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### **Review Series**

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# IKK/NF- $\kappa$ B signaling: balancing life and death — a new approach to cancer therapy

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**IKK kinase/NF- $\kappa$ B (IKK/NF- $\kappa$ B) signaling pathways play critical roles in a variety of physiological and pathological processes. One function of NF- $\kappa$ B is promotion of cell survival through induction of target genes, whose products inhibit components of the apoptotic machinery in normal and cancerous cells. NF- $\kappa$ B can also prevent programmed necrosis by inducing genes encoding antioxidant proteins. Regardless of mechanism, many cancer cells, of either epithelial or hematopoietic origin, use NF- $\kappa$ B to achieve resistance to anticancer drugs, radiation, and death cytokines. Hence, inhibition of IKK-driven NF- $\kappa$ B activation offers a strategy for treatment of different malignancies and can convert inflammation-induced tumor growth to inflammation-induced tumor regression.**

## NF- $\kappa$ B proteins and I $\kappa$ B kinase signaling pathways

The mammalian NF- $\kappa$ B family contains 5 members: NF- $\kappa$ B1 (p105 and p50), NF- $\kappa$ B2 (p100 and p52), c-Rel, RelB, and RelA (p65). These proteins share a Rel homology domain (RHD), which mediates DNA binding, dimerization, and interactions with specific inhibitory factors, the I $\kappa$ Bs, which retain NF- $\kappa$ B dimers in the cytoplasm. Many stimuli activate NF- $\kappa$ B, mostly through I $\kappa$ B kinase-dependent (IKK-dependent) phosphorylation and subsequent degradation of I $\kappa$ B proteins. The liberated NF- $\kappa$ B dimers enter the nucleus, where they regulate transcription of diverse genes encoding cytokines, growth factors, cell adhesion molecules, and pro- and antiapoptotic proteins (1, 2). The IKK complex consists of 2 highly homologous kinase subunits, IKK $\alpha$  and IKK $\beta$ , and a nonenzymatic regulatory component, IKK $\gamma$ /NEMO (3).

Two NF- $\kappa$ B activation pathways exist (Figure 1). The first, the classical pathway, is normally triggered in response to microbial and viral infections or exposure to proinflammatory cytokines that activate the tripartite IKK complex, leading to phosphorylation-induced I $\kappa$ B degradation. This pathway, which mostly targets p50:RelA and p50:c-Rel dimers, depends mainly on IKK $\beta$  activity (4). The other pathway, the alternative pathway, leads to selective activation of p52:RelB dimers by inducing processing of the NF- $\kappa$ B2/p100 precursor protein, which mostly occurs as a heterodimer with RelB in the cytoplasm. This pathway is triggered by certain members of the TNF cytokine family, through selective activation of IKK $\alpha$  homodimers by the upstream kinase NIK (5). Both pathways regulate cell survival and death (6); the classical pathway is responsible for inhibition of programmed cell death (PCD) under most conditions (2, 3). The alternative pathway is important for survival of premature B cells and development of secondary lymphoid organs (7). The antiapoptotic activity of the IKK $\beta$ -driven classical pathway is important for various immu-

noreceptors, including T and B cell receptors, TLR4, and type 1 TNF- $\alpha$  receptor (TNFR1), all of which generate pro-survival and pro-death signals upon ligation (8, 9). Under most circumstances, the survival signals dominate, but under conditions where IKK $\beta$  or NF- $\kappa$ B activities have been compromised, receptor activation results in cell death (10–12).

## The survival function of NF- $\kappa$ B: mechanisms and mediators

*Pathways of cell death.* PCD can be either apoptotic or necrotic. Apoptosis is characterized by membrane blebbing, shrinking, and condensation of the cell and its organelles (13, 14). Two well-established pathways lead to apoptosis: the death receptor (DR) (extrinsic) pathway and the mitochondrial (intrinsic) pathway (15). Both pathways depend on cysteine proteases called caspases (15, 16). However, apoptosis-like PCD can sometimes proceed without caspase activation (17, 18). Furthermore, caspase activation does not always lead to cell death (19), and caspase-8 also has pro-survival functions (20, 21). Necrosis is characterized by swelling of the cell and its organelles, culminating in membrane disruption and cell lysis, often accompanied by inflammation. Failure of energy metabolism and massive generation of ROS are each thought to cause necrosis (22).

