Journal of Pathology
J Pathol 2015; **235:** 355–367
Published online in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/path.4434

INVITED REVIEW

The direct and indirect roles of HBV in liver cancer: prospective markers for HCC screening and potential therapeutic targets

Marc Ringelhan,[1,2,3]* Tracy O'Connor,[1] Ulrike Protzer[1] and Mathias Heikenwalder[1]*

[1] *Institute of Virology, Technische Universität München/Helmholtz Zentrum München, Munich, Germany*
[2] *Second Medical Department, Klinikum Rechts der Isar, Technische Universität München, Munich, Germany*
[3] *German Centre for Infection research (DZIF), Munich Partner Site, Germany*

**Correspondence to: Marc Ringelhan or Mathias Heikenwalder, Institute of Virology, Technische Universität München/Helmholtz Zentrum München, Schneckenburgerstrasse 8, D-81675 München, Germany. E-mail: marc.ringelhan@lrz.tum.de or heikenwaelder@helmholtz-muenchen.de*

Abstract

Chronic hepatitis B virus (HBV) infection remains the number one risk factor for hepatocellular carcinoma (HCC), accounting for more than 600 000 deaths/year. Despite highly effective antiviral treatment options, chronic hepatitis B (CHB), subsequent end-stage liver disease and HCC development remain a major challenge worldwide. In CHB, liver damage is mainly caused by the influx of immune cells and destruction of infected hepatocytes, causing necro-inflammation. Treatment with nucleoside/nucleotide analogues can effectively suppress HBV replication in patients with CHB and thus decrease the risk for HCC development. Nevertheless, the risk of HCC in treated patients showing sufficient suppression of *HBV* DNA replication is significantly higher than in patients with inactive CHB, regardless of the presence of baseline liver cirrhosis, suggesting direct, long-lasting, predisposing effects of HBV. Direct oncogenic effects of HBV include integration in the host genome, leading to deletions, *cis*/*trans*-activation, translocations, the production of fusion transcripts and generalized genomic instability, as well as pleiotropic effects of viral transcripts (HBsAg and HBx). Analysis of these viral factors in active surveillance may allow early identification of high-risk patients, and their integration into a molecular classification of HCC subtypes might help in the development of novel therapeutic approaches.
Copyright © 2014 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.

Keywords: HBV; chronic hepatitis B; HBs antigen; HBx; X-protein; liver cancer; HCC; direct role of viral proteins; hepatocarcinogenesis

Received 22 August 2014; Revised 1 September 2014; Accepted 2 September 2014

No conflicts of interest were declared.

Introduction

Chronic hepatitis B virus (HBV) infection accounts for up to 54% of hepatocellular carcinoma (HCC) cases worldwide [1]. This percentage is lower in developed (23%) than in developing countries (59%), reflecting the variable burden of chronic HBV infection in different areas [2,3]. Worldwide, more than 240–350 million individuals are estimated to be chronically infected with HBV, facing a lifetime risk of 15–40% of developing end-stage liver disease, including cirrhosis, liver failure and HCC [4–7], accounting for more than 600 000 deaths/year [8]. Synergistic effects increasing the risk for hepatocarcinogenesis in CHB are demographic factors, such as Asian or African ancestry, male sex and age, as well as environmental factors, such as alcohol abuse, aflatoxin exposure or non-alcoholic fatty liver disease. Furthermore, viral co-infections (hepatitis C, hepatitis D or HIV co-infections), HBV genotype, viral DNA integration into the host genome or other direct effects of viral proteins might increase the risk for HCC development in CHB [9,10]. Patients chronically infected with HBV risk developing HCC in the absence of inflammation, severe liver damage and cirrhosis, due to direct oncogenic viral factors of HBV [3,9–13]. However, the direct role of HBV in HCC development remains to be elucidated.

HBV virology

HBV is a human blood-borne virus, infecting hepatocytes of humans or humanoid primates due to specific binding of the preS1 domain of the large envelope viral protein to hepatocytes via the bile salt transporter sodium taurocholate co-transporting polypeptide (NTCP) [14,15]. HBV can be transmitted by direct blood contact or sexual contact (horizontal transmission) as well as before or during birth from mother to child (vertical transmission) [16]. A very low number of HBV particles (<10) was shown to be sufficient to establish hepatocyte infection *in vivo* [17,18], demonstrating extremely high infectivity. The individual age at time of infection, as well as the implementation of vaccination programmes, vary greatly between different geographic regions, which at least partially accounts

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

for the different prevalence of CHB and HCC worldwide [1,3,19]. A 90% risk of developing chronic HBV infection is found after vertical transmission, commonly seen in Oceania, South and South East Asia [20–22]. In cases of horizontal transmission, age at time of infection remains the highest risk factor for CHB development. In immune-competent adults the risk for CHB drops below 1%, while young children face a risk of 20–30% of chronic infection [23–25].

HBV, a member of the Hepadnaviridae family, is an enveloped DNA virus replicating via an RNA intermediate with a partially double-stranded relaxed circular DNA (rcDNA) molecule of 3.2 kb [26,27] (see Figure 1). Upon translocation to the nucleus, the rcDNA genome is converted into a covalently closed circular DNA (cccDNA), which serves as a template for viral transcription and allows effective HBV persistence [16,28,29]. The four overlapping open reading frames (ORFs) of the HBV genome encode for preS/S, preCore/Core, Pol and X (see Figure 1). The regulatory elements (enhancer II/basal core promoter, preS1 promoter, preS2/S promoter and enhancer I/X promoter) are also coding sequences for viral proteins within the ORFs [30]. Following transcription, all viral RNAs are translated in the cytosol. The non-structural X-protein (HBx) translated from a small 0.7 kb RNA is essential for viral replication and undergoes multiple interactions in host cells. The structural envelope proteins called small (S) and medium (M; = pre-S2 + S) are translated from a 2.1 kb RNA, and the large envelope protein (L; = pre-S1 + pre-S2 + S) is derived from a 2.4 kb RNA, due to multiple alternative start codons. Envelope protein synthesis far exceeds the amount needed for virion assembly and these proteins are secreted as spherical or subviral non-infectious particles [26,31,32]. The core protein, which forms the nucleocapsid (HBc) and its secreted counterpart HBe, arise from alterative translation initiation sites in the pregenomic RNA (pgRNA, ~3.5 kb). The viral polymerase, which is also translated from the pqRNA, has multiple functions, such as serving as reverse-transcriptase, DNA-dependent DNA polymerase and RNase H. The pgRNA furthermore serves as matrix for viral replication after being incorporated into nucleocapsids and being reverse-transcribed by the viral polymerase into new viral rcDNA [33]. rcDNA is either redelivered into the nucleus for a cccDNA pool or secreted with envelope proteins as infectious virions [32].

CHB and necro-inflammation: key drivers for liver fibrosis and HCC

HBV is a prototype, non-cytopathic virus [34] and in CHB the main liver damage-mediating cells driving HCC are $CD8^+$ T cells [35–37]. The correlation between the strength of HBV-specific T cell responses and virus clearance after HBV infection has been well established. Adaptive immune responses ultimately mediate viral clearance and protective immunity, if the acute HBV infection can be cleared. For efficient control of HBV infection, an interplay between the innate and the adaptive immune response is required. Viral clearance is mediated by an effective adaptive $CD4^+$ and $CD8^+$ T cell response, whereas protective

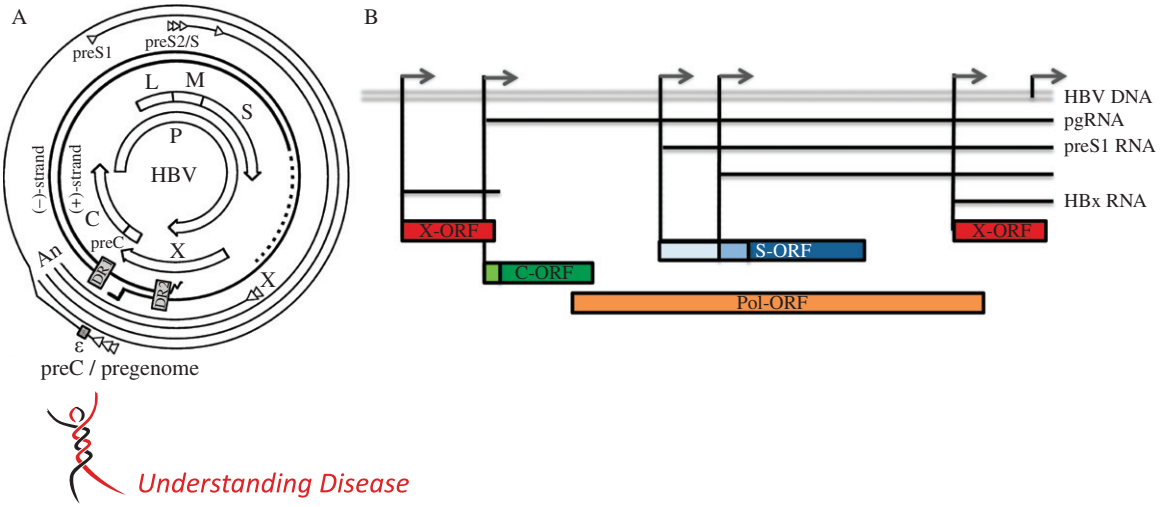

Figure 1. Schematic drawing of the HBV genome. Hepatitis B virus (HBV) is a small, relaxed circular DNA (rcDNA) virus with a partially double-stranded, 3.2 kb genome. The minus strand DNA encodes for four genes and, upon translocation to the nucleus, the *rcDNA* is converted into a covalently closed circular DNA (cccDNA), which serves as template for viral transcription. (A) The four overlapping open reading frames (ORFs) of the HBV genome encode for preS/S, preCore/Core, Pol and X proteins. Regulatory elements, enhancer II/basal core promoter, preS1 promoter, preS2/S promoter and enhancer I/X promoter [indicated by arrows in (B)] are also coding sequences for viral proteins within the ORFs [24]. (B) The transcribed HBV RNAs [pregenomic (pg) RNA ~3.5 kb, PreS1 RNA of 2.4 kb, PreS2 RNA of 2.1 kb and the 0.7 kb X-RNA] encode for all viral proteins [hepatitis B core antigen (HBcAg), its secreted counterpart HBeAg, the viral polymerase (Pol), the structural envelope proteins (HBsAg) called small (S), medium (M; = pre-S2 + S) and large (L; = pre-S1 + pre-S2 + S) proteins and the non-structural protein HBx. Besides serving as template for HBcAg, HBeAg and Pol translation, the pgRNA serves as a matrix for viral replication and is reverse-transcribed by the viral Pol into new viral rcDNA [25]

