Nuclear Factor-κB, an Unappreciated Tumor Suppressor

Fei Chen and Vince Castranova

The Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia

Abstract

The notion that nuclear factor-κB (NF-κB) is a tumor-promoting transcription factor has become a widely accepted dogma in biology. However, recent findings suggest an inhibitory role for NF-κB in carcinogenesis and tumorigenesis. Although the tumor suppressor–like effect of NF-κB remains to be rigorously established by further studies using cellular and animal models, these latest findings warrant caution with respect to blockage of NF-κB activation as a broad strategy in treating cancers. [Cancer Res 2007;67(23):11093–8]

Introduction

Nuclear factor-κB (NF-κB) is a transcription factor important for the expression of numerous genes contributing to inflammation and innate and adaptive immune responses. The most common form of NF-κB is the p50/RelA heterodimer, although other forms of NF-κB dimers, such as p50/p50, p52/p52, p52/RelA, p50/c-Rel, c-Rel/c-Rel, p52/RelB, and p50/RelB, have also been identified in some types of cells (1). In resting state, this transcription factor is inactive and retained in cytoplasm by binding to its inhibitory proteins such as IκBα, IκBβ, and IκBε, among which IκBα is the most abundant. A plethora of intracellular and extracellular cues that influence signaling pathways, which integrate ubiquitination, oligomerization, and phosphorylation, induce activation of IκB kinase (IKK) complexes containing IKKα, IKKβ, and NEMO/IKKγ subunits. Phosphorylation of IκBα by IKK causes ubiquitination and proteasomal degradation of IκBα, leading to liberation and translocation of NF-κB into the nucleus where it regulates gene expression through binding to the κB elements in the enhancer or promoter regions of the genes. Regulation of NF-κB occurs at several different hierarchical levels. The activation of this transcription factor is affected by a number of parallel signaling pathways that cross-talk with NF-κB–activating signal cascades either positively or negatively. In the nucleus, the translocated NF-κB undergoes several different modifications of its subunits, including phosphorylation, acetylation, and ubiquitination. In addition, the nuclear NEMO/IKKγ may be modified by SUMO for the subsequent ATM-dependent phosphorylation and monoubiquitination of NEMO/IKKγ to amplify genotoxic stress–induced NF-κB activation. The transcriptional activity of NF-κB is further influenced by a number of other variables such as local concentrations of different NF-κB dimers, redox status of NF-κB subunits, the nature of its partners, fidelity of canonical κB elements, distance of its binding sites from the start site of transcription, neighboring transcription factor binding sites, the accessibility of κB sites based on the degree of histone H3 and H4 acetylation and methylation in the nearby chromatin structure, the methylation status of proximal CpG islands, and/or other idiosyncrasies of target promoters.

NF-κB and Tumor Promotion

The primary role of NF-κB is to maintain normal cellular functions that range from cell-to-cell communication to cell motility, cell cycle progression, and cell lineage development (1). Earlier evidence suggests that aberrant activation of NF-κB or its upstream signaling pathways is responsible for the initiation of tumorigenesis including evasion of apoptosis, malignant transformation, sustained cell proliferation, metastasis, and angiogenesis (1). A group of antiapoptotic genes, including *Bcl-xL, cIAP1, cIAP2, XIAP, A20, c-FLIP*, and *TRAF-2*, can be up-regulated by NF-κB, leading to protection of the cells from apoptosis in response to a variety of DNA-damaging signals. A sustained activation of NF-κB contributes to the expression of proto-oncogenes including c-myc and cyclin D1, which are accountable for both transformation initiation and tumorigenic proliferation. The capacity of NF-κB to induce expression of cell adhesion molecules, vascular endothelial growth factors (VEGF), and matrix metalloproteinases (MMP) promotes the metastatic potential of tumor cells. The linkage between tumorigenesis and chronic inflammation, in which NF-κB plays a pivotal role in the expression of numerous inflammatory cytokines and other mediators, has been well established (1). Most recently, NF-κB has also been implicated in the transcriptional up-regulation of microRNA-155 (miR-155), an oncogenic miRNA that was derived from B-cell integration cluster (*BIC*) gene and highly expressed in some lymphatic tumors, breast cancer, lung cancer, and colon cancer (2). The majority of miRNAs inhibit expression of genes through siRNA-mediated mRNA silencing, 3-untranslated region–dependent translational interference, and miRNA machinery–induced heterochromatin formation. In contrast, miR-155 stimulates expression of the antiapoptotic cytokines through unknown mechanisms (2). Furthermore, either gain of functional mutations of NF-κB family members or constitutive activation of NF-κB has frequently been observed in many human cancers.

