATR, and Chk2 mediates alternative phosphorylation of RelA (p65), and p53 competes for CBP/p300, resulting in repression of NF-κB prosurvival genes when the ARF-ATR-p53 pathway and proapoptotic target genes are activated (37). Inactivation of this proapoptotic pathway by hypermethylation of *p14ARF*/*p16INK4A* or mutation, or human papillomavirus inactivation of p53, are among the most common alterations in human cancers. Loss of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) expression is associated with increased PI3K/Akt signal activation of NF-κB and c-Myc in prostate carcinoma cells (38).

NF-κB has been shown to regulate many of the genes differentially expressed and implicated in cell proliferation, survival, migration, and tumorigenesis and metastasis in cancer. NF-κB–related gene signatures have been identified and associated with malignant phenotype in squamous cell carcinomas (39–41), Hodgkin's and certain non-Hodgkin's lymphomas (42, 43), and inflammatory breast cancer (44). A number of key genes in cancer have been shown to be targets and transcriptionally regulated by NF-κB. Targets of NF-κB important in cell proliferation and survival include prominent oncogenes such as *cyclin D*, *Bcl-XL*, and *IAP* (45–47). Expression of key angiogenesis factors and adhesion molecules, such as GRO1, interleukin-8 and vascular endothelial growth factor, are directly or indirectly enhanced by NF-κB activation (48). Together, these genes contribute to the increase in proliferation, survival, inflammation, and angiogenesis that leads to rapid tumorigenesis and metastasis.

Clinical-Translational Advances

Inhibition of NF-κB has been found to be an important mechanism of action of steroids, nonsteroidal anti-inflammatory drugs (NSAID), and natural and synthetic compounds that show therapeutic and preventive activity with acceptable safety profiles. Newer agents targeting the proteasome, IKK, and other upstream kinases involved in NF-κB activation have shown anticancer activity in clinical or preclinical studies.

NSAIDs and cancer prevention. NSAIDs, such as aspirin, sulindac, ibuprofen, and celecoxib, have been shown to inhibit NF-κB activation and arachidonic acid inflammatory pathways upstream and downstream of NF-κB (49–52). NSAIDs have shown inhibitory activity against cancer cells *in vitro* and/or *in vivo* models (53). NSAIDs have been shown to reduce

adenoma and colon cancer development in patients with inflammatory bowel disease and hereditary colon cancer, and long-term NSAID use has also been associated with a reduction in risk of colon, breast, and prostate cancer in population-based studies (54–57). Several natural compounds affecting NF-κB (e.g., curcumin, derived from tumeric, the spice in curry) have also shown promise as potential chemopreventive and therapeutic agents (58, 59). Further studies of the efficacy and NF-κB–dependent and NF-κB–independent mechanisms of action of synthetic and dietary NSAIDs are needed. In addition, potential benefits must be compared with possible risks of gastrointestinal or cardiovascular side effects observed with some of the synthetic agents.

Corticosteroids and cytotoxic agents used for therapy. The cytotoxic effects of corticosteroids in combination with other DNA-damaging agents led to the use of steroid-based regimens as a current mainstay of treatment of certain leukemias, lymphomas, and myelomas. Subsequently, corticosteroids were shown to mediate many of their anti-inflammatory and tumor cytotoxic effects through inhibition of NF-κB (60), and these lymphoid malignancies and supporting host responses were found to be exquisitely dependent on NF-κB–regulated survival or inflammatory mechanisms (61). Recently, *cis*-platinum, which has cytotoxic activity and is a standard radiation sensitizer in a wide range of epithelial malignancies, has also been shown to inhibit NF-κB activation and expression of antiapoptotic genes, in addition to its effects as a DNA-damaging agent (62). These agents have found a significant place in current cancer therapy, with supportive measures to manage the hematopoietic and immunosuppressive side effects expected with agents with broad effects on NF-κB, DNA replication, and other functions.

Proteasome inhibitors. A new class of therapeutic agents under development are proteasome inhibitors, which regulate degradation of IκB and inhibit NF-κB, as well as turnover of other cellular proteins (63). The proteasome inhibitor bortezomib (VELCADE, Millennium Pharmaceuticals, Cambridge, MA) has been shown to have significant preclinical and clinical activity and is now approved for treatment of patients with therapy-resistant multiple myeloma (63). Multiple myeloma cells, and the bone marrow stromal interactions on which they depend, exhibit sensitivity to bortezomib through NF-κB–dependent and NF-κB–independent mechanisms. Bortezomib has also recently been shown to have activity in other B cell–related malignancies, including mantle cell lymphoma and Waldenstrom macroglobulinemia (64, 65). Despite evidence for constitutive activation of NF-κB and prosurvival mechanisms in most solid cancers, limited or no activity has been observed with bortezomib monotherapy in Hodgkin's lymphoma, metastatic melanoma, or colon, breast, prostate, and renal cell carcinomas (66–71).

