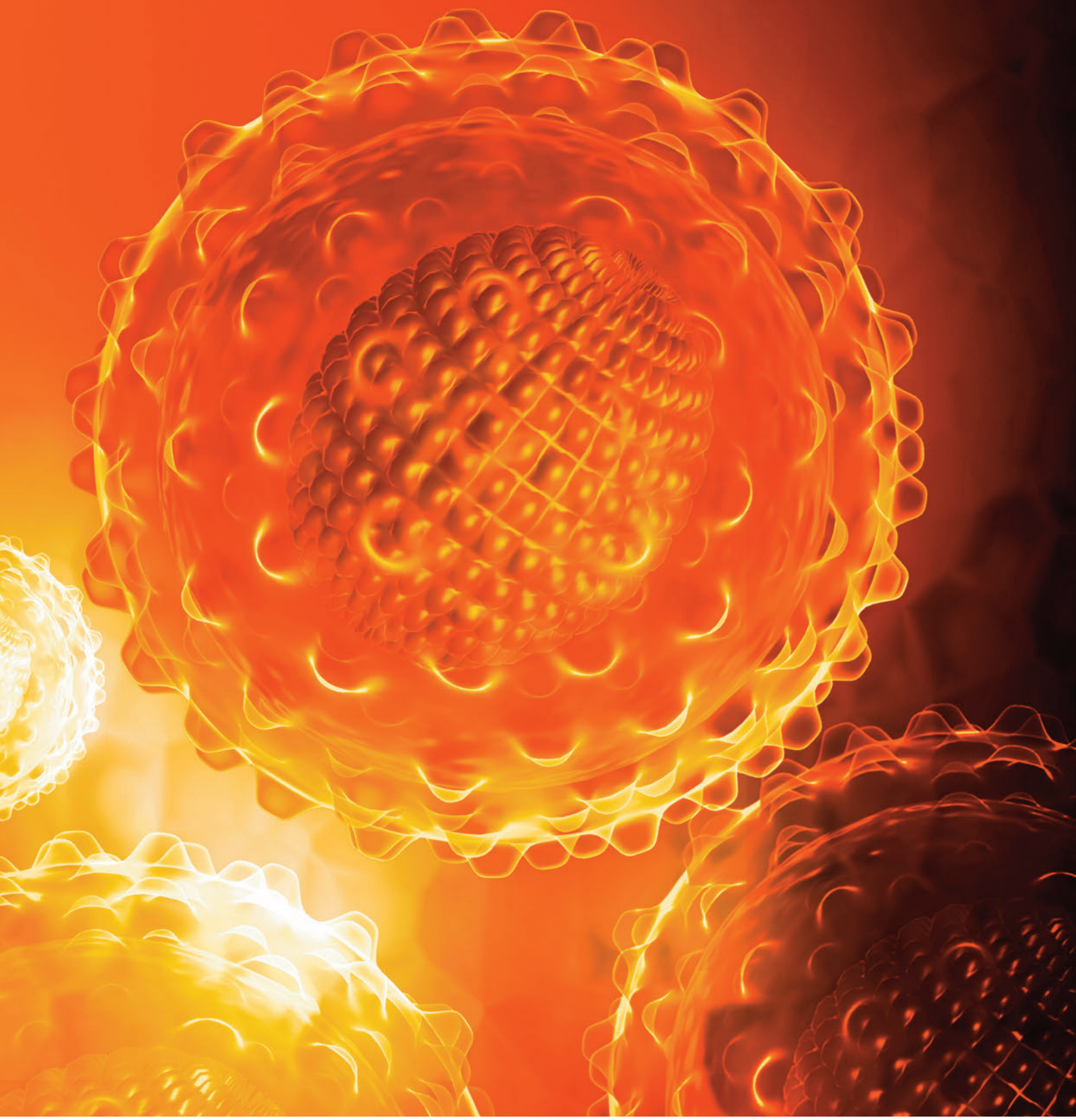
PROMOTING **HEALTH** THROUGH **RESEARCH**



HelmholtzZentrum münchen
German Research Center for Environmental Health

Technische Universität München

The cancer challenge

Commissioner for Health, Tonio Borg supports Europe's concerns about cancer and the huge challenges it presents…

Cancer continues to present a huge challenge for patients and their families, for health policy and for health services across the European Union and indeed beyond.