In addition to the transcriptional regulation of the antiapoptotic genes and growth factors by NF-κB, the upstream kinases of NF-κB, IKKα and IKKβ, are able to modulate cell growth and antiapoptotic responses in a NF-κB–independent manner. IKKα has previously been shown to be capable of phosphorylating histone H3 serine 10 (H3S10p), which is usually associated with the active gene transcription markers, such as trimethylation of the histone H3 lysine 4 (H3K4me3) and acetylation of H3K9 and H3K14. It is generally viewed that active gene transcription is essential for a sustained cell proliferation. IKKα is also capable of phosphorylating SMRT, a transcriptional repressor of Notch-regulated genes, leading to the release of SMRT from the chromatin and the expression of Notch-targeting genes that are important for the growth of the colorectal tumors (3). An additional interesting observation is that IKKα can form a complex with Aurora A and

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Fei Chen, The Health Effects Laboratory Division, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505. Phone: 304-285-6021; E-mail: fchen@cdc.gov.

doi:10.1158/0008-5472.CAN-07-1576

Plk1 kinases at the centrosome (4). Phosphorylation of Aurora A kinase at threonine 288 in the T-loop by IKKα seems to be one of the critical biochemical events required for the completion of mitosis. Unlike IKKα, IKKβ is unable to phosphorylate histone H3, SMRT, and Aurora A. However, phosphorylation of FoxO3a, a pro-apoptotic transcription factor, and HDAC1, a repressive histone deacetylase (HDAC), by IKKβ is regarded as a pivotal mechanism for IKKβ-induced tumorigenesis (5). The phosphorylation of FoxO3a and HDAC1 by IKKβ promotes degradation of these two proteins through the ubiquitin-proteasome pathway. Most recently, IKKβ has also been shown to be capable of phosphorylating tuberous sclerosis 1 (TSC1; ref. 6). TSC1 associates with TSC2 to form a tumor suppressor complex by repressing the mammalian target of rapamycin (mTOR) pathway. Phosphorylation of TSC1 by IKKβ disrupts the formation of TSC1 and TSC2 complex, leading to sustained activation of mTOR signaling that contributes to tumor angiogenesis.

Taken together, it seems to be likely that NF-κB and its upstream activating signals are important contributors to the tumorigenesis by their ability to regulate the expression and/or function of oncogenes, growth factors, antiapoptotic proteins, mitotic kinases, and the transcriptional repressors. Thus, inhibition of NF-κB or its upstream kinases in combination with established cytocidal drugs and therapeutic radiation seems to be a promising strategy for cancer therapy. In addition, such inhibition should also be beneficial in preventing malignant transformation of the normal cells and the uncontrolled growth of the tumor cells in the earlier phases of tumorigenesis.

