Role of nuclear factor κB in liver health and disease

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ABSTRACT

NF-κB (nuclear factor κB) is a heterodimeric transcription factor that is constitutively expressed in all cell types and has a central role as a transcriptional regulator in response to cellular stress. In the present review, we discuss the role of NF-κB signalling in the maintenance of liver homeostasis as well as in the pathogenesis of a wide variety of conditions affecting the liver, including viral hepatitis, steatohepatitis, cirrhosis and hepatocellular carcinoma. Much of the current knowledge of NF-κB signalling in the liver relates to the canonical pathway, the IKK (IκB kinase) complex and the RelA subunit. We explore the weaknesses of the experimental approaches to date and suggest that further work is needed to investigate in detail the discreet functions of each of the Rel subunits in liver physiology and disease.

INTRODUCTION

NF-κB (nuclear factor κB) was first described in 1986 as a nuclear transcription factor required for immunoglobulin kappa light chain transcription in B-cells [1]. Since then it has been demonstrated that NF-κB is constitutively expressed in all cell types and holds a central role as a regulator of the response to cellular stress. Classically, the NF-κB-mediated signalling pathway has been considered as both pro-inflammatory and anti-apoptotic in character and, as such, has been implicated in the pathogenesis of a wide variety of diseases, including inflammatory disorders and tumour development [2]. In the present review, we consider the role of NF-κB in the maintenance of liver homeostasis in health as well as its role in the development of a wide variety of liver pathology and, as such, its potential as a therapeutic target.

The term NF-κB does not refer to a single molecule, but rather a family of dimeric transcription factors consisting of the five Rel subunits, namely p50, p52, RelA (also known as p65), RelB and c-Rel. These subunits can be subdivided into two classes with RelA, RelB and c-Rel being synthesized as mature proteins, whereas p50 and p52 are synthesized as large precursor protein molecules (p105 and p100 respectively) that undergo proteolysis to produce the mature protein forms. Each of these subunits form a variety of functionally distinct hetero- and...
homo-dimers through binding with each other. The most abundant of these is the RelA:p50 heterodimer and it is this dimer which is most commonly referred to by the term NF-κB.

In the resting state, NF-κB dimers are predominately found in the cytoplasm in association with a member of the IκB (inhibitor of κB) family of proteins (IκBα, IκBβ, IκBε, Bcl3 and the C-terminal domains of p105 and p100). IκBα is the most well-characterized inhibitor and prevents NF-κB activation by masking its nuclear localization and DNA-binding domains. Phosphorylation-induced degradation of IκBα increases the rate of nuclear localization of NF-κB and allows DNA binding. The phosphorylation of IκBα is catalysed through the action of IKKs (IκB kinases) known as IKKα and IKKβ (also known as IKK1 and IKK2 respectively) in conjunction with the regulatory subunit NEMO (NF-κB essential modulator) [3]. Traditionally, the activation of NF-κB has been thought of as occurring through the so-called canonical and non-canonical pathways, although more recently other mechanisms of NF-κB activation, such as the response to DNA damage, have been recognized. Both the canonical and non-canonical pathways and their relationship to the maintenance of homoeostasis within the liver will be discussed in more detail below.

NF-κB dimers exert their nuclear effects through binding to 9–10 base pair DNA κB sites (consensus sequence 5′-GGGRRNWYYCC-3′, where R=A or C, N=any nucleotide, W=A or T, and Y=C or T) via the RHD (Rel homology domain) expressed on each of the subunits. Not all of the NF-κB subunits are able to directly influence transcription, with the p50 and p52 subunits lacking a TAD (transcriptional activation domain). The absence of a TAD means that homodimers of p50 and p52 generally function as repressors of transcription, although interactions of these homodimers with transcriptional co-activators can lead to transcriptional activation of a subset of target genes.

**CANONICAL PATHWAY**

The canonical NF-κB activation pathway, also referred to as the classical pathway, is the most well-described NF-κB activation mechanism and it is an intrinsic component of the innate immune response. The canonical pathway can be activated following a wide variety of stimuli, including pro-inflammatory cytokines and PAMPs (pathogen-associated molecular patterns) via their interaction with a variety of receptors belonging to either the TNFR [TNF (tumour necrosis factor) receptor] or TLR (Toll-like receptor)/IL (interleukin)-1 receptor superfamilies [4].

Irrespective of the nature of the receptor–ligand interaction, a common signalling pathway is triggered that leads to NF-κB activation (Figure 1). Upon phosphorylation, IKKβ is activated [5], resulting in phosphorylation of the naturally occurring NF-κB inhibitor IκBα at Ser32 and Ser36. IκBα subsequently undergoes polyubiquitination and degradation by the 26S proteasome [6]. This frees the p50:RelA heterodimer for nuclear translocation and binding to κB DNA motifs. Prior to exerting its transcriptional effects, the NF-κB subunit RelA undergoes further phosphorylation at a variety of sites including Ser276, Ser311, Ser529 and Ser536 [7,8], which serve to facilitate DNA binding, recruitment of co-activators and transactivation [7–11]. The importance of the Ser32 phosphorylation event, in particular, will be discussed later in the present review. It is likely that phosphorylation at these sites acts as a mechanism for fine tuning the NF-κB transcriptional response to a given stimulus [12].

