

## The Biology Behind

# Chemoresistance: Impact of Nuclear Factor (NF)- $\kappa$ B Inhibition by Small Interfering RNA

Commentary re J. Guo *et al.*, Enhanced Chemosensitivity to Irinotecan by RNA Interference-Mediated Down-Regulation of the NF- $\kappa$ B p65 Subunit.  
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Resistance of cancer cells to powerful chemotherapeutic drugs has been an area of intense investigation. Efforts to understand the mechanisms underlying chemoresistance have led to significant progress toward our understanding of signaling pathways that favor cancer cell survival. Now that many survival pathways have been elucidated, one of the challenges ahead is to discover and develop molecularly targeted agents with the potential to enhance tumor cell sensitivity to chemotherapy. Among the signaling pathways that have been shown to impact tumor cell survival, the nuclear factor (NF)- $\kappa$ B pathway has received significant attention. NF- $\kappa$ B is a family of transcription factors composed of structurally related proteins. The five members of this family, c-Rel, RelA (p65), RelB, NF- $\kappa$ B1 (p105 precursor of p50), and NF- $\kappa$ B2 (p100 precursor of p52), exist as either homo- or heterodimers (1). Most NF- $\kappa$ B dimers are activators of transcription, with the p65/p50 heterodimer being best understood as the main regulator of cytokine-induced NF- $\kappa$ B activation. NF- $\kappa$ B activity is under tight control by a family of inhibitors called I $\kappa$ Bs (1). The “classical” mode of activation of p65/p50 requires an upstream kinase complex, the I $\kappa$ B kinase (IKK), in which the IKK $\beta$  subunit phosphorylates I $\kappa$ B on two critical serine residues, leading to degradation of the inhibitor by the ubiquitin proteasome pathway. NF- $\kappa$ B is then free to translocate to the nucleus and activate transcription. An alternative mode of activation relies on the IKK $\alpha$  subunit to induce processing of p100 and nuclear translocation of RelB-p52 dimers. In addition to regulation of nuclear translocation, NF- $\kappa$ B is also controlled at the level of transcriptional activation by phosphorylation of its subunits by several kinases (2).

Until the current decade, most work on the NF- $\kappa$ B pathway focused on the role of the pathway in regulation of immune and inflammatory responses. Whereas a connection between inflammation and cancer had long been suspected, only recently has NF- $\kappa$ B been implicated in the biology of cancer. Some of the earliest evidence for a role for NF- $\kappa$ B in cancer stemmed from the discovery that v-Rel, a NF- $\kappa$ B family member, is the oncoprotein of the avian REV-T retrovirus that causes lymphoma in

birds. Since this observation, numerous cancers have been shown to display constitutive activation of NF- $\kappa$ B, as evidenced by nuclear localization of the proteins or by a pattern of gene expression consistent with NF- $\kappa$ B activation. Cancers that display evidence of NF- $\kappa$ B activation include multiple myeloma, lymphoma, breast cancer, pancreatic cancer, prostate cancer, head and neck squamous cell carcinoma, colon cancer, and others (3–12). NF- $\kappa$ B regulates genes that encode cytokines and chemokines (interleukin-1, interleukin-6, tumor necrosis factor, granulocyte macrophage colony-stimulating factor, interleukin-8, macrophage inhibitory protein-1 alpha, macrophage chemoattractant protein 1, and others), factors that are involved in tumor promotion/proliferation (cyclin D, matrix metalloproteinases, urokinase-type plasminogen activator), angiogenesis (vascular endothelial growth factor), and a plethora of antiapoptotic proteins (*Bcl2* family members, cellular inhibitors of apoptosis protein-1 and -2, tumor necrosis factor receptor-associated factor, and so forth). Thus, multiple genes that are involved in regulating the “hallmarks of cancer” are controlled by NF- $\kappa$ B (13, 14). Finally, NF- $\kappa$ B-mediated chemoresistance can be acquired through the influence of the tumor microenvironment, genetic alterations in the tumor cell, and by activation of NF- $\kappa$ B in response to a therapeutic agent. Several studies have suggested a link between ATM/ATR, p53/p21, and ARF with NF- $\kappa$ B regulation in response to DNA-damaging agents and radiation. Activation of the NF- $\kappa$ B pathway is well established as a mechanism for protection of tumor cells from apoptosis, through induction of antiapoptotic gene expression (15–17).