This is a challenge that can only grow bigger as the population grows older and that's why we need to address it head on. In 2012 alone, 2.6 million European Union citizens were diagnosed with some form of cancer. In the same year, cancer killed close to 1.3 million people in the same European Union.

Given today's incidence rates, we expect that in the European Union, 1 in 3 men and 1 in 4 women will be affected by cancer before reaching 75 years of age.

Cancer is not something that happens only to others. It happens to everybody: our family members, friends, neighbours, colleagues.

This is why cancer is, and must remain, a high priority at all levels.

For over 20 years, the European Commission has contributed towards addressing the cancer challenge. Indeed, the Commission is committed to supporting Member States in the field of cancer.

The Commission launched the European Partnership for Action against cancer back in 2009 and a Joint Action with Member States to implement it. The Joint action provides a framework for identifying and sharing information, capacity and expertise in cancer prevention and control.

This coordination of activities at EU level has helped to pool together expertise, avoid duplication of efforts, and further supported Member States in the development of their national cancer strategies.

Let me briefly mention three examples which highlight the added value of such joint action:

National Cancer Control Strategies

First, we have agreed that by 2014, all EU Member States will have implemented National Cancer Control Strategies or Plans. This will contribute to our ambitious goal to reduce the incidence of cancer by 15% by 2020, as set in our Communication on action against cancer.

Second, the Commission, in co-operation with the International Agency on Research on Cancer, is supporting Member States in implementing screening programmes by developing guidelines for quality assurance for breast, cervical and colorectal cancer screening.

Third, the Commission is currently working on revising the European Code against Cancer, first adopted in 1987. The code is a set of recommendations which seek to get across to citizens two clear measures:

- Certain cancers may be avoided by adopting healthier lifestyles: and here the code includes, for example, the recommendations not to smoke;

- Cancers may be cured, or the prospects of cure greatly increased if they are detected early.

We are confident to launch the revised Code early 2014 and very much hope that patient and medical organisations will play a key role in promoting it. This is particularly important in reaching out to citizens to help prevent cancer, or at least diagnose it at an early stage.

Commissioner for Health
Tonio Borg

The results so far at this Joint Action will be presented in an Open Forum to be held in Ljubljana in November 2014.

Looking further ahead, in 2014 the Commission intends to launch a new Joint Action with Member States on Comprehensive Cancer Control.

The aims of this new Joint action, which we are currently discussing with the Member States, are to identify key elements and quality standards for cancer control in Europe, and to facilitate co-operation among Member States. This includes the exchange of best practices as well as defining key elements to ensure optimal cancer care.

Let me now focus on breast cancer screening, where we have asked the European Commission's Joint Research Centre to update the current European screening and quality assurance guidelines.

The new guidelines – scheduled for 2015 – will help Member States develop and modernise their strategies on breast screening and management, in the best interest of millions of women across the EU.

The new guidelines will form the basis of a set of minimum quality standards for breast cancer services and a voluntary assurance scheme, to be underpinned by accreditation.

This voluntary scheme – the first of its kind in the EU – would place the focus firmly on the patient and would cover all aspects of diagnosis and care.

Let me add that our Joint Research Centre is in the process of setting up a European Cancer Information System, to bring together cancer registries from across Europe. Once again, this work will be taken forward in close cooperation with all relevant stakeholders.

Harmonised data is key to enable better monitoring of the direct effects and benefits of cancer policy interventions, and to allow accurate comparisons to be made across regional and national boundaries. As such, the system will provide a valuable resource for research on cancer, fostering greater understanding of inequalities and related causes.

Still major work to be done

We have come a long way in fighting cancer. There is much work being undertaken and more work is in the pipeline.

It is important to ensure that all this work is taken forward in a co-ordinated and coherent manner. Our success depends very much on sound investments, on sustainable actions, developed in partnership.

To improve the overall governance of our work on cancer, we are considering the creation of a European Union Experts Group on Cancer Control with Member States' representatives and stakeholders, including health professionals and patients organisations. Such a group could help to ensure that a sharp focus is kept on priorities, that actions are effectively co-ordinated and duplication of effort is avoided.