IKKβ-mediated phosphorylation of IκBα is an essential element of the canonical pathway. Mice embryos homozygous for inactivated IKKβ (IKKβ−/−) are unable to reach term due to severe liver degeneration as a result of hepatocyte apoptosis. The regulatory subunit NEMO is also essential for canonical NF-κB activation as has been demonstrated in NEMO-knockout (NEMO−/−) mice, which suffer from fatal liver degeneration during embryonic development. Similar findings are also observed with RelA-knockout (RelA−/−) mice [13–15]. The pro-apoptotic stimulus in these mice is the cytokine TNF-α, since RelA−/− mice which are also TNF-α-deficient go on to have normal embryogenesis [16]. These experiments confirm the key role of NF-κB in regulating the transcription of anti-apoptotic genes and indicate that NF-κB is essential for liver development.

**NON-CANONICAL PATHWAY**

IKKα plays a pivotal role in the non-canonical, or alternative, pathway, which has a more limited repertoire of activators than the canonical pathway and includes BAFF (B-cell-activating factor), CD40 and LTβR [LTβ (lymphotoxin β) receptor]. Upon activation, IKKα phosphorylates p100 which then undergoes ubiquitination and proteasomal processing to generate p52 (Figure 1). This results in the liberation of the RelB:p52 dimer, which can then enter the nucleus to exert its transcriptional effects. The transcriptional targets of RelB differ from those of RelA, and the non-canonical pathway appears to be primarily concerned with the adaptive immune response and lymphoid organ development [4,17,18]. This is reflected in the phenotype of IKKα-deficient (IKKα−/−) mice, which have normal hepatic development but disordered limb and skeletal development [19]. To date, there has been little effort to gain insights into the functions of the non-canonical pathway in liver physiology and disease; however, there
is some evidence to suggest it may be important in modulating the response of the liver to injury and subsequent development of liver fibrosis.

**DNA DAMAGE AND NF-κB ACTIVATION**

NF-κB activation can occur following a variety of insults, including DNA damage in the form of double-strand breaks, which may occur following treatment with chemotherapeutic agents (e.g. camptothecin derivatives), ionizing radiation, errors in DNA replication or other mutagens [20]. A key player in the DNA-damage response is the ATM (ataxia telangiectasia mutated) protein kinase. Cells deficient in ATM are unable to activate NF-κB in response to exposure with camptothecin analogues [21,22]. ATM is normally held in the nucleus as an inactive dimer; however, in the presence of DNA double-strand breaks, the dimer is phosphorylated, liberating free ATM (Figure 1) [23]. NEMO, a key part of the IKK complex, is not sequestered in the cytoplasm, but is able to move freely between the nucleus and cytoplasm. Following genotoxic stress, NEMO forms a complex with PIDD (p53-inducible protein with a death domain) and RIP (receptor-interacting protein), which facilitates the sumoylation of NEMO [24,25]. After sumoylation, NEMO is phosphorylated by ATM prior to its ubiquitination. Following ubiquitination, NEMO forms a heterodimer with ATM, which then moves to the cytoplasm where it activates the IKK complex with resultant freeing of NF-κB dimers [26].

Activation of NF-κB following DNA double-strand breaks is associated with the transcription of anti-apoptotic genes such as Bcl-XL and XIAP (X-linked inhibitor of apoptosis). However, in addition to activating the NF-κB signalling pathway, DNA double-strand breaks also activate the pro-apoptotic p53 signalling pathway. The extent of DNA damage most probably determines the balance between these two signalling pathways and thus determines the fate of cells that are subject to genotoxic stress [23]. It is likely that the NF-κB DNA damage response plays an important role in the development of chemotherapy-associated liver injury, which is often seen in patients treated with
systemic chemotherapy for advanced colorectal cancer. This liver injury is associated with increased morbidity and mortality in patients who undergo potentially curative liver resection [27].

**CHOLESTASIS**

A good example of the role of NF-κB signalling in the maintenance of liver homeostasis is in the setting of cholestasis, which is characterized by the retention of bile within the liver, most commonly due to extra-hepatic biliary obstruction. In mice subjected to BDL (bile duct ligation), there is a marked increase in both ALT (alanine aminotransferase) and AST (aspartate aminotransferase), consistent with hepatocellular injury, at 4 days [28]. There is, however, very little in the way of detectable hepatocyte apoptosis. Furthermore, mRNA levels of various pro-inflammatory cytokines and anti-apoptotic genes under the transcriptional regulation of NF-κB are up-regulated in BDL mice [28]. In vitro exposure of hepatocytes to the bile acid glycochenodeoxycholic acid is associated with a marked increase in apoptosis and no demonstrable induction of NF-κB [28,29]. There is at first glance a contradiction between the in vivo and in vitro situation; however, this can be explained by examining the interaction between the various constituent cell types of the liver.

Cholestasis is associated with portal endotoxaemia as a result of bacterial translocation within the gut. Kupffer cells, the resident macrophages of the liver, are the cells that first come into contact with this bacterial endotoxin and are responsible for initiating the inflammatory response. In mice deplete of Kupffer cells, BDL injury is associated with more severe cholestasis when compared with wild-type mice. The protective effects of Kupffer cells in this model are associated with a marked increase in the expression of IL-6 [30]. It has also been demonstrated that TLR4 expression is raised within the livers of mice subjected to BDL [31]. The interaction of bacterial endotoxin with TLR4 activates the canonical NF-κB signalling pathway. Portal endotoxaemia occurring subsequent to cholestasis most probably results in Kupffer-cell-mediated production of pro-inflammatory cytokines including IL-6. These cytokines can then interact with appropriate receptors on hepatocytes to activate NF-κB signalling with the resultant transcription of anti-apoptotic genes that serve to protect hepatocytes from the toxicity associated with biliary stasis (Figure 2).

