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NF-κB signalling: Embracing complexity to achieve translation

Jayashree Bagchi Chakraborty, Derek A. Mann*

Liver Research Group, Institute of Cellular Medicine, 4th Floor, Catherine Cookson Building, Medical School, Newcastle University, NE2 4HH, UK

NF-κB is a dimeric transcription factor that has emerged as an important regulator of liver homeostasis and is mechanistically implicated in a variety of liver pathologies including hepatitis, steatosis, fibrosis, and hepatocellular carcinoma. The question remains as to whether NF-κB can really be exploited for the development of therapeutics for these pathologies in the diseased human liver. This review casts a critical eye on the experimental evidence gathered to date and in particular questions the rationale for the current focus on components of the upstream IKK complex as therapeutic targets. We make the argument that translation of NF-κB biology to new therapies is more likely to emerge from a re-focus of basic research back to the NF-κB/Rel subunit functions and the complexities of their post-translational modifications and context-dependent co-regulator interactions.

Introduction

The past decade has seen an explosion of new experimental data that suggest important functions for the NF-κB signalling system in liver physiology and disease. Hepatic NF-κB is implicated in at least three normal homeostatic processes: (1) clearance of microbial pathogens, (2) protection of hepatocytes from TNFα-induced cell death and (3) compensatory proliferation of hepatocytes in response to loss of hepatic mass through liver injury. These "healthy" functions of NF-κB contrast with a large and growing body of work suggesting a variety of "unhealthy" activities that span a wide spectrum of pathologies found in chronic liver disease [1]. These include steatosis, insulin resistance, hepatitis, biliary disease, fibrosis, and hepatocellular carcinoma [2–10]. Not surprisingly there is considerable interest for the prospect of targeting NF-κB in liver disease, especially as there are already a vast number of pharmacological and biological inhibitors available (Table 1) [11–25]. However, there are serious caveats to the published experimental work on NF-κB and liver disease that urge caution and possibly even a return to the drawing board. Firstly, studies that describe NF-κB activities and functions in human liver disease are rare and have been hampered by lack of appropriate investigative technologies. Second, the most well cited and popular experimental work is with mouse models of liver disease utilising knockout technology to disarm the IKK complex which is certainly a crucial activator of NF-κB but which has a variety of non-NF-κB targets that can also impact on liver pathology. Furthermore, these mouse models largely rely on the Cre/lox targeting technology which has implications for interpretation and physiological relevance of data. Third, the "healthy" functions of NF-κB are at considerable risk if we were to use currently available inhibitors. While there is literature claiming that mice lacking key components of the hepatic NF-κB system thrive perfectly well [6,9,10,26], one must be alert to the fact that these mice live in a highly controlled environment with strict dietary regimens and protection from environmental pathogens and toxins that would be near impossible to achieve for patients. This review aims to critically reassess where we currently are with respect to translation of NF-κB to therapies for prevention or treatment of liver disease and cancer.

A brief overview of the NF-κB signalling system

Numerous recent reviews provide highly detailed descriptions of the NF-κB system and the signalling pathways that lead to its activation in response to environmental cues [27,28]. As such we will only provide a brief description here but will highlight the complexities that may eventually point the way towards opportunities for therapeutic targeting. NF-κB functions as a dimeric DNA binding complex generated from interactions between the protein products of 5 structurally related members of the Rel gene family, namely relA (or p65), c-rel, relB, nfkb1 (which encodes for the p50 subunit and its precursor p105) and nfkb2 (encoding for p52 and its p100 precursor). Most of what we have learned about NF-κB in the liver has come from studies focused on the so-called canonical activation pathway which results in stimulation of inflammatory gene transcription by the RelA:p50 heterodimer. This dimer cycles between the cytoplasm and the nucleus complexed with an inhibitory protein IκBα which reduces the efficiency of nuclear transport and prevents DNA binding of RelA:p50. Upon interaction with their cell surface receptors, extracellular stimuli such as bacterial LPS and TNFα

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* Corresponding author. Address: Liver Research Group, Institute of Cellular Medicine, 4th Floor, Catherine Cookson Building, Medical School, Newcastle University, Newcastle upon Tyne NE2 4HH, UK. Tel.: +44 (0)191 222 3851; fax: +44 (0)191 222 5455.
E-mail address: derek.mann@ncl.ac.uk (D.AA. Mann).
Abbreviations: NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IKK, IkappaB kinase; CBP, CREB binding protein; RANTES, Regulated upon Activation, Normal T cell Expressed and Secreted.

Table 1. List of inhibitors targeting NF-κB in correlation with liver diseases.