Let me conclude by reassuring you that the Commission remains fully committed to cancer control policy. Our goals remain to prevent cancer where we can, and to improve the prospects of cancer patients obtaining appropriate and timely diagnosis, information and care.

Article taken from a speech given at The European Cancer Congress – 30 September 2014

Hepatocarcinogenesis and future challenges:
Pre-clincial models for virus- or high fat diet-induced liver cancer

Abstract

With more than 80% of HCC cases worldwide, all being associated with chronic HBV – (hepatitis B virus) or chronic HCV (hepatitis C virus) – infection[1], these two hepatotropic virus infections resemble a huge health problem and have to be a central focus in basic HCC research. Chronic inflammation of the liver due to persistence of the virus leads to cell death and compensatory hepatocyte proliferation as well as pro-fibrotic effects being known as key drivers in HCC development [2]. Liver damage is largely due to influx of immune cells and destruction of infected hepatocytes [3]. The different phases of immune response and viral replication exhibit specific risks for subsequent liver damage, necro-inflammation and subsequent HCC development [4]. Numerous mouse models partially reflect this sequence (see above and reviewed in [5]). The host immune response to the virus determines largely the severity of liver inflammation and subsequent liver damage [6]. Secondly, both viruses are known to have direct oncogenic potentials, which can be dissected from inflammatory liver damage. Patients chronically infected with HBV are facing a possible HCC development in the absence of inflammation, severe liver damage and cirrhosis due to direct oncogenic viral factors [4, 7-9] or even in occult HBV infection [10]. However, the exact role of direct viral factors in HCC development remains to be elucidated. Moreover, high fat diet and high sugar diet have come more and more in the focus of liver cancer development. In recent years high fat diet and high sugar diet – driving non-alcoholic liver disease (NASH) – has become a huge problem – causing the fastest growing cancer in the USA and soon in Europe: NASH induced liver cancer.

In industrialised countries high fat diet induced liver cancer is a fast growing cancer type – thus in the coming years of utmost clinical importance. Here, the use of valid mouse models is a key in dissecting and understanding viral factors in hepatocarcinogenesis.

Up to now liver transplantation has been the most effective way to prolong the live-span of patients – but in 50% of the cases cancer is coming back. Various novel drugs have been investigated. Although these regimens are successful approaches to prolong the life span of liver cancer patients – these drugs are rather palliative than curative. HCC do not resemble a single entity but rather a diverse spectrum of cancers in humans. In many cases individual patients carry different liver cancer-subtypes, not only distinct in their morphology, their genetics and epigenetics but – most importantly – also in their responsiveness to therapy. Thus, liver-subtype specific, personalized therapeutic approaches will be needed in the future – depending on the tumor-type, the tumor-stage as well as the individual composition of distinct liver cancers in one and the same patient.

Due to the above outlined problems a systemic, therapeutic approach to treat liver cancer in humans is not available – underlining the urgent need for new targets that can be included basic research and clinical trials. **In the light of the above modeling liver cancer in rodents appears to be very important, but which mouse models resemble what human pathology? A question that needs to be urgently investigated and answered in the future.**

Mouse models for HBV associated HCC

Chronic HBV infection is the most important risk factor for HCC development worldwide [11]. Approximately 240 to 350 million humans worldwide are estimated to be chronically infected with HBV and face hepatitis B related end-stage liver disease and HCC, with up to 54.4% of HCC cases being attributable to HBV infection [1, 12]. The attributed fraction is lower in developed counties (23,3%) than in developing countries (58,8%) reflecting the variable burden of chronic HBV infection between different areas worldwide [4, 13].

Human hepatitis B virus is a member of the hepadnaviridae family, infects the liver of humans and humanoid primates. It is characterized by a very high host and cell tropism and only infects hepatocytes of humans or humanoid primates due to specific binding of the preS1 domain of the large envelope protein to hepatocytes by the bile salt transporter sodium taurocholate co-transporting polypeptide (NTCP) [14, 15]. This finding and characterization of the NTCP will help in generating new mouse models in future.

Numerous transgenic mouse models already exist, which express specific fragments or even all viral proteins encoded by the HBV genome usually under the control of a liver-specific host promoter or the HBV promoter and provide reliable proof on direct effects of viral factors in HBV-induced liver carcinogenesis.

