THEORETICAL ARTICLE

Hepatitis C virus drives the pathogenesis of hepatocellular carcinoma: from immune evasion to carcinogenesis

Miriam Canavese[1], Danushka Wijesundara[2], Guy J Maddern[1], Branka Grubor-Bauk[2] and Ehud Hauben[1]

Persistent hepatitis C virus (HCV) infection is associated with high incidence of hepatocellular carcinoma (HCC), the most common primary malignancy of the liver with over half a million new cases diagnosed annually worldwide. The aryl hydrocarbon receptor (AhR) is a ubiquitously expressed transcription factor and its activation by environmental chemicals and by its endogenous ligand kynurenine (Kyn) has been implicated in a variety of tumour-promoting processes such as transformation, tumorigenesis and in immunosuppression that enables tumour survival and growth. Kyn is generated constitutively by human tumour cells via tryptophan (Trp)-2,3-dioxygenase (TDO), a Trp-degrading enzyme expressed in liver, brain and cancer cells. Notably, it has been shown that TDO-derived Kyn suppresses anti-tumour immune responses, thus promoting tumour-cell survival through activation of the AhR pathway. In the context of HCV infection-associated HCC, it was shown that AhR signalling is increased in HCV-infected hepatocytes, and that modifications in the expression of AhR pathway-specific genes are associated with the progression of HCV infection into HCC. Based on these observations, we present and discuss here the hypothesis that HCV infection promotes HCC by modulation of the TDO–Kyn–AhR pathway, resulting in tumorigenesis as well as in suppression of both anti-HCV and anti-tumour immune responses.

PRESENTATION OF THE HYPOTHESIS

We propose herein that hepatitis C virus (HCV) infection promotes hepatocellular carcinoma (HCC) by augmentation of the tryptophan (Trp)-2,3-dioxygenase-kynurenine-aryl hydrocarbon receptor (TDO–Kyn–AhR) pathway, resulting in suppression of both anti-HCV and anti-tumour immune responses, as well as in tumorigenesis.

The evolution of HCV into HCC was associated with expression of specific AhR pathway genes. Although only few genes were found to be differentially expressed in HCV-induced HCC tumour biopsies compared with paired non-HCC liver sections, pathway analysis revealed strong upregulation of genes involved in AhR signalling in biopsies from HCV-induced HCC tumours.[1–3] In addition, it has been shown that dioxin-induced persistent AhR activation promotes tumour formation, carcinogenicity and clonal expansion of trans-formed cells by inhibiting apoptosis and bypassing AhR-mediated cell cycle arrest.[4] Notably, it has been recently shown that an endogenous ligand promotes the activation of AhR under physiological conditions without the presence of exogenous toxic chemicals.[5] As described by Tian et al.,[6] AhR is a highly evolutionary conserved, ligand-activated transcription factor that is best known to mediate the toxicities of dioxins and dioxin-like compounds. The non-activated AhR resides in

the cytoplasm and, after binding its ligand, translocates to a nucleus where it reacts with its obligatory heterodimer partner AhR nuclear translocator, to become fully competent and bind the promoter element of specific target genes.[6] Functionally, AhR was shown to have an important role in the development and differentiation of lymphocytes.[7]

Opitz et al.[5] have identified the Trp catabolite Kyn as an endogenous ligand of human AhR. Interestingly, these authors also showed that TDO-derived Kyn was able to inhibit anti-tumour effects and promote tumour-cell survival and dissemination through a direct effect on the AhR pathway. They also demonstrated the active presence of the TDO–Kyn–AhR pathway in human brain tumours, which correlated with disease progression and poor survival. This novel association between Trp derivatives and the AhR pathway creates new links among a large body of scientific literature regarding the following: (a) the immune suppressive effects of indoleamine-2, 3-dioxygenase (IDO), a therapeutic target being evaluated in a number of ongoing clinical trials (reviewed in Vachelli et al.,[8] and Munn and Mellor[9]). Interestingly, whereas the level and activity of TDO in HCV or HCC has not been previously investigated, upregulation of hepatic IDO expression and an increased serum Kyn:Trp ratio was

[1]Liver Metastasis Research Group, Discipline of Surgery, The Basil Hetzel Institute for Translational Health Research, The Queen Elizabeth Hospital, University of Adelaide, Adelaide, South Australia, Australia and [2]Virology Laboratory, Discipline of Surgery, The Basil Hetzel Institute for Translational Health Research, The Queen Elizabeth Hospital, University of Adelaide, Adelaide, South Australia, Australia
Correspondence: Dr E Hauben, Liver Metastasis Research Group, Discipline of Surgery, The Basil Hetzel Institute for Translational Health Research, The Queen Elizabeth Hospital, University of Adelaide, 37A Woodville Road, Woodville, Adelaide, South Australia 5011, Australia.
E-mail: ehud.hauben@adelaide.edu.au