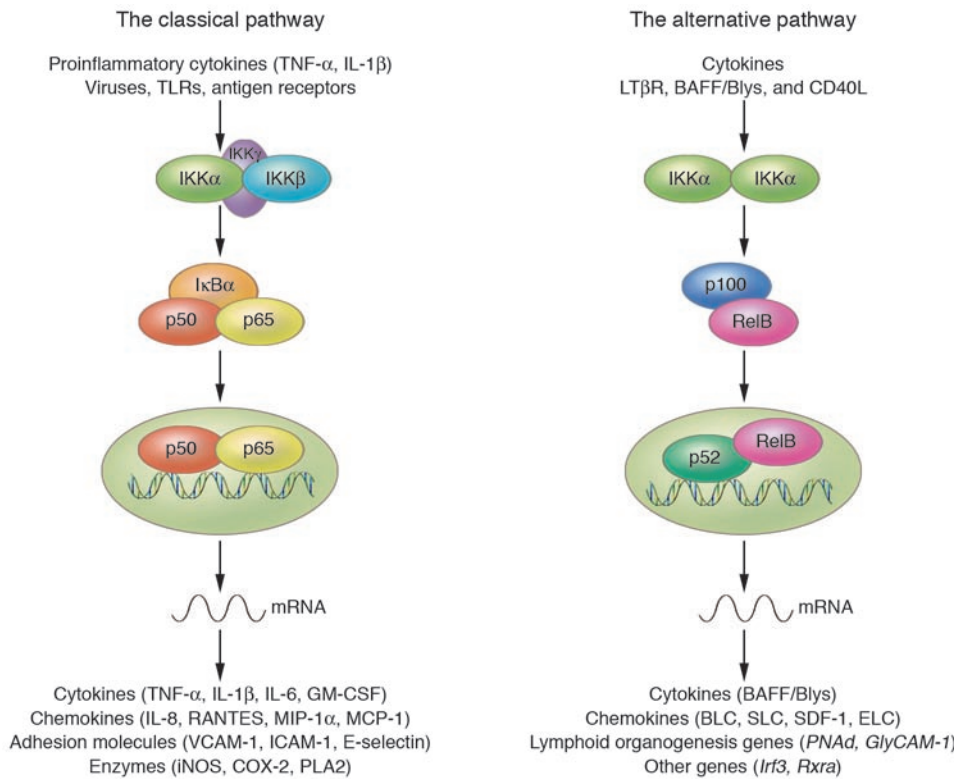
NF- $\kappa$ B suppresses both PCD types, although initially it was thought to antagonize only apoptosis. The first clear evidence for NF- $\kappa$ B as a PCD inhibitor was provided by RelA knockout mice that die mid-gestation by massive liver apoptosis (23). The role of NF- $\kappa$ B in embryonic liver survival, brought about by inhibition of TNFR1-mediated apoptosis (24), is underscored by the very similar phenotypes of mice lacking IKK $\beta$  (4, 25) or IKK $\gamma$  (26). A protective role for NF- $\kappa$ B in adult liver was confirmed in mouse models of liver damage (10, 27, 28) and involves inhibition of both apoptosis and necrosis (9). We will discuss the various mechanisms by which NF- $\kappa$ B suppresses PCD (Figure 2).

*NF- $\kappa$ B and caspases.* There are 2 groups of DRs, based on their signaling complexes. The first group comprises Fas, DR4, and DR5, which directly recruit the death domain-containing (DD-containing) adaptor Fas-associated death domain (FADD), procaspase-8, procaspase-10, and the cellular FLICE-inhibitory protein (FLIP) to form death-inducing signaling complexes (DISCs) (29). The second group comprises TNFR1, DR3, DR6, and ectodysplasin A receptor (EDAR). TNFR1 forms a signaling complex

**Nonstandard abbreviations used:** ATO, arsenic trioxide; DD, death domain; DISC, death-inducing signaling complex; DR, death receptor; FADD, Fas-associated death domain; FHC, ferritin heavy chain; FLIP, FLICE-inhibitory protein; IKK, I $\kappa$ B kinase; MKP, MAPK phosphatase; MnSOD, manganese superoxide dismutase; PCD, programmed cell death; RHD, Rel homology domain; RIP, receptor-interacting protein; SOD, superoxide dismutase; TNFR1, type 1 TNF- $\alpha$  receptor; TRAF, TNFR-associated factor; XIAP, X chromosome-linked inhibitor of apoptosis.

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**Figure 1** IKK/NF-κB signaling pathways. The classical pathway is activated by a variety of inflammatory signals, resulting in coordinate expression of multiple inflammatory and innate immune genes. The alternative pathway is strictly dependent on IKKα homodimers and is activated by lymphotoxin β receptor (LTβR), B cell-activating factor belonging to the TNF family (BAFF), and CD40 ligand (CD40L). The alternative pathway plays a central role in the expression of genes involved in development and maintenance of secondary lymphoid organs. BLC, B lymphocyte chemoattractant; ELC, Epstein-Barr virus-induced molecule 1 ligand CC chemokine; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; PLA2, phospholipase A2; SDF-1, stromal cell-derived factor-1α; SLC, secondary lymphoid tissue chemokine.

(complex I) at the plasma membrane by recruiting the adaptor TNFR1-associated DD protein (TRADD) and the signaling proteins TNFR-associated factor 2 (TRAF2), TRAF5, and receptor-interacting protein 1 (RIP1). After assembly, complex I dissociates from TNFR1, which can then recruit FADD and caspase-8 and trigger an apoptotic response (30).