In this line the group of Chisari could show over 20 years ago that a sequence of liver cell injury, inflammation and compensatory proliferation, can act as an inexorably progresses to hepatocellular carcinoma in a mouse model overexpressing HBV large envelope polypeptide as well as Pre-S, and parts of HBx gene under the control of the Albumin promoter [16]. These (Tg(Alb-lHBV)Bri44) mice exhibit liver cell injury by the age of 4 months and regenerative hyperplasia by 6 months, which drives formation of large adenomas from the age of 12 months with progression to HCC between 12-20 months [16, 17]. Using Tg(Alb-lHBV)Bri44 mice a different study demonstrated after a reconstitution experiment with bone marrow and spleen cells from syngeneic non-transgenic donors that had been previously immunized with recombinant vaccinia virus (HBs-vac) the prominent role of chronic immune-mediated liver cell injury in triggering the development of HCC in the absence of viral transactivation, insertional mutagenesis, and genotoxic chemicals [18]. However, microarray analysis of Tg(Alb-lHBV)Bri44 mice at 3 months of age showed that accumulation of viral proteins overexpressed under the Albumin promoter results in significant difference of expression of 45 genes being associated with proliferation, immune response and several of the genes being associated with apoptosis; concluding that deregulation of apoptosis, could be a mechanism through which HBV directly promotes HCC development [19]. Several studies reported pro-carcinogenic effects of HBV proteins or of their randomly truncated transcripts after integration [4]. Yet, overexpression of the gene for the HBV large envelope protein in a transgenic mouse (50-4), under the control of rat albumin promoter were reported to not show spontaneous HCC until 15 months of age. The mouse strain 50-4 displays liver injury secondary to overexpression of the HBV large envelope protein, which sensitizes livers to chemical carcinogens (i.e aflatoxin and diethylnitrosamine). Adult transgenic mice (50-4) exposed to aflatoxin and diethylnitrosamine (DEN) developed HCC over the age of 15 months with low incidence while dysplastic nodules were found frequently. Microscopically, adenomas and carcinomas were only seen in transgenic mice treated with aflatoxin or DEN and none was found in either control untreated transgenic mice or non-transgenic mice treated with carcinogens [20]. This however provided strong evidence for proposed synergistic effects in HCC development. Induction of increased ER stress and compensatory proliferation by preS2 mutants is suggested to contribute to HCC development. In two HBV transgenic mouse models harboring a mutated preS2 as well as the complete genome of a mutant HBV ("preS2 mutant") the development of HCC was shown by the age of 24 months pointing to a direct carcinogenic effect of preS2 mutants in HCC development [21]. In order to dissect the role of large HBV surface protein (LHBs) and C-terminally truncated middle size surface proteins (MHBs(t)) which show transcriptional activator function, mice transgenic for the PreS2 activator MHBs(t) under the control of an albumin promoter together with a b-globin intron were generated [22] and compared to already established mice transgenic for LHB under the control of the albumin promoter [23]. Transgenic mice for the PreS2 activator MHBst76 showed development of liver tumors/ nodules in over 65% at the age of over 15-18 months in a c-Raf-1/Erk2 dependent manner. Yet, there was no clear examination on the grade of malignancy of the tumors and wildtype mice were as well reported develop nodules in 10% [22].

HBx, a small polypeptide of 154 amino acids of size is usually expressed at low levels during acute and chronic HBV infection and was reported to be frequently detectable in HBV-related HCC tissue [24]. This may be explained by the fact that HBs and HBx may still be expressed from integrated, linear double

Lymphotoxin expression, chronic hepatitis virus infection and liver cancer development

In the past we have tried to identify the inflammatroy signature that is chronically present in patients suffering from infection with Hepatitis viruses. By analysing human tissue specimens from Hepatitis C or B virus infected patients we found one signalling pathway to be strongly turned on in human patients: The Lymphotoxin (LT) pathway. LT, a cytokine of the tumor necrosis factor superfamily acts directly on hepatocytes which express high levels of LTβR but little LT.

