International Reviews of Immunology

ISSN: 0883-0185 (Print) 1563-5244 (Online) Journal homepage: http://www.tandfonline.com/loi/iiri20

Tumor Promoting or Tumor Suppressing of NF-κ B, a Matter of Cell Context Dependency

Fei Chen, Kevin Beezhold & Vince Castranova

To cite this article: Fei Chen, Kevin Beezhold & Vince Castranova (2008) Tumor Promoting or Tumor Suppressing of NF-κ B, a Matter of Cell Context Dependency, International Reviews of Immunology, 27:4, 183-204, DOI: 10.1080/08830180802130327

To link to this article: https://doi.org/10.1080/08830180802130327

International Reviews of Immunology, 27:183–204, 2008
ISSN: 0883-0185 print / 1563-5244 online
DOI: 10.1080/08830180802130327

informa
healthcare

Special Topic: NF-κB, Immunity and Cancer (Invited Review)

Tumor Promoting or Tumor Suppressing of NF-κB, a Matter of Cell Context Dependency

Fei Chen and Kevin Beezhold
Pathology and Physiology Research Branch, The Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA;
Graduate Program in Cancer Cell Biology, West Virginia University, Morgantown, West Virginia, USA

Vince Castranova
Pathology and Physiology Research Branch, The Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA; Department of Physiology and Pharmacology, School of Medicine, West Virginia University, Morgantown, West Virginia, USA

Nuclear factor-κB (NF-κB) and its activating signaling pathways are critical regulators for cell lineage development, growth, differentiation, apoptosis, and tumorigenic transformation. As one of the most important transcription factors, NF-κB has been implicated in the transcriptional upregulation of a number of cytokines, adhesion molecules, growth factors, oncogenes, antiapoptotic proteins, some proapoptotic factors, and even certain viral genes. The role of NF-κB on tumor promoting has been well-documented in the past two decades. However, during the past few years, a considerable number of studies suggested that NF-κB and its activating signaling molecules may act as tumor suppressors under some circumstances. Thus, it is highly possible that tumor promoting or tumor suppressing of NF-κB signaling is determined by the type of cells, stimuli, simultaneous or asynchronous intracellular signals, and other cellular contexts.

Keywords NF-κB, JNK, ROS, tumor suppressing, epigenetics, histone methylation

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

Address Correspondence to Fei Chen, PhD, Tel: 1-304-285-6021. E-mail: LFD3@cdc gov

INTRODUCTION

NF-κB was first discovered as a nuclear factor regulating the transcription of immunoglobulin (Ig) genes during the maturation of B-lymphocytes in 1986 by Sen and Baltimore [1]. By incubating nuclear extract from B cells with a panel of ^{32}P-labeled smaller DNA fragments derived from the Ig μH chain enhancer, the κL chain promoter, and the κL chain intronic enhancer, a new *trans*-acting complex was identified. The *cis*-acting motif recognized by this new *trans*-acting complex encompasses a nucleotide sequence, GGGGACTTTCC, that was found in the κL chain intronic enhancer region and SV40 gene. Because this complex was identified as a nuclear factor regulating expression of Ig κL chain in B cells, it was named as nuclear factor-κB (NF-κB). The abundance of NF-κB in B cells appeared to be associated with the developmental stages of the cell lineage. The mature B cells exhibited a relatively higher level of nuclear NF-κB complex, whereas this complex was hardly detected in pre-B cells. Within a very short period (3 or 4 months) since the first discovery of NF-κB, the original group who identified NF-κB and another group concurrently demonstrated that the activation and activity of NF-κB were inducible [2, 3]. NF-κB was induced by bacterial lipopolysaccharide (LPS) and phorbol ester in pre-B cells, T-cell line (Jurkat), and a nonlymphoid cell line (HeLa). Based on the fact that protein synthesis inhibitors failed to block the induction of NF-κB, it was hypothesized that posttranslational mechanisms might be involved in the activation of NF-κB.

Considering the importance of NF-κB in the innate and possibly adaptive immune responses, investigation of the activating mechanism(s) and function of NF-κB was intensified, which bacame one of the most rapidly growing research areas in the following years. Now it has been well-established that NF-κB can be activated by a wide spectrum of extracellular inducers from bacterial products to inflammatory mediators, environmental pollutants, occupational hazards, and chemical or physical stressors. The key members of NF-κB family include RelA (p65), RelB, c-Rel, p50, and p52. The p50 and p52 proteins are derived from their precursors, p105 (NFκB1) and p100 (NFκB2), respectively, largely through proteasome-dependent posttranslational proteolytic processing and/or co-translational biogenesis [4, 5]. All of these members share an N-terminal Rel homology domain (RHD) and an Ig-like/plexins/transcription factors (IPT) domain with a total size of about 300 amino acids. The RHD and IPT domains are important for dimerization, nuclear translocation, and DNA binding. In addition to the RHD and IPT domains, the p105 and p100 also contain 5 to 7 ankyrin repeat domains at their C-termini. The prototypical NF-κB

complex is the p50/RelA heterodimer. Other dimers, such as p50/p50, p52/p52, p50/c-Rel, p50/RelB, p52/RelA, p52/RelB, and c-Rel/c-Rel, have also been found in many different cell types under certain developmental or differentiation states with or without stimulation. The majority of NF-κB dimers are retained in the cytoplasm by binding with endogenous inhibitors, including IκBα, IκBβ, IκBε, IκBζ (MAIL, INAP), Bcl3, p100, and p105. In response to inflammatory signals, a canonical signaling pathway for NF-κB was activated, in which the IκB was phosphorylated by IκB kinase (IKK) complex containing two catalytic subunits, IKKα and IKKβ, and a scaffold subunit named IKKγ or NEMO. The IKKγ subunit facilitates the assembly of the IKK complex and relays upstream signals to IKKα/β, partially through the formation of K63-form polyubiquitin chains on upstream molecules and IKKγ itself [6]. The phosphorylated IκB proteins are recognized by ubiquitin ligases for further ubiquitination and proteasomal degradation. After degradation of inhibitors, NF-κB dimers are free to translocate to the nucleus. The noncanonical signaling pathway for NF-κB activation is largely mediated by the NF-κB–inducing kinase (NIK) and IKKα dimer in response to signals from CD40, LTβ receptor, and BAFF receptor. IKKα is able to phosphorylate p100, leading to partial degradation of p100 to generate the p52/RelB heterodimer. In the nucleus, NF-κB can bind to the enhancer or promoter region of a number of target genes containing κB-binding site(s) to upregulate their expression.

CONTRIBUTION OF NF-κB SIGNALING TO CARCINOGENESIS

During the past two decades, NF-κB was labeled as a critical factor in many carcinogen-induced human malignancies. The evidence indicating the tumor-promoting effect of NF-κB was largely based on observations showing an aberrant activation or activity of NF-κB in a number of human cancers and cancerous tissues in experimental animals [7, 8]. This notion was further supported by the fact that NF-κB is an essential transcription factor for the expression of genes critical for inflammation, cell survival, cell cycle, angiogenesis, and tumor cell invasion [9]. Inflammation, especially in the form of chronic inflammation, has been linked to several types of cancers including tumors in lung, prostate, breast, gastric system, liver, bladder, and several malignant lymphomatic diseases. Inflammation is a step-by-step pathophysiologic process that includes initial injury, repair, and resolution, which is also considered as an important innate immune response to the invasion of microorganisms. Recruitment of the inflammatory cells, such as neutrophils, monocytes, macrophages, eosinophils, dendritic cells,

mast cells, lymphocytes, and fibroblast cells, to the injury site of the tissue is in a manner of self-amplification, especially if the resolution process is impaired or fails. Persistent release of reactive oxygen species (ROS), cytokines, chemokines, angiogenic factors, prostaglandins, and matric metalloproteinases from these inflammatory cells under such circumstances is not only tissue damaging but also mutagenic for the genome. It is well-known that ROS are able to damage macromolecules including DNA, RNA, lipids, and proteins directly through oxidation, nitration, and halogenation [10]. Oxidation of DNA is highly mutagenic, which causes purine, pyramidine, or deoxyribose modification, single- or double-stranded DNA breaks, protein–DNA cross-links, and several forms of DNA intrastrand adducts. The cytokines, chemokines, angiogenic factors, metalloproteinases, and other inflammatory mediators, on the other hand, provide advantageous conditions for cell growth, migration, and implantation. NF-κB and chronic inflammation can form a deadly liaison, in which NF-κB is arguably the most important transcription factor governing the expression or biosynthesis of the major inflammatory factors, while many inflammatory factors, at the same time, are potent activators for NF-κB.