2

demonstrated in patients with chronic HCV infection and in infected chimpanzees.[10,11] Moreover, hepatic IDO expression decreased in animals that cleared the infection, but remained high in those that progressed to chronicity;[10] (b) the immunosuppressive effects of TDO;[12,13] and (c) the immunosuppressive and tumorigenic effects of the AhR pathway. Based on the above evidence, we propose that manipulation of the TDO–Kyn–AhR pathway by HCV has a pathogenic role in the HCV-infected liver through bystander suppression of anti-tumour immune responses, thus allowing the progression of HCV infection into HCC. Accordingly, we suggest that identification of biological or chemical compounds that inhibit the TDO–Kyn–AhR pathway can lead to the development of new therapeutic strategies for HCV-induced HCC.

CONTEXT OF THE HYPOTHESIS

The Trp to Kyn metabolic pathway is a key regulator of innate and adaptive immunity.[14,15] It has been shown that Trp metabolism is targeted by viruses to escape immune control in a number of chronic viral infections including HCV, hepatitis B virus (HBV), human immunodeficiency virus, herpes and cytomegalovirus (reviewed in Mehraj and Routy[16]). Moreover, a plethora of research evidence suggests that this immune tolerance pathway, driven by the key, rate-limiting enzymes IDO in many tissues and by TDO in the liver, also has a regulatory role in cancer immunity, autoimmunity, transplant rejection and allergy.[12,15] In cancer patients, IDO expression was demonstrated in various types of tumours including prostate, colorectal and pancreatic cancer,[17] and IDO1 expression was associated with poor prognosis in ovarian cancer patients.[18] Based on these findings, drugs targeting the IDO pathway are being tested in clinical trials,[8] with the aim of preventing tumour-induced immunosuppression. However, recent studies[5,13] demonstrating that TDO-mediated Trp metabolism represents an alternative route to IDO activity employed by tumour cells generated growing interest in TDO as therapeutic target for cancer immunotherapy in general and, in particular, liver cancers.[19] Thus, as the available IDO inhibitors do not suppress TDO activity, there could be a need to develop specific TDO inhibitors.[15]

An extensive research effort was dedicated to characterize the regulatory mechanisms of Trp metabolism and of the downstream pathways mediating its effects on immunological functions, including the AhR pathway.[15] Trp catabolites were shown to have an important balancing role in both antimicrobial defence and immune regulation, at least in part through Kyn-mediated AhR activation.[16] Therefore, IDO/TDO-mediated AhR activation generates conflicting effects by (a) depleting L-Trp to starve the invading pathogens and (b) contributing to downregulation of the inflammatory response against microorganisms that survived the acute response.[16] IDO-mediated degradation results in Trp deprivation, affecting various pathogens including certain bacteria, parasites and occasionally viruses.[16] Nevertheless, chronic viral infections result from manipulation of the host immune response via Kyn-mediated AhR activation to create a state of immune tolerance.[16] It is known that various malignancies occur in the context of chronic infection and inflammation,[20] where local immune suppression is sustained by Trp catabolism in the tumour microenvironment.[21] Importantly, Opitz *et al.*[5] implicated Kyn as the endogenous tumour-promoting ligand of the AhR in various cancers. Therefore, these authors showed that activation of the IDO/TDO–Kyn–AhR pathway in response to inflammatory stimuli constitutes a novel link between inflammation and carcinogenesis.
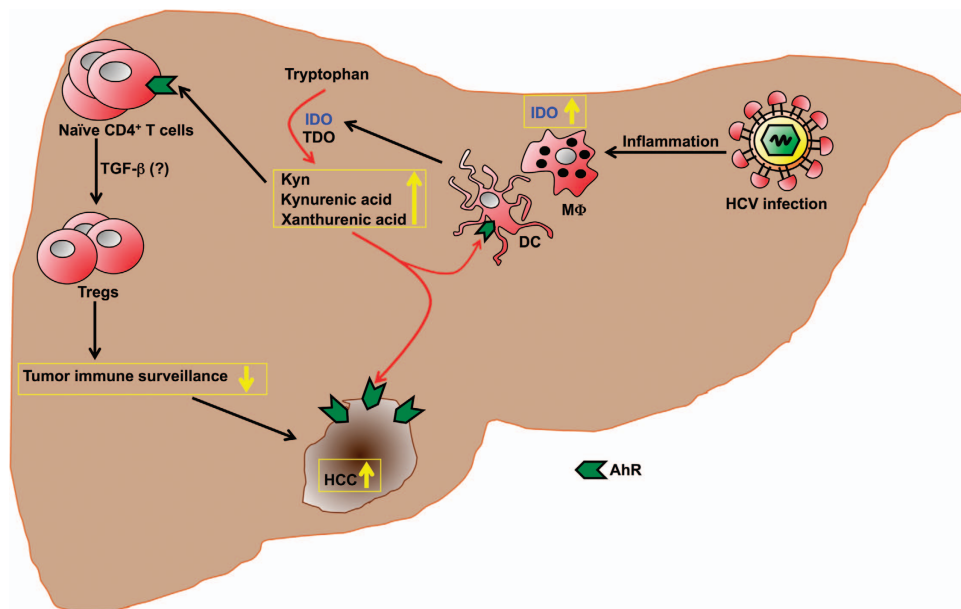