NF-κB as a transcription factor induces genes whose products prevent PCD. An elicitor of NF-κB activation is TNF-α, which is a rather poor inducer of PCD. TNF-α triggers PCD only when new protein or RNA synthesis is inhibited or in NF-κB-deficient cells. NF-κB exerts its pro-survival activity through several anti-apoptotic proteins, including FLIP, Bcl-X<sub>L</sub>, A1/Bfl-1, cellular inhibitor of apoptosis (c-IAP), X chromosome-linked inhibitor of apoptosis (XIAP), TRAF1, and TRAF2 (2, 31). FLIP inhibits apoptosis by interfering with caspase-8 activation (30). c-IAP and XIAP directly bind and inhibit effector caspases, acting downstream of initiator caspases.

**NF-κB and Bcl-2 family members.** NF-κB induces expression of several Bcl-2 family members, most notably Bcl-X<sub>L</sub> and A1/Bfl-1, which prevent apoptosis by inhibiting permeability transition and depolarization of mitochondria, and cytochrome *c* release (2, 31). DRs can trigger apoptosis through different pathways (32). In certain cells, activated caspase-8 directly activates effector caspases, while in cells with poor DISC formation, death signaling requires an additional amplification loop, based on caspase-8-mediated Bid cleavage and generation of truncated tBid that triggers cytochrome *c* release (33, 34) and activation of caspase-9 and caspase-3 (15). This type of DR signaling can be blocked by antiapoptotic Bcl-2 family members, such as Bcl-2 and Bcl-X<sub>L</sub> (15).

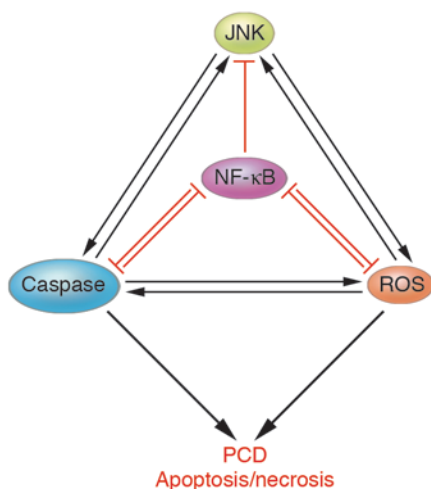
**ROS and the NF-κB-JNK cross-talk.** The role of JNK in PCD has been controversial, because it has both survival and death-enhancing effects. The clearest evidence for JNK as regulator of PCD

comes from analysis of knockout mice: JNK1- or JNK2-deficient mice are relatively resistant to induction of fulminant hepatitis in response to concanavalin A, a pathology that depends on activation of TNFR1 and other DRs (10).

The ratio between JNK and NF-κB activities controls cell survival or death, not only in response to TNFR1 but also in response to other death stimuli (35–37). Whereas TNF-α leads to transient JNK activation in WT cells, it leads to prolonged JNK activation in cells that cannot activate NF-κB (9, 38–40). The pro-survival activity of NF-κB depends on this ability to prevent prolonged JNK activation (9, 38–40). Prolonged JNK activation following concanavalin A administration was also seen in mice lacking IKKβ in liver cells, resulting in massive TNFR1-dependent hepatocyte death (10). In the liver, however, TNFR1 and JNK signaling is also required for regeneration or compensatory hepatocyte proliferation following partial hepatectomy or chemically induced injury (41, 42). Thus, NF-κB may be a critical regulator of cell survival and death through its ability to control the duration of JNK activation (Figure 2).

Prolonged JNK activation in NF-κB-deficient cells implies that NF-κB induces expression of JNK inhibitors. Such a function was proposed for GADD45β (43) and XIAP (39). However, analysis of GADD45β- or XIAP-deficient fibroblasts failed to reveal changes in the kinetics of JNK activation (31, 44), suggesting that NF-κB regulates JNK activation through a different mediator.

ROS are likely the mediators (40). ROS, including H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub><sup>-</sup>, and HO<sup>•</sup> radicals, are generated through many enzymatic pathways, but their major source is leakage of electrons from the mitochondrial respiratory chain (45). ROS activate kinases through oxidation of kinase-interacting molecules. For instance, ROS activate tyrosine kinases by inactivating protein tyrosine phosphatases through oxidation of a highly reactive cysteine residue at their

**Figure 2**

Control of cell survival and death through NF- $\kappa$ B–JNK cross-talk. Positive feedback loops exist between ROS and caspases, caspases and JNK, and JNK and ROS. Negative feedback loops exist between NF- $\kappa$ B and caspases, and NF- $\kappa$ B and ROS. NF- $\kappa$ B functions as a pro-survival transcription factor by inducing the expression of antiapoptotic genes, such as the Bcl-2 family members and caspase inhibitors, and antioxidant genes, such as MnSOD and FHC. Activation of NF- $\kappa$ B also results in inhibition of prolonged JNK activation, mostly through inhibition of ROS accumulation. Inhibition of NF- $\kappa$ B enhances PCD, which can be either apoptotic or necrotic, by removing the negative feedback loops.

catalytic site (46–49). Similarly, ROS mediate the NF- $\kappa$ B–JNK cross-talk through their ability to inactivate various MAPK phosphatases (MKPs) involved in JNK inactivation (45).