One of the key features of malignant transformation of the cells is the evasion of apoptosis, leading to a sustained clonal expansion of cancerous cells. Many cancer cells are capable of generating antiapoptotic proteins autonomously. Bcl2, Bcl-xl, cIAP1, cIAP2, XIAP, IEX1, Bfl1/A1, A20, c-FLIP, and TRAF-2 are potent antiapoptotic proteins that stabilize the mitochondrial membrane, inhibit proapoptotic caspases, and/or induce cell growth. A vast majority of studies have documented the role of NF-κB in the transcription of these antiapoptotic genes. In *in vitro* cellular models, the expression of these antiapoptotic proteins was up-regulated when NF-κB was activated, and vice versa, the expression of these antiapoptotic proteins was repressed with NF-κB inactivation. In the case of human and mouse bcl-xl gene, several NF-κB binding sites were identified in the promoter region of this gene [11–13]. Facilitated by the electrophoretic mobility shift assay (EMSA), our earlier study demonstrated that at least four κB sites were presented in the region of -1010 to -761 of the bcl-xl promoter. All of these κB sites could be recognized by NF-κB p50/p50 homodimer, and three out of these four κB sites could be bound by p50/p65, the most active NF-κB heterodimer. Giving the observations that Bcl-xl was predominately expressed in many types of malignant cells, it is plausible to speculate that the tumor-promoting effect of NF-κB very likely resulted from its regulation on bcl-xl and other antiapoptotic genes.

The strongest evidence linking NF-κB to tumor promotion might be from earlier studies suggesting that gain-of-function mutation of the NF-κB subunits, especially the c-Rel and p100, is transformative for cells [14, 15]. Gene amplification, deletion, and point mutation of c-rel had been noted in human B-cell and T-cell malignancies. Its retroviral counterpart, v-rel produced by avian Rev-T retrovirus, is a well-established avian oncogene that is able to cause lymphoid cell lymphomas and leukemias in infected chickens [16]. In some cases of Hodgkin's and non–Hodgkin's lymphomas, 4 to 75 copies of c-rel locus have been detected. Such gene amplification is obviously responsible for the accumulation of c-rel mRNA and protein. Abnormality in c-rel gene rearrangement, either due to a large deletion on chromosome 2 or the gene translocation to a position near the Ig light chain enhancer, generates c-Rel chimeric proteins that are constitutively nuclear localized. Chromosomal deletion or translocation of p100 gene locus has been intensively investigated in cutaneous B- or T-cell lymphomas, non–Hodgkin's lymphomas, chronic B-cell lymphocytic leukemia, and multiple myelomas. A c-terminal truncated p100 protein with a partial or complete loss of the ankyrin repeats was detected in these malignant cells. The c-terminal truncated p100 is often overexpressed and oncogenic in mouse fibroblasts [17]. In addition to the gain-of-function mutations of the NF-κB family members, loss-of-function mutations of the inhibitory molecules, such as IκBα and CYLD, have also been studied in both malignant cell lines and human lymphomatic diseases. A constitutively active p50/RelA or p50/c-Rel complex was observed in some primary tumor samples from Hodgkin's and Reed–Sternberg lymphomas due to deletion or inactivation of the IκBα gene or the second IκBα gene allele [16, 18]. Genetic mutation of CYLD, a deubiquitinase that specifically removes K63-type polyubiquitin chains conjugated on the upstream signaling molecules for IKK activation, can causes cylindromas, a rare and recessive genetic disorder featured with benign tumors [19]. A study by Bignell et al. [20] demonstrated single nucleotide deletion, insertion, and substitution in the multiple sites of the germ-line CYLD gene. These mutations cause translational frameshifts, premature translational termination, and alternative splicing of the CYLD mRNA. The mutated CYLD gene products are enzymatically inactive and, therefore, tumorigenic due to constitutive activation of NF-κB. Most recently, two teams concurrently revealed genetic alterations of several additional NF-κB signaling molecules, such as TRAF2, TRAF3, cIAP1, CD40, p105 (nfkb1), LTβR, and NIK, in human primary multiple myeloma and other cell lines [21, 22]. It was proposed that the malignant transformation of the plasma cells is a result of promiscuous

mutations of the proteins contributing to both canonical and noncanonical NF-κB activation pathways, leading to a sustained NF-κB activation. This notion was supported by the apoptotic responses of the multiple myeloma cells treated with a small-molecule inhibitor of IKKβ that showed substantial inhibition of NF-κB activation or activity. What is the most interesting in these latest studies is the discovery of outlier expression of NIK as a result of chromosomal translocations between the NIK locus and the IgH locus or the IgL locus. These chromosomal translocations brought the NIK promoter in proximity to the Cλ enhancer, leading to a remarkably increased expression of the NIK gene. Furthermore, another form of chromosomal translocation of the NIK locus was identified in a multiple myeloma cell line (JJN3), which generates an in-frame EFTUD2-NIK fusion protein [21]. Although NIK was viewed as an essential upstream kinase for the alternative activation of NF-κB, it can also activate the canonical pathway of NF-κB when it is overexpressed [23].

TUMOR SUPPRESSION BY NF-κB AND ITS SIGNALING MOLECULES

There is a large volume of original studies and reviews addressing the tumor-promoting effects of NF-κB and/or its related signaling molecules [24–28]. However, challenges to the tumor promoting of NF-κB have been made recently by a considerable numbers of reports [29, 30]. In fact, the involvement of NF-κB in tumor suppression, rather than tumor promotion, was proposed more than one decade ago based on the NF-κB–dependent expression of several proapoptotic genes, including Fas, FasL, Bax, c-myc, DR4, DR5, TRAIL, and Bcl-xs. In a limited number of cell types, NF-κB activation appears to be able to induce cell apoptosis, such as in T cells, HeLa cells, and bone marrow cells. In mature T cells, NF-κB had been shown to be capable of inducing expression of FasL to eliminate those cells with potential genomic mutation due to exposure to DNA damaging signals [31]. In addition, the proapoptotic activity of NF-κB has also been implicated in $CD4^+CD8^+$ double-positive thymocytes [32]. Accordingly, it was believed that NF-κB–mediated apoptosis was an important step for the proper development of T-cell lineages through both positive and negative selection in the thymus. A forced over activation of NF-κB by transfection of c-Rel in HeLa cells caused the onset of apoptosis associated with an inhibition of the E2F DNA binding activity, CDK2 kinase activation, and Rb phosphorylation [33]. The majority of the cells with c-Rel overexpression exhibited cell cycle

arrest at the G1/S phase, which was also correlated with an increased level of $p21^{cip1/waf1}$ (p21) protein, an endogenous inhibitor for CDK2 kinase. Because p21 is a classic target gene of the p53, it is very likely that such an increase in p21 level is a result of p53 activation in these c-Rel overexpression cells. Indeed, an enhanced stability of the p53 protein was noted in the cells where NF-κB was overactivated in some other studies [29, 33]. Because apoptosis is envisioned as one of the options to limit the growth of the tumors, it should not be surprising to link NF-κB with cancer suppression.