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

immunity is mediated by a B cell response, generating neutralizing antibodies [29,38]. Impaired virus control during chronic infection is correlated with functionally exhausted antiviral T cells. Nevertheless, they may still amplify liver injury [35–37,39]. Factors responsible for shifting the balance from immune tolerance to immune clearance are poorly understood. The liver has a unique immunoregulatory function to prevent inadvertent organ damage, which renders the liver an attractive target site for pathogens such as HBV [16]. The innate immune response, early adaptive B and T cell responses, regulatory T cells, hepatocyte-intrinsic factors and the liver microenvironment all seem to play a role in mediating liver damage [39]. For example, modulation of the cytokine response (up-regulation of IL-10 and TGFβ) and a putative role of regulatory T cells (Treg) have been proposed [40–43]. Tregs, for example, mitigate immune-mediated liver damage by down-regulating the activity of effector T cells and limiting cytokine production and cytotoxicity in acute HBV infection [44], yet their actual role in CHB remains elusive. In addition, viral factors of HBV, such as induction of regulatory cytokines, hepatocyte-restricted viral gene expression and high levels of circulating viral antigens, also allow escape from the immune response [16]. After acute damage, the liver has a unique capacity to regenerate via proliferation of normally quiescent hepatocytes, which ultimately restores liver function and size [45]. However, in CHB, persistent viral infection and ineffective T cell responses lead to chronic inflammatory liver damage and repeated compensatory proliferation of hepatocytes (necro-inflammation), which may subsequently lead to liver fibrosis or cirrhosis and HCC [35–37,46–49] (see Figure 2). This sequence was partially dissected in several mouse models, underlining the high importance of compensatory proliferation and deregulated hepatocyte apoptosis [50], chronic inflammation [37] and altered cytokine networks, including NF-κB and other signalling cascades [51,52], as tumour-promoters in the pathophysiology of HCC development (reviewed in [53]). Large-scale longitudinal epidemiological studies have revealed the strong correlation of high viral loads and risk of progression to HCC, pointing to the importance of subsequent inflammation in correlated phases of high viral replication. In CHB, baseline *HBV* DNA levels were even shown to be an independent predictive factor for the development of HCC, even after adjustment for epidemiological (sex, age, alcohol consumption) and other known risk factors, such as severity of liver damage (serum ALT level) [5,54,55]. Nucleoside/nucleotide analogues (NA) controlling HBV replication represent a successful treatment option to overcome this underlying process of chronic inflammatory liver damage and compensatory proliferation.

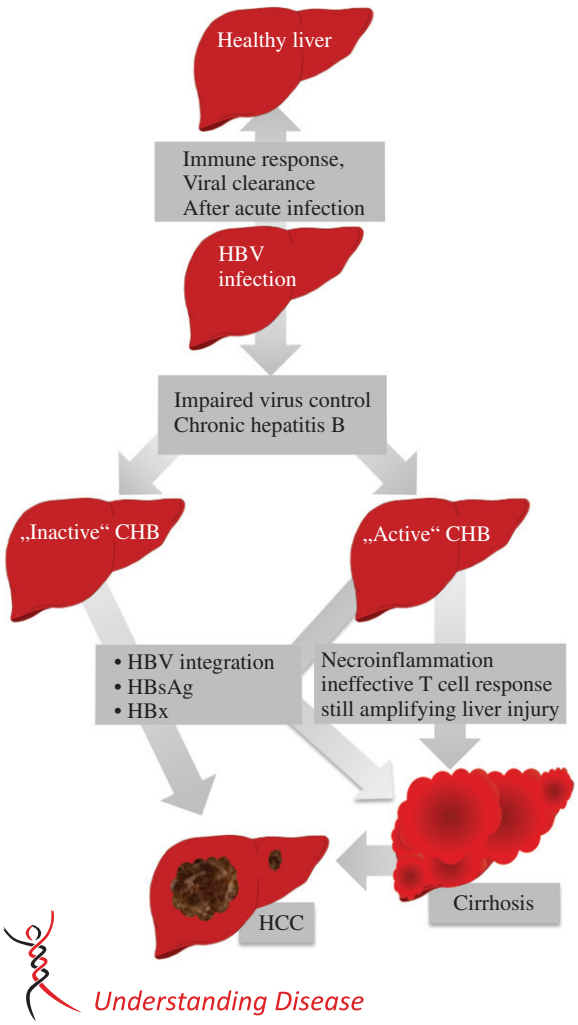

Figure 2. Different sequences from CHB to HCC. After HBV infection, a strong efficient immune response can clear the infection. However, due to pleiotropic effects with an inefficient T cell response, a chronic HBV infection (CHB) might develop. The risk for CHB depends on the route of infection (vertical versus horizontal transmission) and age at time of infection (adults < 1%; young children 20–30%). An inefficient immune response might cause 'active' chronic hepatitis, with severe necro-inflammation, increasing fibrosis and the risk of cirrhosis and subsequent HCC development. *HBV* DNA levels (>2000 IU/ml), ALT levels and fibrosis are typical findings in 'active' CHB. In chronic HBV infection, HCC development also occurs in the absence of cirrhosis and severe prolonged liver damage. Such cases might be 'inactive', asymptomatic carriers and even cases of 'occult' CHB; this is due to direct oncogenic viral factors of HBV, such as *HBV* DNA integration, the viral surface proteins and the non-structural protein HBx

Treatment options for CHB: primary prophylaxis for HCC?

Because cure can hardly be achieved, the current goal of antiviral therapy for hepatitis B is to improve quality of life and survival by preventing the progression of chronic liver disease (CLD) to end-stage liver disease (cirrhosis ± decompensation) and HCC by sustained suppression of HBV replication. Indications for treatment vary between different countries, since therapy is

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

expensive, usually long in duration and not widely available, particularly in many developing countries with the highest prevalence [56]. Algorithms were proposed for treatment stratification by, for example, the American Association for the Study of Liver Diseases (AASLD) [57], the European Association for the Study of the Liver (EASL) [58,59], the Asian Pacific Association for the Study of the Liver (APASL) [60] and several others. According to EASL guidelines, the decision should be based on serum *HBV* DNA levels (>2000 IU/ml/10 000 copies/ml) and/or serum ALT levels (>upper limit of normal) and histology (active necro-inflammation and/or fibrosis; > A1 F1 by METAVIR) [58,59]. During interferon-α (IFNα) treatment, *HBV* DNA levels and HBeAg become undetectable in 30–40% of patients and 10% of patients seroconvert to anti-HBs. However, 5–10% of patients relapse, with full-blown disease after IFN therapy has ended [61,62]. While only a few patients seroconvert to anti-HBs during IFNα therapy, seroconversion rates increase to about 15–20% after the end of treatment [7,27].

NAs block reverse transcription and thus effectively suppress HBV replication. This seems sufficient to decrease necro-inflammation and lower the risk of cirrhosis and HCC development [58,63–65]. As cccDNA remains in the nucleus of infected hepatocytes, however, HBV infection is not eliminated by NA [27]. Treatment benefit with regard to HCC incidence depends on baseline HCC risk, stage of fibrosis/cirrhosis and the type of NA used (high genetic barrier) [57,58,63]. However, antiviral therapy reduces, but does not eliminate, the risk of HCC in chronic hepatitis B patients, regardless of the presence of baseline liver cirrhosis [63,65–67]. The ultimate goal is to achieve a sustained virological response and clearance of cccDNA in CHB. Just recently, a distinct antiviral mechanism that interferes with cccDNA stability driven by antiviral cytokines (IFNα and lymphotoxin-β receptor activation) by up-regulation of APOBEC3A and APOBEC3B cytidine deaminases in hepatocytes was shown to effectively result in cccDNA degradation [27,68]. This might serve as a starting point for new therapeutic approaches that hopefully will eventually be able to cure hepatitis B.

Direct oncogenic effects of HBV in hepatocarcinogenesis

In addition to causing necro-inflammation, HBV has direct oncogenic potential that contributes to the development of HCC, independent of inflammatory liver damage [3,9,11,12]. This is reflected best by the fact that patients chronically infected with HBV face possible HCC development in the absence of inflammation, severe liver damage and cirrhosis, due to direct oncogenic viral factors [13]. The precise role of these direct viral factors in HCC development, however, remains controversial. The association between occult HBV infection (persistence of viral DNA after integration, or as cccDNA in patients negative for HBsAg) and HCC strongly supports the view of direct oncogenic factors of HBV [13]. The current debate on the significance of occult HBV infection in HCC development is ongoing.