NF-κB Acts as a Tumor Suppressor in Collaboration with p53

The topic of NF-κB in tumor promotion has extensively been reviewed in the past (1). However, this general view suggesting that NF-κB is a tumor promoter is increasingly complicated or challenged by recent evidence indicating the tumor suppressor–like effects of NF-κB under many circumstances. Yet the hypothesis that NF-κB is potentially involved in tumor suppression is not completely ill-conceived. One of the earliest suggestions of a possible tumor-suppressing effect of NF-κB was the proapoptotic function of c-Rel and RelA in T cells, B cells, fibroblasts, 293 cells, neuronal cells, and HeLa cells. In these cells, overexpression of these subunits induces apoptosis in response to death stimuli, whereas inhibition of NF-κB protects these cells from apoptosis. It was suggested that NF-κB was capable of inducing expression of p53, Fas, FasL, Bax, c-myc, DR4, DR5, and Bcl-xS, which are actively involved in the promotion of cell apoptosis, thereby facilitating tumor cell death. NF-κB is also implicated in the up-regulation of the tumor suppressor CYLD, a deubiquitinase that terminates NF-κB signaling and enhances cell death through removal of K63-polyubiquitin chains from NEMO/IKKγ and TRAF-6. An intriguing observation is that NF-κB seems to be an essential component in mediating p53-induced cell death in a cellular system where p53 was overexpressed. Inhibition of NF-κB by transfection of the cells with a degradation-resistant IκBα abrogates p53-mediated apoptosis. Further studies suggested that genetic deficiency of NF-κB RelA has similar effects on apoptotic resistance and tumorigenic transformation induced by E1a and Ras as loss of p53. Identifying how NF-κB cooperates with p53 in this apoptotic response seems to be a difficult task due to the fact that p53 has been implicated in the regulation of not only cell apoptosis but cell cycle arrest and metabolism as well. In addition, the hypothesis that NF-κB is required for p53-induced apoptosis had been challenged by the fact that NF-κB signaling antagonizes the function and stability of p53 protein in a variety of cellular models (7). There is also additional evidence showing mutual antagonism between the NF-κB RelA subunit and p53 through competition for limited pools of coactivators, in which IKKα-mediated phosphorylation of cyclic AMP–responsive element binding protein–binding protein (CBP) switches CBP from p53 binding to NF-κB binding. CBP is a p300 homologue histone acetyltransferase that is able to induce transcription initiation by acetylating histone proteins, NF-κB RelA subunit, and other transcription factors. Studies by Perkins and associates (8) implied that the NF-κB family member p52 might be responsible for mediating p53-induced cell death. Activation of p53 has been proposed to tip a switch from NF-κB p52-Bcl-3, an activator complex, to p52-HDAC, a repressor complex. This exchange from activator to repressor can result in the inhibition of cyclin D, a crucial factor promoting cell growth and cell cycle transition. Furthermore, NF-κB p100, which generates mature p52 protein, has been shown to be able to sensitize cells to death receptor–mediated apoptosis through recruitment of the death machinery complexes in several cell lines. In agreement with this notion, NF-κB p52 subunit has also been shown to be capable of interacting with p53 directly to mediate the expression of p53 target genes such as PUMA, DR5, GADD45α, and Chk1, all of which are important for p53-induced apoptosis. Finally, the upstream kinase of NF-κB, IKKα, may be involved in p53-mediated cellular responses by either stabilizing p53 family protein, p73, or fostering expression of *14-3-3σ*, a p53-targeting gene (9, 10). Physical interaction of the nuclear IKKα with p73 leads to accumulation of p73 and an enhanced cell apoptosis induced by cisplatin. The stabilization of p73 by IKKα is dependent on the IKKα kinase activity that is capable of phosphorylating p73 and consequently preventing p73 from ubiquitination and proteasomal degradation. The expression of 14-3-3σ was diminished in $Ikk\alpha^{-/-}$ keratinocytes because of lysine 9 trimethylation of histone H3 and DNA methylation in the promoter region of the *14-3-3σ* gene. Reintroduction of *Ikkα* prevents associations of lysine 9 trimethylase SUV39h1 and DNA methylase Dnmt3a with the *14-3-3σ* locus, leading to derepression of the *14-3-3σ* gene (10). Although the fact that NF-κB or its upstream signaling promotes apoptosis has been relegated to the background by a large volume of literature showing an antiapoptotic effect of NF-κB, a proapoptotic role of NF-κB has been resurrected in several newer studies.

Tumor Suppressor–like Effects of NF-κB in Epidermal Cells

The first evidence directly linking NF-κB to tumor suppression was from experiments with murine and human epidermis transgenic for IκBα protein that inhibits NF-κB (11). Functional blockade of NF-κB in epidermal cells resulted in severe hyperplasia of the skin in transgenic mice, which could be reversed by overexpression of active p50 and RelA subunits of NF-κB, suggesting the tumor-suppressive effect of NF-κB. The epidermal cells from these skin-targeted transgenic mice showed an aggressive growth pattern with a marked increase in DNA synthesis. A follow-up study suggested that the hyperplastic skin lesions, which resembled human squamous cell carcinomas, were possibly a compensatory secondary response to keratinocyte apoptosis owing to permanent inhibition of NF-κB in these cells.

The compensatory proliferation due to apoptosis was further confirmed in retrovirally transduced human keratinocytes expressing NF-κB RelA or IκBα in the presence of active Ha-Ras. Without coexpression of the active Ha-Ras Gly12Val mutant, sustained inhibition of NF-κB by expression of IκBα caused mild hyperplasia. Ras-IκBα coexpression produced invasive large neoplasms, which partially resulted from increased expression of MMP3, cyclin-dependent kinase 4 (CDK4), and telomere maintenance enzyme hTERT. However, several questions remain to be answered. For instance, it is still unclear how NF-κB inhibition increases expression of MMP3, CDK4, and hTERT. Is NF-κB a negative transcriptional regulator for the expression of these genes? What is the underlying mechanism mediating keratinocyte apoptosis-induced compensatory overproliferation?

An inverse correlation between the expression levels of IKKα, an upstream kinase contributing to the NF-κB2/p100 processing–based alternative activation of NF-κB, and the aggressiveness of squamous cell carcinomas was recently shown (12). In a transgenic mouse model, overexpression of IKKα in suprabasal and basal epidermis significantly delays onset and metastasis of carcinogen-induced skin carcinomas, possibly a result of repression in cell mitosis and angiogenesis. An intriguing finding from this study is that IKKα binds to the distal VEGF-A promoter with the consequent inhibition of VEGF-A expression. IKKα had previously been shown to be capable of phosphorylating serine 10 of histone H3 (H3S10p). H3S10p usually acts in collaboration with another active gene transcription marker, trimethyl lysine 4 of histone H3 (H3K4me3), in the euchromatin region of the nucleosome, to regulate gene expression. It will be interesting to study how IKKα binding to the promoter DNA of the VEGF-A gene suppresses the expression of VEGF-A.

