Hypothosis: Targeted Ikkβ deletion upregulates MIF signaling responsiveness and MHC class II expression in mouse hepatocytes

Katherine S Koch
Hyam L Leffert

Hepatocyte Growth Control and Stem Cell Laboratory, Department of Pharmacology, School of Medicine, University of California, San Diego, CA, USA

Abstract: Macrophage migration inhibitory factor (MIF) is causally related to the pathogenesis of chronic liver disease but its hepatocellular mechanisms of action are largely unknown. Scattered reports in the literature hint at functional connections between the expression of MIF and major histocompatibility complex (MHC) Class II molecules. Not surprisingly, these relationships have not yet been explored in hepatocytes because MIF and MHC Class II cell surface receptors are commonly expressed by other cell types including various antigen presenting cells of the immune system. On the other hand, mounting evidence suggests that heteromeric MIF receptors share a common molecule with intracellular MHC Class II complexes, viz., CD74, which also serves as the MHC Class II chaperone; and, while it is unclear what cancer-related role(s) MHC Class II receptors might play, increasing evidence suggests that MIF and CD74 are also implicated in the biology of hepatocellular carcinoma. These reports are provocative for two reasons: firstly, IkkβΔhep mice carrying hepatocyte-targeted deletions of Ikkβ, an IκB kinase complex subunit required for the activation of the transcription factor NF-κB (nuclear factor-κB), have been shown to display heightened susceptibilities to hepatotoxins and chemical hepatocarcinogens; secondly, microarray profiling observations indicate that IkkβΔhep hepatocytes constitutively and “ectopically” overexpress genes, particularly CD74, CD44 (a MIF-receptor subunit) and MHC Class II I-A/E β and I-A α chains, and gene families that regulate host immune process and immune defense responses. These findings together suggest that IkkβΔhep mice might express functional MIF and MHC Class II receptors, leading to increased hepatocellular sensitivity to MIF signaling as well as to the unusual property of antigen presentation; both functions might contribute to the heightened liver disease phenotypes of IkkβΔhep mice. The findings raise questions about the potential existence of cohorts of human patients with genetic abnormalities of Ikkβ that might confer heightened susceptibility to liver disease including hepatocellular carcinoma.

Keywords: hepatocellular toxicity, inflammation, immunity, carcinoma

Introduction

Liver disease is a major cause of suffering worldwide and is associated with a significant financial burden.1–4 In the US alone, based on statistics compiled in 2008, 36,000 people died from liver disease in 2004 and one of every six deaths resulted from primary hepatocellular carcinoma (HCC), one of the most aggressive malignancies known.4 The financial cost of all liver disease was US$13 billion, almost two-thirds of which were accounted for by HCC and viral hepatitis.4 Although HCC is associated with exposure to hepatotoxic environmental carcinogens and toxins,5 with clinical disorders including autoimmune hepatitis, primary biliary cirrhosis and sclerosing cholangitis,6,7 and with a three-fold higher male gender preference,8 its major associations with viral hepatitis infection (particularly...
HBV and HCV) and chronic liver inflammation have been known for many years and widely investigated.9,10

HCC continues to be an intractable human health problem. It is endemic to Southeast Asia and Africa11 and its incidence is rising worldwide, particularly in the US.12 For example, in 2006, the American Cancer Society recorded HCC in roughly 18,000 American men and women, with an overall death rate of 90%.11,12 Of the 7.4 million cancer deaths worldwide, the World Health Organization ranked death rates from HCC as fourth (610,000 deaths) among the five most prevalent malignancies (along with lung, gastric, colorectal and breast). This ranking is 18% higher than for breast cancer (519,000 deaths).13