There is compelling evidence for subgenotypes of HBV being risk factors for early HCC development in genotypes B2–5 [11], and patients bearing genotypes A1, C, Ba and F1 more frequently progress to HBV-related liver disease than genotypes A2 and Bj [3,69–71]. However, further studies focusing on HBV genotypes are needed to dissect direct effects from epidemiological risk factors. Another important risk factor is HBV variants, selected for during the natural course of chronic HBV infection or during treatment. The double substitution A1762T and G1764A in the basal core promoter (BCP) region of HBV and pre-core mutants seems to be an independent risk factor for HCC development associated with high viral titres and increased liver damage, even in the presence of anti-HBe seroconversion [6,9].

The role of HBV integration in HCC development

HBV integration into the host hepatocyte genome is a frequent event in HCC (86.4%) and is also found in adjacent liver tissues (30.7%) [72], which might lead to deletions, *cis*/*trans*-activation, translocations, the production of fusion transcripts and generalized genomic instability [12]. Integration is detrimental for HBV replication, yet it is a dynamic process and was reported over 30 years ago to precede HCC development [73]. However, it is unclear whether HBV integration initiates, or rather is a consequence of, transformation [49]. Hence, the distinct role of *HBV* DNA integration in hepatocarcinogenesis and the attributed risk of occult HBV-infection in HCC development remain controversial. Factors provoking liver cell death and proliferation increase dynamic rates of *HBV* DNA integration [74,75]. HBV integration was initially proposed to be a random event [12,76,77].

More recent high-throughput, next-generation, sequencing approaches identified recurrent sites for integration. Genes reported to be frequently altered are *TERT*, *MLL4*, *CCNE1*, *NTRK2*, *IRAK2* and *p42MAPK1* [72,78,79]. Secondly, viral promoter-driven human transcription and viral–human transcript fusion have been reported [80]. A common effect, increasing with rising numbers of integrations, seems to be increased genomic instability, and a correlation was observed between the number of chromosomal aberrations and the status of tumour suppressor genes (*TP53*, *RB1*, *CDNK2A* and *TP73*) [81]. Lately, the identification of insertion sites within or close to repetitive, non-coding sequences, such as long interspersed nuclear elements (LINEs) or short interspersed nuclear elements (SINEs) has garnered notice [82,83].

Using mathematic algorithms (ViralFusionSeq), the detection of viral–human fusion transcripts was enabled in an unbiased fashion [84] and helped to identify a

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

novel fusion sequence of the human LINE(1) and HBV-encoded HBx [HBx-LINE(1)] after insertion in chr. 8p11. Interestingly, this non-coding fusion RNA (*HBx–LINE1*) was detected in 23.3% of HBV-associated human HCCs, was correlated with poor survival and showed tumour-promoting properties affecting β-catenin activity and epithelial–mesenchymal transition (EMT) [85]. However, integration and low frequencies of fusion transcripts do not suffice to explain the multitude of direct effects of HBV in HCC development. Comparing HBV-associated HCC to other aetiologies shows a significantly more frequently altered p53 pathway (47%), up-regulation of other genes involved in cell-cycle regulation and progenitor phenotypes, as well as specific gene mutations (*HBx*, *TP53*, *IRF2*, *AXIN1* and *CTNNB1*) [86].

HBs antigen and pre-S/S mutants in HCC development: link to UPR

A direct factor driving hepatocarcinogenesis could be the expression level of HBsAg (see Figure 3). In a small cohort of NA-treated patients, hepatocarcinogenesis was observed in patients with high HBsAg levels [≥2000 IU/ml], despite efficient suppression of HBV viraemia as a result of long-term NA therapy [65]. Also, patients from a big Taiwanese cohort, who maintained a high HBsAg load (>1000 IU/ml) showed an elevated risk for HCC development [66]. Furthermore, a subgroup of patients of the REVEAL HBV cohort with HBsAg > 1000 IU/ml, despite spontaneous *HBV* DNA clearance, still showed an HCC risk ratio of 3.86 above baseline [67].

One possible direct carcinogenic effect is the accumulation of HBV surface proteins in the endoplasmic reticulum (ER), leading to the typical histological finding of hepatocytes overloaded with virus protein in HBV-infected livers, referred to as 'ground glass' hepatocytes (GGHs), first described by Hadziyannis and Popper in 1973 [87]. Many studies have shown that GGHs correlate with the expression pattern of HBV antigens and activity of CHB and appear in preneoplastic lesions, harbouring HBsAg with specific pre-S mutants [88]. Type I GGHs harbour mutants with deletions within the pre-S1 region (mutated L protein), whereas type II GGHs contain pre-S2 mutants and are distributed in clusters, showing patterns of proliferative nodules [89]. Over two decades ago, a mouse model over-expressing the HBV large envelope polypeptide (L), PreS1 and parts of HBx [90] was shown to have liver cell injury, inflammation and compensatory proliferation, finally progressing to HCC due to apoptotic dysregulation [91]. This was also found in HBV transgenic mouse models expressing mutated preS2 [92]. Linking different types of GGHs to preS/S mutants identified a direct oncogenic effect of HBV. The emergence of HBV variants carrying mutations in the preS/S genomic region is reportedly a quite frequent event, especially as a consequence of antiviral therapy. Selected changes in the polymerase ORF of HBV in the course of therapy with NA may also cause changes in the structural proteins due to their overlapping ORFs. Some major drug-resistant mutations against NA in the HBV polymerase cause a premature stop codon in the overlapping HBsAg (ie rtA181T → sW172stop, rt184M → sL176stop). These premature stop codons at positions 172 or 181 of the S gene lead to secretory defects and are significantly associated with cirrhosis and HCC [93,94]. However, only alterations that do not compromise viral fitness will be selected. For example, the M surface protein (consisting of preS2 and S), which overlaps a spacer domain of the Pol ORF, is not essential for formation and secretion of virions and tolerates mutations and even large deletions without causing loss of polymerase activity [31,32,95,96]. Of note, viral mutants with antigenically modified surface antigen (HBsAg 'a' escape variants) are potentially infectious to vaccinated patients and may be overlooked by common HBsAg assays in the clinic, thus possibly accounting for cases of 'occult' hepatitis B infection [31].

Accumulation and imbalance of unfolded or misfolded proteins activates intracellular signalling pathways associated with ER stress. This is buffered by the activation of the so-called unfolded protein response (UPR), a homeostatic signalling network recovering ER function, or, if this fails, triggering apoptosis [97]. Additional functions of the UPR described even include a role in innate immunity, metabolism and cell differentiation [98]. UPR has been shown to be an important source of intracellular ROS [99], and oxidative stress caused by increased ROS is known to have a high oncogenic potential by inducing DNA damage and changes in signalling cascades that control proliferation, cell survival and cell death [99,100]. The role of such events in HCC development has been described in many experimental studies [101–103].

Both experimental and human data support an important role of preS/S mutants in the induction of ER stress, with consequent oxidative DNA damage and genomic instability. Pre-S mutants hence may promote hepatocyte proliferation, up-regulate vascular endothelial growth factor-A (VEGF-A), cyclin A and activate Akt–mammalian target of rapamycin (Akt–mTOR) signalling by the induction of UPR [31,35,104–106]. Furthermore, PreS2 mutants can exert oncogenic functions directly by interacting with intracellular signalling pathways [31,104]. A frequent mutant is the C-terminally truncated M protein (see above), also referred to as MHBs(t) [96,104], which can frequently be found integrated in HCC [107]. MHBs(t) may induce TRAIL-induced, caspase-3-dependent apoptosis [108], increase hepatocyte proliferation, trigger PKC-dependent activation of c-Raf-1–Erk2–Ap-1 and NF-κB activation and show *trans*-activation potential [104]. In a meta-analysis of 11 582 patients, the presence of preS mutants was found to correlate with a 3.77-fold higher risk for HCC development [109], underlining the significance of these findings.

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

HBx/HBV X-protein and frequent truncations in the course of HCC development

The HBx protein has been implicated as a direct oncogenic protein [110–112]. Studies using *HBx* transgenic mouse models have shown spontaneous HCC development [113,114]; however, other groups merely observed an increased sensitivity to other known carcinogens [115,116]. HBx, a small polypeptide (154 amino acids), is usually expressed at low levels during HBV infections and is frequently detected at high levels in HBV-related HCCs [117]. HBx can be found in the cytoplasm of infected hepatocytes and at low levels in the nucleus, and has been identified as a *trans*-activating protein [118], playing a crucial role in virus gene expression, viraemia and infectivity [119]. An X-protein-negative clone of woodchuck hepatitis virus failed to establish chronic infection [120]. Severity of liver damage correlates with levels of HBV due to increased integration of HBx in active chronic liver disease and increased hepatocyte proliferation [121,122]. It has been reported that HBx is involved in hepatocarcinogenesis, due to pleiotropic effects on several signalling pathways regulating cell death, proliferation, differentiation (including EMT), oxidative stress and DNA repair, as possible oncogenic factors in hepatocarcinogenesis (see Figure 3) (reviewed in [123–125]). For example, HBx has been reported to drive pro-proliferation [126], induce cell-cycle arrest [127] and prevent [128] or even induce apoptosis [129].