## Evidence for Chemosensitization by Inhibition of NF- $\kappa$ B

The first support for the involvement of NF- $\kappa$ B in cancer therapy-induced apoptosis came from studies using a molecular tool, a mutated form of I $\kappa$ B called the I $\kappa$ B super-repressor. The mutated form of I $\kappa$ B lacks the ability to be phosphorylated by IKK and degraded by the proteasome, thereby preventing NF- $\kappa$ B nuclear translocation. Human tumor cell lines in which NF- $\kappa$ B was bound up by the I $\kappa$ B super-repressor were more readily killed by radiation and the chemotherapy drug daunorubicin than were control tumor cells (18). Subsequent studies used adenovirus-mediated delivery of the I $\kappa$ B super-repressor in tumor-bearing nude mice (19, 20). Treatment of tumor bearing mice with the topoisomerase I inhibitor irinotecan (CPT-11) activated NF- $\kappa$ B in tumors of vector-treated mice, and the activation was blocked by the I $\kappa$ B super-repressor. Remarkably, tumors in the super-repressor-treated mice showed enhanced tumoricidal responses to CPT-11 when compared with vector-treated mice. The inhibition of NF- $\kappa$ B by the I $\kappa$ B super-repressor resulted in increased tumor growth delay and enhanced

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apoptosis. In some of the tumor models used, tumor regression and cures were observed. These studies and many others established that NF- $\kappa$ B activation was an impediment to chemotherapy-induced apoptosis.

The current publication by Guo *et al.* (21), further examines the contribution of NF- $\kappa$ B to a tumor cell's response to chemotherapy. The study used the colorectal tumor cell line HCT116, which is relatively resistant to the cytotoxic effects of CPT-11. The method chosen to block NF- $\kappa$ B signaling was depletion of the p65 NF- $\kappa$ B subunit using small interfering RNA (siRNA). The p65 siRNA specifically blocked p65 expression and inhibited CPT-11-induced activation of NF- $\kappa$ B, as assessed by gel retardation assays and a NF- $\kappa$ B-dependent luciferase reporter. The authors found that whereas loss of p65 did not impact cell viability on its own, the depletion of p65 did increase tumor cell sensitivity to the cytotoxic effects of CPT-11 by severalfold. Importantly, the reduction in viability translated into decreased colony formation in a longer term survival assay.

Guo *et al.* (21) then asked the critical question of whether depletion of p65 could impact the sensitivity of HCT116 cells to CPT-11 in an *in vivo* setting. The studies were performed in two ways. First, tumor cells treated with p65 siRNA *in vitro* were grafted into nude mice and shown to form tumors comparable with those of control-treated mice. When the mice were challenged with CPT-11, tumors from p65 siRNA-treated cells failed to grow in comparison with control tumors in CPT-11-treated mice. A second approach examined whether p65 siRNA could be applied systemically in mice already bearing colorectal tumors. Similar to the previous study, tumors in the p65 siRNA-treated mice grew at comparable rates with tumors in control-treated mice. Again, results from the p65 siRNA-treated mice demonstrated that depletion of p65 enhanced tumor responsiveness to CPT-11.

To gain insight into the molecular mechanism of the increased sensitivity to CPT-11, Guo *et al.* (21) measured the effects of p65 depletion on CPT-11-induced apoptosis and found that p65 siRNA decreased expression of two cellular inhibitors of apoptosis that are known to be regulated by NF- $\kappa$ B, c-IAP1 and c-IAP2, and subsequently increased the ability of CPT-11 to activate caspase-3 activity. These results are in keeping with the previously defined role for NF- $\kappa$ B in preventing CPT-11-mediated apoptosis.

### Potential Opportunities for NF- $\kappa$ B-Targeted Therapies

The above findings suggest that NF- $\kappa$ B inhibition combined with CPT-11 holds therapeutic promise. However, the multiplicity of signaling events that impinge on NF- $\kappa$ B demands that additional work be done to clarify the best strategies. The upstream events responsible for NF- $\kappa$ B activation by DNA-damaging agents including CPT-11 are not well understood. In cell lines, several signaling pathways have been implicated in the direct or indirect activation of the IKK complex. Studies have suggested various oncogenic signaling pathways, such as Ras-Raf-mitogen-activated protein kinase kinase kinase, the phosphatidylinositol 3'-kinase pathway, and receptor tyrosine kinases, as potential signaling mechanisms for NF- $\kappa$ B activation through the IKK complex (22). Recent studies have also sug-