A few animal studies of NF-κB function in tumor suppression are worth mentioning. The first evidence of NF-κB in tumor suppression was provided by transgenic expression of IκBα, an inhibitor of NF-κB, in human and murine epidermis [34]. In the basal epithelial layer of normal epidermis, NF-κB appeared to be inactive as evidenced by the exclusively cytoplasmic location. In contrast, NF-κB was nuclear translocated in those nonproliferative and terminally differentiated cells in the suprabasal layer. Transgenic expression of a degradation-resistant form of IκBα to block the activation of NF-κB in the suprabasal layer cells fostered development of epidermal hyperplasia featured by the thickness and invaginations of the suprabasal squamous layer. The hyperplasia, which resembled human squamous cell carcinomas, was reversed by the transgenic expression of the active NF-κB subunits, p50 and RelA. A similar observation was made in the transgenic expression of IKKα, an upstream kinase contributing to the noncanonical NF-κB activation, in mouse skin cells [35]. A significantly delayed onset and metastasis of carcinogen-induced skin carcinomas was observed in suprabasal and basal epidermis where IKKα was overexpressed.

Recent evidence also indicates a role of NF-κB in tumor suppression in chemical carcinogen–induced hepatocellular carcinoma (HCC) in mouse models. A hepatocyte-specific knockout of the Ikkβ gene enhanced the formation of HCC induced by diethylnitrosamine, which correlated with an increased ROS generation and JNK activation [36]. The development of HCC is much faster and more severe when the Ikkγ gene was disrupted specifically in hepatocytes in mice [37]. Both IKKβ and IKKγ are the key components of the IKK complex responsible for the canonical activation of NF-κB. Deficiency in either IKKβ or IKKγ blocks the basal and signal-induced activation of NF-κB. Clearly, a signaling pathway that maintains normal activation of NF-κB is required for the protection of the liver from spontaneous or carcinogen-induced tumorigenesis, at least in experimental animals.

MOLECULAR MECHANISMS OF NF-κB–MEDIATED TUMOR SUPPRESSION

Unlike its role in tumor promotion, the mechanism of tumor suppression of NF-κB is far from clear. In both studies of skin and liver cancer, a compensatory secondary response, proliferation of bystander cells in response to apoptosis of the cells with a permanent inhibition of NF-κB, was hypothesized [29, 36]. If this hypothesis is truly authentic, the antiapoptotic activity of NF-κB appears to be no longer tumor promoting as widely assumed, but rather tumor suppressing. New questions then arise: How does apoptosis trigger overproliferation of the bystander nonapoptotic cells? Does apoptosis potentiate tumor burden through inducing bystander cell proliferation in a given tumor undergoing chemotherapy? Why would some routine apoptotic processes, such as lineage-specific development of the cells and tissue/organ remodeling, not trigger tumorigenesis through compensatory bystander cell proliferation? The theory of compensatory secondary response is exclusively focused on the role of NF-κB in antiapoptotic responses. Although this hypothesis made a reasonable reconciliation with the widely accepted dogma of NF-κB in apoptosis, other factors beyond apoptosis appear to be overlooked. Obviously finding these factors of NF-κB–mediated tumor suppression will aid in a better understanding of the molecular mechanisms of carcinogenesis. We believe that the following findings emphasizing the tumor suppressing activity of NF-κB warrant further discussion.

NF-κB Signaling Inhibits JNK

The cross-talk between NF-κB and JNK was extensively studied in the 1990s [38]. Such cross-talk occurs at almost every level of the signaling pathways leading to the activation of NF-κB and JNK. There are many upstream signaling molecules, such as interleukin 1 receptor–associated kinases, TNFα receptor–associated proteins, mitogen-activated protein/extracellular signal–regulated kinase kinase kinase, protein kinase Cζ, transforming growth factor-β, and scaffold protein Act1, that are shared by both NF-κB and JNK activation pathways. The downstream products of NF-κB and JNK also appear to be mutually affected, for example, some NF-κB–induced cytokines and proapoptotic proteins are activators of JNK, whereas JNK-regulated β-TrCP is essential for NF-κB activation. Furthermore, there are a considerable number of genes whose expression depends on both NF-κB and JNK.

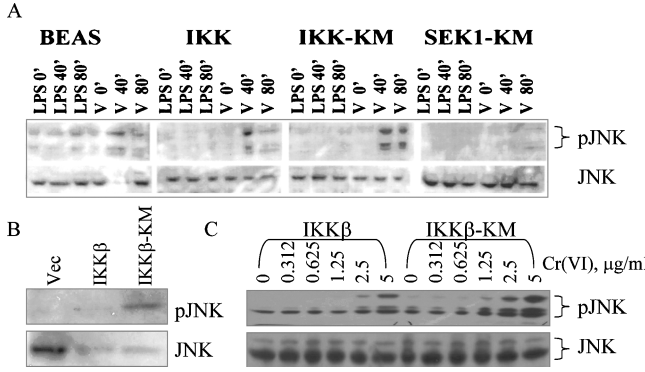

FIGURE 1 Historical experiment data showing enhanced JNK activation in NF-κB–inhibited cells. (A) Enhanced JNK activation in human bronchial epithelial cells (BEAS-2B) transfected with a kinase-mutated IKKβ (IKKβ-KM) and treated with LPS or vanadate (V) for the indicated times. The JNK activation was prevented by expression of a kinase-mutated JNK kinase, SEK1-KM. (B) Basal JNK activation was elevated in BEAS-2B cells transfected with IKKβ-KM transiently. (C) An enhanced JNK induction by chromium (VI)[Cr(VI)] was observed in the IKKβ-KM–transfected cells.

In the late 1990s, we were attempting to determine whether some occupational toxic metals induced human diseases through NF-κB signaling. In several transfection experiments using human wild-type (WT) or kinase-mutated IKKβ (IKKβ-KM) that inhibits NF-κB activation, we frequently noted an enhancement of JNK activation by chromium (VI) or vanadate (V) in the NF-κB–inhibited cells (Fig. 1A, C). In one experiment, we even noted that transient transfection of IKKβ-KM itself induced an appreciable JNK activation compared with control cells or cells transfected with WT-IKKβ (Fig. 1B). These preliminary results were totally unexpected for us as we originally assumed that impairment of NF-κB signaling might weaken the JNK activation based on the fact that both JNK and NF-κB share some common upstream regulators. Disbelief was expressed when we discussed our preliminary findings with some prominent scientists in the fields of NF-κB and JNK. After an additional 1 or 2 years of painstaking investigation, these observations were partially reported [39]. In that report, we used human bronchial epithelial cell line (BEAS-2B) as a cellular model to monitor the mutual regulation of NF-κB and JNK. The IKK kinase activity and the consequent NF-κB activation were abrogated when the cells were transfected with IKKβ-KM. The activation of MAP kinases was monitored by the treatment of these transfected cells with

arsenic, a potent activator of Erk, JNK, and p38. In agreement with our preliminary observation, we noted that inhibition of IKK and NF-κB remarkably enhanced arsenic-induced activation of Erk and JNK in a dose-dependent manner. The activation of p38 appeared to be the same between the cells expressing wild-type IKKβ and the cells expressing IKKβ-KM, indicating that the NF-κB signaling only affects activation of Erk and JNK, but not p38. Intriguingly, a further time-course study suggested that JNK activation was undetected after 8 to 24 h of arsenic treatment in the cells expressing wild-type IKKβ. In contrast, in addition to the enhanced JNK activation at 1 and 4 h of arsenic treatment, the JNK activation was still detectable after 8 and 24 h of arsenic treatment in the cells transfected with IKKβ-KM. Thus, these data, along with our preliminary results depicted in Figure 1, clearly indicate that NF-κB inhibition not only potentiates but also prolongs JNK activation. In other words, NF-κB and its activating signaling are inhibitory for JNK activation. In retrospect, our paper may have provided the first evidence showing that NF-κB serves as a negative regulator for JNK activation.