One proposed possible direct carcinogenic effect of HBx is the induction of regional hypermethylation or

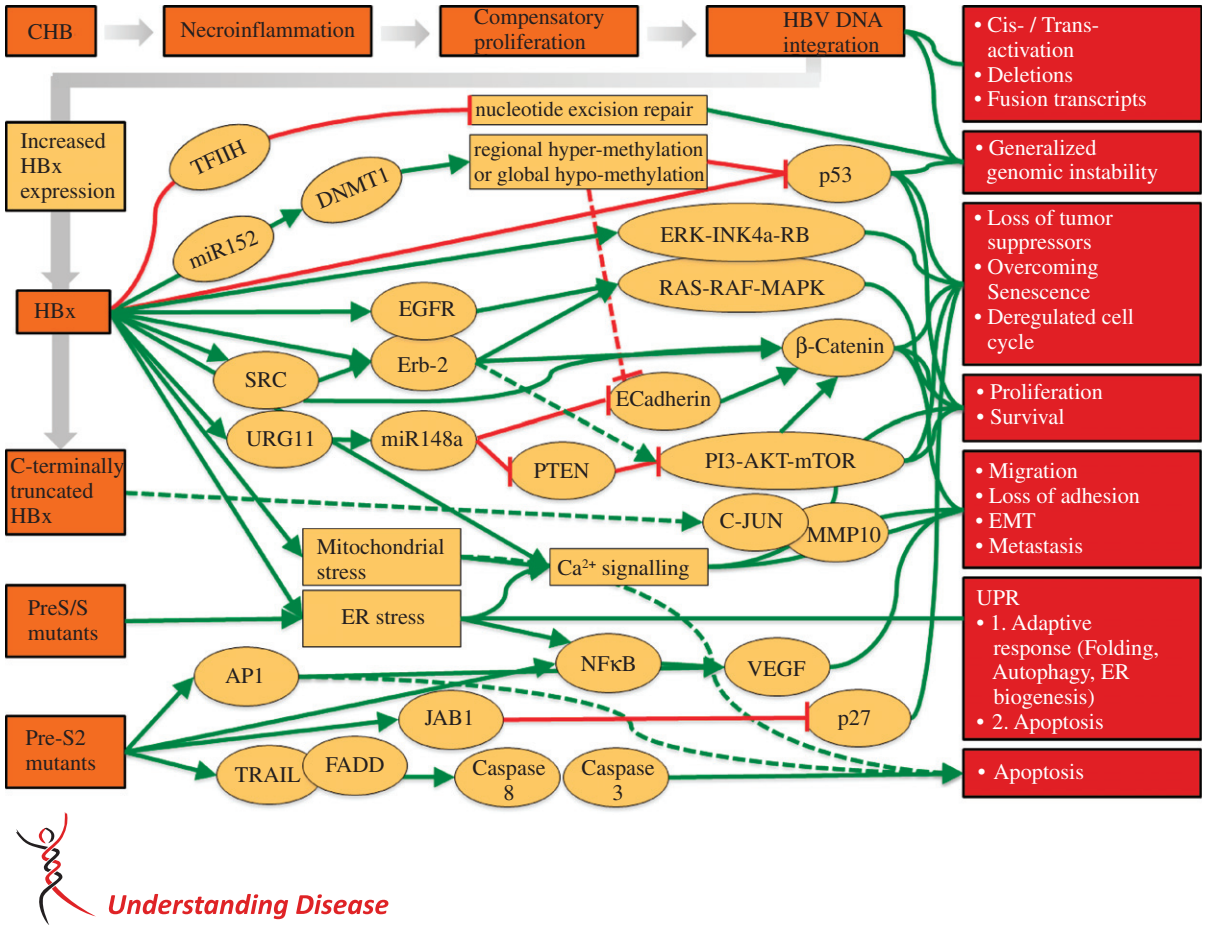

Figure 3. Examples of proposed, direct HBV effects driving hepatocarcinogenesis. The key players of HBV integration, HBx, c-terminally truncated HBx and PreS/S mutants, as well as their proposed signalling pathways in HBV-associated hepatocellular carcinoma (HCC), are shown. These factors directly alter the expression and activity of selected signalling pathways and drive malignant transformation. Secondly, these factors can drive metastatic disease as well as worsen overall survival by making HCC more aggressive: CHB, chronic hepatitis B; TFIIH, transcription factor II H; miR, microRNA; DNMT1, DNA methyltransferase 1; ERK, extracellular signal-regulated kinases; INK4a, also known as p16/cyclin-dependent kinase inhibitor 2A; RB, retinoblastoma protein; RAS, protein family class of small GTPases; RAF, family of three serine/threonine-specific protein kinases; MAPK, mitogen-activated protein kinases; EGFR, epidermal growth factor receptor (also known as ErbB-1/HER1); ErbB-2, also known as CD340/proto-oncogene Neu/human epidermal growth factor receptor 2 HER2/neu; SRC, c-Src, a non-receptor protein tyrosine kinase; *URG11*, up-regulated gene 11; PTEN, phosphatase and tensin homologue; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; AKT, also known as protein kinase B (PKB); mTOR, mammalian target of rapamycin; MMP10, matrix metalloproteinase 10; ER, endoplasmic reticulum; AP1, activator protein 1; NF-κB, nuclear factor-κ light chain enhancer of activated B cells; VEGF, vascular endothelial growth factor; JAB1, JAK-binding protein; p27/Cip1, cyclin-dependent kinase inhibitor 1B; TRAIL, TNF-related apoptosis-inducing ligand; FADD, Fas-associated protein with death domain; EMT, epithelial–mesenchymal transition; UPR, unfolded protein response)

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

global hypomethylation, eg by inducing DNA methyltransferase 1 (*DNMT1*) expression, silencing tumour suppressor genes and inducing chromosomal instability [130]. Accordingly, HBx was reported to complex with wild-type p53 protein, limiting p53-specific tumour suppressor functions (for review, see [131]) by inhibiting sequence-specific DNA binding and transcriptional activation, as well as suppressing nucleotide excision repair [132,133]. In the same direction, HBx was shown to activate the RAS–RAF–MAPK [134] and ERK–INK4a–RB pathways [135]. This abrogates expression of tumour suppressors and overcomes senescence, which is usually triggered due to chronic inflammation and oxidative stress to protect from carcinogenesis [136]. Next, HBx was reported to overcome senescence by suppression of cyclin-dependent kinase inhibitors INK4a (p16), as well as p21 Cip1/CDKN1A) [137] and to deregulate the G_2–M checkpoint, resulting in a rescue from HBx-mediated apoptosis [138]. Another proposed key player in suppressing DNA repair and tumour suppressors is directly by HBx-up-regulated *URG11*, altering expression of multiple microRNAs. Resulting up-regulation of *miR-148a* has been shown to drive cell cycle progression and cell migration by suppressing PTEN, thus increasing Akt–mTOR signalling [139]. Furthermore, other microRNAs are possibly also directly targeted by HBx-like *miR-122a*, which targets cyclin G1 [140] and inhibits HBV replication, as well as inhibiting expression of p27 [121].

Other effects attributed to HBx are enhanced oxidative stress, increased levels of ROS and calcium signalling by interaction with the ER [141] and mitochondria [123,142,143]. Various studies point to sustained higher cytosolic calcium levels and a stimulating effect on HBV replication, in addition to other possible oncogenic effects, such as activating src and ras signalling [144] (reviewed in [143]). HBx was shown to activate FOXO4, which enhances resistances to ROS-induced cell death [145], activating autophagy as a pro-survival factor facilitating virus persistence and hepatocarcinogenesis [100,146]. Additionally, hypoxia induced by necro-inflammation induces binding and stabilizing hypoxia-induced factor 1a (HIF1a) by HBx, promoting angiogenesis and cell migration by up-regulation of VEGF and matrix metalloproteinases [147–149].

Increased EGFR signalling and up-regulation of ErbB2 (Her2/Neu) and other members of the epidermal growth factor receptor tyrosine kinases, as well as loss of inhibitor proteins, have been reported in a subset of HCCs, creating autocrine/paracrine activation loops [150–153]. ErbB2 up-regulation in CHB and in some HCCs correlated with HBx expression [154,155]. Targeting *ERBB2* mRNA by a specific siRNA not only reduced ErbB2 expression but also reduced the expression of β-catenin [154]. This links the ErbB family to the multitude of effects reported by activation of β-catenin signalling in HCC, including proliferation, migration, EMT and loss of adhesion. β-Catenin activation occurs in many cancers, mostly driven by activating mutations in β-catenin itself or by inherited and sporadic mutations in regulatory proteins such as the tumour suppressor APC and AXIN, which normally complex and inactivate β-catenin in the OFF-state of canonical WNT signalling, together with E-cadherin and other factors [156–158]. In human HCC, mutations in WNT/β-catenin signalling are common (~15–33% *CTNNB1*, ~15% *AXIN1* and 1–2% *APC*) [159–161]. However, these mutations were mostly reported in HBV-negative HCCs and frequent β-catenin activation in CHB-driven HCC must rely on other modes of activation. Consistent with this idea, up-regulation of *URG11* through HBx leads to up-regulation of β-catenin [138]; inhibitory binding of β-catenin by E-cadherin is facilitated by HBx through DNA methylation of the E-cadherin promoter [162] or inhibition of miR-373 and up-regulation of SNAIL [163] – all driving increased WNT-independent activation of β-catenin. Furthermore, HBx was reported to activate the proto-oncogene Src, which can also activate β-catenin signalling [164–166].

The analysis of *HBx* mutants naturally induced by integration in the host genome has helped to define the functional relevance of protein domains of HBx. For example, HBx amino acids 116–140 were shown to be required for mitogenic pathway activation [167]. HBx variants were reported to play an important role in the course of hepatocarcinogenesis [148,167–170] and were reported to be independent predictors for survival after HCC resection [171]. C-terminally truncated HBx, in particular, is frequently found in HCC [81,86,168,169]. A recent study reported detection of C-terminally truncated HBx in 46% of HBV-HCC [168], which was significantly correlated with venous invasion, and *in vitro* data showed increased cell invasion, C-Jun activity and expression of MMP10 [168].

At the same time, multiple functions of HBx, as well as changes in host gene expression mediated by HBx, seem to be essential for virus replication and hepatocyte survival or protection from the immune response, consequently benefiting the virus and hence promoting HCC development [121]. Some of the proposed oncogenic effects of HBx mentioned above were reported from *in vitro* experiments or mouse models with very high expression levels of the HBx protein. This points to a potent function of HBx also in already established HCC, since the highest accumulation of viral *HBx* RNA was found in HCC tissue [110,172]. Secondly, the anti-apoptotic and pro-apoptotic effects of the HBx protein seem to be dependent on the status of hepatocyte differentiation [173].

