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BEYOND THE JOURNAL

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Die another day

Hepatocyte-specific inhibition of NF-κB leads to apoptosis after TNF treatment, but not after partial hepatectomy. Chaisson ML, Brooling JT, Ladiges W, Tsai S, Fausto N. Department of Pathology, University of Washington, Seattle, USA

One of the earliest TNF-dependent events to occur during liver regeneration is the activation of the transcription factor NF-κB through TNF receptor type 1. NF-κB activation in the liver can have both antiapoptotic and proliferative effects, but it is unclear which liver cell types, hepacytes or nonparenchymal cells (NPCs), contribute to these effects. To specifically evaluate the role of hepatocyte NF-κB, we created GLVP/ΔNIκB(α) transengenic mice, in which expression of a deletion mutant of IκB(α) (ΔNIκB(α)) was induced in hepatocytes after injection of mifepristone. In control mice, injection of 25 μg/kg TNF caused NF-κB nuclear translocation in virtually all hepatocytes by 30 min and no detectable apoptosis, while in mice expressing ΔN-IκB(α), NF-κB nuclear translocation was blocked in 45% of hepatocytes, leading to apoptosis 4 h after TNF injection. In contrast, expression of ΔN-IκBα in hepatocytes during the first several hours after partial hepatectomy did not lead to apoptosis or decreased proliferation. As NF-κB activation was not inhibited in liver NPCs, it is likely that these cells are responsible for mediating the proliferative and antiapoptotic effects of NF-κB during liver regeneration.

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The transcription factor NF-κB plays a key role in cell survival during inflammation and proliferation. In most cells, NF-κB is predominantly composed of a p65:p50 heterodimer. In quiescent cells, NF-κB is maintained in the cytoplasm by binding to its inhibitor IκB. Three different IκB isoforms exist (IκBα, -β, -ε). Current thinking is that NF-κB is activated in response to cytokines like tumor necrosis factor alpha (TNFα) and interleukin-1 (IL-1). Activation occurs when inhibitory protein IκB, is phosphorylated at specific serine residues. This results in the release of IκB from the p65 subunit of NF-κB which exposes a nuclear localization sequence on the p65 subunit permitting translocation of NF-κB to the nucleus. Phosphorylated IκB is ubiquitinated and degraded in proteasomes. In the nucleus, NF-κB binds to κB binding sites in promoters of target genes and induces transcription of these genes. NF-κB activity is regulated at different levels [1,2]. (1) Phosphorylation of IκB is accomplished after a series of intermediate phosphorylation steps, involving kinases like NIK (NF-κB inducing kinase), and IKK (IκB kinase). Several isoforms of IKK exist with non-identical, but partly overlapping functions [1,2]. (2) IκBα is itself under the control of NF-κB [1,2]. After resynthesis, IκB recomplexes with p65 subunits terminating NF-κB activation and preventing prolonged NF-κB activation. It is postulated that phosphorylation of IκBα mediates rapid NF-κB activation, whereas IκBβ and IκBε respond more slowly to IKK activation. The latter IκBs function to dampen the response of the NF-κB signaling pathway to sustained TNF input [3]. (3) IκB itself can also shuttle between cytoplasm and nucleus, thus regulating NF-κB activity in the nucleus as well [4,5]. (4) The p65 subunit of NF-κB is subject to phosphorylation resulting in enhanced DNA binding activity of NF-κB [6]. Furthermore, acetylation of p65 inhibits NF-κB export from the nucleus and increases DNA binding activity [7,8]. (5) Other signal transduction pathways modulate NF-κB activation, including the JNK pathway, the PI-3-kinase/Akt pathway and Protein kinase C-ζ pathway [1,2,9].

Targets of the transcription factor NF-κB are genes which are induced during inflammation such as TNFα, iNOS and COX-2. Many NF-κB-regulated genes are survival or anti-apoptotic genes that protect cells against harmful compounds released during inflammation. Examples of these genes are the superoxide radical scavenger Mn-SOD, iNOS, the Bcl-2 family member A1/Bfl-1 and members of the IAP family of caspase inhibitors, such as