In addition to these grim statistics, the precise molecular and cellular mechanisms that cause HCC have been difficult to identify, not only because of the complexities inherent in dissecting the initiation and progression of malignant disease, but also because of the limited development of experimental laboratory models which accurately and reliably reflect various human HCC etiologies. However, recent work at the basic science level suggests these difficulties are being addressed in meaningful ways. For example, increasing genomics analyses have identified many genetic signaling systems that govern hepatocellular carcinogenesis, and which can be corroborated in several murine model systems.14,15 In relation to these genetic findings, the mouse model of HCC induced by the procarcinogen diethylnitrosamine (DEN), has been suggested to reflect a course etiologically comparable with that of human HCC.15

**Ikkβ<sub>Δhep</sub> mice: A model system for studies of augmented susceptibility to hepatotoxicity, DEN induction of HCC and hepatocyte growth advantages**

One promising mouse model system, notable for its concomitant use of related but distinct gene-targeted strains to reveal intrahepatic cell-cell interactions involved in DEN-induced hepatocarcinogenesis, has focused on the Ikkβ gene.36 Ikkβ encodes an 87 kDa protein kinase subunit (IKKβ) of the IκB complex (IKK).17 As an IKK member, Ikkβ is ubiquitously required for activation of nuclear factor-κB (NF-κB).17,18 A multifunctional transcription factor that governs innate and adaptive immunity, cell survival and cellular susceptibility to carcinogenesis.19,20 However, Ikkβ also has other signaling roles in many different types of cells.17–21

Parallel in vivo and in vitro investigations have led to the hypothesis that hepatic Ikkβ has a bifunctional role as an hepatocellular growth and tumor suppressor,16,21 and as an NF-κB-dependent regulator of liver nonparenchymal cell (NPC) cytokine secretion.16,21–23 Paradoxical aspects of this hypothesis have been supported by experiments with two different strains of conditional knockout mice. Both strains are on a C57BL/6 Ikkβ<sup>F/F</sup> background, in which the Ikkβ exon 3 and most of the kinase domain are flanked by Cre-recombinase lox sites, the span of which is targeted for deletion by the specific promoter-driven expression of Cre-recombinase. The Alb-Cre-driven strain, in which augmentation of DEN-induced HCC formation occurs, is characterized by Ikkβ<sup>Δhep</sup> in hepatocytes alone (Ikkβ<sup>Δhep</sup> mice), because deletion is targeted by an albumin promoter which controls Cre-recombinase expression. In the sister strain, Mx1-Cre, in which DEN-induced HCC formation is attenuated, the Mx1-Cre promoter, in response to poly(IC) treatment, drives expression of Cre-recombinase to delete Ikkβ in hepatocytes as well as in intra- and extrahepatic myeloid cells (Ikkβ<sup>Δhep</sup> mice).16

Initial findings with Ikkβ<sup>Δhep</sup> mice indicated that, compared with control Ikkβ<sup>F/F</sup> mice, hepatotoxicity was augmented significantly by treatments with Con-A or LPS/galactosamine.24 Augmented toxicities in Ikkβ<sup>Δhep</sup> mice, attributed to requirements for Ikkβ for the prevention of hepatocyte apoptosis, were mediated by T-cell bound TNFα activation of hepatocyte TNFα-receptor 2, with subsequent reactive oxygen species (ROS) formation, leading to the inhibition of mitogen-activated protein kinase (MAPK) phosphatases, and thus resulting in sustained JNK1 activation.25 Working with Ikk<sub>2.Δhep</sub> mice deleted in Ikkβ exons 6–7 on a different strain background,26,27 others subsequently reported that Ikkβ<sup>Δhep</sup> did not sensitize TNFα-induced hepatocyte apoptosis;26 rather, hepatocyte survival in Ikk<sub>2.Δhep</sub> mice depended upon Ikkα/γ subunits.28 These differences remain unclarified28 but might reflect different TNFα injection protocols; alternatively, delayed S-phase peaks after 70% partial hepatectomy (PH)17,30 might also suggest that Ikk<sub>2.Δhep</sub> mice are differentially affected by exon 6–7 targeting.