Future perspective

Identification of new therapeutic targets in HCC

Potentially curative treatment options for HCC, such as liver transplantation and surgical interventions, are limited to early stages [174]. However, many patients face a recurrence of HCC after resection, or are only eligible for palliative treatment due to advanced

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

tumour stage. Sorafenib, a pan-kinase inhibitor, remains the only approved systemic, standard-of-care drug, improving overall survival by only 2–3 months and with significant side-effects [175,176]. A recent study described that sorafenib not only directly inhibits tumour cell growth but also modifies the crosstalk between tumour-associated macrophages and natural killer cells, thus slowing down HCC growth [177]. The poor prognostic outcome of late-stage HCC results from human HCCs showing a diverse spectrum of cancer subtypes, without common oncogene addiction loops [174,178]. Establishing a comprehensive molecular classification of HCC remains a challenge, due to inter- and intratumour heterogeneity [159,179–181]. The different aetiologies of HCC, and especially the direct carcinogenic effects by HBV, can contribute synergistically or even alone as carcinogens. In this 'non-Vogelstein-type' carcinogenesis, a molecular classification consisting of six HCC subgroups was proposed [182], pointing to a specific role of low or high copy number of HBV (termed 'G1/G2' tumours) with specific oncogene patterns [160]. Even though the role of certain viral proteins, as well as HBV integration, seems overly complex, compelling evidence exists for their direct role in hepatocarcinogenesis and they should be further evaluated in carcinogenic-risk stratification and therapeutic approaches. Thus, the integration of a molecular classification, intratumour diversity and aetiological backgrounds, including direct oncogenic factors of HBV, are missing. This might explain the failure of some promising Phase II and Phase III trials using kinase inhibitors, eg sunitinib, brivanib, erlotenib and linifanib and the mTor inhibitor, everolimus [178]. In order to understand which patients might profit most from certain therapeutics, a personalized fingerprinting of HCC is likely to be needed in future clinical trials.

Active surveillance and preventing HCC development in CHB

Hepatocarcinogenesis remains a major risk for chronically HBV-infected patients, despite the existence of potent antiviral NAs with high genetic barriers. Active surveillance programmes can significantly reduce morbidity and mortality from HCC [1,62] because early HCC detection enables potentially curative resection. *HBV* DNA levels have been confirmed as the most important predictor of disease progression and HCC development [5]. However, HBsAg levels and increased baseline HCC risk should be taken into account for active surveillance, even in *HBV* DNA-negative patients undergoing NA treatment, since over-expression and truncation of HBsAg increase the risk for HCC [31,62,65,110,172]. Thus, non-invasive markers for risk stratification are necessary to identify patients at high risk for HCC, allowing early diagnosis and treatment. Certain viral proteins (ie HBs, HBx and their mutants), as well as integration of *HBV* DNA, should be evaluated for inclusion in a screening of a personal risk prediction for HCC.

Acknowledgements

MR received a clinical leave stipend funded by the German Centre for Infection Research (DZIF); MH was supported by the Foundation for Experimental Biomedicine Zürich, Switzerland.

Contributions

MR, UP, TO and MH wrote the text.

Abbreviations

AKT, protein kinase B (PKB); anti-HBc, antibodies against HBcAg; anti-HBe, antibodies against HBeAg; anti-HBs, antibodies against HBsAg; AP1, activator protein 1; BCP, basal core promoter; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; CLD, chronic liver disease; DNMT1, DNA methyltransferase 1; EGFR, epidermal growth factor receptor (also known as ErbB-1/HER1); EMT, epithelial–mesenchymal transition; ER, endoplasmic reticulum; ErbB-2, CD340/proto-oncogene Neu/human epidermal growth factor receptor 2; ERK, extracellular signal-regulated kinases; FADD, Fas-associated protein with death domain; GGHs, 'ground glass' hepatocytes; HBc, hepatitis B core protein; HBeAg, hepatitis B virus envelope antigen; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HBx, hepatitis B virus X protein; HCC, hepatocellular carcinoma; INK4, p16/cyclin-dependent kinase inhibitor 2A; JAB1, JAK-binding protein; LINE, long interspersed nuclear elements; MAPK, mitogen-activated protein kinases; miR, microRNA; MMP10, matrix metalloproteinase 10; mTOR, mammalian target of rapamycin; NA, nucleoside/nucleotide analogues; NF-κB, nuclear factor-κ light chain enhancer of activated B cells; NTCP, sodium taurocholate co-transporting polypeptide; p27/Cip1, cyclin-dependent kinase inhibitor 1B; pgRNA, pregenomic RNA; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PTEN, phosphatase and tensin homologue; RAF, family of three serine/threonine-specific protein kinases; RAS, protein family class of small GTPases; RB, retinoblastoma protein; rcDNA, relaxed circular DNA; ROS, reactive oxygen species; SINE, short interspersed nuclear elements; SRC, c-Src, a non-receptor protein tyrosine kinase; TFIIH, transcription factor II H; TRAIL, TNF-related apoptosis-inducing ligand; Treg, regulatory T cells; UPR, unfolded protein response; *URG11*, up-regulated gene 11; VEGF, vascular endothelial growth factor.

References

1. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142:** 1264–1273 e1261.
2. Parkin DM. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; **118:** 3030–3044.

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

3. Ringelhan M, Heikenwalder M, Protzer U. Direct effects of hepatitis B virus-encoded proteins and chronic infection in liver cancer development. *Dig Dis* 2013; **31:** 138–151.
4. Fattovich G, Olivari N, Pasino M, *et al.* Long-term outcome of chronic hepatitis B in Caucasian patients: mortality after 25 years. *Gut* 2008; **57:** 84–90.
5. Chen CJ, Yang HI. Natural history of chronic hepatitis B REVEALed. *J Gastroenterol Hepatol* 2011; **26:** 628–638.
6. Yang HI, Yeh SH, Chen PJ, *et al.* Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100:** 1134–1143.
7. Kim BK, Han KH, Ahn SH. Prevention of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *Oncology* 2011; **81**(suppl 1): 41–49.
8. World Health Organization. Hepatitis B. WHO Fact Sheet No. 204: http://www.who.int/mediacentre/factsheets/fs204/en/WHO 2012
9. McMahon BJ. The natural history of chronic hepatitis B virus infection. *Hepatology* 2009; **49:** S45–55.
10. Lin CL, Kao JH. Risk stratification for hepatitis B virus related hepatocellular carcinoma. *J Gastroenterol Hepatol* 2013; **28:** 10–17.
11. Chan HL, Hui AY, Wong ML, *et al.* Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. *Gut* 2004; **53:** 1494–1498.
12. Bonilla Guerrero R, Roberts LR. The role of hepatitis B virus integrations in the pathogenesis of human hepatocellular carcinoma. *J Hepatol* 2005; **42:** 760–777.
13. Shi Y, Wu YH, Wu W, *et al.* Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis. *Liver Int* 2012; **32:** 231–240.
14. Yan H, Zhong G, Xu G, *et al.* Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife* 2012; **1:** e00049.
15. Nkongolo S, Ni Y, Lempp FA, *et al.* Cyclosporin A inhibits hepatitis B and hepatitis D virus entry by cyclophilin-independent interference with the NTCP receptor. *J Hepatol* 2014; **60:** 723–731.
16. Protzer U, Maini MK, Knolle PA. Living in the liver: hepatic infections. *Nat Rev Immunol* 2012; **12:** 201–213.
17. Jilbert AR, Miller DS, Scougall CA, *et al.* Kinetics of duck hepatitis B virus infection following low dose virus inoculation: one virus DNA genome is infectious in neonatal ducks. *Virology* 1996; **226:** 338–345.
18. Asabe S, Wieland SF, Chattopadhyay PK, *et al.* The size of the viral inoculum contributes to the outcome of hepatitis B virus infection. *J Virol* 2009; **83:** 9652–9662.
19. Ott JJ, Stevens GA, Groeger J, *et al.* Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine* 2012; **30:** 2212–2219.
20. Mast EE, Margolis HS, Fiore AE, *et al.* A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: immunization of infants, children, and adolescents. *MMWR Recomm Rep* 2005; **54:** 1–31.
21. Ott JJ, Stevens GA, Wiersma ST. The risk of perinatal hepatitis B virus transmission: hepatitis B e antigen (HBeAg) prevalence estimates for all world regions. *BMC Infect Dis* 2012; **12:** 131.
22. Chen HL, Lin LH, Hu FC, *et al.* Effects of maternal screening and universal immunization to prevent mother-to-infant transmission of HBV. *Gastroenterology* 2012; **142:** 773–781, e772.
23. Liaw YF, Chu CM. Hepatitis B virus infection. *Lancet* 2009; **373:** 582–592.
24. Mast EE, Alter MJ, Margolis HS. Strategies to prevent and control hepatitis B and C virus infections: a global perspective. *Vaccine* 1999; **17:** 1730–1733.
25. Chu CM, Hung SJ, Lin J, *et al.* Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. *Am J Med* 2004; **116:** 829–834.
26. Quasdorff M, Protzer U. Control of hepatitis B virus at the level of transcription. *J Viral Hepat* 2010; **17:** 527–536.
27. Lucifora J, Xia Y, Reisinger F, *et al.* Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science* 2014; **343:** 1221–1228.
28. Ganem D, Prince AM. Hepatitis B virus infection – natural history and clinical consequences. *N Engl J Med* 2004; **350:** 1118–1129.
29. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* 2005; **5:** 215–229.
30. Seeger C, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000; **64:** 51–68.
31. Pollicino T, Cacciola I, Saffioti F, *et al.* Hepatitis B virus preS/S gene variants: pathobiology and clinical implications. *J Hepatol* 2014; **61:** 408–417.
32. Bruss V. Hepatitis B virus morphogenesis. *World J Gastroenterol* 2007; **13:** 65–73.
33. Nassal M. Hepatitis B viruses: reverse transcription a different way. *Virus Res* 2008; **134:** 235–249.
34. Protzer U, Schaller H. Immune escape by hepatitis B viruses. *Virus Genes* 2000; **21:** 27–37.
35. Chisari FV, Klopchin K, Moriyama T, *et al.* Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. *Cell* 1989; **59:** 1145–1156.
36. Guidotti LG, Chisari FV. Immunobiology and pathogenesis of viral hepatitis. *Annu Rev Pathol* 2006; **1:** 23–61.
37. Nakamoto Y, Guidotti LG, Kuhlen CV, *et al.* Immune pathogenesis of hepatocellular carcinoma. *J Exp Med* 1998; **188:** 341–350.
38. Ganem D, Prince AM. Hepatitis B virus infection – natural history and clinical consequences. *N Engl J Med* 2004; **350:** 1118–1129.
39. Bauer T, Sprinzl M, Protzer U. Immune control of hepatitis B virus. *Dig Dis* 2011; **29:** 423–433.
40. Billerbeck E, Bottler T, Thimme R. Regulatory T cells in viral hepatitis. *World J Gastroenterol* 2007; **13:** 4858–4864.
41. Manigold T, Racanelli V. T-cell regulation by CD4 regulatory T cells during hepatitis B and C virus infections: facts and controversies. *Lancet Infect Dis* 2007; **7:** 804–813.
42. Stoop JN, van der Molen RG, Baan CC, *et al.* Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. *Hepatology* 2005; **41:** 771–778.
43. Franzese O, Kennedy PT, Gehring AJ, *et al.* Modulation of the $CD8^+$ T-cell response by $CD4^+$ $CD25^+$ regulatory T cells in patients with hepatitis B virus infection. *J Virol* 2005; **79:** 3322–3328.
44. Stross L, Gunther J, Gasteiger G, *et al.* $Foxp3^+$ regulatory T cells protect the liver from immune damage and compromise virus control during acute experimental hepatitis B virus infection in mice. *Hepatology* 2012; **56:** 873–883.
45. Ringelhan M, Schmid RM, Geisler F. The NF-κB subunit RelA/p65 is dispensable for successful liver regeneration after partial hepatectomy in mice. *PLoS One* 2012; **7:** e46469.
46. Fattovich G, Stroffolini T, Zagni I, *et al.* Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127:** S35–50.
47. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol* 2008; **48:** 335–352.
48. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379:** 1245–1255.
49. Bouchard MJ, Navas-Martin S. Hepatitis B and C virus hepatocarcinogenesis: lessons learned and future challenges. *Cancer Lett* 2011; **305:** 123–143.