It was interesting to note that our findings of JNK inhibition by NF-κB was independently confirmed several months later by two additional groups who used mouse embryo fibroblast cells derived from wild-type and relA and/or Ikkβ gene knockout mice [40, 41]. Both groups confirmed that inhibition of NF-κB by relA or Ikkβ gene deficiency enhanced TNFα-induced JNK activation. Studies by De Smaele et al. [40] indicated that NF-κB–dependent expression of GADD45β was capable of inhibiting the activation of JNK. An appreciable decrease in GADD45β expression was noted in the relA$^{-/-}$ mouse embryo fibroblasts (MEFs) in which JNK activation was greatly enhanced by TNFα. Based on the observation that transfection of gadd45β gene reduced JNK activation in a T-cell line, it was assumed that JNK inhibition by NF-κB is through NF-κB–dependent expression of GADD45β. At the same time, Tang et al. [41] observed a marginal decrease in the expression of XIAP, another NF-κB–regulated gene, in relA$^{-/-}$ MEFs where JNK activation was enhanced. Transient transfection of the relA$^{-/-}$ cells with an expression vector harboring the xiap gene partially reduced JNK activation at the 60-min, but not the 15-min, time point of TNFα treatment, implying that XIAP might be involved in mediating the JNK inhibition by NF-κB.

A number of studies had demonstrated that a sustained activation of JNK gives rise to a growth advantage of the cells [42]. In mammalian cells, at least 10 isoforms of JNK have been identified. These isoforms are derived from the alternative splicing of JNK mRNAs of the jnk1,

jnk2, and jnk3 genes. It is noteworthy that JNK2 may play the most important role in cell growth regulation. In a mouse skin cancer model, genetic disruption of the jnk2 gene reduced tumor incidence and delayed tumor growth in response to DMBA along with TPA treatment [43]. Ablation of JNK2 by antisense oligonucleotides blocked EGF-stimulated proliferation and anchorage-independent growth of the lung cancer cells [44]. Accordingly, the antagonism of NF-κB on JNK may be an important measure to keep the activation of JNK under check. Loss of this antagonistic mechanism, therefore, will cause a sustained JNK activation and, consequently, the malignant transformation of the cells.

NF-κB is An Inhibitor of ROS Generation

Reactive oxygen species (ROS), which include superoxide, hydroxyl free radicals, and hydrogen peroxide, are highly reactive due to the presence of unpaired valence shell electrons. The main sources of ROS are mitochondria, cytochrome p450, peroxomes, and NADPH oxidases. Endogenous ROS are constitutively produced even under normal physiologic conditions. However, these ROS are either removed or neutralized by cellular antioxidant systems, mainly the superoxide dismutases (SOD), catalases, glutathione peroxidases, and some small antioxidant molecules, before they cause any damage to other macromolecules. Under many pathologic circumstances, the balance between ROS generation and antioxidant defense systems is changed, leading to oxidative stress responses that are usually associated with oxidative damage to DNA, proteins, and lipids.

During the period of 1998 and 2000, when we were demonstrating that JNK activation was promoted by NF-κB inhibition, we also noted a substantial increase in ROS generation in the cells transfected with IKKβ-KM or the cells derived from IKKβ gene knockout mice. At that time, the assumption that ROS activate NF-κB was dominant among researchers working in the areas of oxidative stress and signal transduction [45]. Although studies showed involvement of NF-κB in the regulation of superoxide dismutases (SODs), this effect of NF-κB was viewed as pro-oxidative because of the formation of H_2O_2 by the SODs [46]. Thus, we were very cautious to reach a conclusion and actively communicating such issue with a well-established scientist who has been working on IKK and JNK for some time. Despite two Nature papers [40, 41] in late 2001 claiming that NF-κB inhibits JNK through GADD45β and XIAP, respectively, we still strongly believed that the major factors connecting NF-κB and JNK were the ROS. In our own hands, we failed to see a difference in the expression of XIAP

and GADD45β between wild-type and Ikk$\beta^{-/-}$ MEFs. Accordingly, we hypothesized that the prolonged JNK activation in the cells with deficiency in NF-κB signaling was caused by excessive ROS generation. Our hypothesis was later supported by Sakon et al. [47] who reported that ROS are accountable for the enhanced JNK activation by TNFα in relA$^{-/-}$ cells. It was very interesting that both Sakon's [47] and our paper [48] reached precisely the same conclusion, that is, inhibition of NF-κB by disrupting either relA or Ikkβ gene fosters a prolonged JNK activation due to increased generation of ROS. To determine why ROS were overproduced in the cells with a deficiency in NF-κB signaling, we examined the expression of some redox-regulating molecules in both wild-type and Ikk$\beta^{-/-}$ MEFs. The gene for p450 CYP1B1 was highly expressed, whereas the genes encoding both metallothionein I (MT1) and metallothionein II (MTII) were significantly decreased in the Ikk$\beta^{-/-}$ cells relative to that in wild-type cells. It has been known for decades that p450 members catalyze a large spectrum of oxidation of endobiotics and xenobiotic chemicals, leading to ROS generation [49]. The MT1 and MT2 are cysteine-rich metal binding proteins capable of detoxifying metal ions and scavenging ROS. Our finding of reduced MT1 and MT2 expression in Ikk$\beta^{-/-}$ cells was recently reproduced and extended by Xia's team, which demonstrated that ROS generation and the enhanced JNK activation could be reversed by ectopic expression of MT1 in Ikk$\beta^{-/-}$ cells [50].

The elevation in ROS generation in the cells with genetic deficiency in NF-κB signaling suggests that NF-κB is important in maintaining a normal redox status to prevent oxidative stress, at least in normal cells. Without such a protective mechanism, genomic DNA will be in danger of mutation due to excessive ROS generation. At the same time, the DNA repairing machinery and checkpoint proteins might be inactivated by ROS-mediated oxidation, leading to further accumulation of genomic damage and evasion of the cells from apoptosis. Consequently, the cells acquire features of malignant transformation and tumorigenesis.

NF-κB Signaling Protects Cells from Epigenetic Abnormality

Epigenetics is a term that refers to some genetic events that cannot be explained by the established genetic principles. The word *epigenetics* was first created by Conrad Hal Waddington, an encyclopedic scientist in development biology, paleontology, genetics, embryology, philosophy, and, by today's definition, system biology at the University of Edinburgh from the 1940s to 1970s [51]. Because of the polymathic

nature of his research interests in diverging areas, his name may not be recognized by many life scientists who are working in a narrow discipline today. The theory of epigenetics is perhaps the most memorable contribution of his to the modern biology or life sciences. The term *epigenetics* first appeared in his book entitled *Introduction to Modern Genetics* in 1939 [52]. In his original version, epigenetics is a process linking genetics to phenotype in a random manner to determine the fate of a given cell.