Names	Pharmacological origin	Mode of action
Sulfasalazine	Derivative of mesalazine (5-aminosalicylic acid)	IKKα and IKKβ inhibitor [11]
Gliotoxin	*Aspergillus fumigatus*	Prevent IκBα degradation [12]
Curcumin	Polyphenol from *Curcuma longa*	Induce oxidative stress and inhibits NF-κB activation [13]
(−)-Epigallocatechin-3-gallate	Antioxidant present in *Camellia sinensis*	Abrogates p300-induced p65 acetylation [14]
Resveratrol	Phytoalexin from *Polygonum cuspidatum*	Prevent nuclear translocation of NF-κB [15]
Silymarin	Flavonoid isolated from *Silybum marianum*	Inhibits NF-κB activation by inhibiting upstream kinases [16]
Captopril	Angiotensin-converting enzyme (ACE) inhibitor	Inhibits NF-κB activation [17]
Pyrrolidine dithiocarbamate	Synthetic	Inhibits IκB release [18]
Over-expression of IκBα	Synthetic I-κBα superrepressor	Mutation at 32nd and 36th amino acid,prevent phosphorylated degradation of IκB [19]
Thalidomide	Synthetic derivative of Glutamic acid	Inhibit degradation of IκB [20]
Proteasome inhibitor (a) Bortezomib (b) NPI-0052	Synthetic molecule	Inhibits IκB degradation [21]
Cell-permeable peptides	Synthetic peptides	Block the association of IKKγ/NEMO with IKKβ and thereby inhibit NF-κB activation [22]
(a) Glucocorticoids (e.g. dexamethasone; prednisolone) (b) Selective estrogen receptor modulator	Corticosteroids	Glucocorticoid when binds to its receptor it can interact with NF-κB transcription factors, forming transexpression complexes or induces IκBa expression and retain NF-κB in the cytoplasm [23]
Decoy oligonucleotides	Synthetic double stranded oligonucleotides	Contains the consensus sequence and thus inhibits NF-κB binding to its promoter region [24]
Small interfering ribonucleic acid	Double stranded RNA molecule	Inhibit NF-κB protein synthesis [25]

activate RelA:p50 via a complex series of signalling events that are channelled through the IKK complex. The IKK complex is comprised of three major components known as IKK1 (or IKKα), IKK2 (or IKKβ) and NEMO (or IKKγ). The general consensus is that the catalytic component IKK2 and the scaffold component NEMO transduce signals to the canonical pathway. IKK2 catalyses phosphorylation of N-terminal serine residues of IκBα which leads to polyubiquitination and degradation by the 26S proteasome to generate "free" RelA:p50. Loss of IKK2 or NEMO results in diminished activation of RelA:p50 and reduced expression of pro-inflammatory cytokines, this explains the intense focus on these molecules in experimental mouse studies [2,10]. However, because NF-κB is a regulator of so many other important physiological processes (immunity; cell differentiation, growth and life-span; metabolism etc.), it is critical that additional regulatory checkpoints help control its activity. For example, for RelA:p50 to be fully transcriptionally active the RelA subunit undergoes a series of post-translational modifications including phosphorylation and acetylation [29]. Details of the kinases, phosphatases, acetyltransferases and deacteylases that control these modifications of RelA are emerging but are not well understood. At many genes RelA:p50 competes for its DNA target motif (5′-GGGPuNNNPyPyCC-3′) with the p50:p50 homodimer which lacks transcriptional activity and when in association with the histone deacteylase HDAC1 can actively repress transcription [30]. How p50 and RelA interactions are regulated is poorly defined, as are the molecular events that control competition for DNA binding between RelA:p50 and p50:p50. Discovering the answers to these questions may enable selective experimental control over the type of NF-κB that is recruited to inflammatory, fibrogenic and tumour-regulating genes.

The IKK complex and liver disease

Initial efforts to investigate the canonical NF-κB pathway in the mouse liver were problematic since non-conditional knockout of RelA, IKK2 or NEMO resulted in massive and fatal TNF-mediated death of hepatocytes during foetal development [31–34]. This led to the use of Cre/lox technology to target knockout of the canonical pathway to specific liver cell types. Unfortunately the data that has emerged from different laboratories that have used this technology to target IKK/NEMO has generated unclear and often contradictory conclusions [35]. This confusion may be explained by different environmental conditions in the investigator laboratories and/or may also directly relate to the Cre/lox technology itself. As described in more detail elsewhere, Cre is associated with cellular toxicity including induction of DNA damage and growth arrest which unfortunately were often not appropriately controlled for in many high profile studies. If we ignore these caveats, then the current popular opinion is that targeted knockout of IKK2 in hepatocytes removes a cytoprotective property of RelA:p50 which prevents excessive cell death in response to toxic damage of the liver. When the toxin is also a carcinogen such as diethylnitrosamine the high degree of cell death occurring in the absence of IKK2 leads to compensatory proliferation of hepatocytes including transformed cells [10]. As a result, mice lacking hepatocyte IKK2 develop liver cancer more rapidly than wild type mice. By contrast, if IKK2 knockout is targeted to the myeloid cell lineage (including macrophages) then the expression of hepatomitogens by these cells is blunted and the outcome is attenuated development of liver cancer [10]. Targeted knockout of NEMO to parenchymal liver cells using an Alfp-Cre system was reported to result in spontaneous steatohepatitis and fibrosis