

Inflammatory Tales of Liver Cancer

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Cell culture studies have established NF- κ B's critical role in cancer cell survival and proliferation and led to the clinical use of NF- κ B inhibitors. However, a paper in this issue of *Cancer Cell* reveals an anti-cancer function for NF- κ B in a mouse model where NF- κ B activity is lost specifically in hepatocytes. These studies suggest careful examination of NF- κ B inhibitors as a therapeutic modality for cancer.

Epidemiological studies have long established a link between chronic inflammation and cancer. Chronic infections with hepatitis B and hepatitis C viruses and with *Helicobacter pylori* are major risk factors for hepatocellular carcinomas. Similarly, chronic inflammatory diseases like ulcerative colitis increase the risk of colorectal cancer by approximately 1% per year. By some accounts, 15%–20% of cancer deaths are due to chronic inflammation. How inflammatory mediators activate proliferative programs remains unclear, but recent studies point to important, yet seemingly contradictory roles of the NF- κ B signaling pathway.

The transcription factor NF- κ B, whose activity was first identified during B cell activation, is well known as a major regulator of inflammatory as well as antiapoptotic gene expression (Hoffmann and Baltimore, 2006; Li and Verma, 2002). Indeed, abnormally high nuclear NF- κ B activity is a clinical hallmark of chronic inflammation and has been found in many different types of cancer cells. Drugs that dampen the effect of NF- κ B activity have been found to be useful additions to the chemotherapy regimens of a variety of cancers. Steroids, the mainstay of the anti-inflammatory regimen in the clinic and often a component of cancer therapies, inhibit NF- κ B-activated gene expression. Similarly, proteasome inhibitors like bortezomib, recently approved to treat multiple myelomas, block NF- κ B activation.

This issue of *Cancer Cell* contains a provocative but thorough study that explores the role of NF- κ B in

liver carcinogenesis in a mouse model system (Luedde et al., 2007). The authors used a conditional knockout approach to eliminate NF- κ B activity in hepatocytes. Surpris-

ingly, they found that lack of NF- κ B resulted in elevated inflammatory cytokine expression and spontaneous carcinogenesis in every animal within a year. Luedde et al. therefore

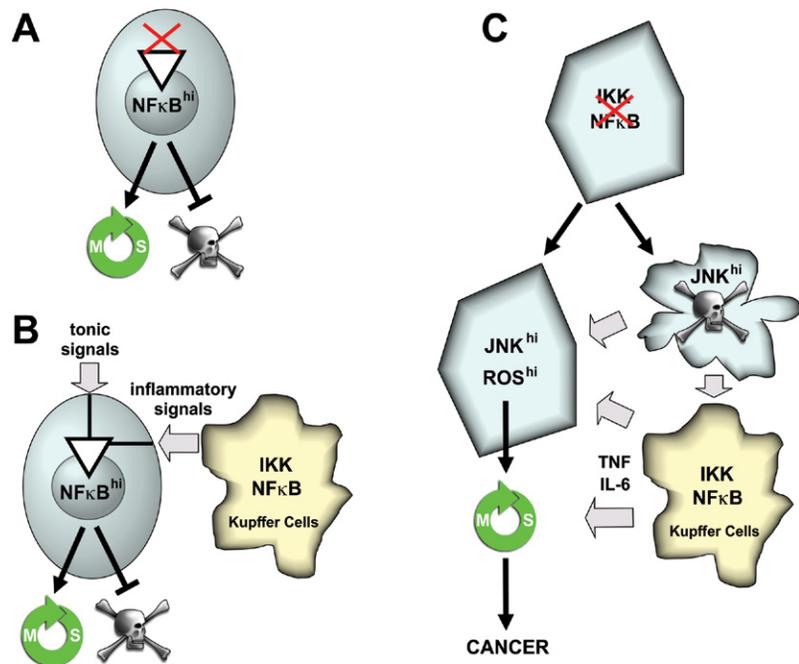


Figure 1. NF- κ B in Different Cancer Models

(A) Cell culture models often involve cancer cells in which genetic or biochemical lesions (red X) lead to an amplification (triangle) of constitutive NF- κ B activity in a cell-autonomous manner. Elevated NF- κ B activity inhibits apoptosis (skull and crossbones) and drives cellular proliferation (green circle indicating cell cycle).