Today, epigenetics generally describes heritable phenomena occurring in the genome without changes in DNA sequence, which includes the accessibility of the genome, chromosome structure, genomic stability, position effect variegation (PEV), and gene imprinting. Studies of epigenetics were largely centered on DNA methylation during the past three or four decades [53]. Until recently, extensive studies have been made on the modification of histone proteins, such as methylation, acetylation, and ubiquitination, among which methylation of lysine residues may be arguably the most critical in affecting the epigenetic landscape and genome function. The states of histone methylation, especially on the N-terminal tails of histones H3 and H4, determines the accessibility of genes in the condensed packed chromatin environment. The lysine residues in histones H3 and H4 can be modified by mono-, di-, and trimethylation. It has been generally viewed that trimethylation of lysines 4, 36, and 79 of histone H3 is associated with an active transcription of the genes in the decondensed chromatin (euchromatin) region. In contrast, trimethylation of lysines 9 (H3K9me3) and 27 (H3K27me3) is refractory for gene expression due to the formation of the highly condensed heterochromatin architecture [54]. A number of histone methytransferases have been identified, among which human EZH2 and SUV39hl are the best characterized methytransferases responsible for the trimethylation of lysines 27 and 9, respectively, on histone H3 [55]. Histone methylation was traditionally regarded as a permanent mark that was irreversible. Since the first discovery of histone lysine specific demethylases-1 (LSD1) in 2004 [56], such a view has changed recently. Since 2006, several groups independently identified a JmjC family of histone demethylases that demethylate di- or trimethylated lysines 9 and 27 on histone H3 [57–60]. In addition to regulating transcriptional accessibility of the genome, histone methylation may also affect DNA replication, recombination, and damage repair [61]. The addition of a methyl group to lysine is unable to change the charges that affect the chromatin structure due to the nature of the low molecular weight of the methyl group. It is very likely that methylation of the side chains of lysine residues creates a binding code for some regulatory proteins.

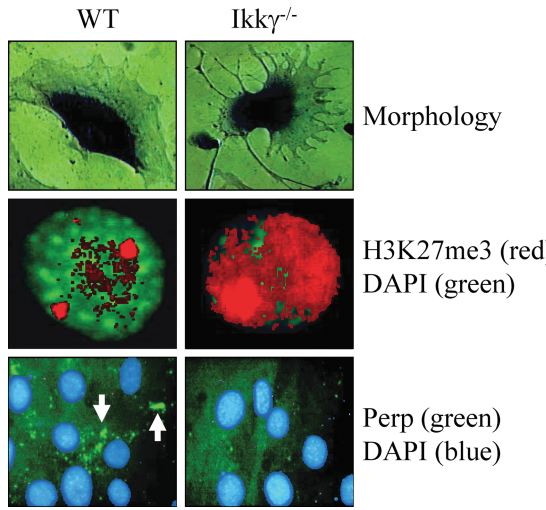

FIGURE 2 Differences in cell morphology, level of H3K27me3, and Perp expression (pointed by white block arrows) in wild-type (WT) and Ikk$\gamma^{-/-}$ cells treated with 10 μM arsenic (As^{3+}) for 12 h.

The involvement of NF-κB signaling in epigenetic regulation was recently revealed in mouse bone marrow–derived macrophages based on the demonstration of NF-κB–dependent induction of Jmjd3, a demethylase for the trimethyl histone H3 lysine 27 (H3K27me3) [62]. The demethylase activity of Jmjd3 is indispensable for the removal of parental imprints and nuclear reprogramming of the embryonic cells. In somatic cells, H3K27me3 along with H3K9me3 facilitates the formation of repressive chromatin structure to form constitutive, facultative, and focal heterochromatins. The expression of some tumor suppressor genes is silenced due to the abundant occurrence of H3K27me3 on the nucleosomes surrounding the transcription start sites. Accordingly, histone demethylase, such as Jmjd3, is required for erasing the repressive histone markers and initiating the expression of these tumor suppressors. Six conserved NF-κB binding sites, five in the first intron and one in 3-UTR, were found in both the mouse and human Jmjd3 gene. The functional authenticity of some of these NF-κB binding sites was validated by chromatin immunoprecipitation (ChIP) using an antibody against RelA. Blockage of NF-κB activation by expression of a degradation-resistant IκBα or knock-in deletion of the Ikkγ gene strongly impaired Jmjd3 induction by inflammatory stimulation.

In our ongoing studies, we also noted an association between IKKγ deficiency and the alteration of H3K27me3 in mouse embryonic

fibroblast (MEF) cells. The level of H3K27me3 was downregulated by arsenic, a potent stress inducer, in wild-type MEFs. In contrast, H3K27me3 was not decreased, but appreciably increased in the Ikk$\gamma^{-/-}$MEFs treated with arsenic (Fig. 2). To investigate whether such a change in H3K27me3 correlates with Jmjd3, the protein level of Jmjd3 in both wild-type and Ikk$\gamma^{-/-}$cells was compared. It is interesting to note that arsenic induces accumulation of Jmjd3 protein in wild-type MEFs in a dose-dependent manner, whereas it causes a dose-dependent reduction of Jmjd3 in Ikk$\gamma^{-/-}$cells.

We had previously demonstrated that MEFs derived from Ikk$\beta^{-/-}$ mice exhibited an altered cell morphology, along with enhanced stress fiber formation, cell motility, and proliferation [63]. These characteristics of Ikk$\beta^{-/-}$ cells are suggestive for potential malignant transformation of the cells. Recent studies by Chanda's group not only confirmed but further extended our conclusion indicating that deficiency in NF-κB signaling, such as gene disruption of Ikkβ, is tumorigenic [64]. Earlier studies by others have reported a protein-protein interaction of IKKα and Aurora A kinase, a critical serine/threonine kinase regulating the location, maturation, separation, and function of centrosomes in HeLa and COS7 cells [65]. The studies by Chanda and colleagues [64] also revealed an association of IKKβ with Aurora A kinases in mitotic HeLa cells. An abnormality in centrosome distribution and spindle morphology was observed in Ikk$\beta^{-/-}$ cells. The wild-type MEFs were not tumorigenic in inoculated nude mice. In contrast, tumor formation was observed after inoculation with Ikk$\beta^{-/-}$cells. In our current studies, we found that the Ikk$\gamma^{-/-}$MEFs resemble those of Ikk$\beta^{-/-}$MEFs in morphology (Fig. 2). Considering the observed alterations in H3K27me3 and Jmjd3, we believe that the changes in cell morphology, mobility, and tumorigenecity might be directly linked to the genes regulated by H3K27me3. Through gene profiling and ChIP, we were able to show that the expression of Perp, a possible tumor suppressor involved in p53-induced cell apoptosis [66], was silenced in the Ikk$\gamma^{-/-}$ cells (Fig. 2). Analyses of the histone methylation profiles demonstrated that H3K27me3 is abundant in the region encompassing the proximal promoter and the transcriptional start site of the perp gene. The Perp protein had also been implicated in the assembly of the cytoskeleton and desmosome complex. Thus, we assume that the morphologic changes of Ikk$\gamma^{-/-}$cells may result from repression of Perp expression, which possibly is linked to the enhanced cell motility and tumorigenic transformation.