Subsequent HCC-induction studies using both Ikkβ<sup>Δhep</sup> and Ikkβ<sup>Δhep/LIV</sup> strains showed that, compared with Ikkβ<sup>F/F</sup> mice, several DEN-dependent events were augmented in Ikkβ<sup>Δhep</sup> mice: ROS formation, hepatocyte apoptosis and necrosis; prolonged activation of hepatic c-jun nuclear kinase 1 (JNK1); release of signaling and growth regulatory molecules from hepatocytes and liver NPCs, respectively; compensatory hepatocyte proliferation (CHP); and, eventual multinodular HCCs presumably derived from Ikkβ<sup>Δhep</sup> hepatocytes.16 Further studies with DEN indicated that IL-1α, an “alarmin” released...
from necrotic hepatocytes, stimulated Kupffer cells (KCs) to secrete IL-6, which, in turn, augmented JNK1 activation, proliferation of DEN-initiated Ikkβhep hepatocyte survivors and subsequent progression to HCC.22 These observations positioned augmented ROS formation and hepatocyte death as major determinants of augmented HCC during hepatocarcinogenesis in Ikkβhep mice.

However, it was also reported that, compared with IkkβF/F hepatocytes, Ikkβhep hepatocytes in primary culture showed cell-autonomous growth advantages including enhanced recovery efficiency, precocious cyclin D1 expression, elevated S-phase BrdU-labeling indices (LIIs), and enhanced growth-factor sensitivity to hepatocyte mitogens like TGFα and TNFα.21 The same study showed that, compared with IkkβF/F hepatocytes, Ikkβhep hepatocytes also displayed in vivo growth advantages during liver regeneration following 70% PH; these consisted of precocious elevations in PCNA, cyclin D1 and S-phase LIIs (ie, shortened G0→G1 transitions), and elevated numbers of mitotic figures. Most recently, a series of syngeneic transplantation and pharmacologic studies31 has suggested that the augmented intrinsic capacity of cell-autonomous proliferation reflected in hepatocyte proliferative advantages conferred by Ikkβ deletion21 or Ikkβ inhibition, or the proliferative disadvantages conferred by IKKβ overexpression, are also determinants of HCC promotion and progression in IkkβF/F and Ikkβ mice.21,31

This conclusion is consistent with the well established fact that chemical hepatocarcinogenesis requires cell proliferation during the phase of tumor “initiation”, when cell selection generates survivors that propagate carcinogen-induced growth-altering mutations, and during the phases of tumor promotion and progression, which allow further proliferative expansion and evolution of tumor cell populations that have escaped normal growth controls. Current hypotheses emphasize ROS formation and cell death as major causes of KC activation and CHP, rather than intrinsic growth advantages of Ikkβhep hepatocytes, both because both former processes were inhibited by the anti-oxidant butylated hydroxyanisole (BHA) when administered over consecutive two-day intervals, before and after HCC-initiation by DEN, and because selective depletion of KCs by GdCl₃ injections 24 hours prior to DEN treatment reduced subsequently elevated CHP, as well as KC-generated increases of TNFα, IL-6 and HGF mRNAs.16,22

While there is little doubt that ROS formation and ROS-mediated KC activation are etiologically involved in DEN-induced HCC, as proposed from observations with this Ikkβ deletion model,16,22,23,25 and from findings in mice specifically ablated in hepatocyte NEMO/IKKγ regulatory subunits of IKK, that develop spontaneous HCC,32 it has also been shown that (a) BHA concomitantly blocked many cellular processes in standard DEN-induction models;16,22 and that, (b) liver repletion of KCs occurs shortly after GdCl₃ treatment.33 Thus, along with BHA inhibition of ROS formation, hepatocyte necrosis and apoptosis, reduction by BHA of serum ALT levels, and the ability of BHA to blunt significantly hepatocyte DNA-adduct formation,34 BHA treatment almost completely inhibited DEN-induced hepatocyte proliferation, in a quantitatively greater percentage than its inhibitory effects on HCC tumor number and tumor size.16,22

Consequently, to understand more fully the major causes of augmented HCC formation in Ikkβhep mice, consideration is given here to other underlying causes of hepatocellular growth advantage, and to other potential cytokine-dependent mechanisms of macrophage activation (e.g., interferon γ [IFN-γ]).35 Here we propose new research directions to identify other contributing, but perhaps fundamental, mechanisms of DEN-induced HCC in Ikkβhep mice.