TNF- $\alpha$  induces ROS accumulation in many cell types, and these ROS are important mediators of PCD (22). TNF- $\alpha$ -induced ROS accumulation is seen in NF- $\kappa$ B-deficient cells, but not in NF- $\kappa$ B-competent cells (9, 40). Treatment of cells with the antioxidant butylated hydroxyanisole (BHA) has no effect on transient JNK activation triggered by TNF- $\alpha$ , but it suppresses prolonged JNK activation and PCD in TNF- $\alpha$ -treated NF- $\kappa$ B-deficient cells (9, 40). This protective effect is due to BHA's ability to prevent oxidation of MKPs, ensuring transient JNK activation (9). Expression of dominant-negative mutants of MKPs leads to prolonged JNK activation and allows killing by TNF- $\alpha$  of NF- $\kappa$ B-competent cells, which otherwise are TNF- $\alpha$ -resistant (9).

The loss of NF- $\kappa$ B activity results in ROS accumulation because NF- $\kappa$ B induces expression of several antioxidant genes such as manganese superoxide dismutase (MnSOD), ferritin heavy chain (FHC), glutathione-S-transferase, and metallothionein (50). Overexpression of mitochondrial MnSOD protects cells from TNF- $\alpha$ -induced cytotoxicity (9, 51). Overexpression of FHC also suppresses TNF- $\alpha$ -induced PCD along with attenuation of prolonged JNK activation (52). Another interesting observation is that TNF- $\alpha$  induces expression of a number of cytochrome p450 family members, such as CYP1B1, that enhance ROS production in NF- $\kappa$ B-deficient fibroblasts (37). Taken together, these findings show that NF- $\kappa$ B protects cells from oxidative stress by activating expression of various antioxidant systems, whose failure enhances TNF- $\alpha$ -induced PCD.

The mechanism of TNF- $\alpha$ -induced ROS production is unclear. One possible source of ROS is the cytosolic phospholipase A2 (53). However, several lines of evidence suggest that mitochon-

dria are the main source of ROS during TNF- $\alpha$ -induced PCD (22). Compared with our understanding of DR-induced caspase activation, the mechanism of DR-induced ROS production is obscure. TNF- $\alpha$  does not induce ROS accumulation and programmed necrosis in FADD- or RIP1-deficient cells, indicating essential roles for FADD and RIP1 (54). In contrast to the established function of RIP1 as an adaptor molecule in NF- $\kappa$ B activation, its kinase activity is necessary for Fas-induced necrosis, which mostly occurs in caspase-8-deficient cells (55). Interestingly, inhibition of caspases potentiates ROS accumulation and cell death (53, 56). Although DRs can induce ROS accumulation without caspase activation in certain cell types, caspase activation can also lead to mitochondrial damage and ROS accumulation (33, 34, 57–59). Thus, caspase-dependent and -independent mechanisms might be involved in ROS accumulation.

JNK activation may also enhance ROS accumulation, potentiating TNF- $\alpha$ -stimulated necrosis (60). Although the mechanism by which JNK potentiates ROS accumulation is unclear, a positive feedback loop between ROS accumulation and JNK activation may exist (Figure 2). Such a loop may also involve caspase activation. Although caspases are not involved in TNF- $\alpha$ -induced prolonged JNK activation in NF- $\kappa$ B-deficient cells (40), caspase-mediated cleavage of upstream MAP3Ks may cause constitutive JNK activation (61). JNK activation also contributes to caspase activation, an effect mediated through enhanced cytochrome *c* release, during UV-induced apoptosis (62). Alternatively, JNK causes caspase activation through jBid formation during TNFR1 signaling (63). Importantly, NF- $\kappa$ B suppresses all of these amplification loops by inducing expression of caspase inhibitors, Bcl-2 family members, and antioxidants (Figure 2). Interestingly, negative feedback loops exist between NF- $\kappa$ B and various death-promoting proteins. Caspase-mediated cleavage of RelA and IKK $\beta$  can prevent NF- $\kappa$ B activation (64, 65). Caspases can also cleave I $\kappa$ B to generate a degradation-resistant NF- $\kappa$ B inhibitor (66). Oxidation of a cysteine residue in the RHD of RelA prevents its binding to DNA (67), whereas oxidation of another cysteine within the activation loop of IKK $\beta$  interferes with its activation (68). It is unlikely, however, that all of these regulatory loops and modifications take place simultaneously, and a major challenge for the future is to sort out the events that do take place during different physiological and pathophysiological conditions. It is possible to use some of these regulatory loops in designing drugs and therapeutic strategies to kill cancer cells. Fas and TNF- $\alpha$  can induce both apoptosis and necrosis, and so do anticancer drugs. In L929 cells, for instance, TNF- $\alpha$  triggers mostly necrosis, whereas Fas can induce necrosis only when the apoptotic pathway is suppressed (69). FADD and RIP play central roles in controlling the choice between the 2 death pathways (70). NF- $\kappa$ B activation also inhibits programmed necrosis, in addition to its role in prevention of apoptosis.

