Published in final edited form as:

Cancer Cell. 2012 June 12; 21(6): 711–712. doi:10.1016/j.ccr.2012.05.034.

CYLD and HCC: When Being Too Sensitive to Your Dirty Neighbors Results in Self-Destruction

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Abstract

Hepatocellular carcinoma is the outcome of ongoing cycles of cell death and regeneration in chronic liver disease. In this issue of *Cancer Cell*, Nikolaou et al. show that the deubiquitinating enzyme CYLD is critical for controlling the balance between hepatocyte loss, regeneration, and malignant progression.

The liver is the quintessential regenerative organ in mammals as first documented in the Greek mythology. It has been molded through evolution to endure extreme challenges by environmental pollutants, toxic metabolites, infections, and all kinds of cellular stress. The liver is equipped with an astonishing capacity for cellular repopulation even after massive cell death. Despite the obvious benefits of these regenerative properties, they come at a high cost. Chronic liver injury and the sustained regeneration it induces provide the perfect breeding grounds for one of the deadliest cancers, hepatocellular carcinoma (HCC). Chronic liver disease is supported and sustained by unresolved fibrotic and inflammatory responses. It is in this context where NF-κB signaling has acquired a title role in the liver cancer field despite its Janus-like character.

NF-κB signaling in Kupffer cells and other immune cell types is critical in propagation of liver inflammation. However, hepatocyte-specific NF-κB deficiency caused by ablation of either IκB kinase β (IKKβ) or its regulatory subunit IKKγ/NEMO can either augment chemically induced liver carcinogenesis (Maeda et al., 2005) or lead to spontaneous liver damage, inflammation, and tumorigenesis (Luedde et al., 2007). Inactivation of hepatocyte NF-κB results in critical over-activation of JNK and ablation of *Jnk1* in $Ikk\beta^{\Delta hep}$ mice prevents enhanced susceptibility to hepatic carcinogens (Sakurai et al., 2006). In addition, defective expression of IKKγ/NEMO and TAK1 also results in spontaneous tumorigenesis with persistent JNK activation (Luedde et al., 2007; Inokuchi et al., 2010; Bettermann et al., 2010). Treatment of $Ikk\beta^{\Delta hep}$ and $Ikk\gamma/Nemo^{\Delta hep}$ mice with an antioxidant or ablation of *TnfrI* in $Tak1^{\Delta hep}$ mice suppressed JNK over-activation and HCC formation. These results

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underscore that HCC formation in these mice is caused mainly by compensatory hepatocyte proliferation following massive hepatocyte death with enhanced reactive oxygen species production and JNK activation due to TNF over-production combined with loss of protective NF-κB activity and expression of antioxidant proteins. Paradoxically, however, NF-κB is a key driver of spontaneous HCC development in *Mdr2*$^{-/-}$ mice. Indeed, inhibition of NF-κB or titration of TNF in these mice inhibited HCC development (Pikarsky et al., 2004). In this issue of *Cancer Cell*, Nikolaou et al. (2012) show that specific deletion of the cylindromatosis tumor suppressor gene (*Cyld*) in hepatocytes results in HCC formation. Surprisingly, the authors found a procarcinogenic effect for TAK1, NF-κB, and JNK in *Cyld*$^{\Delta hep}$ mice.