Tumor Suppressor–like Effects of NF-κB in Other Type of Cells

Most studies revealing a tumor-suppressive effect of NF-κB were from transgenic inhibition of NF-κB in skin cells, giving the impression that this tumor suppressor–like function of NF-κB occurred predominantly in epidermal cells. Since 2006, however, several groups reported additional evidence implying that NF-κB signaling or activity is indeed involved in tumor suppression in other experimental systems including endothelial cells (13), mouse embryonic fibroblasts (MEF; ref. 14), and *Drosophila* tracheal epithelial cells (15). The basic issue addressed by Kisseleva et al. (13) is how NF-κB regulates endothelial cell function, angiogenesis, and tumor metastasis based on endothelial cell specific transgenic expression of IκBα in mice. Despite the observation indicating that such inhibition of NF-κB sensitizes endothelial cells to lipopolysaccharide-induced apoptosis, the endothelial cells in which NF-κB activation was abrogated exhibited an increased angiogenic potential. This effect seemed to produce a marked increase in tumor burden and metastasis of the inoculated melanoma due to an extensive tumor vasculature in the transgenic mice. A major drawback of animal models is the intertwining complexity of interactions between the transgenic cells and the surrounding cells or tissues. It is usually difficult, therefore, to differentiate the autonomous response of transgenic cells from the compensatory reaction of bystander cells. This problem can be possibly circumvented by the use of homologous cells. Indeed, using MEFs derived from wild-type (wt) and *Ikkβ* gene knockout mice, our recent study suggests that $Ikk\beta^{-/-}$ MEFs exhibit a high rate of proliferation, migration, and invasion (14). The $Ikk\beta^{-/-}$ cells also showed alterations in cytoskeleton rearrangement and cell morphology, similar to the cells undergoing epithelial-mesenchymal transition during tumorigenesis. IKKβ is a key upstream kinase responsible for the canonical activation of NF-κB. Deficiency in the *Ikkβ* gene results in severe inhibition of the basal or inflammation-induced activation of NF-κB. Thus, NF-κB or its upstream kinase seems to be important for the maintenance of normal cell morphology and function. Accordingly, cells with interrupted NF-κB signaling acquire transformed phenotypes spontaneously. One argument is that MEFs themselves are genetically unstable and heterogeneous. They are derived from mesenchymal stem cells, which are multipotent and capable of differentiating into either mesoderm-type lineages or non–mesoderm-type lineages. The question to be asked is why it is that although MEFs are pluripotent, only $Ikk\beta^{-/-}$ MEFs, but not wt MEFs, acquire characteristics of transformative cells during culture and passage. In fact, this transformative phenotype occurred not only in $Ikk\beta^{-/-}$ MEFs but in $relA^{-/-}$ MEFs as well (16), indicative of the role of NF-κB in maintaining genomic stability. The transformative potential of the *relA*-deficient MEFs seems to be partially dependent on the compensating expression of NF-κB p50/p50 homodimers among different $relA^{-/-}$ isolates (16). The $relA^{-/-}$ MEFs with a relatively higher level of p50/p50 homodimer are capable of undergoing transformation, whereas the $relA^{-/-}$ MEFs with a lower level of p50/p50 homodimer seem to be morphologically normal. Furthermore, a striking similarity in changes of cell morphology, cytoskeleton, and mobility was seen in *Drosophila* S2 cells and tracheal epithelial cells in which IKKε, a possible alternative kinase for activating *Drosophila* NF-κB Dorsal/Dif system, was diminished (15). IKKε inactivation, either by expression of a kinase-mutated IKKε or transfection with IKKε siRNA, fosters cell morphology alterations as seen in $Ikk\beta^{-/-}$ and $relA^{-/-}$ MEFs.