Recent work employing IKKα, IKKβ or dual IKKα/β knockouts targeted to hepatic parenchymal cells has revealed that the absence of either one of the kinases results in the remaining IKK activating the canonical pathway in response to either bacterial LPS (lipopolysaccharide) or TNF-α [32]. In this context, the IKKs therefore display a degree of functional redundancy. However, absence of both IKKα and IKKβ leads to significant hepatocyte apoptosis and the development of hepatic failure as a result of diminished RelA nuclear binding. Interestingly, IKKα/β-knockout mice also develop cholangitis as a result of bile duct destruction, predominantly affecting the small portal bile ducts. Although IKKα deficiency alone is associated with impaired tight junction formation in biliary epithelial cells, it was only in the absence of both kinases, and, hence, impaired RelA activity, that mice developed cholangitis. These findings demonstrate that the canonical NF-κB pathway not only determines the response to cholestasis, but also plays a key role in the maintenance of bile duct integrity [32].

Control of NF-κB activity is regulated at multiple levels and the response to a given insult can be modulated by a number of factors. One example of this is the FXR (farnesoid X receptor), which is a bile acid receptor that plays key roles in bile acid homeostasis and modulation of bile acid toxicity. Recent evidence has emerged that activation of FXR serves to negatively regulate the production of pro-inflammatory cytokines as a result of canonical NF-κB signalling without having an impact on the expression of anti-apoptotic factors [33]. This therefore demonstrates how, through intricate control of NF-κB in response to cholestasis, the liver strives to maintain homeostasis by promoting cell survival whilst minimizing the inflammatory insult.

**NAFLD (NON-ALCOHOLIC FATTY LIVER DISEASE)**

NAFLD is a disease entity that has increased in incidence over recent years and has therefore become the focus of much attention in the medical research arena. The term NAFLD covers a broad spectrum of disease, ranging from simple hepatic steatosis to steatohepatitis, and occasionally cirrhosis and end-stage liver disease [34]. NAFLD affects up to 25% of the North American population; its incidence is increasing across Europe and it is linked to the increasing prevalence of obesity [35,36]. The development of steatosis has been linked to the so-called ‘metabolic syndrome’, which is associated with obesity, Type 2 diabetes mellitus, hyperlipidaemia and insulin resistance [37,38]. Insulin resistance alone, however, is not sufficient for the progression from steatosis to steatohepatitis. A popular theory is that the steatotic liver is subject to a ‘second hit’, such as oxidative stress, endotoxaemia or cytokine exposure, which results in the progression to steatohepatitis [39].

Hepatic steatosis in NAFLD is thought to result from disordered expression of cytokines and adipokines from adipose tissue. Rather than being a simple fat-storing tissue, adipose tissue is now clearly acknowledged
Figure 2  Canonical NF-κB signalling in cholestasis

Cholestasis results in enteric bacterial translocation and subsequent portal endotoxaemia with binding of bacterial LPS to the TLR4 ligand on Kupffer cells. These cells produce pro-inflammatory cytokines through activation of NF-κB and nuclear localization of RelA. These cytokines, in turn, stimulate nuclear localization of RelA within hepatocytes which results in a subsequent increase in transcription of anti-apoptotic genes serving to protect the hepatocyte from toxic biliary stasis within the liver.

CIAP1, cellular inhibitor of apoptosis protein 1.

to be capable of secreting various pro-inflammatory cytokines such as TNF-α and IL-6, as well as fat hormones including leptin, resistin and adiponectin, all of which are capable of regulating metabolism in distant tissues [40]. Serum levels of pro-inflammatory cytokines are elevated in patients with NAFLD. These cytokines, through interaction with other factors, such as SREBP (sterol-regulatory-element-binding protein)-1c, result in hepatocyte insulin resistance and steatosis.

As already discussed, the pro-inflammatory cytokines TNF-α and IL-6 are both activators of, and end products of, the NF-κB signalling pathway. As such, it is no surprise that NF-κB has been implicated in the development of hepatic steatosis. Mice fed a high-fat diet, and Zucker fatty rats, have increased hepatic NF-κB and IKKβ activity compared with lean control animals [41,42]. Selective inhibition of hepatic NF-κB signalling prevents the development of hepatic steatosis when rodents are fed a high-fat diet [41,43,44]. Supporting the role of increased NF-κB-mediated transcription in the development of steatohepatitis, increased nuclear localization of RelA has been detected in liver biopsies of obese patients undergoing bariatric surgery [45].

Hepatocyte insulin resistance occurs as a result of defective insulin receptor signalling. The SOCS (suppressor of cytokine signalling)-3 protein is capable of preventing tyrosine phosphorylation of the insulin receptor upon ligand binding. Increased IL-6 expression, as a result of NF-κB signalling, results in increased hepatocyte expression of SOCS-3 [46,47]. In addition to its effects on the insulin receptor, SOCS-3 also mediates hepatocyte insulin resistance through increased expression of SREBP-1c by inhibition of the STAT (signal transducer and activator of transcription)-3 signalling pathway [48]. SREBP-1c regulates the expression of FAS (fatty acid synthase), which is responsible for the synthesis of de novo fatty acids [49,50].