Nikolaou et al. (2012) deleted *Cyld* specifically in hepatocytes to analyze its effects on the liver. CYLD is a negative regulator of NF-κB signaling, being a key deubiquitinase that removes K63-linked ubiquitin chains from several key effectors of the pathway, including TAK1 and IKKγ/NEMO. Since some of these effectors control the activation of MAP kinases (MAPK), the absence of CYLD also results in upregulation of MAPKs, including JNK. When *Cyld*$^{\Delta hep}$ mice are born, their liver appears normal. However after 25 days of age, periportal hepatocytes start dying. The authors demonstrate that this spontaneous cell death is due to prolonged JNK activation. This finding prompted Nikolaou and colleagues (2012) to identify the upstream effector responsible for JNK activation. Because TAK1 activation requires K63-linked ubiquitination and CYLD counteracts this modification in CYLD-deficient livers, TAK1 is spontaneously activated. Although previous reports showed that deletion of TAK1 in hepatocytes also results in spontaneous cell death, chronic TAK1 activation leads to sustained JNK activation which eventually promotes cell death even in the presence of NF-κB. To better investigate this point, the authors generated *Cyld* and *Tak1* double knockout mice. These animals exhibit a complete reversion of periportal hepatocyte death accompanied by a reduction in fibrosis and JNK activity, similar to those found in *Tak1*$^{\Delta hep}$ single mutants. Remarkably, periportal cell death and fibrosis in *Cyld*$^{\Delta hep}$ mice, with time, are extended toward the central vein region. This is accompanied by increased infiltration of inflammatory cells and TNF production on postnatal day 45 when hepatic NF-κB activation becomes detectable. This result prompted the authors to hypothesize that there are two different phases in the life of the CYLD-deficient liver. Initially, CYLD-devoid periportal hepatocytes suffer spontaneous cell death, provoking a second phase of injury expansion mediated by immune cells through TNF-mediated death receptor signaling. Ablation of the *Tnfr1* gene in *Cyld*$^{\Delta hep}$ mice demonstrated that expansion of the damage is TNF-dependent, but the persistent periportal damage pinpoints the TNF-independent nature of the initial defect. Progressive and sustained liver injury and fibrosis eventually lead to development of HCC after 12 months of age.

The work of Nikolaou et al. (2012) offers novel insights into the role of NF-κB signaling in liver homeostasis and disease. So far, most of our knowledge of NF-κB signaling in liver pathophysiology is derived from studies in which NF-κB activation or activity is inhibited. The authors used the opposite approach trying to activate NF-κB by removing CYLD. Surprisingly, only hepatocytes in close vicinity to the portal triad suffer spontaneous apoptosis but only after postnatal day 10, arguing against a cell autonomous promotion of

cell death in the absence of CYLD. If this is the case, what triggers the periportal cell death from postnatal day 10 onward? Pertinently, the authors point to commensal bacteria in the gut. During embryogenesis and shortly after birth, the gut is sterile and thus, the portal circulation to the liver is free of bacteria or microbial products. However, after the first postnatal days, the microflora start colonizing the gut, and an influx of microbial components, such as lipopolysaccharide (LPS) reaches the liver. At this point, if the hepatocytes are devoid of CYLD, an important attenuator of NF-κB signaling, they misinterpret the amount of LPS and other TLR agonists to which they are exposed. Invariably, this would lead to programmed cell death in hepatocytes that overreact to TLR agonists. Of note, CYLD is expressed in a gradient, being higher in the periportal region and lower close to the central vein, supporting an important role for CYLD in signal fine-tuning in the area where TLR agonist concentration can fluctuate the most. New investigations using germ-free *Cyld*$^{\Delta hep}$ mice would provide an important test of this hypothesis. After this initial trigger, the CYLD-deficient liver starts on a self-destruction spiral. The initial death of periportal hepatocytes triggers an inflammatory response mediated by Kupffer cells. These cells produce TNF and other death cytokines that act on hyper-responsive hepatocytes, causing more cell death and more inflammation, eventually enhancing compensatory proliferation. Interestingly, Dapito et al. (2012) recently reported that HCC promotion is affected by intestinal microbiota through TLR4 signaling in the liver. Considering that in several human liver diseases there is an increase in intestinal permeability and consequently an increase of microbial components in the portal circulation, the studies of Nikolaou et al. (2012) and Dapito et al. (2012) establish a new paradigm in liver disease in which intestinal microbiota can determine hepatocyte cellular survival and death. Thus, the intestinal barrier and commensal microflora further influence the NF-κB-JNK interplay that is already known to play a central role in the control of liver pathophysiology.

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