Evidence from preclinical and clinical studies suggest that combination with other cytotoxic therapies, or inhibitors of other prosurvival pathways, may enhance antitumor activity of bortezomib in some cancers. In preclinical and an ongoing phase I clinical study, we have found that bortezomib has NF-κB inhibitory, antiproliferative, proapoptotic, and radiation-sensitizing activity in head and neck squamous cell carcinoma (72). In combination with re-irradiation, partial clinical response and stabilization of disease was observed in a subset of patients. Dose-limiting hypotension and hyponatremia was observed with 6 weeks of twice weekly bortezomib (0.6 and 0.9 mg/M^2/dose) but has been reduced with amendment of the schedule to include a mid-course treatment break and i.v. fluid support, and dose escalation is ongoing. A combination, including bortezomib, gemcitabine, and carboplatinum, has shown activity and prolonged survival in patients with non–small cell lung cancer (73). Combinations with platinum or taxanes have also shown clinical responses in patients with advanced malignancies in phase I studies (74–76). The possible potentiation of effects of combining proteasome and NF-κB inhibition together with DNA-damaging agents, or with agents targeting other pathways, is a subject of current interest. The role of proteasome inhibitors in combination with other standard or molecular targeted therapies in solid malignancies, and role of NF-κB, awaits determination in larger phase II studies.

IKK inhibitors. IKKβ antagonists that more specifically inhibit IκB and the NF-κB classic pathway initially implicated in cancers have been the subject of intensive development and preclinical studies (2, 77). This is based largely on evidence that IKKβ and p65 knockout sensitize cells to apoptosis and cytotoxic therapies, and anticipation that fewer side effects may be encountered with more specific agents. The IKKβ-selective antagonist PS-1145 or ML120b derivatives (Millennium Pharmaceuticals) have shown antiproliferative, cytotoxic, and antitumor activity in preclinical studies with multiple myeloma cells, diffuse large B-cell lymphoma, chronic myelogenous leukemia, and prostate carcinoma cells (78–80). Another IKKβ antagonist, BMS-345541 (Bristol-Myers Squibb, Princeton, NJ), has shown apoptotic and antitumor activity in melanoma xenograft models (81). These agents exhibit IC_{50}s for their molecular targets in the submicromolar range and for inhibition of sensitive cells in the 5 to 30 micromolar range (78, 81). To date, no clinical trials with IKK inhibitors have been reported.

It is not clear that targeting IKKβ and the classic pathway according to the current paradigm will provide sufficient blockade of NF-κB signal pathways. Our finding that IKKβ inhibitors only partially inhibit NF-κB activation and are not cytotoxic in head and neck cancers led us to the discovery that IKKα and IKKβ as well as protein kinase CK2 (formerly casein kinase 2) contribute to activation of NF-κB (25). Several other studies indicate IKKα may contribute to activation of both NF-κB1 and NF-κB2 and survival of cancer cells (82–84), suggesting that alternative and classic NF-κB pathway activation may promote cell survival in other cancers. These findings suggest there is a need to re-examine the paradigm upon which IKKβ drug development has been based, and to identify inhibitors of both IKKα and IKKβ, CK2, other kinases, or ubiquitin ligases that specifically regulate NF-κB transactivation (Fig. 1).

NF-κB as Proapoptotic or Antiapoptotic Signal in Cancer?