One direction might focus on genetic and biochemical investigations of potential intergenotypic differences in the formation, types and stability of hepatocyte DNA-adducts in DEN-treated IkkβF/F and Ikkβhep mice. Thus far, however, quantitative differences in DNA-adduct formation have not been detected between the two genotypes.16 Another might explore the possible selection by DEN for the emergence of premalignant liver stem or liver cancer stem cells16–38 in Ikkβhep mice. However, apart from critical experimental issues surrounding the identification of such stem cells, as well as the necessity to combine studies of fate-mapping, fluctuation analysis, cell-cell fusion and systematic dilution of transplanted cells35 it should be noted that past and current evidence16–18,35,38 suggests that mature Ikkβ hepatocytes are HCC-progenitors in both models of DEN-induced HCC.

**New hypothesis and research directions concerning HCC etiology: Preliminary evidence and general background**

Recent observations from our laboratory have revealed unusual and unexpected constitutive phenotypes of hepatocytes in Ikkβhep mice.39 Comparative microarray profiling studies suggest that, in comparison with IkkβF/F hepatocytes, Ikkβhep hepatocytes express very high mRNA levels of CD74 (the MHC class II chaperone)39 and MHC class II I-A/E β and I-A α chains; significant overexpression of genes encoding large families of molecules involved in innate and adaptive...
immunity, including CD44, CIITA, signal transducer and activator of transcription (STAT) 1, cathepsin S and co-activator CD86, as well as IFN-γ regulation have also been observed. Examples of high scoring results are shown in Table 1. Western blot and immunohistochemical findings support observations of Ikkβ<sup>−/−</sup> hepatocyte-restricted expression of CD74 and MHC Class II molecules (unpublished observations).

Based upon these observations, we propose the hypothesis that augmented susceptibility of Ikkβ<sup>−/−</sup> mice to HCC requires functional hepatocellular expression of CD74, MIF<sup>41,42</sup> receptors, IFN-γ regulatory proteins, and antigen-presentation by MHC class II receptors. We refer to this apparently unique phenotypic cluster as “hepatic immunologic ectopia” (see Figure 1). One or more of these functions, if activated, might lead to hepatocellular antigen presentation, proliferation and/or death (via apoptosis or necrosis). Various MAPK and STAT pathways may be called into play as mediators of these responses.

An extensive experimental and clinical literature lends support to several aspects of this hypothesis. CD74 is a key mediator of the toxic and proinflammatory effects of MIF.<sup>41–43</sup> The 9.2-kb mouse CD74 gene resides on chromosome 18; it consists of nine exons which generate six mRNA splice variants by alternative splicing.<sup>40</sup> Two CD74 isoforms, polypeptides of 31 kDa and 41 kDa (which contain chondroitin sulfate side chains), generate Type II membrane glycoproteins with 30 amino acid cytoplasmic tails.<sup>40,41,44</sup> CD74 is widely expressed by B-cells, monocytes, and macrophages<sup>41–45</sup> including hepatic stellate cells (HSCs);<sup>45</sup> a rare and sparse perportal subpopulation of CD74<sup>+</sup> hepatocytes has been reported in wild-type B10. BR mice.<sup>46</sup> Notably, CD74 is associated with two reported biochemical mechanisms unrelated to its intracellular chaperone function (Figure 1). Firstly, in DNA-mediated transfection studies with human 293 cells, regulated intracellular proteolysis (RIP) of CD74 releases a 10 kDa cytoplasmic fragment that migrates into the nucleus where it activates p65 and NF-κB.<sup>47</sup> And, secondly, although MIF has also been reported to be a noncognate ligand of CXCR2 and CXCR4 chemokine receptors in monocytes and T cells,<sup>48</sup> perhaps of more relevance to our hypothesis are plasma membrane complexes of CD74/CD44 which have been shown to mediate MIF signaling in most cell systems studied thus far (viz., CD74/CD44 complexes are considered to be the major MIF receptor acting via one or more MAPK pathways).<sup>49–52</sup>