### Proapoptotic functions of NF- $\kappa$ B?

*NF- $\kappa$ B may induce apoptosis in a cell type- and stimulus-dependent manner.* Most commonly, NF- $\kappa$ B activation inhibits PCD, as evidenced by several knockout mouse models (4, 23, 26, 71). However, under certain circumstances activation of NF- $\kappa$ B may promote cell death. For instance, NF- $\kappa$ B may mediate doxorubicin-induced cell death in N-type neuroblastoma cells (72). NF- $\kappa$ B is also required for anti-CD3-induced apoptosis of double-positive thymocytes (73). Apoptosis in HL-60 cells induced by etoposide or





1- $\beta$ -D-arabinofuranosylcytosine correlates with NF- $\kappa$ B activation (74). Human melanoma cells were protected from UV-induced apoptosis by NF- $\kappa$ B downregulation (75). More recently, it was reported that NF- $\kappa$ B induced by UV light or daunorubicin/doxorubicin is functionally distinct from the response elicited by TNF- $\alpha$ , and under such conditions NF- $\kappa$ B may become a repressor of antiapoptotic genes (76). Furthermore, UV light and daunorubicin inhibit TNF- $\alpha$ -induced NF- $\kappa$ B transcriptional activity, which is antiapoptotic, by enhancing association of RelA with histone deacetylases (76). These results suggest that NF- $\kappa$ B may mediate apoptosis under certain conditions. However, the pathophysiological relevance of these observations is not clear, and it remains to be demonstrated that NF- $\kappa$ B has proapoptotic functions *in vivo*. It appears that those agents or stimuli that were reported to induce apoptosis by activating NF- $\kappa$ B are neither strong nor typical NF- $\kappa$ B activators, as opposed to TNF- $\alpha$ , IL-1, or LPS, and that they also activate another signaling pathway(s), which may be more relevant to cell killing than NF- $\kappa$ B.

*Tumor suppressors interact with NF- $\kappa$ B pathway.* Suppression of cell proliferation, induction of premature senescence, and/or induction of apoptosis are some mechanisms through which tumor suppressors inhibit cancer development. In general, NF- $\kappa$ B acts antagonistically to tumor suppressors, based on its ability to promote cell survival, inhibit PCD, and enhance cell proliferation (77). However, in some cases NF- $\kappa$ B may collaborate with, rather than antagonize, certain tumor suppressors.

Although p53 stabilization decreases upon NF- $\kappa$ B activation (78), under special circumstances apoptosis induced by p53 may involve activation of NF- $\kappa$ B (79). Similar to situations in which NF- $\kappa$ B activation promotes apoptosis, NF- $\kappa$ B induction by p53 does not involve classical IKK activation and I $\kappa$ B degradation. Instead, p53 may stimulate the serine/threonine kinase ribosomal S6 kinase 1 (RSK1), which in turn phosphorylates RelA (80). The lower affinity of RSK1-phosphorylated RelA for I $\kappa$ B $\alpha$  decreases I $\kappa$ B $\alpha$ -mediated nuclear export, prolonging RelA nuclear residence (80). NF- $\kappa$ B also plays an essential role in activation of p53 to initiate proapoptotic signaling in response to ROS accumulation. Consequently, NF- $\kappa$ B-dependent p53 activity induces p53-regulated genes, such as Puma and p21<sup>waf1</sup> (81). However, a more common observation, seen *in vivo*, is that NF- $\kappa$ B activation counteracts p53-induced apoptosis by destabilizing p53, perhaps through enhanced Mdm2 expression (78, 82).

Another tumor suppressor, BRCA1, can bind RelA to serve as a coactivator (83). Treatment of 293T cells with TNF- $\alpha$  induces an interaction between endogenous RelA and BRCA1, mediated by the RHD of RelA and the N-terminal region of BRCA1. Forced BRCA1 expression significantly enhances the ability of TNF- $\alpha$  or IL-1 $\beta$  to induce NF- $\kappa$ B target genes, and inhibition of NF- $\kappa$ B by the chemical inhibitor SN-50 blocks this effect (83). Nonetheless, it remains to be seen whether any of these responses documented *in vitro* occurs *in vivo*.