Figure 1 IDO/TDO-based HCC development and progression following persistent HCV infection. Persistent HCV infection leads to chronic inflammation, which recruits immune cells expressing IDO such as dendritic cells (DCs) and macrophages (MΦ) to the liver. This increases the levels of IDO in the liver, which along with TDO participates in the catabolism of Trp into metabolites such as Kyn, kynurenic acid and xanthurenic acid that can activate AhR signalling. Elevated levels of AhR ligands, in particular Kyn, engage with AhR on naive CD4+ T cells present in the liver. AhR signalling possibly coupled with signalling from cytokines such as transforming growth factor (TGF)-β leads to differentiation of naive CD4+ T cells to Tregs that can suppress immune responses that can prevent formation of HCC. We hypothesize that this immune suppression pathway leads to the establishment of HCC. HCC also express AhR, which following activation contributes to HCC progression. Furthermore, DC also express AhR and activation of this receptor on DC is known to increase IDO expression, which can also contribute to immune suppression using the illustrated pathway. The yellow arrows indicate up/downregulation of metabolites or a process. Furthermore, red arrows indicate catabolic enzymatic reaction of Trp, which leads to elevated AhR signalling, and the black arrows are now exclusively present to indicate the direction of the flow of the diagram.

HCC is the most prevalent primary malignancy of the liver; it is the fifth most common cancer in men and seventh among women, with over 500 000 new cases diagnosed worldwide each year;[22–24] yet, effective therapeutic options for advanced HCC are limited. Therefore, characterization of the mechanisms mediating the progression to HCC in HCV-infected patients is a worldwide medical priority. Cirrhosis due to chronic HBV or HCV infection is the most critical pathogenic factor for HCC.[25] Indeed, global epidemiology of HCC is associated with the prevalence of viral hepatitis and the age it is acquired in. HCV is an RNA virus, which does not integrate its genome into the DNA of host cells. In most cases, failure to clear the virus results in chronic infection associated with progressive risk of hepatic fibrosis, cirrhosis and HCC.[26] The DNA damage and oxidative stress induced by chronic hepatic inflammation are the major correlated causes of HCV-associated HCC.[27–29] The large body of research evidence supporting the direct role of HCV in promotion of liver cancer is summarized in Figure 1.[30–33]

In chronic liver disease, altered gene expression can be mediated by epigenetic changes, by inhibition of DNA repair and/or by differential expression of microRNAs. These alterations include constitutive upregulated expression of 'stemness' factors, suggesting that both HCV and HBV contribute to HCC by promoting stemness,[34] referring to the capability to self-renew and differentiate into other tumour cell types.[35] Notably, HCV- and HBV-encoded proteins regulate cancer-associated host gene expression patterns and cellular phenotypes. These changes can result in growth factor-independent proliferation, drug resistance, tissue invasion and metastasis.[36] Chronic inflammation can also promote somatic mutations in tumour cells. The contribution of HCV to HCC involves the core protein and non-structural proteins NS3 and NS5A, which have been shown to contribute to oncogenic transformation.[34,37,38] Changes in host gene expression that promote tumorigenesis also seem to support virus replication and/or protect virus-infected hepatocytes from immune-mediated damage or destruction. Therefore, tumour-promoting changes in local immunity seem to simultaneously sustain chronic viral infection and promote HCC carcinogenesis. Thus, the pathogenesis of HCC is closely correlated with virus persistence in infected cells over a long period of time.[34] Nevertheless, HCV persistence does not include DNA integration and the virus maintains itself as an endoplasmic reticulum-associated episome. Therefore, progressive chronic liver disease is associated with increased proportion of virus-infected liver cells that acquire certain characteristic of malignant cells.[34,39] As a result, persistent infections can accelerate the pathogenesis of HCC through common necro-inflammatory pathways or by direct oncogenic activity.[40]