MIF, a 12.5 kDa proinflammatory cytokine, is associated with multisystemic disease.<sup>53–55</sup> MIF is secreted as a soluble homotrimer from preformed pools by T-, B- and epithelial cells,<sup>41–43,54</sup> as well as by liver KCs,<sup>55</sup> in response to toxins, endotoxins, and cytokines including Con-A, LPS, TNFα and IFN-γ. MIF expresses intrinsic enzymatic activities, and it induces cyclin D1, mitogenesis and nitric oxide production.<sup>52,53,60</sup> MIF is causally related to the pathogenesis of chronic liver disease,<sup>61</sup> HBV infection<sup>62</sup> and acute liver failure following alcoholic hepatitis;<sup>63,64</sup> it is found in hepatocytes from fibrotic livers of thioacetamide- and ConA-treated mice;<sup>55,66</sup> and it is causally involved in Bacille-Calmette-Guerin-primed LPS-induced T-cell-mediated acute liver failure (BLTLF).<sup>59</sup> Of potential clinical importance, anti-MIF Abs and MIF anti-sense cDNA block BLTLF and LPS-induced liver injury, respectively;<sup>55,67</sup> anti-MIF antibodies

| Table 1 Overexpression of key genes in Ikkβ<sup>−/−</sup> hepatocytes revealed by microarray profiling |
| Description | E<sup>+</sup>/R<sup>−</sup> |
| CD74 antigen (invariant polypeptide) | 38.3/4.6 |
| Interferon gamma inducible protein 47 (Ifi47) | 7.2/3.2 |
| Immunity-related GTPase family, M (Irmg) | 6.5/3.7 |
| TAP binding protein (Tapb) | 5.2/2.3 |
| CD44 antigen (Cd44) | 4.3/1.9 |
| Histocompatibility 2, T region locus 10 (H2-T10) | 4.2/3.2 |
| Transporter 1, ATP-binding (MDR/TAP) (Tap1) | 4/1.9 |
| Toll-like receptor 2 (Tlr2) | 4/1.7 |
| Complement component 1, C1qc | 4/1.4 |
| Chemokine (C-C motif) receptor 5 (Ccr5) | 3.9/2.1 |
| Lysozyme (Lyz) | 3.9/1.7 |
| Histocompatibility 2, class II antigen A, beta | 22.5/2.5 |
| Histocompatibility 2, class II antigen E beta | 18.2/2.1 |
| Histocompatibility 2, class II, locus DMA (H2-DMA) | 15.8/3.1 |
| Histocompatibility 2, class II antigen A, alpha | 14.4/2.3 |
| T-cell specific GTPase (Tgtp) | 13.3/4.8 |
| Guanylate nucleotide binding protein 1 (Gbp1) | 11/4.3 |
| Guanylate nucleotide binding protein 3 (Gbp3) | 9/3.9 |
| Guanylate nucleotide binding protein 2 (Gbp2) | 7/2.9 |
| Chemokine (C-X-C motif) ligand 9 (Cxcl9) | 6.5/1.3 |
| CD274 antigen (Cd274) | 5.5/2.9 |
| Proteasome (prosome, macropain) (Psmb9) | 5.3/1.6 |
| Interferon regulatory factor 1 (Irf1) | 4.2/2.1 |