*NF- $\kappa$ B and proapoptotic genes.* NF- $\kappa$ B has been implicated as a transcriptional activator of some proapoptotic genes, such as Fas/CD95 (84), FasL (85), DR4, and DR5 (86). FasL is expressed in activated T cells and represents a major cytotoxic effector through which T cells kill their targets. FasL expression is under the stringent control of various transcription factors, including NF- $\kappa$ B (85). Recently, it was reported that certain types of cancer cells also express FasL, which may contribute to their ability to escape immune surveillance and resist immunotherapy. Overexpression

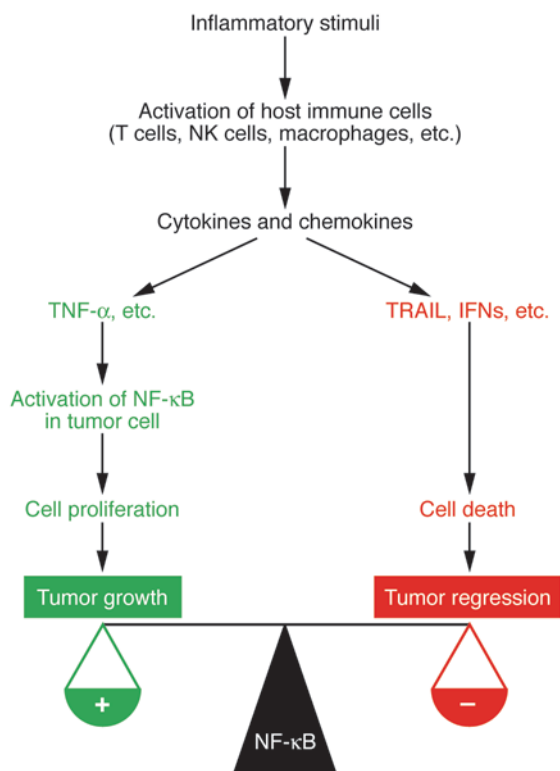
of the Myc family member Max in non-small cell lung cancer cell lines markedly increases basal FasL promoter activity and enhances NF- $\kappa$ B-mediated FasL induction. Thus, high levels of Max and stress-induced NF- $\kappa$ B activation may elevate FasL expression in human lung cancer cells (85). TNF- $\alpha$  combined with IFN- $\alpha$  accelerates NF- $\kappa$ B-mediated apoptosis by enhancing Fas expression in human colon adenocarcinoma RPMI4788 cells (84). However, there may be another explanation for these results, as type I IFNs and related cytokines, such as IL-10, may actually function as NF- $\kappa$ B inhibitors (87). Another TNF family member, TRAIL, triggers apoptosis through engagement of DR4 and DR5. The c-Rel subunit of NF- $\kappa$ B induces expression of both receptors, while a degradation-resistant mutant of I $\kappa$ B $\alpha$  (I $\kappa$ B super-repressor) or a transactivation-deficient mutant of c-Rel reduces DR expression (86). However, NF- $\kappa$ B was shown to be a major impediment to TRAIL-mediated tumor killing (88).

### IKK/NF- $\kappa$ B and cancer

*IKK/NF- $\kappa$ B links inflammation to cancer.* Based on many functions of NF- $\kappa$ B target genes, a close relationship between NF- $\kappa$ B and cancer was proposed (89) and recently reviewed (89–94). The association of NF- $\kappa$ B activation with inflammation-associated tumor promotion, progression, and metastasis is well documented and was demonstrated in several mouse models (88, 95, 96). The IKK $\beta$ -dependent NF- $\kappa$ B activation pathway is a critical molecular link between inflammation and colon cancer in a mouse model (95). Activation of IKK $\beta$  in enterocytes, which give rise to the malignant component of this tumor, suppresses apoptosis of preneoplastic cells, whereas its activation in myeloid cells promotes production of various cytokines that serve as growth factors for the transformed enterocytes. Inhibition of 1 of these factors, IL-6, interferes with tumor growth but has no effect on tumor cell survival (97). Conversely, inactivation of IKK $\beta$  in enterocytes results in a dramatic decrease in tumor number due to increased apoptosis but has no effect on proliferation of transformed enterocytes or tumor growth (95).

The role of NF- $\kappa$ B in inflammation-associated cancer was also demonstrated in Mdr2-deficient mice, which develop cholestatic hepatitis followed by hepatocellular carcinoma (96). In this model, the inflammatory process triggered chronic activation of NF- $\kappa$ B in hepatocytes, most likely through enhanced production of TNF- $\alpha$  by adjacent endothelial and inflammatory cells. Switching NF- $\kappa$ B off in Mdr2<sup>-/-</sup> mice from birth to 7 months of age had no effect on the course of hepatitis or early phases of tumorigenesis (96). By contrast, suppressing chronic NF- $\kappa$ B activation at later stages resulted in the apoptotic death of transformed hepatocytes and failure to progress to hepatocellular carcinoma (96).