Hepatic non-parenchymal cells, such as Kupffer cells, play key roles in the progression from simple steatosis to steatohepatitis. The importance of Kupffer cell NF-κB signalling for the development of steatohepatitis was demonstrated in mice lacking IKKβ in myeloid cells (IKKβΔmye) [51]. These animals had improved hepatic and global insulin sensitivity when fed a high-fat diet compared with control animals. It is likely that the inflammatory response generated by myeloid cells such as Kupffer cells serves as an initial inflammatory response that subsequently drives the insulin resistance seen in hepatocytes by activating the canonical pathway within these cells [51].

PPAR (peroxisome-proliferator-activated receptor)-α is a nuclear-bound, ligand-activated, transcription factor...
which recognizes PPREs (PPAR response elements) in the promoter region of target genes. Its main role is in the control of lipid metabolism, in particular the β-oxidation of non-esterified (‘free’) fatty acids [52]. In addition, PPAR-α is able to act as a direct inhibitor of NF-κB-mediated pro-inflammatory signalling both through direct interaction with RelA as well as through the up-regulation of IkBa [53,54]. In OLETF fatty rats, which overeat and develop steatosis, PPAR-α expression is significantly reduced compared with control animals [55]. In addition, PPAR-α-deficient mice develop hepatic steatosis [56]. These findings led to the suggestion that the PPAR-α agonists such as bezafibrate and fenofibrate may be of benefit in the prevention and treatment of NAFLD both through improved hepatic lipid metabolism and through attenuation of NF-κB-mediated pro-inflammatory signalling. This hypothesis has been upheld in animal studies, where mice fed an MCD diet (methionine and choline-deficient diet) had increased expression of factors associated with fatty acid β-oxidation alongside decreased expression of pro-inflammatory cytokines, such as TNF-α and IL-6, when treated with bezafibrate [57]. This reduction in cytokine expression was associated with less steatosis and inflammation on histological examination of the liver [57]. Further work is now needed, in the form of clinical trials, to establish the role of the PPAR-α agonists in the treatment of patients with NAFLD.

**VIRAL HEPATITIS**

Viral hepatitis represents a major global health problem with approx. 350 million people worldwide chronically infected with the HBV (hepatitis B virus) and 180 million with HCV (hepatitis C virus) (http://www.who.int/topics/hepatitis/en/index.html). HBV infection often has a benign course; however, in a significant proportion of patients it can progress to cirrhosis and the development of HCC (hepatocellular carcinoma). Similarly with HCV, up to 35% of patients will develop significant liver pathology over the course of their disease, with 20% of patients developing cirrhosis within 20 years of infection [58]. There is a growing body of evidence that suggests that hepatic NF-κB is activated in patients with viral hepatitis and may play a key role in the development of the complications associated with chronic infection.

HBV contains a 3.2 kb strand of circular DNA which replicates in hepatocytes and secretes a variety of antigens such as HBsAg (hepatitis B surface antigen), HBeAg (hepatitis B e antigen) and HBXAg (hepatitis B X antigen). HBXAg is a regulatory protein responsible for viral gene expression and has been implicated in NF-κB activation in HBV infection. As early as 1992, it was demonstrated that transfection of HepG2 cells with HBXAg was associated with increased NF-κB-mediated transcription [59]. Subsequent work in HeLa cells showed that HBXAg transfection is associated with the degradation of IkBα [60]. It has been suggested that increased RelA-mediated transcription of anti-apoptotic factors may help protect infected hepatocytes from cell death and thus promote carcinogenesis [61].

In contrast with HBV, HCV is a single-strand positive-sense RNA virus and thus does not integrate into the host genome. The HCV genome encodes several structural and non-structural peptides, and these can play a role in NF-κB activation by the virus. Transfection of HepG2 cells with HCV structural proteins, in particular the core protein, leads to nuclear translocation of both RelA:p50 heterodimers and p50 homodimers, and resistance to TNF-α-mediated apoptosis, which was reversed with the NF-κB inhibitor PDTC (pyrrolidine dithiocarbamate) [62]. The HCV core protein is able to interact with both LTβR and TNFR1, and it is likely that interaction with these receptors activates canonical and non-canonical forms of NF-κB [63,64]. The HCV core protein is capable of interacting with IKKβ in a macrophage cell line, although in this setting it acts as a suppressor of NF-κB. Whether the same holds true in infected hepatocytes is not known [65]. In addition to the role of the core protein, the non-structural protein NS3 induces NF-κB activity in both HepG2 and HuH7 cells through enhanced IKKα activity and proteolytic breakdown of IkBα [66].

In an attempt to clear both HBV and HCV, the Fas ligand of cytotoxic T-cells binds to the Fas receptor on hepatocytes with subsequent activation of pro-apoptotic signalling pathways through the activation of caspase 8 [67,68]. HepG2 cells transfected with HBXAg are resistant to anti-Fas-mediated killing in an NF-κB-dependent manner [69]. This resistance to Fas-mediated apoptosis occurs, in part, through increased expression of the novel gene URG7 (up-regulated gene 7), which appears to block the activation of caspases 3 and 8. Transcription of URG7 appears to be regulated by NF-κB [70,71].

Activation of NF-κB by both HBV and HCV can therefore make a significant contribution to the persistence of chronic infection and its sequelae. One of the most commonly employed therapeutic strategies in patients with chronic active viral hepatitis is IFN-α (interferon-α), which is able to block NF-κB activation in HBXAg-transfected HuH7 cells despite the high level expression of HBXAg [72].