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

50. Weber A, Boger R, Vick B, *et al.* Hepatocyte-specific deletion of the antiapoptotic protein myeloid cell leukemia-1 triggers proliferation and hepatocarcinogenesis in mice. *Hepatology* 2010; **51:** 1226–1236.
51. Pikarsky E, Porat RM, Stein I, *et al.* NF-κB functions as a tumour promoter in inflammation-associated cancer. *Nature* 2004; **431:** 461–466.
52. Haybaeck J, Zeller N, Wolf MJ, *et al.* A lymphotoxin-driven pathway to hepatocellular carcinoma. *Cancer Cell* 2009; **16:** 295–308.
53. Fausto N, Campbell JS. Mouse models of hepatocellular carcinoma. *Semin Liver Dis* 2010; **30:** 87–98.
54. Chen CJ, Yang HI, Su J, *et al.* Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *J Am Med Assoc* 2006; **295:** 65–73.
55. Iloeje UH, Yang HI, Su J, *et al.* Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; **130:** 678–686.
56. Kim BK, Han KH, Ahn SH. Prevention of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *Oncology* 2011; **81**(suppl 1): 41–49.
57. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50:** 661–662.
58. European Association for the Study of the L. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; **50:** 227–242.
59. European Association for the Study of the L. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57:** 167–185.
60. Liaw YF, Leung N, Kao JH, *et al.* Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008; **2:** 263–283.
61. Wong DK, Cheung AM, O'Rourke K, *et al.* Effect of α-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. *Ann Intern Med* 1993; **119:** 312–323.
62. Honer Zu Siederdissen C, Cornberg M. The role of HBsAg levels in the current management of chronic HBV infection. *Ann Gastroenterol* 2014; **27:** 105–112.
63. Cho JY, Paik YH, Sohn W, *et al.* Patients with chronic hepatitis B treated with oral antiviral therapy retain a higher risk for HCC compared with patients with inactive stage disease. *Gut* 2014; DOI: 10.1136/gutjnl-2013-306409 (in press).
64. Cornberg M, Protzer U, Petersen J, *et al.* [Prophylaxis, diagnosis and therapy of hepatitis B virus infection – the German guideline]. *Z Gastroenterol* 2011; **49:** 871–930.
65. Kawanaka M, Nishino K, Nakamura J, *et al.* Quantitative levels of hepatitis B virus DNA and surface antigen and the risk of hepatocellular carcinoma in patients with hepatitis B receiving long-term nucleos(t)ide analogue therapy. *Liver Cancer* 2014; **3:** 41–52.
66. Tseng TC, Liu CJ, Yang HC, *et al.* Serum hepatitis B surface antigen levels help predict disease progression in patients with low hepatitis B virus loads. *Hepatology* 2013; **57:** 441–450.
67. Liu J, Yang HI, Lee MH, *et al.* Spontaneous seroclearance of hepatitis B seromarkers and subsequent risk of hepatocellular carcinoma. *Gut* 2014; **63**: 1648–1657.
68. Xia Y, Lucifora J, Reisinger F, *et al.* Virology. Response to Comment on 'Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA'. *Science* 2014; **344:** 1237.
69. Tanwar S, Dusheiko G. Is there any value to hepatitis B virus genotype analysis? *Curr Gastroenterol Rep* 2012; **14:** 37–46.
70. McMahon BJ. The influence of hepatitis B virus genotype and subgenotype on the natural history of chronic hepatitis B. *Hepatol Int* 2009; **3:** 334–342.
71. Wai CT, Chu CJ, Hussain M, *et al.* HBV genotype B is associated with better response to interferon therapy in $HBeAg^+$ chronic hepatitis than genotype C. *Hepatology* 2002; **36:** 1425–1430.
72. Sung WK, Zheng H, Li S, *et al.* Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. *Nat Genet* 2012; **44:** 765–769.
73. Shafritz DA, Shouval D, Sherman HI, *et al.* Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. Studies in percutaneous liver biopsies and post-mortem tissue specimens. *N Engl J Med* 1981; **305:** 1067–1073.
74. Kremsdorf D, Soussan P, Paterlini-Brechot P, *et al.* Hepatitis B virus-related hepatocellular carcinoma: paradigms for viral-related human carcinogenesis. *Oncogene* 2006; **25:** 3823–3833.
75. Chemin I, Zoulim F. Hepatitis B virus induced hepatocellular carcinoma. *Cancer Lett* 2009; **286:** 52–59.
76. Brechot C, Gozuacik D, Murakami Y, *et al.* Molecular bases for the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). *Semin Cancer Biol* 2000; **10:** 211–231.
77. Brechot C. Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. *Gastroenterology* 2004; **127:** S56–61.
78. Murakami Y, Saigo K, Takashima H, *et al.* Large scaled analysis of hepatitis B virus (HBV) DNA integration in HBV related hepatocellular carcinomas. *Gut* 2005; **54:** 1162–1168.
79. Paterlini-Brechot P, Saigo K, Murakami Y, *et al.* Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene. *Oncogene* 2003; **22:** 3911–3916.
80. Jiang Z, Jhunjhunwala S, Liu J, *et al.* The effects of hepatitis B virus integration into the genomes of hepatocellular carcinoma patients. *Genome Res* 2012; **22:** 593–601.
81. Jiang S, Yang Z, Li W, *et al.* Re-evaluation of the carcinogenic significance of hepatitis B virus integration in hepatocarcinogenesis. *PLoS One* 2012; **7:** e40363.
82. Heikenwalder M, Protzer U. LINE(1)s of evidence in HBV-driven liver cancer. *Cell Host Microbe* 2014; **15:** 249–250.
83. Ding D, Lou X, Hua D, *et al.* Recurrent targeted genes of hepatitis B virus in the liver cancer genomes identified by a next-generation sequencing-based approach. *PLoS Genet* 2012; **8:** e1003065.
84. Li JW, Wan R, Yu CS, *et al.* ViralFusionSeq: accurately discover viral integration events and reconstruct fusion transcripts at single-base resolution. *Bioinformatics* 2013; **29:** 649–651.
85. Lau CC, Sun T, Ching AK, *et al.* Viral-human chimeric transcript predisposes risk to liver cancer development and progression. *Cancer Cell* 2014; **25:** 335–349.
86. Amaddeo G, Cao Q, Ladeiro Y, *et al.* Integration of tumour and viral genomic characterisations in HBV-related hepatocellular carcinomas. *Gut* 2014; DOI: 10.1136/gutjnl-2013-306228 (in press).
87. Hadziyannis S, Gerber MA, Vissoulis C, *et al.* Cytoplasmic hepatitis B antigen in 'ground-glass' hepatocytes of carriers. *Arch Pathol* 1973; **96:** 327–330.
88. Su IJ, Wang HC, Wu HC, *et al.* Ground glass hepatocytes contain pre-S mutants and represent preneoplastic lesions in chronic hepatitis B virus infection. *J Gastroenterol Hepatol* 2008; **23:** 1169–1174.
89. Wang HC, Wu HC, Chen CF, *et al.* Different types of ground glass hepatocytes in chronic hepatitis B virus infection contain specific pre-S mutants that may induce endoplasmic reticulum stress. *Am J Pathol* 2003; **163:** 2441–2449.
90. Dunsford HA, Sell S, Chisari FV. Hepatocarcinogenesis due to chronic liver cell injury in hepatitis B virus transgenic mice. *Cancer Res* 1990; **50:** 3400–3407.
91. Barone M, Spano D, D'Apolito M, *et al.* Gene expression analysis in HBV transgenic mouse liver: a model to study early events related to hepatocarcinogenesis. *Mol Med* 2006; **12:** 115–123.
92. Na B, Huang Z, Wang Q, *et al.* Transgenic expression of entire hepatitis B virus in mice induces hepatocarcinogenesis independent of chronic liver injury. *PLoS One* 2011; **6:** e26240.