NF- $\kappa$ B activation also plays a critical role in inflammation-driven tumor progression as demonstrated in a syngeneic colon and mammary cancer xenograft mouse model (88). Cancer cells in this model were introduced into syngeneic immunocompetent mice to form metastatic growths in the lungs. Once the metastases were established, the mice were given a sublethal dose of LPS to elicit systemic inflammation, which stimulated tumor growth. Remarkably, inhibition of NF- $\kappa$ B in cancer cells converted LPS-induced tumor growth to LPS-induced tumor regression without affecting the ability of the cancer cells to migrate to the lung and establish metastatic growths (88). Further investigation revealed that inflammation-induced tumor growth in this model was mediated by TNF- $\alpha$  produced by host immune cells, whereas LPS-induced



regression of NF- $\kappa$ B-deficient tumors was mediated by TRAIL, whose synthesis is induced by IFNs in host inflammatory cells in response to LPS-mediated activation of TLR4 (Figure 3). These results indicate that NF- $\kappa$ B is a major mediator of inflammation-induced tumor progression through overcoming the potential tumor-killing by TRAIL induction (88). Given that NF- $\kappa$ B activation in cancer cells may be a major hindrance to TRAIL-induced apoptosis, NF- $\kappa$ B or IKK inhibitors may potentiate the activity of either recombinant TRAIL or TRAIL inducers, such as type I and type II IFNs, to achieve enhanced tumor killing (Figure 3).

Whereas the role of NF- $\kappa$ B in inflammation-induced tumor promotion, growth, and progression is becoming clear (88, 95, 96), its role in tumor initiation is still ambiguous (98–101). As NF- $\kappa$ B regulates a large group of genes that have different functions, some of which display cell-type specificity, NF- $\kappa$ B may have distinct roles in different cell types. For instance, in normal epidermal keratinocytes, NF- $\kappa$ B proteins are present in the cytoplasm of basal cells but are nuclear in more differentiated suprabasal cells, suggesting that NF- $\kappa$ B activation is linked to growth arrest (98, 102). Indeed, inhibition of NF- $\kappa$ B signaling in the murine epidermis results in an increased apoptosis, hyperproliferation of surviving cells, and spontaneous development of squamous cell carcinomas (99, 103). Correspondingly, application of a pharmacological NF- $\kappa$ B inhibitor to mouse skin induced epidermal hyperplasia (98). In contrast, overexpression of active NF- $\kappa$ B subunits in transgenic epithelium produced hypoplasia and growth inhibition (98). Contrary to the requirement of an intact IKK/NF- $\kappa$ B pathway for H-ras-mediated fibroblast transformation (104), NF- $\kappa$ B inhibition synergized with oncogenic H-ras to induce transformation of primary human keratinocytes (100). Congruously, activation of NF- $\kappa$ B in normal human epidermal keratinocytes triggered cell-cycle arrest (100). These results suggest that the IKK $\beta$ -dependent NF- $\kappa$ B pathway

### Figure 3

Inhibition of NF- $\kappa$ B in cancer cells converts inflammation-induced tumor growth to tumor regression. Activation of the innate and adaptive immune system can have profound influence on tumor growth and development. In addition to its role in activation of immune cells, NF- $\kappa$ B within the malignant cell is a major modulator of the tumor response to inflammation. Activation of NF- $\kappa$ B promotes tumor growth and confers resistance to death cytokines, such as TRAIL. Conversely, inhibition of NF- $\kappa$ B prevents inflammation-stimulated tumor growth and enhances inflammation-induced tumor regression mediated by TRAIL.

in epidermal keratinocytes promotes keratinocyte growth arrest and differentiation to maintain the barrier function of the epidermis, whose perturbation may result in severe inflammation (105). Interestingly, formation of mouse squamous cell carcinomas in response to a chemical carcinogen (106) and following inhibition of NF- $\kappa$ B (107) is dependent on TNF- $\alpha$ . Similar observations were recently made in a mouse model of chemically induced hepatocellular carcinoma, where deletion of IKK $\beta$  in hepatocytes promoted tumor development by enhancing compensatory proliferation, whereas an additional deletion of IKK $\beta$  in liver myeloid cells prevented tumor development by depriving the transformed hepatocytes of essential growth factors (108).

*NF- $\kappa$ B inhibitors in cancer therapy.* The pivotal role of the IKK $\beta$ /NF- $\kappa$ B signaling pathway in inhibition of PCD, tumor promotion, and tumor progression, together with the occurrence of constitutively activated NF- $\kappa$ B in various solid and hematopoietic malignancies, strongly suggests that IKK $\beta$  and/or NF- $\kappa$ B inhibitors would be useful in cancer therapy. In fact, much effort is currently invested in developing various IKK $\beta$  and/or NF- $\kappa$ B inhibitors and testing their efficacy in both animal models and human cancer (109, 110). Many inhibitors currently available are not specific for either IKK $\beta$  or NF- $\kappa$ B. These include antiinflammatory agents such as sulfasalazine and trans-resveratrol, NSAIDs such as aspirin and sulindac sulfide, cyclopentenone prostaglandins, proteasome inhibitors, and glucocorticoids (90, 109–112). However, specific IKK $\beta$  inhibitors are being developed, and a few publications have documented their efficacy in triggering apoptosis in cancer cell lines in combination with either death-inducing cytokines or chemotherapeutic drugs (113–115).