Infection-induced inflammation triggers catabolism of Trp via IDO/AhR.[16] In human, Trp catabolism through the Kyn pathway is regulated by three distinct enzymes: IDO-1, IDO-2, which are inducible in many tissues, and TDO, which is expressed in liver, brain and in cancer cells.[41,42] In physiological conditions, TDO is the main enzyme degrading Trp (reviewed in Platten et al.[15] and Mehraj and Routy[16]), while in the context of infections: IDO-1 is induced and becomes the major intracellular enzyme mediating Trp degradation. Thus, in chronic viral infections IDO induction is considered the main cause of decreased serum Trp levels.[16] It has been shown that both natural and induced human T regulatory (Treg) cells have an important role in the progression of HCV infection to HCC and are associated with the severity of viral recurrence after liver graft transplantation.[43,44] Although nothing is known with regards to the specific impact of HCV on these two Treg cell populations, Ouaguia et al.[45] have suggested that HCV promotes recruitment and induction of Treg cells in the infected liver, mediating their phenotype and suppressive activity to induce immune tolerance and allow the progression of liver disease. This suggestion was based on the findings showing that HCV infection (a) induces Treg cell-mediated anergy, (b) promotes the recruitment of Treg cells by HCV-infected hepatocytes and (c) induces significant increase in the expression of Treg markers, such as CD25 and FOXP3, thus potentiating Treg suppressive function. Moreover, the authors demonstrated that HCV promotes conversion of naive T lymphocytes into induced Treg type 1 (Tr1) cells, which could act as another mechanism for HCV to induce immune tolerance towards viral antigens. Notably, the fact that HCV can potentiate the suppressive function of natural Treg cells as well as induce Tr1 cells may help to explain the cellular mechanism by which HCV escapes the immune system to promote the progression of hepatitis C infection to cirrhosis and HCC.[45] However, the molecular mechanisms mediating HCV immune evasion, potentially including IDO/TDO, Kyn and AhR activation, remain to be further characterized.

It has been previously demonstrated that AhR activation induces antigen-specific active tolerance through direct and dendritic cell-mediated effects on Treg cell survival and function.[46] In addition, more recent work done by Mascanfroni et al.[47] reported that a metabolic programme controlled by the transcription factor hypoxia-inducible factor-1α (HIF-1α) and by AhR promotes the differentiation of Tr1 cells. Notably, this work showed that the balance between HIF-1α and AhR provides a pathway for modulating the immune response in response to immunological, metabolic and environmental signals. Thus, AhR induces the degradation of HIF-1α and subsequently regulates the differentiation of Tr1 cells from naive T cells. On the other hand, extracellular ATP and hypoxia, linked to inflammation, trigger AhR inactivation by HIF-1α and inhibit Tr1 cell differentiation. Therefore, the cross-talk between HIF-1α and AhR provides a system for immune modulation based on the bioenergetic needs of specific cell subsets.[47] Moreover, dimerization of HIF-1β or AhR nuclear translocator with HIF-1α is involved in various aspects of carcinogenesis, including proliferation and survival under hypoxic conditions: to this end, Choi et al.[48] have recently demonstrated suppression of tumour cell invasion and migration in HIF-1β-silenced HCC cell lines, suggesting that HIF-1β expression is essential for tumour cell survival under hypoxic conditions in HCC. The competition between tissue resident cells and circulating immune cells on the limited locally available resources can result in 'natural selection' of immune phenotypes at the site of inflammation.[49]

Therefore, through its interactions with both immune and metabolic systems, HCV activates multiple direct and indirect pathogenic pathways, including liver fibrogenic pathways, cellular and survival pathways.[50]

Cozzi et al.[51] showed that patients with chronic HCV infection have lower serum Trp concentrations compared with healthy donors. IDO, the rate-limiting enzyme of the Kyn pathway, produces several metabolites, which are also AhR ligands.[52] Kyn is one such catabolite that regulates immune functions by acting as an AhR agonist, at least in part by promoting the induction and activation of immuno-suppressive Treg cells.[53,54] Indeed, it has been shown that IDO1-expressing dendritic cells exert broad and robust immuno-suppressive effects such as directly suppressing the proliferation and effector functions of cytotoxic T lymphocytes, NK cells and plasma cells,[8,55,56] promoting the induction of CD4+CD25+FOXP3+ Treg cells from naive CD4+ T cells[57,58] and triggering immunosuppressive activity in neighbouring IDO1-expressing dendritic cells.[57,59] Notably, the upregulation of IDO1 in plasmocytoid dendritic cells[60] has been

4

shown to contribute to the immunosuppressive activity of human immunodeficiency virus-1.[61,62] Taken together, these findings reinforce the idea that IDO/TDO–Kyn–AhR pathway mediates robust immunosuppressive effects in both physiological and pathological contexts.