(B) Animal models have highlighted the importance of intercellular signaling. Macrophage-specific genetic inhibition of NF- κ B revealed that tissue-resident macrophages (Kupffer Cells) play a major role in controlling antiapoptotic and proliferative activity of early cancerous cells by providing inflammatory signals (Greten et al., 2004; Maeda et al., 2005; Pikarsky et al., 2004). When such early cancerous cells are also subject to tonic developmental signals that are mediated by the noncanonical NF- κ B pathway, the resulting signaling crosstalk may promote cellular proliferation and/or tumor development (Basak et al., 2007).

(C) The study in this issue of *Cancer Cell* (Luedde et al., 2007) presents an animal model that also involves cell-cell interactions: NF- κ B-inhibition in hepatocytes causes apoptosis in many cells, which stimulates compensatory proliferation. Kupffer cells respond to apoptotic bodies with inflammatory cytokines that act as growth factors. ROS signaling and JNK activity, both elevated due to the loss of NF- κ B, further stimulate cell proliferation and may drive genetic instability.

conclude that NF- κ B is a tumor suppressor, which is in direct contradiction to the previously accepted model that posits NF- κ B as a cancer-driving transcription factor.

To study molecular mechanisms of human carcinogenesis, a large number of experimental model systems have been developed. Apparent discrepancies in the role of NF- κ B in cancer may be understandable when considering the specifics of the cancer model systems.

Cell culture constitutes the simplest model system. Transformed human or mouse cells lines, or cell lines derived from specific human cancers, have revealed a role for NF- κ B in blocking spontaneous apoptosis in both chemoresistance and proliferation (Figure 1A). Indeed, constitutive NF- κ B activity in such cells is often correlated with the aggressiveness of the cancer, and the cells have become addicted to NF- κ B. In this model system, cells are transformed and function autonomously without tissue interactions. The relevance of these findings to later cancer stages was demonstrated in mouse model systems, in which induction of I κ B super-repressor expression prevented liver cancer progression (Pikarsky et al., 2004), or IKK deletion reduced colitis-associated tumor incidence due to increasing epithelial apoptosis during tumor promotion (Greten et al., 2004).

However, mouse cancer models have also revealed the importance of intercellular signaling and cell-cell interactions within an organ. Interestingly, tissue-resident macrophages were shown (by a tissue-specific knockout of IKK or expression of nondegradable I κ B mutant) to provide inflammatory mediators that act as growth signals for chemically or genetically induced colorectal and liver cancer cells (Greten et al., 2004; Maeda et al., 2005; Pikarsky et al., 2004) (Figure 1B). These studies began to dissect the cellular and molecular signaling network that forms the basis for the epidemiological link between chronic inflammation and cancer. How inflammatory mediators such as TNF or IL-6 acti-

vate proliferative programs remains unclear, but a recent study points to the potential importance of crosstalk between canonical and noncanonical signaling pathways within the NF- κ B signaling system, through which inflammatory stimuli may alter the processing of "harmless" tonic developmental signals to have disease-causing effects (Basak et al., 2007).

But now, in the present study (Luedde et al., 2007), a similar tissue-specific knockout leads to the opposite conclusions. In this study, NF- κ B is genetically blocked only in hepatocytes, rendering them highly susceptible to apoptosis. However, liver, more than many other organs, has capacity for regeneration. As many, but not all, cells die as a result of NF- κ B inhibition, survivors are able to compensate by undergoing hepatic compensatory proliferation (Figure 1C). As in HBV-infected patients, compensatory hepatocyte proliferation increases the risk for cancer. In a similar study, partial inhibition of NF- κ B led to liver cancer following chemically induced liver damage (Maeda et al., 2005). Thus, these studies emphasize the role of tissue maintenance and homeostasis in carcinogenesis. They also show that, although hepatocyte proliferation and survival involves NF- κ B (Lavon et al., 2000), these processes can also occur in the absence of NF- κ B. In this model, liver cancer cells have been selected to survive in the absence of NF- κ B-dependent survival gene expression, though it is likely that they remain more chemosensitive than NF- κ B-competent controls.