NF-κB Acts as a Tumor Suppressor in Liver Cancer

Recent studies on the mouse models of hepatocellular carcinoma (HCC) have taken a step further in defining the tumor-suppressive or tumor-promoting functions of NF-κB. Mice with hepatocyte-specific *Ikkβ* gene knockout exhibited a marked increase in HCC induced by diethylnitrosamine, a chemical carcinogen, correlating with enhanced reactive oxygen species (ROS) production and c-Jun NH_2-terminal kinase (JNK) activation (17). The importance of JNK signaling in carcinogen-induced HCC has previously been shown based on the fact that hepatocyte-specific deletion of c-Jun or JNK1 minimized the carcinogenic potential of chemical carcinogens. In agreement with this notion, treatment of the mice carrying hepatocyte-specific *Ikkβ* gene deletion with butylated hydroxyanisole, an established antioxidant that removes ROS and inhibits JNK activation, ameliorated the onset of HCC. Surprising results have also been obtained from studies on mice with hepatocyte-specific deletion of NEMO/IKKγ (18). The time scale of HCC development in mice with hepatocyte-specific deficiency in NF-κB signaling seems to be correlated with the degree of NF-κB inhibition. Partial inhibition of NF-κB by hepatocyte-specific deletion of *Ikkβ* did not cause spontaneous HCC. The HCC, as well as JNK activation, in mouse liver could be seen only after applying a chemical carcinogen to these mice for several months. On the other hand, severe or complete inhibition of NF-κB in parenchymal cells, as achieved by hepatocyte-specific

deletion of NEMO/IKKγ, resulted in spontaneous HCC development and JNK activation without additional carcinogen treatment (18). Thus, these latest findings clearly support the hypothesis that NF-κB can play a tumor suppressor–like role in MEFs, mouse or human skin cancer models, and mouse liver cancer models.

Tumor Suppression by NF-κB Is Attributed to JNK Inhibition

How might loss of function of NF-κB actually foster early abnormal cell growth and/or clonal expansion in the early phase of tumorigenesis? It is difficult to identify molecules mediating the tumor suppressor–like function of NF-κB. These molecules possibly affect several aspects of cell cycle, cell death, signal transduction, or carcinogenic transformation. In addition, a number of other transcription factors, structural proteins, metabolic enzymes, and proteins involved in the biochemical operation of the cells have been shown to be effectors of NF-κB activation. One of the very likely targets of NF-κB in tumor suppression is JNK, a versatile kinase that is able to phosphorylate a number of proteins contributing to both apoptotic and antiapoptotic responses. A sustained activation of JNK gives rise to a cellular growth advantage, resulting in progressive and uncontrolled proliferation, a general feature of tumor cells. The earliest evidence supporting JNK inhibition by NF-κB is from transient transfection of the kinase-mutated IKKβ into the human bronchial epithelial cells, in which an enhanced JNK activation was observed following IKKβ-NF-κB inhibition (19). This observation was supported by later reports showing elevation of stress signal–induced JNK activation in either $Ikk\beta^{-/-}$ or $relA^{-/-}$ MEFs. A substantial enhancement of JNK activation by tumor necrosis factor α (TNFα) was observed in the cells where NF-κB was deficient due to genetic interruption of the *Ikkβ* or *relA* gene. Reexpression of the corresponding