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

93. Lai MW, Yeh CT. The oncogenic potential of hepatitis B virus rtA181T/surface truncation mutant. *Antivir Ther* 2008; **13:** 875–879.
94. Warner N, Locarnini S. The antiviral drug selected hepatitis B virus rtA181T/sW172* mutant has a dominant negative secretion defect and alters the typical profile of viral rebound. *Hepatology* 2008; **48:** 88–98.
95. Locarnini S. Hepatitis B viral resistance: mechanisms and diagnosis. *J Hepatol* 2003; **39**(suppl 1): S124–132.
96. Locarnini S, McMillan J, Bartholomeusz A. The hepatitis B virus and common mutants. *Semin Liver Dis* 2003; **23:** 5–20.
97. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 2007; **8:** 519–529.
98. Moenner M, Pluquet O, Bouchecareilh M, *et al.* Integrated endoplasmic reticulum stress responses in cancer. *Cancer Res* 2007; **67:** 10631–10634.
99. Haynes CM, Titus EA, Cooper AA. Degradation of misfolded proteins prevents ER-derived oxidative stress and cell death. *Mol Cell* 2004; **15:** 767–776.
100. Sosa V, Moline T, Somoza R, *et al.* Oxidative stress and cancer: an overview. *Ageing Res Rev* 2013; **12:** 376–390.
101. Hagen TM, Huang S, Curnutte J, *et al.* Extensive oxidative DNA damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. *Proc Natl Acad Sci USA* 1994; **91:** 12808–12812.
102. Hung JH, Su IJ, Lei HY, *et al.* Endoplasmic reticulum stress stimulates the expression of cyclooxygenase-2 through activation of NF-κBand pp38 mitogen-activated protein kinase. *J Biol Chem* 2004; **279:** 46384–46392.
103. Block TM, Mehta AS, Fimmel CJ, *et al.* Molecular viral oncology of hepatocellular carcinoma. *Oncogene* 2003; **22:** 5093–5107.
104. Hildt E, Munz B, Saher G, *et al.* The PreS2 activator MHBs(t) of hepatitis B virus activates c-raf-1/Erk2 signaling in transgenic mice. *EMBO J* 2002; **21:** 525–535.
105. Wang HC, Chang WT, Chang WW, *et al.* Hepatitis B virus pre-S2 mutant upregulates cyclin A expression and induces nodular proliferation of hepatocytes. *Hepatology* 2005; **41:** 761–770.
106. Yang JC, Teng CF, Wu HC, *et al.* Enhanced expression of vascular endothelial growth factor-A in ground glass hepatocytes and its implication in hepatitis B virus hepatocarcinogenesis. *Hepatology* 2009; **49:** 1962–1971.
107. Kekule AS, Lauer U, Meyer M, *et al.* The preS2/S region of integrated hepatitis B virus DNA encodes a transcriptional transactivator. *Nature* 1990; **343:** 457–461.
108. Liang X, Du J, Liu Y, *et al.* The hepatitis B virus protein MHBs(t) sensitizes hepatoma cells to TRAIL-induced apoptosis through ERK2. *Apoptosis* 2007; **12:** 1827–1836.
109. Liu S, Zhang H, Gu C, *et al.* Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; **101:** 1066–1082.
110. Paterlini P, Poussin K, Kew M, *et al.* Selective accumulation of the X transcript of hepatitis B virus in patients negative for hepatitis B surface antigen with hepatocellular carcinoma. *Hepatology* 1995; **21:** 313–321.
111. Pollicino T, Saitta C. Occult hepatitis B virus and hepatocellular carcinoma. *World J Gastroenterol* 2014; **20:** 5951–5961.
112. Ou DP, Tao YM, Tang FQ, *et al.* The hepatitis B virus X protein promotes hepatocellular carcinoma metastasis by upregulation of matrix metalloproteinases. *Int J Cancer* 2007; **120:** 1208–1214.
113. Kim CM, Koike K, Saito I, *et al. HBx* gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* 1991; **351:** 317–320.
114. Yu DY, Moon HB, Son JK, *et al.* Incidence of hepatocellular carcinoma in transgenic mice expressing the hepatitis B virus X-protein. *J Hepatol* 1999; **31:** 123–132.
115. Terradillos O, Billet O, Renard CA, *et al.* The hepatitis B virus X gene potentiates c-myc-induced liver oncogenesis in transgenic mice. *Oncogene* 1997; **14:** 395–404.
116. Slagle BL, Lee TH, Medina D, *et al.* Increased sensitivity to the hepatocarcinogen diethylnitrosamine in transgenic mice carrying the hepatitis B virus X gene. *Mol Carcinog* 1996; **15:** 261–269.
117. Hwang GY, Lin CY, Huang LM, *et al.* Detection of the hepatitis B virus X protein (HBx) antigen and anti-HBx antibodies in cases of human hepatocellular carcinoma. *J Clin Microbiol* 2003; **41:** 5598–5603.
118. Misra KP, Mukherji A, Kumar V. The conserved amino-terminal region (amino acids 1–20) of the hepatitis B virus X protein shows a transrepression function. *Virus Res* 2004; **105:** 157–165.
119. Lucifora J, Arzberger S, Durantel D, *et al.* Hepatitis B virus X protein is essential to initiate and maintain virus replication after infection. *J Hepatol* 2011; **55:** 996–1003.
120. Zoulim F, Saputelli J, Seeger C. Woodchuck hepatitis virus X protein is required for viral infection *in vivo*. *J Virol* 1994; **68:** 2026–2030.
121. Arzumanyan A, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer* 2013; **13:** 123–135.
122. Jin YM, Yun C, Park C, *et al.* Expression of hepatitis B virus X protein is closely correlated with the high periportal inflammatory activity of liver diseases. *J Viral Hepat* 2001; **8:** 322–330.
123. Feitelson MA, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. *Cancer Lett* 2007; **252:** 157–170.
124. Feitelson MA. Mechanisms of HBx mediated liver cancer: multiple pathways and opportunities. In Lau JWY, ed. *Hepatocellular Carcinoma – Basic Research*, Lau JWY (ed.). InTech, 2012; 287–312: www.intechopen.com
125. Neuveut C, Wei Y, Buendia MA. Mechanisms of HBV-related hepatocarcinogenesis. *J Hepatol* 2010; **52:** 594–604.
126. Madden CR, Slagle BL. Stimulation of cellular proliferation by hepatitis B virus X protein. *Dis Markers* 2001; **17:** 153–157.
127. Bouchard M, Giannakopoulos S, Wang EH, *et al.* Hepatitis B virus HBx protein activation of cyclin A-cyclin-dependent kinase 2 complexes and G1 transit via a Src kinase pathway. *J Virol* 2001; **75:** 4247–4257.
128. Elmore LW, Hancock AR, Chang SF, *et al.* Hepatitis B virus X protein and p53 tumor suppressor interactions in the modulation of apoptosis. *Proc Natl Acad Sci USA* 1997; **94:** 14707–14712.
129. Su F, Schneider RJ. Hepatitis B virus HBx protein sensitizes cells to apoptotic killing by tumor necrosis factor-α. *Proc Natl Acad Sci USA* 1997; **94:** 8744–8749.
130. Jung JK, Arora P, Pagano JS, *et al.* Expression of DNA methyltransferase 1 is activated by hepatitis B virus X protein via a regulatory circuit involving the p16INK4a–cyclin D1–CDK 4/6–pRb–E2F1 pathway. *Cancer Res* 2007; **67:** 5771–5778.
131. Meek DW. Tumour suppression by p53: a role for the DNA damage response? *Nat Rev Cancer* 2009; **9:** 714–723.
132. Wang XW, Forrester K, Yeh H, *et al.* Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc Natl Acad Sci USA* 1994; **91:** 2230–2234.
133. Chan C, Wang Y, Chow PK, *et al.* Altered binding site selection of p53 transcription cassettes by hepatitis B virus X protein. *Mol Cell Biol* 2013; **33:** 485–497.
134. Benn J, Schneider RJ. Hepatitis B virus HBx protein activates Ras–GTP complex formation and establishes a Ras, Raf, MAP kinase signaling cascade. *Proc Natl Acad Sci USA* 1994; **91:** 10350–10354.
135. Tarn C, Lee S, Hu Y, *et al.* Hepatitis B virus X protein differentially activates RAS–RAF–MAPK and JNK pathways in X-transforming