**LIVER REGENERATION**

The liver holds a privileged position within the human body in that it is the only solid organ able to regenerate following injury without the involvement of stem cells. This regenerative capacity of the liver is key to its
ability to recover from a variety of insults such as inflammation and injury with restoration of functional hepatic parenchyma. The ability of the liver to regenerate has been exploited in modern liver surgical practice in the resection of both primary and metastatic liver tumours. Liver regeneration can be significantly impaired in the presence of underlying liver disease. In this section, however, we will focus on the role of NF-κB in the regeneration of the normal liver.

Liver regeneration is a highly orchestrated process that begins immediately following injury and loss of liver mass [73]. Initially, non-parenchymal cells play a key role in releasing autocrine and paracrine growth factors that then stimulate hepatocytes, through activation of various cell signalling pathways, to undergo mitosis in a co-ordinated fashion. Many of these growth factors, such as cell-cycle regulators and inflammatory cytokines (e.g. TNF-α and IL–6), are subject to transcriptional regulation by NF-κB and, therefore, it would appear logical that NF-κB plays a key role in the regulation of liver regeneration. Various groups have tried to unpick the role of NF-κB in liver regeneration and this has resulted in the publication of what, at face value, appears to be conflicting and somewhat confusing results, some of which are discussed below.

As far back as 1994, it was demonstrated that in rats subjected to partial hepatectomy there is activation of the RelA:p50 dimer in whole-liver nuclear extracts [74]. It is generally accepted that the priming signal for hepatocyte NF-κB activation is TNF-α production by Kupffer cells, which subsequently binds to TNFR1 thereby activating the canonical pathway. Adenoviral transfection with a super-repressor form of the IκBα gene (ΔN-IκBα) reduces TNF-α mediated increases in IL–6, STAT-3 and cMyc mRNA with impairment of DNA synthesis and hepatocyte replication [75,76]. Furthermore, mice treated with the fungal metabolite gliotoxin (an inhibitor of TNF-α-dependent NF-κB activation) 4 h prior to partial hepatectomy had reduced NF-κB activation during liver regeneration with associated increased hepatocyte apoptosis, reduced DNA synthesis and impaired cell-cycle progression [77].

Transgenic mice which express ΔN-IκBα upon stimulation with a progesterone antagonist had impaired NF-κB activation, but this was not associated with perturbed hepatocyte regeneration or increased hepatocyte apoptosis. Indeed, there is increased transcription of the anti-apoptotic gene Bcl-XL in the absence of NF-κB during liver regeneration, suggesting the activation of other anti-apoptotic pathways may be important [78]. Similarly, pre-treatment of rats undergoing partial hepatectomy with either pentoxifylline or PDTC (which disrupt the activation of NF-κB) does not result in impaired liver regeneration or DNA synthesis [79].

Mice with hepatocyte-specific IKKβ knockout (IKKβΔHEP) have also been utilized in partial hepatectomy studies to determine the role of this kinase in liver regeneration. As might be expected, deletion of IKKβ results in delayed nuclear translocation of p65 within hepatocytes at early time points following partial hepatectomy, being detectable at 1 h in wild-type animals compared with 3 h in IKKβΔHEP animals [80]. However, rather than being associated with impaired liver regeneration, the IKKβΔHEP phenotype is associated with earlier entry of hepatocytes into the cell cycle and enhanced liver regeneration. This has been attributed to a stronger acute-phase response to surgery in these animals [80]. Interestingly, mice treated peri-operatively with the systemic IKKβ inhibitor AS602868 had no differences in liver regeneration compared with untreated animals [80].

As already mentioned, it is thought that non-parenchymal cells, such as Kupffer cells, provide the stimulus for hepatocyte regeneration. Using transgenic mice that express EGFP (enhanced green fluorescent protein) upon NF-κB activation, it has been shown that there is significant activation of NF-κB within Kupffer cells following partial hepatectomy [81]. The importance of Kupffer cells has, however, been called into question in studies utilizing gadolinium chloride prior to partial hepatectomy to eradicate macrophages in the liver. In these animals, gadolinium chloride administration actually results in enhanced liver regeneration [82]. On the other hand, studies in hepatectomized mice utilizing liposome-encapsulated dichloromethylene diphosphonate, a selective depleter of Kupffer cells, demonstrate impaired liver regeneration and, in particular, a significant reduction in whole liver RelA nuclear localization [83].

Given the contradictory findings concerning the role of NF-κB in the regenerating liver, it is currently difficult to draw definitive conclusions. The methodologies applied in these studies may underpin some of the differences seen. For example, a key target of NF-κB in regenerating hepatocytes is IL-6; however, the administration of adenoviral vectors alone can increase IL-6 transcription therefore suggesting that perhaps this negates the effect of administration of the IkBα super-repressor [82]. Similarly, gadolinium chloride administration results in cytokine activation and may negate the effect of Kupffer cell depletion [83]. However, a wide variety of signalling pathways play a role in liver regeneration and thus far it has not been demonstrated that blocking any one of these pathways can result in a failure of liver regeneration. This implies that this process is of such importance that evolution has provided a variety of alternative mechanisms through which liver regeneration can proceed should one pathway fail.