We could show in this study that LTα, β and their receptor (LTβR) are increased in intrahepatic lymphocytes and hepatocytes of patients with chromic HBV- or HCV-induced chronic hepatitis or HCC.

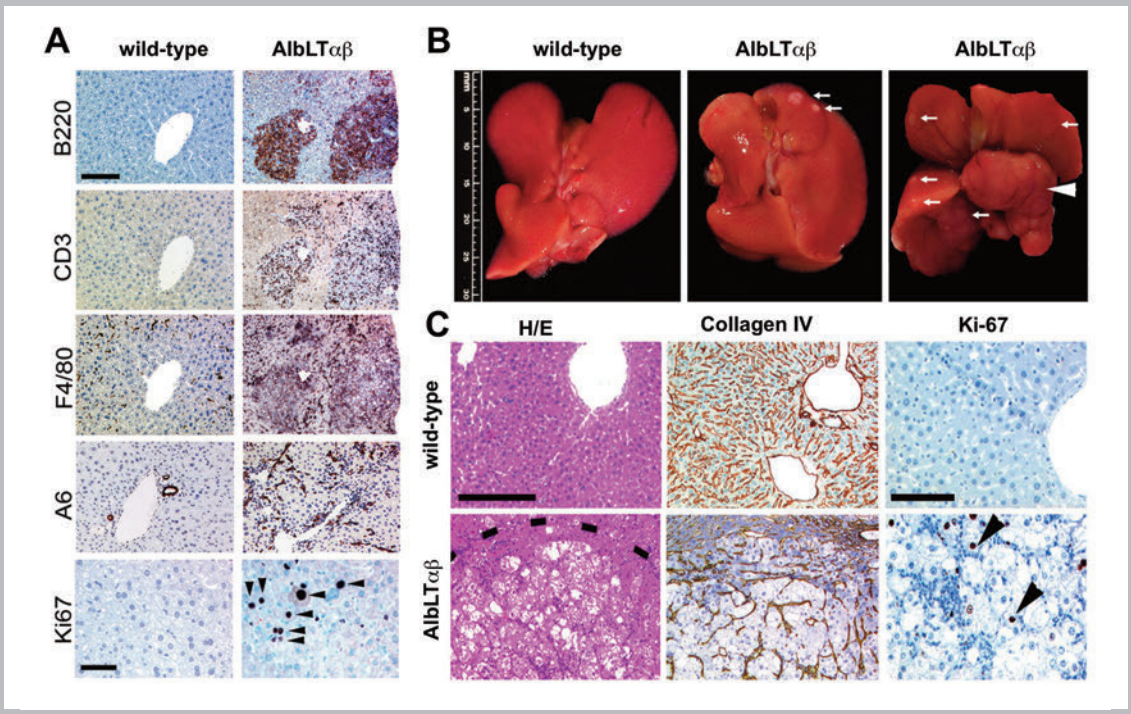
Thus, the question remained whether LT expression in mice suffices to induce liver inflammation and HCC. Indeed, transgenic expression of LT specifically in the liver recapitulated some features of human pathology seen in patients with chronic hepatitis B or C. AlbLTαβ mice developed hepatitis and HCC (Figure 1). Furthermore, suppression of LTβR signalling reduced the incidence of chronic hepatitis and abolished liver cancer development (Figure 2).

stranded DNA. However, the role of HBx in HCC development remains controversial in in-vitro as well as different transgenic mouse models. The mouse strain CD1 transgenic for HBx under its own transcription enhancer [25] as well as C57BL/6xDBA mice transgenic for HBx plus a portion of pre C-C under the HBV transcriptional enhancer [26] were reported to spontaneously develop neoplastic nodules/adenomas at the age of 6 to 10 months and HCC starting from 11 to 18 months [25, 26]. However, mice transgenic for HBx (nt 1376-1840) under the control of the human alpha-1-antitrypsin regulatory elements (ATX mice [27]) as well as PEX lineage that express HBx in the liver under control of different viral regulatory elements (enhancer I, X promoter [27]) did not show spontaneous HCC development, indicating no direct carcinogenic effect of HBx [28, 29]. Yet HBx expression sensitized ATX mice to other known carcinogens (DEN) [28]. Mice liver-specifically expressing of c-myc driven by woodchuck hepatitis virus regulatory sequences causes liver cancer in all animals. These mice were crossed with ATX and PEX lineages, showing an earlier tumor development (2 to 3 months earlier) in bitransgenic animals. This as well proofed HBx to act as a synergistic tumor promoter by deregulates the hepatocyte growth control in vivo, yet not driving HCC by its own [29]. The latter findings of an increased sensitivity to HCC development by expression of HBx was supported by another mouse model being transgenic for the oncogene c-myc together with a truncated HBx protein [30]. This two-hit hypothesis might reflect the human situation where full-length HBx is expressed in low levels together with other viral proteins and HCC development is rather dependent on inflammation and other synergistic factors.