Collectively, we believe that one aspect of the tumor-suppressing effects of NF-κB is achieved through epigenetic regulation that affects

the lysine methylation states of histone proteins. In wild-type cells, activation of NF-κB contributes to the expression of Jmjd3, a demethylase that is able to erase the repressive histone marker, H3K27me3. In response to stress or DNA-damaging signals, genomic reprogramming occurs in the tumor suppressor gene loci, such as Perp, by Jmjd3-mediated removal of H3K27me3. Consequently, transcription of these tumor suppressors is initiated. The transformation of the cells is effectively prevented or delayed by the elevated tumor suppressors. In the cells where NF-κB signaling is deficient, such as those of Ikk$\beta^{-/-}$cells, Ikk$\gamma^{-/-}$ cells, and relA$^{-/-}$cells, the expression of Jmjd3 is impaired, leading to a sustained occupancy of H3K27me3 in the gene loci of tumor suppressors. Because of the silencing of tumor suppressor expression, these cells are prone to be tumorigenetic.

TUMOR PROMOTION OR TUMOR SUPPRESSION BY NF-κB MAY BE CELL CONTEXT DEPENDENT

NF-κB or its activation signaling in tumor initiation and promotion has been documented for almost two decades [24]. The biochemical and pathophysiologic roles of NF-κB in carcinogenesis are confounded by reports of tumor suppression activity of this ubiquitous transcription factor. In addition to a large volume of circumstantial evidence demonstrating constitutively active NF-κB in many types of cancer, the underlying mechanism of the tumor-promoting effect of NF-κB has been intensively explored. In contrast, how NF-κB or its activation signaling contributes to tumor suppression remains a terra incognita. Recent evidence showing tumorigenesis or malignant transformation of the tissues or cells with genetic deficiency in Ikkα, Ikkβ, Ikkγ, and relA clearly suggests that tumor suppression by NF-κB is not a fabled phenomenon. It may be difficult to reconcile these two obvious opposite roles of NF-κB (i.e., tumor promotion and tumor suppression) at present. However, the differences in the experimental systems applied by researchers may provide valuable insights into whether NF-κB is tumor promoting or tumor suppressing. The involvement of NF-κB in tumor promotion was largely demonstrated by overexpression of NF-κB subunits, observation of NF-κB activity in tumor samples, and experiments in immortalized cell lines with transformative or tumorigenic potential. On the other hand, tumor suppression by NF-κB was mainly inferred from genetic deficiency of the functional subunits of NF-κB or IKK complexes in normal tissues or cells. Thus, it may be plausible to hypothesize that a normal level of NF-κB in developmentally normal tissues or cells is tumor suppressive (Fig. 3). This normal level of NF-κB

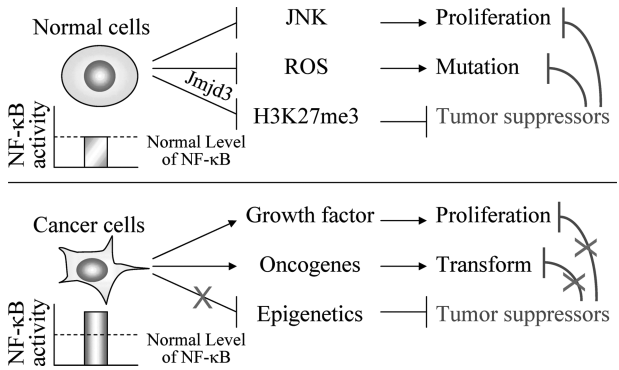

FIGURE 3 The possible mechanisms involved in tumor suppression and tumor promotion by NF-κB in a system with different cell context (see text for details).

is capable of antagonizing aberrant JNK activation, ROS generation, and epigenetic alterations. Furthermore, this normal level of NF-κB in the normal tissues or cells is sufficient to maintain expression of Jmjd3 to prevent overaccumulation of H3K27me3 in the gene loci of tumor suppressors, leading to de-repression of the tumor suppressors. An occasional over activation of NF-κB in normal cells might be non-tumorigenic because of the intact signaling pathways leading to tumor suppressor production, antioxidant defense, and JNK inhibition. One of the key characteristics of the transformed or tumor cell is the multiple mutations accumulated in the genome and aberrant alteration of the epigenic landscape of the chromosomes. The NF-κB is usually overactivated due to sustained activation of some upstream kinases in tumor cells. The activation of NF-κB may be tumor promoting rather than tumor suppressing in these transformed or tumor cells, because of the loss of tumor suppressor expression, constitutive synthesis of cyclins and other cell cycle regulating proteins, and a prolonged production of growth factors for cell proliferation. An additional point in this regard is that tumor suppression or tumor promotion is determined by the extent or duration of NF-κB activation in any given cells. It is conceivable that under a certain cellular context, the level of NF-κB may be lowered below the threshold necessary for malignant transformation but remains high enough to execute its normal function. These normal functions include but are not limited to tumor-suppressor production, antioxidant defense, and JNK inhibition to prevent initiation of tumorigenic transformation.

CONCLUSION

The cell-context dependency of NF-κB in tumor promotion or tumor suppression will certainly shed light on the design of NF-κB–based therapy in either inflammatory diseases or cancer. It appears to be desirable to develop reagents that remove excessively activated NF-κB but maintain a normal level of NF-κB. At experimental levels, more than 700 inhibitors for NF-κB have been identified to date, which target the upstream kinases, proteasomal degradation, nuclear translocation, DNA binding, and some cofactors required for the functioning of NF-κB [67]. Intriguingly, a considerable number of existing drugs clinically used for human diseases have been implicated in the inhibition of NF-κB, such as aspirin and other nonsteroidal anti-inflammatory drugs. The major limitation of these currently available NF-κB inhibitors is that almost all of these inhibitors are nonspecific with effects beyond NF-κB signaling. It is a real challenge to identify an inhibitor targeting a specific module of NF-κB signaling because of the degree of complexity. For example, a single molecule is shared by two or more overlapping, parallel, or cross-talk signaling pathways. However, one may expect the development of a regime in which several inhibitors targeting different signaling points of NF-κB are combined and applied in a localized manner, such as airway instillation or topical administration, to avoid systematic and nonspecific effects.

REFERENCES

[1] R. Sen and D. Baltimore, Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* **46**: 705–716, 1986.
[2] M.L. Atchison and R.P. Perry, The role of the kappa enhancer and its binding factor nf-kappa b in the developmental regulation of kappa gene transcription. *Cell* **48**: 121–128, 1987.
[3] R. Sen and D. Baltimore, Inducibility of kappa immunoglobulin enhancer-binding protein nf-kappa b by a posttranslational mechanism. *Cell* **47**: 921–928, 1986.
[4] L. Lin and S. Ghosh, A glycine-rich region in nf-kappab p105 functions as a processing signal for the generation of the p50 subunit. *Mol Cell Biol* **16**: 2248–2254, 1996.
[5] L. Lin, G.N. DeMartino, and W.C. Greene, Cotranslational biogenesis of nf-kappab p50 by the 26s proteasome. *Cell* **92**: 819–828, 1998.
[6] F. Chen, D. Bhatia, Q. Chang, and V. Castranova, Finding nemo by k63-linked polyubiquitin chain. *Cell Death Differ* **13**: 1835–1838, 2006.
[7] F. Chen, V. Castranova, and X. Shi, New insights into the role of nuclear factor-kappab in cell growth regulation. *Am J Pathol* **159**: 387–397, 2001.
[8] F. Chen, V. Castranova, X. Shi, and L.M. Demers, New insights into the role of nuclear factor-kappab, a ubiquitous transcription factor in the initiation of diseases. *Clin Chem* **45**: 7–17, 1999.