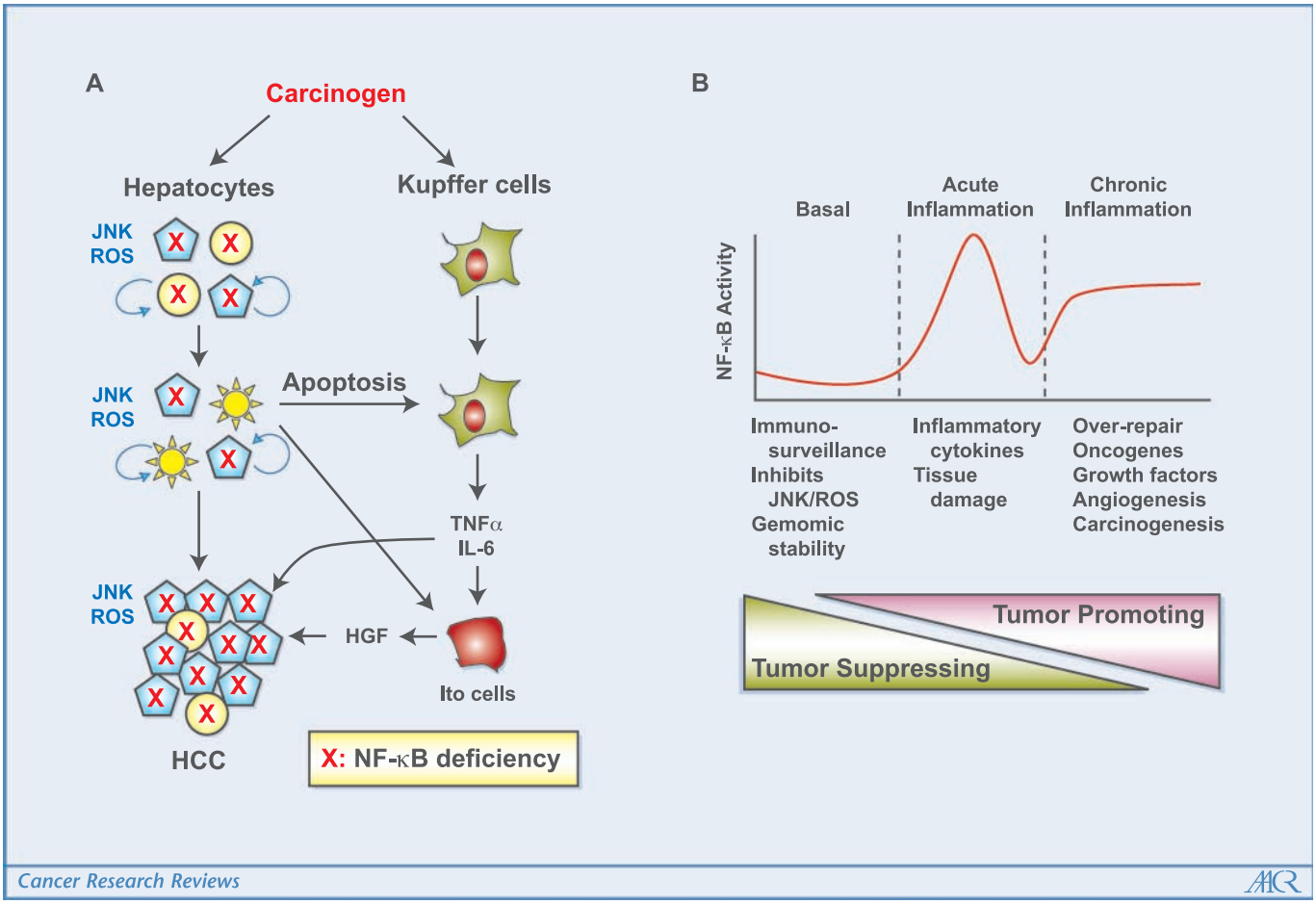

Figure 1. *A,* NF-κB inhibition induces carcinogenesis in liver or other organs. In the liver cancer model, hepatocye-specific deficiency (depicted by a *red X*) in *relA, Ikkβ*, or *Ikkγ* enhances JNK activation and ROS generation in these cells. In response to a carcinogen, a subpopulation of NF-κB–deficient hepatocytes undergo apoptosis because of a weakened antiapoptotic response. The apoptotic cells trigger release of hepatomitogens such as TNFα and IL-6 from Kupffer cells and hepatocyte growth factor (*HGF*) from Ito cells in which NF-κB is normal. These hepatomitogens, along with the enhanced JNK activation and ROS generation, foster uncontrolled proliferation and carcinogenic transformation of the remaining hepatocytes. These proliferative hepatocytes may be those cells that have either escaped from apoptosis despite deficiency in NF-κB or differentiated into a stage that differs from that of cells prone to apoptosis (as denoted by a different shape). *B,* the tumor-suppressive or tumor-promoting effect of NF-κB might be determined by the degree or duration of NF-κB activation and other cellular contexts. The basal-level NF-κB may be tumor suppressive by maintaining the functions of immunosurveillance, inhibiting JNK or ROS generation and guarding genomic stability. During acute inflammation, transient acute NF-κB activation is important for inflammatory cytokine production. These cytokines may be involved in innate immune response against both invading microorganisms and tissue damage. A sustained activation of NF-κB, such as in the case of chronic inflammation, is tumorigenic because of over-repair of the damaged tissues, expression of oncogenes and growth factors, and the release of a number of angiogenic factors.

deficient genes reversed the enhancement of JNK activation in these cells. Whereas it has not been rigorously established how NF-κB inhibits JNK, GADD45β and XIAP were originally proposed as key regulators that impede TNFα-induced JNK activation. However, later studies by several other groups including ourselves suggest that ROS may be the key mediators of TNFα- or arsenic-induced JNK activation in the cells where NF-κB is inhibited (19). NF-κB seems to be essential to protect the cells from oxidative stress by curtailing ROS generation and consequent JNK activation. Conversely, defects in NF-κB activation or activity make it possible to unleash the process of ROS generation and, consequently, a sustained JNK activation. Earlier studies indicated that NF-κB is an important transcription factor regulating the expression of antioxidant enzymes including superoxide dismutases (SOD1 and SOD2) and glutathione peroxidase (GPx). In $Ikk\beta^{-/-}$ MEFs, an appreciable reduction in the expression of metallothionein 1 (MT1) and metallothionein 2 (MT2) was noted (19). Both MT1 and MT2 are low molecular weight metal binding proteins characterized by a high content of cysteine residues and lack of aromatic amino acids. The abundance of cysteine residues makes both proteins capable of detoxifying metal ions and scavenging ROS. Indeed, the ectopic expression of MT1 in $Ikk\beta^{-/-}$ MEFs could attenuate the cytotoxic response to arsenic, indicating the importance of MT1 in metal detoxification and redox regulation (20). Thus, the increased ROS generation and JNK activation in the cells where NF-κB signaling is deficient very likely result from a reduction in the expression of the genes such as SODs, GPx, MT1, and MT2, which are involved in protective responses against oxidative stress. Conversely, a normal level of NF-κB activation or activity may be pivotal to keep the ROS in check and prevent sustained JNK activation that otherwise will be tumorigenic.