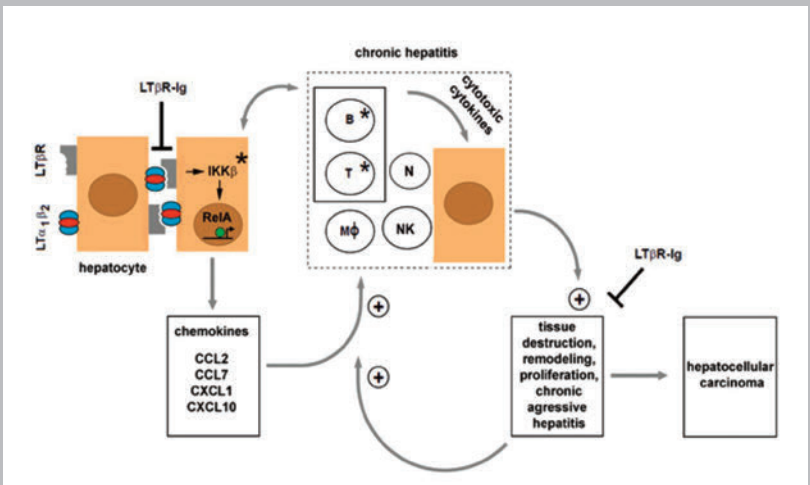
Hepatitis C Virus

Approximately 180 million people worldwide are estimated to be chronically infected with HCV. The risk for chronically HCV-infected patients to develop HCC is 17- to 20-fold higher in comparison to HCV-negative controls [1], with up to 31.1% of HCC cases being attributable to HCV infection [13]. HCV-associated cirrhosis is the major risk factor for hepatocarcinogenesis. Once established, the annual incidence of HCC is 1% to 4% independent of an interferon therapy [31, 32].

Numerous transgenic mouse lines exist expressing different HCV proteins (i.e. structural proteins: E1, E2, p7 and non-structural proteins: NS2, NS3, NS4A,B, NS5A,B). Some of these transgenic mouse models, in which the HCV proteins are expressed, helped to elucidate a direct pathogenicity of HCV, including oncogenic activities [33]. A direct oncogenic potential



Chronic inflammation-induced liver damage and HCC in AlbLTαβ mice. (A) Immunohistochemistry of representative livers from 9-months-old C57BL/6 and AlbLTαβ mice. B220+ B cells, CD3+ T cells, F4/80+ macrophages, Kupffer cells (scale bar: 150 μm). Ki67+, proliferating hepatocytes (arrowheads) or inflammatory cells are indicated (scale bar: 50 μm). (B) Macroscopy of C57BL/6 and AlbLTαβ livers. White arrows indicate tumour nodules. White arrowhead indicates a complete liver lobe affected by HCC. (C). Liver histology of 12-month-old C57BL/6 and AlbLTαβ mice. H/E demarcates a HCC nodule. Collagen IV staining highlights the broadening of the liver cell cords, loss of collagen IV networks indicate HCC (scale bar: 200 μm). High numbers of Ki67+ proliferating hepatocytes (arrowheads) in AlbLTαβ HCC (scale bar: 100 μm).