[9] W.W. Lin and M. Karin, A cytokine-mediated link between innate immunity, inflammation, and cancer. *J Clin Invest* **117**: 1175–1183, 2007.
[10] S.P. Hussain, L.J. Hofseth, and C.C. Harris, Radical causes of cancer. *Nat Rev Cancer* **3**: 276–285, 2003.
[11] F. Chen, L.M. Demers, V. Vallyathan, Y. Lu, V. Castranova, and X. Shi, Involvement of 5'-flanking kappab-like sites within bcl-x gene in silica-induced bcl-x expression. *J Biol Chem* **274**: 35591–35595, 1999.
[12] E.P. Dixon, D.T. Stephenson, J.A. Clemens, and S.P. Little, Bcl-xshort is elevated following severe global ischemia in rat brains. *Brain Res* **776**: 222–229, 1997.
[13] H.H. Lee, H. Dadgostar, Q. Cheng, J. Shu, and G. Cheng, Nf-kappab-mediated up-regulation of bcl-x and bfl-1/a1 is required for cd40 survival signaling in b lymphocytes. *Proc Natl Acad Sci USA* **96**: 9136–9141, 1999.
[14] J. Houldsworth, S. Mathew, P.H. Rao, K. Dyomina, D.C. Louie, N. Parsa, K. Offit, and R. S. Chaganti, Rel proto-oncogene is frequently amplified in extranodal diffuse large cell lymphoma. *Blood* **87**: 25–29, 1996.
[15] S. Liptay, T. Seriu, C.R. Bartram, and R.M. Schmid, Germline configuration of nfkb2, c-rel and bcl3 in childhood acute lymphoblastic leukemia (all). *Leukemia* **11**: 1364–1366, 1997.
[16] G. Courtois and T.D. Gilmore, Mutations in the nf-kappab signaling pathway: Implications for human disease. *Oncogene* **25**: 6831–6843, 2006.
[17] P. Ciana, A. Neri, C. Cappellini, F. Cavallo, M. Pomati, C.C. Chang, A.T. Maiolo, and L. Lombardi, Constitutive expression of lymphoma-associated nfkb-2/lyt-10 proteins is tumorigenic in murine fibroblasts. *Oncogene* **14**: 1805–1810, 1997.
[18] K.M. Wood, M. Roff, and R.T. Hay, Defective ikappabalpha in hodgkin cell lines with constitutively active nf-kappab. *Oncogene* **16**: 2131–2139, 1998.
[19] T.R. Brummelkamp, S.M. Nijman, A.M. Dirac, and R. Bernards, Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating nf-kappab. *Nature* **424**: 797–801, 2003.
[20] G.R. Bignell, W. Warren, S. Seal, M. Takahashi, E. Rapley, R. Barfoot, H. Green, C. Brown, P.J. Biggs, S.R. Lakhani, C. Jones, J. Hansen, E. Blair, B. Hofmann, R. Siebert, G. Turner, D.G. Evans, C. Schrander-Stumpel, F.A. Beemer, A. van Den Ouweland, D. Halley, B. Delpech, M.G. Cleveland, I. Leigh, J. Leisti, and S. Rasmussen, Identification of the familial cylindromatosis tumour-suppressor gene. *Nat Genet* **25**: 160–165, 2000.
[21] C.M. Annunziata, R.E. Davis, Y. Demchenko, W. Bellamy, A. Gabrea, F. Zhan, G. Lenz, I. Hanamura, G. Wright, W. Xiao, S. Dave, E.M. Hurt, B. Tan, H. Zhao, O. Stephens, M. Santra, D.R. Williams, L. Dang, B. Barlogie, J.D. Shaughnessy, Jr., W.M. Kuehl, L.M. Staudt, Frequent engagement of the classical and alternative nf-kappab pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell* **12**: 115–130, 2007.
[22] J.J. Keats, R. Fonseca, M. Chesi, R. Schop, A. Baker, W.J. Chng, S. Van Wier, R. Tiedemann, C. X. Shi, M. Sebag, E. Braggio, T. Henry, Y.X. Zhu, H. Fogle, T. Price-Troska, G. Ahmann, C. Mancini, L.A. Brents, S. Kumar, P. Greipp, A. Dispenzieri, B. Bryant, G. Mulligan, L. Bruhn, M. Barrett, R. Valdez, J. Trent, A.K. Stewart, J. Carpten, and P.L. Bergsagel, Promiscuous mutations activate the noncanonical nf-kappab pathway in multiple myeloma. *Cancer Cell* **12**: 131–144, 2007.
[23] J.D. Woronicz, X. Gao, Z. Cao, M. Rothe, and D.V. Goeddel, Ikappab kinase-beta: Nf-kappab activation and complex formation with ikappab kinase-alpha and nik. *Science* **278**: 866–869, 1997.

[24] M. Karin, Nuclear factor-kappab in cancer development and progression. *Nature* **441**: 431–436, 2006.
[25] F. Chen, Endogenous inhibitors of nuclear factor-kappab, an opportunity for cancer control. *Cancer Res* **64**: 8135–8138, 2004.
[26] N.D. Perkins, The rel/nf-kappa b family: Friend and foe. *Trends Biochem Sci* **25**: 434–440, 2000.
[27] A.S. Baldwin, Control of oncogenesis and cancer therapy resistance by the transcription factor nf-kappab. *J Clin Invest* **107**: 241–246, 2001.
[28] T.D. Gilmore, Multiple myeloma: Lusting for nf-kappab. *Cancer Cell* **12**: 95–97, 2007.
[29] F. Chen and V. Castranova, Nuclear factor-kappab, an unappreciated tumor suppressor. *Cancer Res* **67**: 11093–11098, 2007.
[30] N.D. Perkins, Nf-kappab: Tumor promoter or suppressor? *Trends Cell Biol* **14**: 64–69, 2004.
[31] S. Kasibhatla, T. Brunner, L. Genestier, F. Echeverri, A. Mahboubi, and D.R. Green, DNA damaging agents induce expression of fas ligand and subsequent apoptosis in t lymphocytes via the activation of nf-kappa b and ap-1. *Mol Cell* **1**: 543–551, 1998.
[32] T. Hettmann, J. DiDonato, M. Karin, and J.M. Leiden, An essential role for nuclear factor kappab in promoting double positive thymocyte apoptosis. *J Exp Med* **189**: 145–158, 1999.
[33] J. Bash, W.X. Zong, and C. Gelinas, C-rel arrests the proliferation of hela cells and affects critical regulators of the g1/s-phase transition. *Mol Cell Biol* **17**: 6526–6536, 1997.
[34] C.S. Seitz, Q. Lin, H. Deng, and P.A. Khavari, Alterations in nf-kappab function in transgenic epithelial tissue demonstrate a growth inhibitory role for nf-kappab. *Proc Natl Acad Sci U S A* **95**: 2307–2312, 1998.
[35] B. Liu, E. Park, F. Zhu, T. Bustos, J. Liu, J. Shen, S.M. Fischer, and Y. Hu, A critical role for i kappab kinase alpha in the development of human and mouse squamous cell carcinomas. *Proc Natl Acad Sci USA* **103**: 17202–17207 2006.
[36] S. Maeda, H. Kamata, J.L. Luo, H. Leffert, and M. Karin, Ikkbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. *Cell* **121**: 977–990, 2005.
[37] T. Luedde, N. Beraza, V. Kotsikoris, G. van Loo, A. Nenci, R. De Vos , T. Roskams, C. Trautwein, and M. Pasparakis, Deletion of nemo/ikkgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. *Cancer Cell* **11**: 119–132, 2007.
[38] Y. Zhang and F. Chen, Reactive oxygen species (ros), troublemakers between nuclear factor-kappab (nf-kappab) and c-jun nh(2)-terminal kinase (jnk). *Cancer Res* **64**: 1902–1905, 2004.
[39] F. Chen, Y. Lu, Z. Zhang, V. Vallyathan, M. Ding, V. Castranova, and X. Shi, Opposite effect of nf-kappa b and c-jun n-terminal kinase on p53-independent gadd45 induction by arsenite. *J Biol Chem* **276**: 11414–11419, 2001.
[40] E. De Smaele, F. Zazzeroni, S. Papa, D.U. Nguyen, R. Jin, J. Jones, R. Cong, and G. Franzoso, Induction of gadd45beta by nf-kappab downregulates pro-apoptotic jnk signalling. *Nature* **414**: 308–313, 2001.
[41] G. Tang, Y. Minemoto, B. Dibling, N.H. Purcell, Z. Li, M. Karin, and A. Lin, Inhibition of jnk activation through nf-kappab target genes. *Nature* **414**: 313–317, 2001.
[42] L.E. Heasley and S.Y. Han, Jnk regulation of oncogenesis. *Mol Cells* **21**: 167–173, 2006.