Notes: E = X-fold higher expression (Ikkβ<sup>−/−</sup> > Ikkβ<sup>−/−</sup>); and, R = ratio of expression (isolated hepatocytes)/[whole liver tissue]. See Koch and Leffert<sup>40</sup> for details of microarray profiling.
Hepatocytes, IKKβ, HCC and hepatic immunologic ectopia

also attenuate experimentally-induced murine colitis;67 MIF-knockout mice are also protected from LPS-,68 ConA-,66 and acetaminophen-mediated69 liver injuries. And surprisingly, as mentioned above, in comparison with expression in IkkβF/F hepatocytes, numerous immune process and host defense genes are highly and constitutively overexpressed in Ikkβ∆hep hepatocytes–phenotypes that, along with growth advantages, might also augment Ikkβ∆hep susceptibility to MIF and HCC.

CD74 and MIF are also implicated in the biology of human HCC. A role for MIF was first suggested when transplanted hepatoma ascites cell lines, obtained from DEN-treated guinea pigs, were rejected by what appeared to be adherent intraperitoneal macrophage aggregates.29 Subsequent reports showed that MIF and CD74 expression were altered or heightened in many malignancies including HCC.61,62,71–73 CD74 serves a triple role: as a component of the MIF receptor along with CD44 (at plasma membrane sites); as a source of RIP-mediated fragments that migrate into the nucleus where they activate p65 (RelA)/NF-κB-mediated transcription; and, as the intracytoplasmic MHC Class II chaperone in a complex with MHC Class II heterodimers that binds processed antigen (the antigen is then presented extracellularly). Depending upon the intrahepatic microenvironment, the separate or collective functions of these molecules are postulated to lead to cellular immune system-mediated anti-hepatocyte responses, hepatocyte proliferation and/or hepatocyte death. The annotations and symbols are defined in the figure (see text and references for further details).

Figure 1 Hepatic immunologic ectopia: a constellation of constitutive immunophenotypes associated with Ikkβ∆hep hepatocytes. The diagram illustrates elements of the basic hypothesis, viz., that compared to IkkβF/F hepatocytes, Ikkβ∆hep hepatocytes ectopically express MIF receptors associated with MAPK signaling pathways, IFN-γ regulatory proteins which facilitate hepatocellular responses to IFN-γ (an activator of hepatic macrophages, as well as several immune system cells that may be situated in hepatotoxic livers), and antigen-presentation-competent MHC class II receptors (which respond to DEN-associated antigens and elicit specialized intrahepatic CD4+ and CD8+ T cell responses). CD74 serves a triple role: as a component of the MIF receptor along with CD44 (at plasma membrane sites); as a source of RIP-mediated fragments that migrate into the nucleus where they activate p65 (RelA)/NF-κB-mediated transcription; and, as the intracytoplasmic MHC Class II chaperone in a complex with MHC Class II heterodimers that binds processed antigen (the antigen is then presented extracellularly). Depending upon the intrahepatic microenvironment, the separate or collective functions of these molecules are postulated to lead to cellular immune system-mediated anti-hepatocyte responses, hepatocyte proliferation and/or hepatocyte death. The annotations and symbols are defined in the figure (see text and references for further details).

Abbreviations: MAPK, mitogen-activated protein kinase; MHC, major histocompatibility complex; MIF, macrophage migration inhibitory factor; RIP, regulated intramembrane proteolysis.