Scheme of chronic inflammation-induced liver carcinogenesis in AlbLTαβ mice. Hepatocytes (brown) express LTα, LTβ and induce chemokine production (e.g., CCL2, CCL7, CXCL1, CXCL10) in the presence of IKKβ and intrahepatic lymphocytes. Chemoattraction, activation of myeloid cells and lymphocytes expressing chemokine receptors (e.g., CXCR3, CXCR2, CCR2, CCR1) causes hepatitis. Activated, infiltrating immune cells secrete cytotoxic cytokines (e.g., IL6, IL1β, TNFα, IFNγ, LTaβ) causing tissue destruction, hepatocyte proliferation, cell death and tissue remodelling. Tissue destruction and remodelling supports the infiltration of activated inflammatory cells (e.g., myeloid cells) leading to a feed-forward loop towards chronic aggressive hepatitis and HCC. * Genetic depletion of components (IKKb; T and B cells) which leads to a block in chronic hepatitis and HCC development. Blocking LTβR signalling with LTbR-Ig in 9-month-old AlbLTαβ mice reduces chronic hepatitis incidence and prevents HCC. RelA is schematically depicted as a green circle, inducing transcription of NF-κB target genes (arrow). B and T: B and T cells; MØ: macrophages; N: neutrophils; NK: NK cells. Adapted from Haybaeck et al., Cancer Cell, 2009.

a HCV protein was first shown in two lines (C21 and C49) of HCV core (genotype 1b) transgenic mice under the control of an exogenous promoter. These mice development hepatic steatosis beginning at 3 months, adenomas from 12-16 months of age and HCC with an incidence of 25% in C21 and 30% in C49 animals at the age of 16 months [34]. In a proteomics approach a sequence of changes as possible steps in direct HCV-dependent hepatocarcinogenesis were shown in livers of latter mice (C21 and C49): after 6 months suppression of apoptotic proteins, after 12 months up-regulation of proteins related to respiration, the electron-transfer system, and anti-oxidation and after 16 months suppression of proteins related to defense, beta-oxidation, and apoptosis [35]. Koike et al. reported no tumor development in mice transgenic for HCV envelope gene (E1-E2) under the control of a regulatory element from hepatitis B virus until the age of 18 months and no evidence of tissue pathology in the mouse liver up to 16 months of age, suggesting that E1 and E2 proteins may not have direct pathogenic effects [36]. Another study compared mice expressing HCV core, E1 and E2 (Core-E1-E2 Tg) under the albumin promoter to previously published mice expressing HCV core alone under the control of regulatory element from hepatitis B (core tg [34, 37]). In contrast to earlier findings, Kamegaya et al. reported no spontaneous HCC development in either Core-E1-E2 Tg or core Tg animals, which was concluded to might be due to the mixed FVB/C57Bl/6 background [38]. After DEN treatment Core-E1-E2 Tg mice developed larger tumor nodules as compared to control DEN-treated animals after 32 weeks. Taken this finding, Kamegaya et al. conclude a potential direct carcinogenic effect of HCV envelope proteins E1 and E2 in conjunction with core by suppression of apoptosis as a two hit model in HCV associated hepatocarcinogenesis [38]. A direct carcinogenic effect of HCV structural but as well an possible role of non-structural proteins was confirmed in mice transgenic for full-length HCV polyprotein (FL-N strain) and transgenic mice for structural protein (S-N strain). These mice show with age increasing steatose, and HCC development in the FL-N strain starting at 13 months and in the S-N strain starting at 18 months of age in the absence of inflammation due to being transgenic for HCV proteins. Thus, expression of the structural proteins enhances steatosis additional low level expression of nonstructural proteins increases the risk of cancer [39].

High fat diet and cancer

Importantly, only recently – in line with epidemiological studies – it was shown that dietary and genetic obesity promote non-alcoholic steatohepatitis (NASH; (pathologic hepatic lipid deposition and hepatitis) and tumorigenesis. Moreover, combination of high fat, high sugar diet and little exercise additionally worsen this problematic development. Currently, mouse models are urgently needed to investigate this disease with a mainly dietary origin, to compare them with the human disease and to use them as preclinical mouse models. Given the effort that has been invested in the past to understand and treat HCC – either induced by viruses, high fat diet or chronic alcohol consumption – clinical success has been extremely small. **Only good pre-clinical mouse models will pave the way to change this in the future and to be able to deal with the coming problems of NASH induced liver cancer.**