[43] N. Chen, M. Nomura, Q.B. She, W.Y. Ma, A.M. Bode, L. Wang, R.A. Flavell, and Z. Dong, Suppression of skin tumorigenesis in c-jun nh(2)-terminal kinase-2-deficient mice. *Cancer Res* **61**: 3908–3912, 2001.

[44] F. Bost, R. McKay, M. Bost, O. Potapova, N.M. Dean, and D. Mercola, The jun kinase 2 isoform is preferentially required for epidermal growth factor-induced transformation of human a549 lung carcinoma cells. *Mol Cell Biol* **19**: 1938–1949, 1999.

[45] R. Schreck, K. Albermann, and P.A. Baeuerle, Nuclear factor kappa b: An oxidative stress-responsive transcription factor of eukaryotic cells (a review). *Free Radic Res Commun* **17**: 221–237, 1992.

[46] K.C. Das, Y. Lewis-Molock, and C.W. White, Activation of nf-kappa b and elevation of mnsod gene expression by thiol reducing agents in lung adenocarcinoma (a549) cells. *Am J Physiol* **269**: L588–602, 1995.

[47] S. Sakon, X. Xue, M. Takekawa, T. Sasazuki, T. Okazaki, Y. Kojima, J.H. Piao, H. Yagita, K. Okumura, T. Doi, and H. Nakano, Nf-kappab inhibits tnf-induced accumulation of ros that mediate prolonged mapk activation and necrotic cell death. *EMBO J* **22**: 3898–3909, 2003.

[48] F. Chen, V. Castranova, Z. Li, M. Karin, X. Shi, Inhibitor of nuclear factor kappab kinase deficiency enhances oxidative stress and prolongs c-jun nh2-terminal kinase activation induced by arsenic. *Cancer Res* **63**: 7689–7693, 2003.

[49] F.P. Guengerich, Common and uncommon cytochrome p450 reactions related to metabolism and chemical toxicity. *Chem Res Toxicol* **14**: 611–650, 2001.

[50] Z. Peng, L. Peng, Y. Fan, E. Zandi, H.G. Shertzer, and Y. Xia, A critical role for ikappab kinase beta in metallothionein-1 expression and protection against arsenic toxicity. *J Biol Chem* **282**: 21487–21496, 2007.

[51] J.M. Slack, Conrad Hal Waddington: The last renaissance biologist? *Nat Rev Genet* **3**: 889–895, 2002.

[52] C.H. Waddington, *Intruduction to Modern Genetics.* London: George Allen & Unwin, 1939.

[53] R. Holliday Epigenetics: A historical overview. *Epigenetics* **1**: 76–80, 2006

[54] J.R. Whetstine, A. Nottke, F. Lan, M. Huarte, S. Smolikov, Z. Chen, E. Spooner, E. Li, G. Zhang, M. Colaiacovo, and Y. Shi: Reversal of histone lysine trimethylation by the jmjd2 family of histone demethylases. *Cell* **125**: 467–481, 2006.

[55] A.J. Bannister and T. Kouzarides, Reversing histone methylation. *Nature* **436**: 1103–1106, 2005.

[56] Y. Shi, F. Lan, C. Matson, P. Mulligan, J.R. Whetstine, P.A. Cole, R.A. Casero, and Y. Shi, Histone demethylation mediated by the nuclear amine oxidase homolog lsd1. *Cell* **119**: 941–953, 2004.

[57] P.A. Cloos, J. Christensen, K. Agger, A. Maiolica, J. Rappsilber, T. Antal, K.H. Hansen, and K. Helin, The putative oncogene gasc1 demethylates tri- and dimethylated lysine 9 on histone h3. *Nature* **442**: 307–311, 2006.

[58] B.D. Fodor, S. Kubicek, M. Yonezawa, R.J. O'Sullivan, R. Sengupta, L. Perez-Burgos, S. Opravil, K. Mechtler, G. Schotta, and T. Jenuwein, Jmjd2b antagonizes h3k9 trimethylation at pericentric heterochromatin in mammalian cells. *Genes Dev* **20**: 1557–1562, 2006.

[59] Y. Tsukada, J. Fang, H. Erdjument-Bromage, M.E. Warren, C.H. Borchers, P. Tempst, and Y. Zhang, Histone demethylation by a family of jmjc domain-containing proteins. *Nature* **439**: 811–816, 2006.

[60] K. Yamane, C. Toumazou, Y. Tsukada, H. Erdjument-Bromage, P. Tempst, J. Wong, and Y. Zhang, Jhdm2a, a jmjc-containing h3k9 demethylase, facilitates transcription activation by androgen receptor. *Cell* **125**: 483–495, 2006.

[61] Y. Huyen, O. Zgheib, R.A. Ditullio, Jr., V.G. Gorgoulis, P. Zacharatos, T.J. Petty, E.A. Sheston, H.S. Mellert, E.S. Stavridi, and T.D. Halazonetis, Methylated lysine 79 of histone h3 targets 53bp1 to DNA double-strand breaks. *Nature* **432**: 406–411, 2004.

[62] F. De Santa, M.G. Totaro, E. Prosperini, S. Notarbartolo, G. Testa, and G. Natoli, The histone h3 lysine-27 demethylase jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. *Cell* **130**: 1083–1094, 2007.

[63] F. Chen, Y. Lu, V. Castranova, Z. Li, M. Karin, Loss of ikkbeta promotes migration and proliferation of mouse embryo fibroblast cells. *J Biol Chem* **281**: 37142–37149, 2006.

[64] J.T. Irelan, T.J. Murphy, P.D. DeJesus, H. Teo, D. Xu, M.A. Gomez-Ferreria, Y. Zhou, L. J. Miraglia, D.R. Rines, I.M. Verma, D.J. Sharp, V. Tergaonkar, and S.K. Chanda, A role for ikappab kinase 2 in bipolar spindle assembly. *Proc Natl Acad Sci U S A* **104**: 16940–16945, 2007.

[65] S. Prajapati, Z. Tu, Y. Yamamoto, and R.B. Gaynor, Ikkalpha regulates the mitotic phase of the cell cycle by modulating aurora a phosphorylation. *Cell Cycle* **5**: 2371–2380, 2006.

[66] R.A. Ihrie, M.R. Marques, B.T. Nguyen, J.S. Horner, C. Papazoglu, R.T. Bronson, A.A. Mills, and L.D. Attardi, Perp is a p63-regulated gene essential for epithelial integrity. *Cell* **120**: 843–856, 2005.

[67] T.D. Gilmore, and M. Herscovitch, Inhibitors of nf-kappab signaling: 785 and counting. *Oncogene* **25**: 6887–6899, 2006.