One glaring deficiency in studies investigating NF-κB and liver regeneration to date is the lack of information regarding the nature of the NF-κB complexes that are recruited to genes involved either in the priming of the response of non-parenchymal cells or those controlling
DNA synthesis and the cell cycle in the hepatocyte. Chromatin immunoprecipitation studies combined with subunit-specific knockout mice would supply such information and lead to an improved understanding of the role of NF-κB in the regenerative response of the injured liver.

**HEPATIC I/R (ISCHAEMIA/REPERFUSION) INJURY**

Hepatic I/R injury occurs following a period of liver oxygen deprivation with subsequent reperfusion. It is characterized by the generation of ROS (reactive oxygen species) and results in an acute inflammatory response within the liver which is accompanied by hepatocellular death [84]. Clinically, this problem is encountered during hepatic resection when the hepatic inflow is deliberately occluded to minimize blood loss during parenchymal transection. It is also commonly seen in transplanted organs which are subject to prolonged periods of ischaemia. In addition, this mechanism of liver injury is seen in patients who experience prolonged systemic hypotension, e.g. during haemorrhagic or septic shock.

Activation of NF-κB has been demonstrated in animal models of I/R injury and orthotopic liver transplantation; however, there is much debate within the literature as to whether this activation serves to promote I/R injury due to the pro-inflammatory nature of NF-κB or to ameliorate the effect of I/R injury because of its anti-apoptotic effects [85,86]. It has been suggested that, in this setting, NF-κB activation occurs as a direct result of ROS-mediated activation of Src tyrosine kinases which are able to directly phosphorylate IκBs without the involvement of IKKα and IKKβ [87]. If this were the case, however, one would expect that deletion of NEMO would not have an effect on NF-κB activation following I/R, which is not the case [88]. Using a conditional hepatocyte-specific NEMO-knockout mouse, it has been demonstrated that there is an absence of RelA activation within hepatocytes following I/R, whereas there is extensive RelA activation within the non-parenchymal cells of the liver which is associated with a greater inflammatory response when compared with the livers of control animals [88]. The NEMO-knockout mice in this model also have more marked hepatocyte necrosis in response to I/R injury, suggesting that hepatocyte NF-κB activation in this context serves not only an anti-apoptotic role, but also to downregulate the inflammatory response generated in non-parenchymal cells [88].

The results generated from the conditional NEMO-knockout mouse is, however, contradicted by a similar study by the same group using the hepatocyte-specific IKKβ-knockout (IKKβ\(^{ΔIHEP}\)) mouse, which had reduced hepatocyte necrosis and hepatic inflammation following I/R [89]. This was associated with impaired nuclear translocation of RelA [89]. Similar effects were also observed in mice treated with the pharmacological IKKβ inhibitor AS602868 prior to I/R injury [89]. Likewise, work from another group utilizing adenoviral transfection of hepatocytes and Kupffer cells with a super-repressor form of IκBα observed impaired nuclear translocation of RelA in response to I/R injury, as well as reduced hepatic inflammation and hepatocyte necrosis [90]. Although the IKKβ\(^{ΔIHEP}\) and IκBα super-repressor models reduce RelA nuclear translocation compared with control animals, they do not, however, completely abrogate it, unlike the conditional NEMO-knockout mouse [88]. It has been suggested that the low level of RelA seen in these latter models may be sufficient to mediate an anti-apoptotic effect of NF-κB. It does, however, have to be borne in mind in all of these studies that the upstream kinases in the NF-κB signalling pathway are able to cross-talk with a wide variety of other signalling pathways and, as such, their inhibition does not equate to a specific inhibition of NF-κB alone.

The concept that hepatocyte-specific activation of NF-κB can serve to promote cell survival in the face of I/R injury is supported by several other studies. For example, a short period of ischaemic pre-conditioning in mice prior to I/R injury results in increased activation of NF-κB signalling thereby priming hepatocytes to enter the cell cycle early, through increased expression of cyclin D1, with a subsequent reduction in hepatocyte necrosis compared with non-preconditioned animals [91]. Mice subjected to I/R injury under conditions of profound hypothermia (\(<33^\circ\)C\) had increased levels of RelA nuclear translocation within hepatocytes, but diminished nuclear translocation within Kupffer cells compared with normothermic animals [92]. Those animals subjected to I/R at body temperatures below 33°C correspondingly have reduced levels of hepatic inflammation and necrosis compared with normothermic controls and, indeed, those animals subjected to I/R at temperatures below 29°C failed to show any evidence of liver injury [92]. The increase in hepatocyte RelA nuclear translocation in hypothermic animals occurs independently of IκB degradation and has been attributed to increased Pin1 expression. Pin1 is a regulator of phosphorylation-mediated protein activation and, interestingly, Pin1-knockout (Pin1\(^{−/−}\)) mice have increased hepatocyte death without any effect on the inflammatory response to I/R injury [93]. Of note, Pin1\(^{−/−}\) mice have reduced nuclear RelA translocation within hepatocytes but not Kupffer cells following I/R injury. Moreover, Pin1 does not appear to be capable of regulating NF-κB within Kupffer cells [93].

On balance, the effect of NF-κB on the response to I/R injury needs to be viewed in relation to individual cell types within the liver rather than the whole organ. In response to I/R injury, NF-κB activation within Kupffer cells results in a predominantly
pro-inflammatory phenotype with a detrimental effect on the liver. Within hepatocytes NF-κB activation imparts a survival advantage in the face of I/R injury with a subsequent reduction in hepatocyte necrosis. The exact details of the mechanisms which lead to NF-κB activation following I/R injury are unclear and need to be examined in more detail, but it may be that these mechanisms also differ between parenchymal and non-parenchymal cells. Cell-specific targeting of NF-κB signalling may be necessary before therapeutic advances can be made in reducing the impact of I/R injury on the liver.