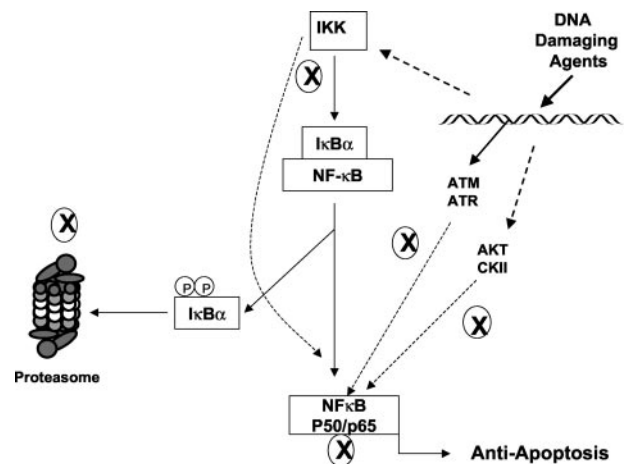


Fig. 1 DNA-damaging agents regulate nuclear factor- $\kappa$ B activity via several pathways, many of which converge onto p65/p50 heterodimers. The X marks potential sites for therapeutic intervention where an inhibitor potentially could overcome tumor cell chemoresistance by blocking nuclear factor- $\kappa$ B-driven expression of antiapoptotic genes.

gested IKK-independent means of activating NF- $\kappa$ B. One study examined doxorubicin activation of NF- $\kappa$ B in IKK-deficient embryonic fibroblasts. Whereas a large portion of NF- $\kappa$ B activation in these cells is IKK $\beta$  dependent, a small fraction of NF- $\kappa$ B activation is independent of the IKK complex (23). Several signaling pathways can directly impinge on the transactivation potential of p65. For example, protein kinase A can bind to p65, phosphorylate Ser<sup>276</sup>, and activate NF- $\kappa$ B-dependent transcription independently of I $\kappa$ B degradation. Additional kinases, including casein kinase II, AKT, and protein kinase C  $\zeta$ , have shown the ability to phosphorylate p65 at distinct residues and to increase transcriptional activation (reviewed in Ref. 22). The physiological relevance of these IKK-independent kinases to NF- $\kappa$ B activation has yet to be determined. Because they all lead to activation of NF- $\kappa$ B directly through p65, signaling through p65 is likely to be a critical component in regulating NF- $\kappa$ B activity. Taken together with the results obtained by Guo *et al.* (21), both the IKK complex and p65 itself appear to be attractive targets for therapeutic intervention in cancer (Fig. 1).

Major efforts to develop inhibitors of the NF- $\kappa$ B pathway are taking place in the pharmaceutical industry, as reviewed in Ref. 2. Whereas much effort is focused on the discovery of selective small molecule inhibitors of IKK $\beta$ , other potential approaches merit consideration. These include regulation of NF- $\kappa$ B protein expression (siRNA), DNA binding (NF- $\kappa$ B decoys), nuclear translocation, interference with IKK complex formation, blockade of upstream activators, and inhibition of NF- $\kappa$ B transcriptional activity (p65 kinases). For many of these approaches, development of inhibitors with drug-like properties is feasible, especially in the kinase arena. Other lines of attack will be more challenging. The study by Guo *et al.* (21) demonstrates that delivery of siRNA to tumor cells *in vivo* is possible and suggests that blocking NF- $\kappa$ B-mediated transcription by p65 siRNA may have therapeutic potential. Despite these promising findings, gene-specific knockdown at the RNA level is a

relatively new technology and will require significant refinement before clinical utility can be considered. Major hurdles to overcome include efficiency of delivery, duration of action, improved specificity, and establishment of safety (24, 25).

A novel therapeutic strategy for preventing NF- $\kappa$ B activation is to interfere with the degradation of I $\kappa$ B by inhibition of the proteasome. The proteasome inhibitor bortezomib (Velcade) was approved by the United States Food and Drug Administration for clinical use in 2003 for relapsed multiple myeloma that is refractory to conventional therapy (26, 27). Because proteasome inhibition impacts many signaling pathways, it is not clear whether the therapeutic effects of bortezomib are mediated by inhibition of NF- $\kappa$ B activation. Nonetheless, numerous preclinical studies with bortezomib have shown that proteasome inhibition blocks activation of NF- $\kappa$ B and enhances the effects of chemotherapeutic drugs, including CPT-11 (28). Bortezomib is currently undergoing further clinical development in hematological malignancies and solid tumors, as a single agent and in combination with conventional chemotherapeutic drugs and new agents.

In closing, the results provided by Guo *et al.* (21) serve to further pique the interest in potential therapeutic strategies that interfere with NF- $\kappa$ B activation in cancer. Targeting NF- $\kappa$ B should be approached with a healthy degree of caution because inhibition of NF- $\kappa$ B activation is likely to have far-reaching consequences in many organ systems (29). Additional advances in our understanding of the biology of NF- $\kappa$ B will no doubt have a significant impact on whether the therapeutic approaches prove to be successful.

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