Is the Tumor Suppressor–like Effect of NF-κB Achieved through Antiapoptosis?

The importance of apoptosis in attenuating tumor burden is well known. Apoptosis has been a highly desired effect in cancer therapy. However, the role of NF-κB in apoptosis and its role in carcinogenesis or tumorigenesis remain unresolved. In the mouse liver cancer models, two groups attempted to reconcile the antiapoptotic role of NF-κB with the carcinogenic effect of NF-κB inhibition (17, 18). The central concept of these models is that NF-κB is still antiapoptotic as shown by an overwhelming number of studies. However, loss of NF-κB in hepatocytes sensitizes these cells for spontaneous and stress-induced hepatocyte apoptosis, which triggers release of hepatomitogens such as interleukin 6 (IL-6) and TNFα from the bystander Kupffer cells in which NF-κB signaling is normal. The hepatomitogens, in turn, initiate a compensatory proliferation of a subset of hepatocytes that either escape apoptosis despite the deficiency of NF-κB or differentiate into a stage that differs from that of those cells prone to apoptosis (Fig. 1*A*). An alternative possibility is that hepatomitogens promote aberrant clonal expansion and differentiation of the progenitor cells in humans or oval cells in rodents, which are the precursor cells of the hepatocytes located in the periphery of the biliary tract, leading to HCC. It is fair to say that this explanation is intuitively plausible for NF-κB deficiency–induced HCC, although much remains to be learned. If apoptosis is indeed the initiator for HCC, what is the role of apoptosis in other types of cancer that undergo therapy-induced apoptosis? It is known that almost all types of cancers are cytologically heterogeneous and abundant in cancer stem cells and poorly differentiated cells. Can these cells also undergo an uncontrolled proliferation by apoptosis? If so, preventing apoptosis, rather than inducing apoptosis, might be desirable for cancer therapy. Another daunting question is whether these latest mouse liver cancer models are truly representative of human HCC. The most common etiologies of human HCC are HBV or HCV infection and nonalcoholic fatty liver disease. There is no available evidence indicating that the development of human HCC is a result of defects in NF-κB signalings in hepatocytes. In fact, previous reports had suggested that NF-κB activation was instrumental in inducing human HCC. Our recent studies indicate that the expression levels of three IKK kinase subunits, IKKα, IKKβ, and NEMO/IKKγ, are much higher in human HCC relative to the surrounding normal liver tissues.[1]

Future Perspectives

Although NF-κB does not meet the established criteria for a tumor suppressor, such as loss of functional mutation and frequent deletion in tumor cells, rapidly emerging data strongly indicate that normal levels of NF-κB activity are fundamentally important to impede aberrant JNK activation and ROS generation. The sustained JNK activation and ROS generation may be tumorigenic through both genetic and epigenetic mechanisms. The genetic influences, such as ROS-induced genomic mutations and JNK-induced expression of oncogene or growth factors that are intimately involved in cancer development, have been established beyond doubt. The link between JNK or ROS and epigenetic regulation, in contrast, is currently underemphasized. Epigenetic abnormalities in cancer development, mainly the altered methylation states on both histones and genomic DNA, have been gathering interest in the last two years. Involvement of JNK in polycomb signaling that catalyzes lysine 27 methylation of histone H3 and links to DNA methylation had previously been shown in the developmental regulation of *Drosophila*. It is highly possible that both JNK and ROS are important participants in epigenetic modulation in human cancer. Therefore, a tumor suppressor–like effect of NF-κB might be achieved by both genetic activation of target genes and by effects on epigenomics. The functional outcome of NF-κB in tumor suppression or tumor promotion may be determined by the cell types, stimulators, duration of activation, and other accompanying signals (Fig. 1*B*). Deciphering the web of NF-κB, JNK, and ROS in tumor suppression or promotion will be invaluable to define better therapeutic strategies for cancers.

Acknowledgments

Received 4/30/2007; revised 8/13/2007; accepted 8/20/2007.

Grant support: Intramural research grants from the National Institute for Occupational Safety and Health (F. Chen and V. Castranova).