Even nonspecific IKK $\beta$ /NF- $\kappa$ B inhibitors may be effective when used as adjuvants with conventional anticancer treatments. As many signaling pathways may be simultaneously activated and/or inactivated in a given malignant cell, collectively contributing to its neoplastic phenotype, nonspecific IKK $\beta$ /NF- $\kappa$ B inhibitors may affect several signaling pathways at once and lead to much more effective killing of such cells. The anticancer drug arsenic trioxide (ATO), which is useful for treating promyelocytic leukemia (116) and possibly multiple myeloma (117), is a noteworthy example. ATO is not a specific inhibitor for IKK $\beta$  or NF- $\kappa$ B and may have several molecular targets, since it was found that trivalent arsenicals, a chemical class to which ATO belongs, are potent JNK activators (118) as well as IKK $\beta$  inhibitors (68). JNK activation in this case is mostly due to the ability of trivalent arsenicals to directly interact with the catalytic cysteine of JNK phosphatases, whereas in the case of IKK $\beta$  the target is the aforementioned reactive cysteine within the activation loop. An additional effect of ATO on JNK activity may be due to NF- $\kappa$ B inhibition and accumulation of ROS (35). Thus, by inhibiting IKK and activating JNK, ATO may trigger apoptosis in many different types of cancers. Since NF- $\kappa$ B inhibi-



tion usually does not result in spontaneous apoptosis, it is unlikely that even specific IKK $\beta$ /NF- $\kappa$ B inhibitors would be functional as monotherapeutic agents in most cancers. Indeed, using Jurkat cells as a model, the IKK $\beta$  inhibitor AS602868 was not cytotoxic on its own but strongly potentiated killing by TNF- $\alpha$  (113). Based on our analysis of knockout mice and tumor models, we predict that IKK $\beta$ /NF- $\kappa$ B inhibitors will be useful adjuvants for conventional chemotherapeutic drugs, ionizing radiation, or tumoricidal cytokines, such as IFNs or TRAIL (Figure 3).

NF- $\kappa$ B regulates PCD through a cross-talk with JNK, ROS, and caspases, and an important pro-survival factor regulated by NF- $\kappa$ B is the antioxidant enzyme MnSOD (9, 51). Thus, MnSOD2 inhibitors may target a particular NF- $\kappa$ B function, the suppression of ROS production and PCD, while leaving other functions, such as innate immunity, intact. In fact, inhibition of superoxide dismutase (SOD) in human leukemia cells caused accumulation of O $_2^-$ , which was followed by ROS-mediated mitochondrial damage, cytochrome c release, and apoptosis (119). Given its regulation by NF- $\kappa$ B, whose activity is elevated in most types of cancer, it is likely that MnSOD expression is higher in malignant cells than in normal cells, and therefore the former may be more sensitive to SOD inhibitors. In fact, certain estrogen derivatives, acting as SOD inhibitors, selectively kill human leukemia cells but not normal lymphocytes (119). In case such compounds are not sufficiently potent on their own, they need to be tested as adjuvants for more conventional chemotherapeutic and radiotherapeutic approaches.

### Concluding remarks

The inhibition of IKK $\beta$ /NF- $\kappa$ B appears to be a promising strategy for cancer therapy when combined with established cytotoxic drugs, death cytokines, or therapeutic radiation. Certain anti-

cancer drugs may work much better with IKK $\beta$ /NF- $\kappa$ B inhibitors than others. For instance, the combined application of TRAIL or TRAIL inducers, such as IFNs (Figure 3), with anti-inflammatory or anti-TNF- $\alpha$  therapy alongside IKK $\beta$ /NF- $\kappa$ B inhibitors may result in selective killing of malignant cells not achieved by either agent alone (88). An important advantage of IKK $\beta$ /NF- $\kappa$ B inhibitors over conventional therapeutics is their ability to block NF- $\kappa$ B activation also in infiltrating inflammatory cells, which are an important source of tumor growth and survival factors. It should be noted, however, that, given the critical role of NF- $\kappa$ B in innate and adaptive immune responses, there may be a certain amount of risk due to induced immunodeficiency caused by long-term use of IKK $\beta$ /NF- $\kappa$ B inhibitors. Hence, alternative approaches should be considered. For instance, an approach based on selective inhibition of antiapoptotic targets of NF- $\kappa$ B, without affecting target genes required for immune responses, would be particularly attractive.

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