But how is the cancer risk increased in the mouse model used by Luedde et al.? There appear to be three factors that warrant consideration. First, the present study blocks NF- κ B activation in one cell type but leaves liver-resident monocytes (Kupffer cells) intact. As Kupffer cells sense the apoptotic hepatocytes, they produce inflammatory cytokines, such as TNF or IL-6, that may act to stimulate proliferation of remaining hepatocytes. Indeed, the present study shows that, when apoptosis in hepatocytes is blocked

genetically, carcinogenesis is also blocked. Second, NF- κ B is known to antagonize the activity of JNK, the other major stress-inducible signaling pathway, which is known both as a proapoptotic and as a proliferative regulator. Indeed, JNK activation in NF- κ B or IKK-deleted cells is enhanced, contributing to both the apoptotic sensitivity of hepatocytes as well as proliferative response of survivors. (Similarly, elevated JNK activity was shown to mediate hyperproliferation of keratinocytes [Zhang et al., 2004].) Antioxidant treatment inhibits JNK and attenuates liver carcinogenesis (Luedde et al., 2007; Maeda et al., 2005), and genetic deletion of JNK unambiguously established the requirement for JNK in the ensuing carcinogenesis (Sakurai et al., 2006). Finally, we may imagine that NF- κ B deficiency may cause decreased genomic stability by lowering cellular antioxidant capacity, thus allowing for cancer mutations to arise more quickly. Primary murine embryonic fibroblasts deficient in NF- κ B are more easily immortalized by repeated passage and are more likely to assume a pretransformed state than their wild-type counterparts (Gapuzan et al., 2005).

Does the present study provide a reason to abandon all anti-NF- κ B therapeutic strategies, or can the results be considered an artifact of a cell-type-specific knockout strategy within an organ that contains multiple cell types? The importance of cell-cell interactions and tissue homeostasis, and the ensuing complexity when analyzing cell-type-specific transgenes, has previously been noted in the skin (Schmidt-Ullrich et al., 2001; van Hogerlinden et al., 1999). However, the degree of inhibition upon administering an NF- κ B therapeutic is by no means equal in all cells of an organ but dependent on cell-type-specific metabolic networks and location-specific pharmacokinetics. The good news is that the recent work has drawn attention to intercellular signals that represent druggable therapeutic opportunities. The challenge lies in incorporating the information gleaned from

diverse cancer model systems to examine the safety and effectiveness of NF- κ B therapeutics. In that regard, computational modeling may prove useful in integrating the complexity of cell-intrinsic and intercellular molecular mechanisms, tonic and responsive signaling, and pharmacokinetics and drug metabolism to predict or evaluate the effectiveness of therapeutic agents.

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A Radical Role for p38 MAPK in Tumor Initiation

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It is established that p38 MAPK can negatively regulate tumorigenesis, but the mechanism is incompletely understood. A new study in this issue of *Cancer Cell* shows that p38 MAP kinase plays a selective role in tumor initiation mediated by oxidative stress.

Cells sense changes in their environment by activating signal transduction pathways that direct biochemical programs to mediate proliferation, differentiation, and survival. The mitogen-activated protein kinase (MAPK) family represents an important group of signaling proteins that can regulate these fundamental cellular processes. The extracellular signal-regulated kinase (ERK) MAPK pathway primarily directs a program of proliferation and survival, while the cJun NH₂-terminal kinase (JNK) pathway can promote either proliferation or apoptosis (Kennedy and Davis, 2003). Conversely, the p38 MAPK pathway is activated upon cellular stress and often engages pathways that can block proliferation or promote apoptosis (Bulavin and Fornace, 2004).

The importance of MAPK pathways to cell proliferation and death is highlighted by the observation that dysregulation of these kinase cascades can result in cell transformation and cancer. Activated ERK and JNK pathways can lead to increased proliferation and survival, although loss of JNK in some instances may also promote tumorigenesis (Kennedy and Davis, 2003). In contrast, the p38 MAPK pathway is implicated in suppression of tumorigenesis because it can inhibit cell growth by decreasing the expression of cyclin D (Lavoie et al., 1996), inhibit the activity of Cdc25 phosphatases (Manke et al., 2005), and engage the p16/Rb and p19^{ARF}/p53 tumor suppressor pathways (Bulavin et al., 2002, 2004). The p38 MAPK pathway can also cause apoptosis by a mechanism that is incom-

pletely understood but may involve the phosphorylation of members of the Bcl2 family and activation of the mitochondrial apoptotic pathway (Figure 1). The selectivity of the p38 MAPK signaling pathway in tumor suppression is unclear. However, a new study by Dolado et al. (2007) reported in this issue of *Cancer Cell* now demonstrates that p38 MAPK selectively functions as a sensor of oxidative stress during the initiation of tumorigenesis.

Dolado et al. examined the properties of fibroblasts isolated from p38 α ^{-/-} mice when transformed by an activated HRasV12 oncogene. They report that p38 α deficiency caused increased proliferation, an increased number of foci, an increased ability to form colonies in soft agar, and decreased apoptosis. The p38 α -deficient cells also