Differing results between human cancers reviewed previously and among several experimental animal models indicate that the mechanism or stage of carcinogenesis, affected cell subpopulation, or other molecular changes may determine whether NF-κB acts as a repressor or promoter of malignancy. Some of these findings have raised questions about whether targeting NF-κB for prevention could have deleterious rather than beneficial effects. These experimental animal studies

examined effects of tissue targeted alterations in expression of IκB, IKKβ, or NF-κB on cancer development. Consistent with a role of IKK/NF-κB in promotion and pathogenesis of cancer, inhibition of NF-κB by targeted IKKβ knockout in colonic epithelia exposed to bacteria in an inflammatory bowel disease model repressed colon carcinogenesis (85). Pilarsky et al. found that inhibition of NF-κB by targeted overexpression of IκB signal mutant or treatment with anti-TNF antibodies was associated with a decrease in NF-κB activation and tumorigenesis in mdr-2 knockout mice that develop inflammation-associated hepatocellular carcinomas (86). By contrast, targeted disruption of the classic pathway by IKKβ knockout in hepatocytes during exposure to carcinogen DEN increased tumorigenesis (87). TNF and c-Jun NH_2-terminal kinase 1 pathway activations were implicated in necrosis-induced inflammation and promotion of the increase in carcinogenesis in this model (87, 88).

Apparent differences in role of NF-κB have also been observed in human squamous cell carcinoma and among some murine models. Constitutive activation of NF-κB has been found with progression of squamous cell carcinoma derived from skin of BALB/c and α-catenin knockout mice (89, 90) and in squamous dysplasias and carcinomas of the cervix and the head and neck (15, 17, 91). Inhibition of NF-κB by IκB repressor, steroids, or proteasome inhibitors promotes apoptosis and inhibits tumorigenesis of these cancers *in vitro* and *in vivo* (40, 72, 91, 92). By contrast, in transgenic mouse models targeting squamous epithelia, selective expression of an IκB repressor of NF-κB during carcinogen exposure or with expression of *ras* oncogene resulted in increased carcinogenesis of squamous cell carcinoma of the skin (93, 94). With inhibition of NF-κB in that setting, increased TNF, c-Jun NH_2-terminal kinase, and activator protein pathway activation were shown to contribute to the increase in carcinogenesis (95). These findings suggest there may be greater complexity and differences in the mechanism of activation of NF-κB during carcinogenesis in human and murine cancers than are thus far reflected in some of these targeted murine models.

Several molecular alterations that occur with cancer development have been identified that could help explain the apparent differences in function of NF-κB in these different contexts. First, most knockout models to date do not account for the multiple positive signal mechanisms activated during cancer development that can contribute to constitutive activation of the classic or alternative NF-κB pathways. As reviewed above, multiple growth factor and cytokine receptors, PI3K, Akt, CK2, IKKα and IKKβ, other signal kinases, and cofactors may be coactivated in cancer and contribute to transactivation of NF-κB1 and/or NF-κB2. In addition, most cancers exhibit inactivation or loss of expression of tumor suppressors that have been shown to negatively regulate NF-κB activation. Perkins et al. have shown that in embryonic fibroblasts or malignant cells deficient in functional p14ARF, p53, and proapoptotic gene expression, NF-κB acts as an activator of prosurvival genes, such as *cyclin D1* and *Bcl-XL* (37, 96). However, re-expression and/or activation of functional ARF, ATR, Chk2, and p53 tumor suppressor pathway components can alter NF-κB to become a functional repressor. ARF, ATR, and Chk2 were shown to mediate phosphorylation of Thr^{505} of RelA (p65), and p53 was shown to compete for the coactivator CBP/p300, resulting in association with histone deacetylases and inactivation of NF-κB and prosurvival genes. Consistent with this model, others have found that NF-κB overexpression in immortalized keratinocyte cell lines with mutant p53 exhibits increased malignant potential and tumorigenesis (97). Mice with targeted deletion of α-catenin, another tumor suppressor commonly inactivated in human cancer, also exhibit increased NF-κB activation, inflammation, proliferation, and malignant transformation (90). Together, these observations may help account for the apparent disparities in role of NF-κB in different experimental knockout models and human SCC above because the *p14ARF/p16INK4a* locus encoding p14ARF and p53, and α-catenin frequently undergo inactivation or mutation in human and murine cancers in which constitutive NF-κB activation has been shown.

Conclusions

The alterations in multiple signal pathways shown to positively and negatively regulate NF-κB activation are consistent with its frequent activation and important functional role in cancer. The redundancy in alterations that may lead to activation can also explain in part why drugs targeting receptors and kinases further upstream are effective primarily in cancers in which limited alterations and a dominant role of these molecules is established. Agents with broad activities that include inhibition of NF-κB, such as NSAIDS, corticosteroids, and proteasome inhibitors, have already shown evidence of efficacy and safety in prevention or therapy of certain cancers. The important role of CK2, IKKs, ubiquitin ligase βTrCP, and proteasome in integrating these signals and the final steps of activation have made these important molecules for study as potential targets for prevention and therapy.

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