Hepatocytes, IKKβ, HCC and hepatic immunologic ectopia is correlated with augmented cyclin D1 expression (a hallmark of activated hepatocyte proliferation,81 tumor size and metastasis,82 and tumor angiogenesis),83,84 whereas reduced MIF expression upregulates the cell-cycle CDK inhibitor, p27.85 Serum levels of MIF are high in cirrhotic patients with HCC.61,62,71 MIF is directly mitogenic in several systems51,52,60 including human HepG2 cells.86,87 Circulating levels of MIF increase following human liver resection,88 and if secreted, might explain reduced intrahepatic levels of MIF after 70% PH.89 Microarray profiling suggests complex associations between hepatic CD74 expression and the intrahepatic recurrence of human HCC.76

In a provocative study designed to investigate the roles of IFN-γ (a macrophage activator produced by NK, NKT and dendritic cells, and by CD4+ Th1- and CD8+ T-cells)80 and of IFN-α/β as mediators of DEN-induced HCC, quantitative measurements of intrahepatic cytokine mRNA expression in adult 129SV mice, chronically fed DEN in their drinking water, indicated that, compared with levels in vehicle-fed wild-type mice, MIF (which was elevated at 1 month, and

Dovepress

submit your manuscript | www.dovepress.com                                                                 43

Dovepress

Hepatocytes, IKKβ, HCC and hepatic immunologic ectopia

Figure 1 Hepatic immunologic ectopia: a constellation of constitutive immunophenotypes associated with Ikkβ∆hep hepatocytes. The diagram illustrates elements of the basic hypothesis, viz., that compared to IkkβF/F hepatocytes, Ikkβ∆hep hepatocytes ectopically express MIF receptors associated with MAPK signaling pathways, IFN-γ regulatory proteins which facilitate hepatocellular responses to IFN-γ (an activator of hepatic macrophages, as well as several immune system cells that may be situated in hepatotoxic livers), and antigen-presentation-competent MHC class II receptors (which respond to DEN-associated antigens and elicit specialized intrahepatic CD4+ and CD8+ T cell responses). CD74 serves a triple role: as a component of the MIF receptor along with CD44 (at plasma membrane sites); as a source of RIP-mediated fragments that migrate into the nucleus where they activate p65 (RelA)/NF-κB-mediated transcription; and, as the intracytoplasmic MHC Class II chaperone in a complex with MHC Class II heterodimers that binds processed antigen (the antigen is then presented extracellularly). Depending upon the intrahepatic microenvironment, the separate or collective functions of these molecules are postulated to lead to cellular immune system-mediated anti-hepatocyte responses, hepatocyte proliferation and/or hepatocyte death. The annotations and symbols are defined in the figure (see text and references for further details).

Abbreviations: MAPK, mitogen-activated protein kinase; MHC, major histocompatibility complex; MIF, macrophage migration inhibitory factor; RIP, regulated intramembrane proteolysis.

Hepatocytes, IKKβ, HCC and hepatic immunologic ectopia is correlated with augmented cyclin D1 expression (a hallmark of activated hepatocyte proliferation,81 tumor size and metastasis,82 and tumor angiogenesis),83,84 whereas reduced MIF expression upregulates the cell-cycle CDK inhibitor, p27.85 Serum levels of MIF are high in cirrhotic patients with HCC.61,62,71 MIF is directly mitogenic in several systems51,52,60 including human HepG2 cells.86,87 Circulating levels of MIF increase following human liver resection,88 and if secreted, might explain reduced intrahepatic levels of MIF after 70% PH.89 Microarray profiling suggests complex associations between hepatic CD74 expression and the intrahepatic recurrence of human HCC.76