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

versus non-transforming AML12 hepatocytes. *J Biol Chem* 2001; **276:** 34671–34680.
136. Collado M, Serrano M. The senescent side of tumor suppression. *Cell Cycle* 2005; **4:** 1722–1724.
137. Park SH, Jung JK, Lim JS, *et al.* Hepatitis B virus X protein overcomes all-trans retinoic acid-induced cellular senescence by downregulating levels of p16 and p21 via DNA methylation. *J Gen Virol* 2011; **92:** 1309–1317.
138. Lee S, Tarn C, Wang WH, *et al.* Hepatitis B virus X protein differentially regulates cell cycle progression in X-transforming versus nontransforming hepatocyte (AML12) cell lines. *J Biol Chem* 2002; **277:** 8730–8740.
139. Yuan K, Lian Z, Sun B, *et al.* Role of miR-148a in hepatitis B associated hepatocellular carcinoma. *PLoS One* 2012; **7:** e35331.
140. Gramantieri L, Ferracin M, Fornari F, *et al.* Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res* 2007; **67:** 6092–6099.
141. Wu HC, Tsai HW, Teng CF, *et al.* Ground-glass hepatocytes co-expressing hepatitis B virus X protein and surface antigens exhibit enhanced oncogenic effects and tumorigenesis. *Hum Pathol* 2014; **45:** 1294–1301.
142. Clippinger AJ, Bouchard MJ. Hepatitis B virus HBx protein localizes to mitochondria in primary rat hepatocytes and modulates mitochondrial membrane potential. *J Virol* 2008; **82:** 6798–6811.
143. Yang B, Bouchard MJ. The hepatitis B virus X protein elevates cytosolic calcium signals by modulating mitochondrial calcium uptake. *J Virol* 2012; **86:** 313–327.
144. Bouchard MJ, Wang LH, Schneider RJ. Calcium signaling by HBx protein in hepatitis B virus DNA replication. *Science* 2001; **294:** 2376–2378.
145. Srisuttee R, Koh SS, Park EH, *et al.* Up-regulation of Foxo4 mediated by hepatitis B virus X protein confers resistance to oxidative stress-induced cell death. *Int J Mol Med* 2011; **28:** 255–260.
146. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat Rev Mol Cell Biol* 2012; **13:** 89–102.
147. Yoo YG, Oh SH, Park ES, *et al.* Hepatitis B virus X protein enhances transcriptional activity of hypoxia-inducible factor-1α through activation of mitogen-activated protein kinase pathway. *J Biol Chem* 2003; **278:** 39076–39084.
148. Liu LP, Hu BG, Ye C, *et al.* HBx mutants differentially affect the activation of hypoxia-inducible factor-1α in hepatocellular carcinoma. *Br J Cancer* 2014; **110:** 1066–1073.
149. Yoo YG, Na TY, Seo HW, *et al.* Hepatitis B virus X protein induces the expression of MTA1 and HDAC1, which enhances hypoxia signaling in hepatocellular carcinoma cells. *Oncogene* 2008; **27:** 3405–3413.
150. Neo SY, Leow CK, Vega VB, *et al.* Identification of discriminators of hepatoma by gene expression profiling using a minimal dataset approach. *Hepatology* 2004; **39:** 944–953.
151. Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. *Hepatology* 2008; **48:** 1312–1327.
152. Berasain C, Ujue Latasa M, Urtasun R, *et al.* Epidermal growth factor receptor (EGFR) crosstalks in liver cancer. *Cancers (Basel)* 2011; **3:** 2444–2461.
153. Fujimoto A, Totoki Y, Abe T, *et al.* Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. *Nat Genet* 2012; **44:** 760–764.
154. Liu J, Ahiekpor A, Li L, *et al.* Increased expression of ErbB-2 in liver is associated with hepatitis Bx antigen and shorter survival in patients with liver cancer. *Int J Cancer* 2009; **125:** 1894–1901.
155. Ito Y, Takeda T, Sakon M, *et al.* Expression and clinical significance of erb-B receptor family in hepatocellular carcinoma. *Br J Cancer* 2001; **84:** 1377–1383.
156. Moon RT, Kohn AD, De Ferrari GV, *et al.* WNT and β-catenin signalling: diseases and therapies. *Nat Rev Genet* 2004; **5:** 691–701.
157. Rubinfeld B, Souza B, Albert I, *et al.* Association of the APC gene product with β-catenin. *Science* 1993; **262:** 1731–1734.
158. Clevers H. Wnt/β-catenin signaling in development and disease. *Cell* 2006; **127:** 469–480.
159. Villanueva A, Llovet JM. Liver cancer in 2013: Mutational landscape of HCC – the end of the beginning. *Nat Rev Clin Oncol* 2014; **11:** 73–74.
160. Zucman-Rossi J. Molecular classification of hepatocellular carcinoma. *Dig Liver Dis* 2010; **42**(suppl 3): S235–241.
161. Guichard C, Amaddeo G, Imbeaud S, *et al.* Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat Genet* 2012; **44:** 694–698.
162. Lee JO, Kwun HJ, Jung JK, *et al.* Hepatitis B virus X protein represses E-cadherin expression via activation of DNA methyltransferase 1. *Oncogene* 2005; **24:** 6617–6625.
163. Arzumanyan A, Friedman T, Kotei E, *et al.* Epigenetic repression of E-cadherin expression by hepatitis B virus x antigen in liver cancer. *Oncogene* 2012; **31:** 563–572.
164. Lara-Pezzi E, Roche S, Andrisani OM, *et al.* The hepatitis B virus HBx protein induces adherens junction disruption in a src-dependent manner. *Oncogene* 2001; **20:** 3323–3331.
165. Lara-Pezzi E, Serrador JM, Montoya MC, *et al.* The hepatitis B virus X protein (HBx) induces a migratory phenotype in a CD44-dependent manner: possible role of HBx in invasion and metastasis. *Hepatology* 2001; **33:** 1270–1281.
166. Klein NP, Schneider RJ. Activation of Src family kinases by hepatitis B virus HBx protein and coupled signaling to Ras. *Mol Cell Biol* 1997; **17:** 6427–6436.
167. Li H, Chi CY, Lee S, *et al.* The mitogenic function of hepatitis B virus X protein resides within amino acids 51 to 140 and is modulated by N- and C-terminal regulatory regions. *J Virol* 2006; **80:** 10554–10564.
168. Sze KM, Chu GK, Lee JM, *et al.* C-terminal truncated hepatitis B virus x protein is associated with metastasis and enhances invasiveness by C-Jun/matrix metalloproteinase protein 10 activation in hepatocellular carcinoma. *Hepatology* 2013; **57:** 131–139.
169. Lee JH, Han KH, Lee JM, *et al.* Impact of hepatitis B virus (HBV) x gene mutations on hepatocellular carcinoma development in chronic HBV infection. *Clin Vaccine Immunol* 2011; **18:** 914–921.
170. Wang Q, Zhang T, Ye L, *et al.* Analysis of hepatitis B virus X gene (HBx) mutants in tissues of patients suffered from hepatocellular carcinoma in China. *Cancer Epidemiol* 2012; **36:** 369–374.
171. Xie Y, Liu S, Zhao Y, *et al.* X protein mutations in hepatitis B virus DNA predict postoperative survival in hepatocellular carcinoma. *Tumour Biol* 2014; **35:** 10325–10331.
172. Paterlini P, Poussin K, Kew M, *et al.* Selective accumulation of the X transcript of hepatitis B virus in patients negative for hepatitis B surface antigen with hepatocellular carcinoma. *Hepatology* 1995; **21:** 313–321.
173. Bouchard MJ, Schneider RJ. The enigmatic X gene of hepatitis B virus. *J Virol* 2004; **78:** 12725–12734.
174. Villanueva A, Hernandez-Gea V, Llovet JM. Medical therapies for hepatocellular carcinoma: a critical view of the evidence. *Nat Rev Gastroenterol Hepatol* 2013; **10:** 34–42.
175. Llovet JM, Ricci S, Mazzaferro V, *et al.* Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359:** 378–390.
176. Cheng AL, Kang YK, Chen Z, *et al.* Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk

carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10:** 25–34.
177. Sprinzl MF, Reisinger F, Puschnik A, *et al.* Sorafenib perpetuates cellular anticancer effector functions by modulating the crosstalk between macrophages and natural killer cells. *Hepatology* 2013; **57:** 2358–2368.
178. Llovet JM, Hernandez-Gea V. Hepatocellular carcinoma: reasons for phase III failure and novel perspectives on trial design. *Clin Cancer Res* 2014; **20:** 2072–2079.
179. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996; **87:** 159–170.
180. Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. *Nat Genet* 2002; **31:** 339–346.
181. Nault JC, Zucman-Rossi J. Genetics of hepatocellular carcinoma: the next generation. *J Hepatol* 2014; **60:** 224–226.
182. Boyault S, Rickman DS, de Reynies A, *et al.* Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 2007; **45:** 42–52.

75 Years ago in the *Journal of Pathology…*

The effect of cholesterol and of sera contaminated with bacteria on the hæmolysins produced by hæmolytic streptococci

L. F. Hewitt and E. W. Todd

The production of hæmolysin and peroxide by hæmolytic streptococci in relation to the non-hæmolytic variants of group A

A. T. Fuller and W. R. Maxted

An investigation of the histological changes in the subcutaneous tissues of mice during the induction of sarcoma by carcinogenic hydrocarbons

John W. Orr

The morphology of 160 tumours induced by carcinogenic hydrocarbons in the subcutaneous tissues of mice

G. M. Bonser and J. W. Orr

To view these articles, and more, please visit:
www.thejournalofpathology.com

Click 'ALL ISSUES (1892 - 2011)', to read articles going right back to Volume 1, Issue 1.

The Journal of Pathology
Understanding Disease

Journal of
The Pathological Society

Copyright © 2014 Pathological Society of Great Britain and Ireland.
Published by John Wiley & Sons, Ltd. www.pathsoc.org.uk