**LIVER FIBROSIS/CIRRHOsis**

Irrespective of aetiology, the end stage of virtually all chronic liver disease is fibrosis and progression to cirrhosis. HSCs (hepatic stellate cells) are a major source of fibrogenic myofibroblasts which underpin the development of hepatic fibrosis. Activated myo-fibroblastic HSCs express high levels of constitutively active NF-κB which can be elevated further in response to environmental stimuli such as ligand binding to CD40 [94]. Treatment of activated rat HSCs with the fungal metabolite gliotoxin results in inhibition of NF-κB DNA binding and apoptosis. Using the carbon tetrachloride model of liver fibrosis, work from our laboratory has shown that administration of a single dose of gliotoxin results in HSC apoptosis in vivo with subsequent decreases in liver fibrosis [95]. Furthermore, when gliotoxin is selectively targeted to activated HSCs it can induce reversal of fibrosis despite ongoing liver injury [96].

More recently, we have shown that administration of the IKK inhibitor sulphasalazine to rats with established fibrosis results in a significant improvement in fibrosis pathology scores, as well as decreased expression of the pro-fibrogenic genes α-SMA (α-smooth muscle actin), TIMP-1 (tissue inhibitor of metalloproteinases-1) and pro-collagen I [97]. Additional studies have revealed that the elevated constitutive NF-κB activity is responsible for the high resistance of human HSC-derived myofibroblasts to apoptosis [98]. This constitutive NF-κB activity results from transcriptional suppression of IkBa expression [99] and constitutive phosphorylation of Ser536 on the RelA subunit [100]. Phosphorylation of RelA at Ser536 is essential for the survival of human and rodent HSC-derived myofibroblasts and is maintained by an autocrine renin–angiotensin signalling pathway. This pathway activates IKKβ, which phosphorylates RelA at Ser536, enabling its nuclear translocation, stimulating the expression of anti-apoptotic genes including Bcl2 and Gadd45β (growth-arrest and DNA-damage-inducible protein 45β). Angiotensinogen, the substrate for the renin–angiotensin pathway, is itself an NF-κB-responsive gene and as a result a positive feedback loop is established that ensures survival of the myofibroblast and progression of fibrosis. Blockade of IKK, ACE (angiotensin-converting enzyme) or AT1 receptor (angiotensin II type 1 receptor) in myofibroblasts results in apoptosis and regression of established fibrosis [100]. Treatment of patients with liver fibrosis secondary to chronic hepatitis C with the AT1 receptor antagonist losartan for 18 months resulted in regression of liver fibrosis in 50% of patients [100]. Immunohistochemical analysis of tissue obtained from these patients demonstrated that those who responded to this therapy had higher numbers of RelA phosphorylo-

**INFLAMMATION–FIBROSIS–CANCER AXIS**

It is increasingly recognized that NF-κB signalling plays a pivotal role in driving the progression from hepatic inflammation to the development of HCC. As already described, chronic inflammation is associated with activation of HSCs and Kupffer cells in an NF-κB-dependent manner. These activated non-parenchymal cells secrete multiple hepatomimetogens, such as TNF-α, IL-6 and HGF (hepatocyte growth factor), which promote the growth of transformed hepatocytes [102,103]. NF-κB activation has been demonstrated in hepatocytes adjacent to areas of inflammation and is, at least in part, likely to be responsible for the development of HCC in these regions. This tumour-promoting property of NF-κB is probably a result of cytokine secretion by non-parenchymal cells which drive the inflammatory process [104].

The traditional view that NF-κB acts in an anti-apoptotic manner would suggest that, in the context of HCC, it would serve to promote tumour growth
and development; however, recent evidence obtained from mice with hepatocyte-specific IKKβ deletion (IKKβΔHEP) suggests that this is not the case and that in fact, at least in the context of the hepatocyte, NF-κB functions as a tumour suppressor [105]. In the DEN (diethylnitrosamine) model of chemically induced HCC, it has been observed, somewhat surprisingly, that in IKKβΔHEP mice there is a 3-fold increase in the number of tumours seen compared with wild-type mice; however, when NF-κB activity is also blocked in non-parenchymal cells, such as Kupffer cells, there is a 4-fold decrease in the number of tumours which develop compared with wild-type mice [105]. This appears to suggest that NF-κB in non-parenchymal cells drives the production of cytokines and other factors associated with HCC development, whereas in hepatocytes its activation serves as a tumour suppressor [105]. In hepatocytes, a key role of NF-κB may be to negatively regulate JNK (c-Jun N-terminal kinase) activity. Using the DEN model, IKKβΔHEP mice crossed with mice deficient in JNK1 (IKKβΔHEP/JNK−/−) have a 3-fold lower tumour number compared with IKKβΔHEP/JNK+/+ mice [106]. Indeed, IKKβΔHEP/JNK−/− mice have similar HCC formation following DEN exposure compared with wild-type mice, strongly suggesting that one major role of NF-κB in modulating HCC development is JNK regulation within hepatocytes [106]. Recent in vitro work has suggested that NF-κB acts as a negative regulator of growth in hepatocytes again adding weight to the argument that it functions as a tumour suppressor in the context of HCC development (Figure 4) [107].