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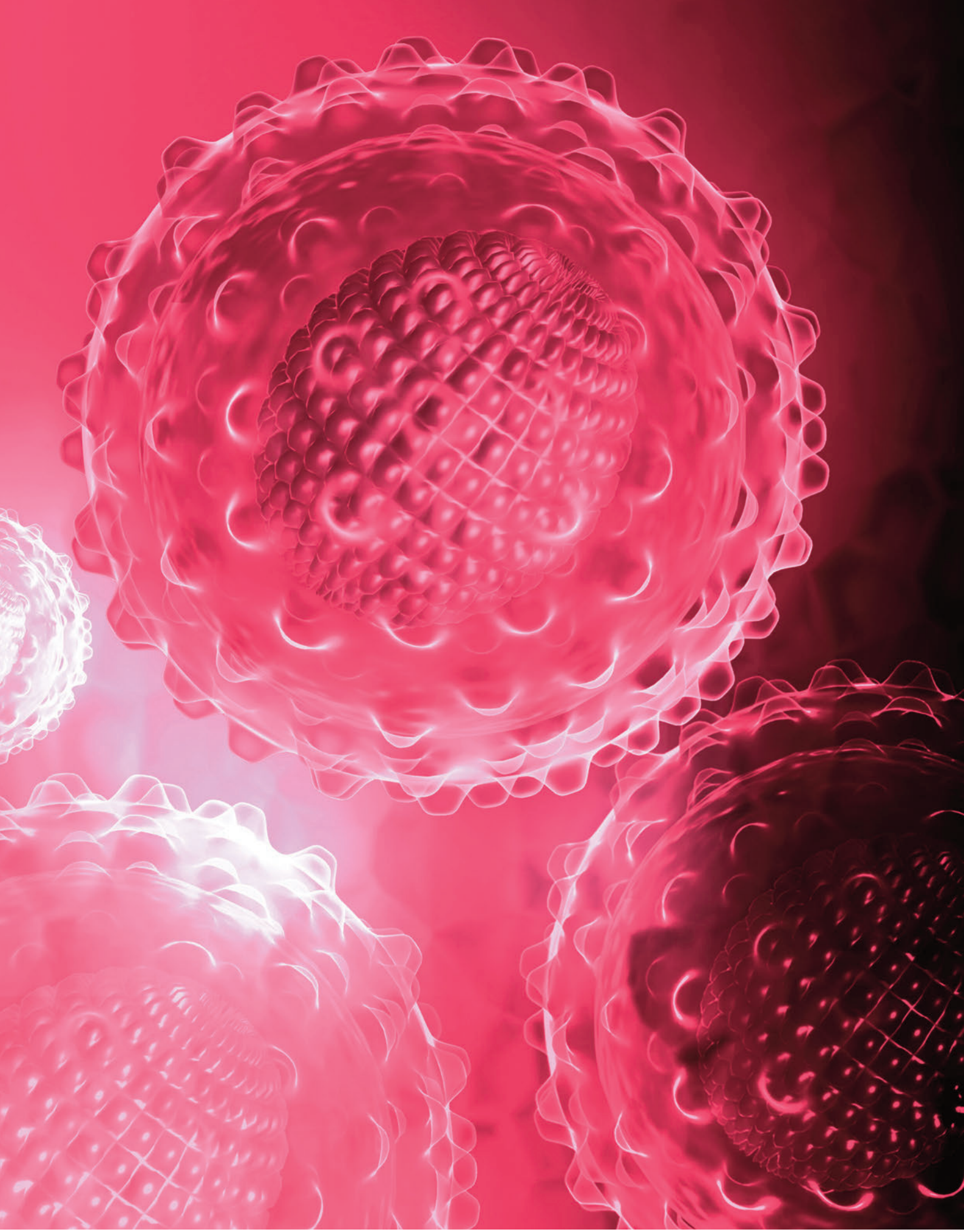
Heikenwalder Mathias
Institute of Virology, Technische Universität München,
Schneckenburgerstr. 8, 81675 München
Institute of Virology, Helmholtz Zentrum München,
German Research Center for Environmental Health (GmbH), Institute of Virology, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

E-mail: heikenwaelder@helmholtz-muenchen.de
Tel: +49 89 4140 7440
Fax: +49 89 4140 7444
Homepage: http://www.virologie.med.tu-muenchen.de



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