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HIAP (rat: cIAP2) [10]. Therefore, exposure of cells to inflammatory cytokines in the absence of NF-κB activation leads to apoptosis. This has been demonstrated in hepatocytes [10]. The role of NF-κB in hepatocytes in vivo has also been addressed previously. However, techniques to inhibit NF-κB activity in vivo lack organ and cell specificity or the method itself activates NF-κB. In models of acute liver failure such as endotoxin or exposure to TNFα in the presence of a transcriptional inhibitor like actinomycin D or D-galactosamine, all transcription is inhibited. Therefore these models are not specific for blocking NF-κB-regulated transcription [11]. Specific inhibition of NF-κB-mediated transcription is achieved by adenoviral overexpression of a dominant negative IκB mutant. However, the use of adenoviruses in vivo has disadvantages: at high doses, the adenovirus itself causes inflammation and TNFα release [12,13]. Furthermore, all these methods do not distinguish between different organs or different cell types within one organ. Although adenoviruses readily infect hepatocytes, other cell types are also vulnerable to infection. Titrating the dose of adenovirus to limit infection to a specific cell population is hardly feasible. To achieve cell type-specific adenoviral infection, targeting of these viruses to cell type-specific receptors (in combination with cell type specific promoters) could be a solution.

Chaisson et al. now report a very elegant method which is able to specifically inhibit NF-κB activation in one cell type, the hepatocyte, using a clever transgenic approach. In this approach, a deletion mutant of IkBα is under the control of an inducible and hepatocyte specific promoter in mice [14]. In these animals, NF-κB is inhibited exclusively in hepatocytes and not in other organs or in other cell types in the liver. The limitation is that this method inhibits NF-κB activation in only 50% of the hepatocytes. Yet, the results unequivocally demonstrate that NF-κB plays an essential role in the protection against apoptosis: in transgenic mice unable to activate NF-κB in hepatocytes, TNFα exposure leads to massive apoptosis of hepatocytes. In contrast to the endotoxin/D-galactosamine model, there was no mortality in the transgenic model. The explanation for this difference may be related to the fact that in the transgenic model only the liver (hepatocytes) is affected, but not other organs. Mortality in the endotoxin/D-galactosamine model is usually attributed to complications like shock and acute respiratory stress. In addition, the proliferative response of hepatocytes is not affected, indicating that inhibition of NF-κB activation in hepatocytes does not abolish their capacity to proliferate. The proliferative signals probably originate from non-parenchymal cells and may include cytokines like IL-6 [15] or TNFα [16]. The authors also investigated the effect of inhibiting NF-κB in hepatocytes after partial hepatectomy (PH). Surprisingly, the authors did not observe hepatocyte apoptosis after PH, which is in contrast to the findings of Iimuro et al. using adenoviral constructs [12]. Chaisson et al. demonstrate that the lack of apoptosis is due to a less severe apoptotic stress after PH compared to TNFα administration, indicated by a lack of TNFα release and a lack of IκB degradation. On the other hand, NF-κB activation determined by electrophoretic mobility shift assay is similar after PH and TNFα administration and the authors go on to show that lower TNFα levels also lead to full activation of NF-κB. Alternatively, NF-κB may be activated by other cytokines, like IL-1. They also suggest that after PH non-NF-κB-regulated anti-apoptotic genes are induced, such as Bcl-xl as shown in this study, or members of the IAP family.

In summary, in this very extensive and elegant study, Chaisson et al. demonstrate that NF-κB plays a key role in the survival of hepatocytes during inflammation but not after PH and that NF-κB activation is not essential to allow proliferation of hepatocytes. What's next? The authors suggest that cytokines produced by non-parenchymal cells are essential for the proliferation of hepatocytes, in particular IL-6 and TNFα [15,16]. The genes for many of these cytokines, e.g. TNFα, are under the control of NF-κB. It will be a challenge to devise a similar approach to specifically inhibit NF-κB activation in non-parenchymal cells. It could be speculated that in the absence of proliferation signals from non-parenchymal cells, hepatocyte proliferation will be retarded after PH. In addition, in this condition, no apoptosis will occur after endotoxin administration (or in acute hepatitis) since no TNFα is released. These experiments have to take into account the capacity of bile duct epithelial cells to produce cytokines, including TNFα, in the absence of functional Kupffer cells, as shown by the group of A.M. Diehl [17]. Then, it will also become clear whether it is beneficial to inhibit NF-κB activation in non-parenchymal cells in acute liver failure or acute hepatitis to prevent exposure of hepatocytes to high levels of cytokines, including TNFα. What is needed are promoters specific for non-parenchymal cells. Chaisson et al. have pointed out the right direction to answer these questions.

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