Mice with hepatocyte-specific NEMO deletion (NEMOΔHEP) spontaneously develop steatohepatitis, hepatocyte apoptosis and formation of regenerative nodules by 6 months of age and HCC by the age of 9–12 months [108]. NEMOΔHEP mice also have constitutive JNK activation, supporting the observations described above. Interestingly, the development of hepatic pathology in these mice was ameliorated by treatment with antioxidants. This phenotype is not observed when IKKβ alone is inhibited, perhaps because IKKα provides an alternative means for IkBα phosphorylation in this setting [108]. More recently, it has been demonstrated that the NEMOΔHEP phenotype is associated with increased hepatocyte-mediated sensitivity to TRAIL (TNF-related apoptosis-inducing ligand)-mediated apoptosis, at least in part through the activation of hepatic natural killer cells [109]. Mice with hepatocyte-specific deletion of RelA (RelAΔHEP) similarly have increased sensitivity to TNF-α-mediated apoptosis. It is of note, however, that the RelAΔHEP mouse fails to display the liver disease phenotype associated with the NEMOΔHEP mouse. This discrepancy raises the possibility that NEMO deletion is not specifically inhibiting NF-κB-mediated signalling, but may also exert effects on other signalling pathways influencing liver physiology [110].

Much of the work related to the role of NF-κB in HCC development and progression has concentrated on the role of the RelA subunit. Immunohistochemical analysis of tumour tissue from patients has demonstrated, however, that there is increased nuclear localization of p50/p52 and this is linked to the overexpression of Bcl-3, a member of the IkBa family of proteins [111]. It therefore appears likely that the precise role of the NF-κB family of proteins in the development and progression of HCC is not straightforward and much more work still needs to be done to unravel the complex molecular mechanisms underpinning the development of this disease [111].

**CONCLUSIONS**

It is now clear that NF-κB is an important regulator of normal and pathological events in the liver and there is real potential for the transcription factor and its system of regulators to be exploited in the treatment and prevention of liver disease and cancer. However, many deficiencies in our knowledge remain and, indeed, we are still unclear as to the precise role of NF-κB in processes such as liver regeneration and HCC, where
Figure 4 Inflammation–fibrosis–cancer axis

Chronic liver injury results in hepatocyte apoptosis with subsequent activation of non-parenchymal cells, such as HSCs and Kupffer cells, which results in the production of pro-inflammatory cytokines and hepatic mitogens that serve to activate NF-κB signalling within hepatocytes. In addition to its traditional anti-apoptotic roles, nuclear localization of p65 is thought to have a tumour-suppressor role through decreased transcription of JNK1. Nuclear localization of p50/p52 results in the overexpression of Bcl-3, a member of the IκB family of proteins. HGF, hepatocyte growth factor.

Contradictory or controversial results prevail. Although there has been a dramatic increase in our knowledge of the role NF-κB plays in the maintenance of liver homoeostasis and the development of hepatic pathology, this knowledge predominantly relates to the canonical pathway, the IKK complex and the RelA subunit. The other Rel subunits will also exert influences on the response to liver injury, perhaps through the modulation of the NF-κB response. For example, work in our laboratory has shown that NF-κB1-knockout (NF-κB1−/−) mice develop severe inflammation and hepatic fibrosis in response to chronic administration of carbon tetrachloride [112]. This is associated with the expression of TNF-α in activated HSCs, a cytokine not normally produced by this cell type. TNF-α expression is normally curtailed by p50 homodimeric products of the NF-κB1 gene which recruit the transcriptional co-repressor HDAC1 (histone deacetylase 1) to the TNF-α promoter [112]. We have also recently reported the attenuation of inflammation, fibrogenesis and hepatocyte proliferation in c-Rel-knockout mice with intrinsic defects in the functions of non-parenchymal and parenchymal cells [113]. These studies highlight the need to recognize and investigate in detail the discreet functions of the Rel subunits in liver physiology and disease.

Manipulation of the NF-κB signalling pathway has the potential to be a therapeutic strategy in the management of liver fibrosis, and the use of specific inhibitors of NF-κB may become a feature in the management of patients with chronic liver disease. One must, however, exercise caution with this strategy because of the varied roles of the NF-κB subunits. For example, although it may be attractive to inhibit NF-κB in HSCs of the fibrotic liver, inhibition of NF-κB in hepatocytes may have deleterious results in terms of tumour development in these patients. Therefore it may be that therapy to augment NF-κB signalling needs to be cell-type-specific and that the use of systemic NF-κB agonists/antagonists is not advisable.

One also needs to be careful at what level in the NF-κB signalling cascade therapy is targeted. The actions of IKKα and IKKβ are not limited to the NF-κB signalling pathway, but have an impact on a wide variety of other cellular processes; for example, IKKα activation results in increased cyclin D1 expression, whereas IKKβ...
can have effects on insulin receptor signalling (for a review of the functions of the IKKs, see [114]). It must therefore be borne in mind that upstream inhibition of members of the NF-κB signalling cascade does not equate precisely to inhibition of NF-κB signalling. It may be that the most effective strategy for therapeutically modulating NF-κB responses is to target post-translational modifications of the individual Rel subunits (e.g. Ser536 of RelA), enabling more specific therapy and minimizing the effects on other cell signalling pathways. In order for this to be done effectively, much more work is still needed to characterize the roles of each of the Rel subunits in hepatic pathophysiology and the regulation of their activities by post-translational modification.

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