In a provocative study designed to investigate the roles of IFN-γ (a macrophage activator produced by NK, NKT and dendritic cells, and by CD4+ Th1- and CD8+ T-cells)80 and of IFN-α/β as mediators of DEN-induced HCC, quantitative measurements of intrahepatic cytokine mRNA expression in adult 129SV mice, chronically fed DEN in their drinking water, indicated that, compared with levels in vehicle-fed wild-type mice, MIF (which was elevated at 1 month, and
peaked at 3 months along with HCC infiltrating monocytes and macrophages), IFN-γ and IFN-β (which peaked at 4–5 months), and TNFα (which peaked at 3–4 months) were all induced by DEN treatment, concomitantly with HCC onset at 2–3 months; HCC incidence reached 100% between 4–5 months. In contrast, although tumor diameters did not differ among groups, the numbers of DEN-induced HCCs/liver in IFN-γ-receptor knockout (IFN-γR KO) mice fell by 60% compared with DEN-induced IFN-α/βR KO and wild-type mice. Immunohistochemical studies showed that the numbers of infiltrating mononuclear cells were greatly reduced only in the livers of IFN-γR KO mice, consistent with the observations that, in comparison with that in wild type controls, HCC-induced intrahepatic cytokine expression and oxidative DNA damage were also diminished in the IFN-γR KO mice. Although the chronic DEN feeding regimen, strain background, and G_{hepatocyte} status differ from the single injection standard models in which either neonatal host or donor hepatocyte proliferation occur actively on C57BL/6 backgrounds, the findings with the chronic feeding regimen model strongly suggest that DEN might also induce similar cytokine expression phenotypes in standard models of DEN-induced HCC.

Lastly, ectopic MHC class II expression and competent antigen presentation, as shown by the MHC-dependent induction of proliferation of CD4^+ Th1 and CD8^+ T cells (the peptide-free MHC class II molecular structure shown in Figure 1 is taken from reference 91), have been observed in human HCC cell lines (± IFN-γ treatment) and in antigen-presentation competent hepatocytes from CIITA-transgenic mice, the livers of which did not show constitutive autoimmunity. These findings, plus a report of DEN suppression of humoral immunity in adult mice, and our preliminary results, further raise the intriguing possibilities that innate and adaptive immune responses may preferentially occur and also affect HCC formation and fate in DEN-treated Ikkβ<sup>Δhep</sup> mice.

Conclusions and clinical implications

New findings in Ikkβ<sup>Δhep</sup> hepatocytes are provocative because CD74, CD44 and MHC Class II molecules, which hepatocytes do not usually express, have all been linked, along with heightened MIF responsiveness, to precancerous and malignant HCC. Thus, new investigations in Ikkβ<sup>Δhep</sup> mice may well supply new explanations of enhanced human susceptibility to HCC. If obtained, such direct evidence may provide rational frameworks for translational therapies to develop novel clinical and pharmacologic interventions to prevent or attenuate the morbidity and mortality associated with human HCC.

For example, anthracycline derivatives such as epirubicin, daunorubicin and adriamycin are considered to be some of the most potent chemotherapeutic drugs used to treat inoperable human HCC. Not unexpectedly, one of the greatest problems with chemotherapy involves the nonspecific and toxic side effects of the administered drugs. Targeted drug delivery is therefore a major rational goal of chemotherapy of human HCC. The availability of transplantable primary HCC tissues or HCC cell lines, derived from human or murine biopsies of Ikkβ<sup>Δhep</sup> tumors that express cell surface CD74, would provide in vivo and in vitro model systems to evaluate the targeted effects of anti-CD74 antibodies conjugated to anthracycline derivatives. Such conjugates exist, and have already been reported to cure human B-cell lymphoma xenografts in SCID mice. Thus, based on the preliminary observations we have made in model system studies with Ikkβ<sup>Δhep</sup> mice, the hypothesis advanced here might provide a rationale for new experimental HCC treatment paradigms with translational potential for treatment of human HCC, particularly should cohorts of human patients exist with genetic abnormalities of Ikkβ that confer hepatic immunologic ectopia and heightened susceptibility to liver disease including HCC.

Acknowledgments

This publication was made possible by grants from the National Institute of Allergy and Infectious Diseases (R21AI067354), the National Cancer Institute (R01CA113602) and the National Institute of Environmental Health Sciences (P42ES010337).

Disclosures

The authors report no conflicts of interest in this work.

References

Hepatocytes, IKKβ, HCC and hepatic immunologic ectopia


34. Leng L, Bucala R. Insight into the biology of macrophage migration inhibitory factor (MIF) revealed by the cloning of its cell surface receptor. Cell. 2006;126:162–168.