We thank Dr. Murali Rao of the Pathology and Physiology Research Branch of National Institute for Occupational Safety and Health/Centers for Disease Control and Prevention for critical reading of the manuscript.

We apologize to the authors for our inability to cite their original works in this brief review. Detailed citation of the references can be found online in Supplementary data.

Disclaimer: The opinions expressed in this manuscript are those of the authors and do not necessarily represent the views of the U.S. National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

[1] Q. Chang, Y. Zhang, K.J. Beezhold, H. Zhao, J. Chen, V. Castronova, X. Shi, F. Chen, unpublished observations.

References

1. Karin M. Nuclear factor-κB in cancer development and progression. Nature 2006;441:431–6.
2. Kluiver J, van den Berg A, de Jong D, et al. Regulation of pri-microRNA BIC transcription and processing in Burkitt lymphoma. Oncogene 2007;26:3769–76.
3. Fernandez-Majada V, Aguilera C, Villanueva A, et al. Nuclear IKK activity leads to dysregulated notch-dependent gene expression in colorectal cancer. Proc Natl Acad Sci U S A 2007;104:276–81.
4. Prajapati S, Tu Z, Yamamoto Y, Gaynor RB. IKKα regulates the mitotic phase of the cell cycle by modulating Aurora A phosphorylation. Cell Cycle 2006; 5:2371–80.
5. Hu MC, Lee DF, Xia W, et al. IκB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a. Cell 2004;117:225–37.
6. Lee DF, Kuo HP, Chen CT, et al. IKKβ suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. Cell 2007;130:440–55.
7. Tergaonkar V, Pando M, Vafa O, Wahl G, Verma I. p53 stabilization is decreased upon NFκB activation: a role for NFκB in acquisition of resistance to chemotherapy. Cancer Cell 2002;1:493–503.
8. Perkins ND. NF-κB: tumor promoter or suppressor? Trends Cell Biol 2004;14:64–9.
9. Furuya K, Ozaki T, Hanamoto T, et al. Stabilization of p73 by nuclear IKK-α mediates cisplatin-induced apoptosis. J Biol Chem 2007;282:18365–78.
10. Zhu F, Xia X, Liu B, et al. IKKα shields 14-3-3σ, a G(2)/M cell cycle checkpoint gene, from hypermethylation, preventing its silencing. Mol Cell 2007;27: 214–27.
11. Seitz CS, Lin Q, Deng H, Khavari PA. Alterations in NF-κB function in transgenic epithelial tissue demonstrate a growth inhibitory role for NF-κB. Proc Natl Acad Sci U S A 1998;95:2307–12.
12. Liu B, Park E, Zhu F, et al. A critical role for I κB kinase α in the development of human and mouse squamous cell carcinomas. Proc Natl Acad Sci U S A 2006;103: 17202–7.
13. Kisseleva T, Song L, Vorontchikhina M, Feirt N, Kitajewski J, Schindler C. NF-κB regulation of endothelial cell function during LPS-induced toxemia and cancer. J Clin Invest 2006;116:2955–63.
14. Chen F, Lu Y, Castranova V, Li Z, Karin M. Loss of Ikkβ promotes migration and proliferation of mouse embryo fibroblast cells. J Biol Chem 2006;281:37142–9.
15. Oshima K, Takeda M, Kuranaga E, et al. IKK epsilon regulates F actin assembly and interacts with *Drosophila* IAP1 in cellular morphogenesis. Curr Biol 2006;16:1531–7.
16. Gapuzan ME, Schmah O, Pollock AD, Hoffmann A, Gilmore TD. Immortalized fibroblasts from NF-κB RelA knockout mice show phenotypic heterogeneity and maintain increased sensitivity to tumor necrosis factor α after transformation by v-Ras. Oncogene 2005;24: 6574–83.
17. Maeda S, Kamata H, Luo JL, Leffert H, Karin M. IKKβ couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 2005;121:977–90.
18. Luedde T, Beraza N, Kotsikoris V, et al. Deletion of NEMO/IKKγ in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. Cancer Cell 2007;11:119–32.
19. Zhang Y, Chen F. Reactive oxygen species (ROS), troublemakers between nuclear factor-κB (NF-κB) and c-Jun NH(2)-terminal kinase (JNK). Cancer Res 2004;64: 1902–5.
20. Peng Z, Peng L, Fan Y, Zandi E, Shertzer HG, Xia Y. A critical role for IκB kinase β in metallothionein-1 expression and protection against arsenic toxicity. J Biol Chem 2007;282:21487–96.

Nuclear Factor-κB, an Unappreciated Tumor Suppressor

Fei Chen and Vince Castranova

Cancer Res 2007;67:11093-11098.