The direct and indirect links between the AhR pathway and HCC tumorigenesis were further outlined by functional characterization of the AhR nuclear translocator, also known as HIF-1β, which is widely expressed in human cells, including hepatocytes.[48] When responding to different extracellular stimuli, AhR nuclear translocator can form a heterodimeric complex with AhR, HIF-1α and its homologous factors (HIF-2α and HIF-3α), to mediate various biological actions such as hypoxia reaction,[47] xenobiotic metabolism and immunosuppression,[1,63] other than being an important regulator of HCC growth and metastasis, and therefore a promising prognostic candidate in HCC patients.[1] In line with our hypothesis, preclinical studies have suggested that AhR is responsible for mediating, at least in part, the immunosuppressive effects of cancer-derived Trp metabolites[5,54] and, therefore, represents a logical pharmaceutical target for cancer immunotherapy.[15] Specifically, in the context of HCV-associated HCC, it was shown that AhR signalling is impaired in HCV-infected hepatocytes, and that changes in mRNA expression of specific genes in the AhR pathway are linked to progression of HCV infection to HCC.[2,3] Briefly, the Kyn pathway is regulated by IDO1, IDO2 and TDO.[41,42] These three enzymes actively deplete Trp from the circulation, thus producing several catabolites, known as Kyns.[64] Some of these metabolites such as kynurenic acid and quinolinic acid regulate neuronal functions,[65] whereas the amino acid L-Kyn has immunosuppressive effects, through the AhR and other pathways as well. Notably, the immunomodulatory AhR ligand L-Kyn is the first stable Trp catabolite in this pathway, whereas some of its downstream derivatives including 3-hydroxykynurenine, anthranilic, kynurenic, quinaldic, xanthurenic and 3-hydroxyanthranilic have been shown to have a role in neurodegenerative disorders.[65]Therefore, based on the above findings we propose herein that HCV infection promotes HCC tumour survival and growth by activating theTDO–Kyn–AhR pathway, resulting in the following: (a) tumorigenesis, (b) suppression of the anti-HCV immune response and (c) bystander suppression of anti-tumour immune responses. Moreover, the proposed hypothesis supports the development of therapeutic interventions specifically targeting the TDO–Kyn–AhR pathway in HCV-infected patients.

TESTING THE HYPOTHESIS

Based on this hypothesis, we propose that the TDO–Kyn–AhR pathway can be characterized in blood samples from patients with acute HCV infections, persistent HCV infections and no HCC, persistent HCV-associated HCC and healthy controls. The aim of discovery efforts could be to identify AhR pathway-related targets and predictive biomarkers representing molecular checkpoints in the progression of HCV infection into HCC. In the second phase, the capacity of biological/chemical AhR pathway modulators to regulate the expression and/or function of relevant AhR pathway targets will be evaluated, with the aim of developing novel therapeutic strategies for HCV and HCV-induced HCC. This approach could reveal molecular mechanisms involved in HCV-persistent infection and progression into HCC, and thus facilitate the development of prognostic surveillance approach in HCV patients for early detection of HCC, valuable research tools such as predictive *in vitro* assays and AhR antagonist compounds, as well as new therapeutic avenues.

IMPLICATIONS OF THE HYPOTHESIS

In summary, the identification and characterization of the link among TDO, Kyn and AhR, including its negative feedback mechanisms,[66] may pave the way for targeted therapeutic interventions to allow abrogation of HCV immune evasion mechanisms and bystander suppression of anti-HCC immune responses. New directions include further examination into development and clinical testing of Trp immune-metabolic pathway inhibitors, AhR pathway inhibitors, as well as the possibility of combination therapy with non-redundant immune checkpoint inhibitors, such as those targeting the programmed death-1, T-cell immunoglobulin mucin receptor 3 and cytotoxic T-lymphocyte-associated protein 4 pathways.[8,67] Such immunological approach in patients with chronic viral infections using immune checkpoint inhibitors and/or interleukin-7 may result in different safety profiles as compared with similar interventions in cancer patients.[68–71] Marra et al.[72] characterized the induction of HCC by viral factors and identified disease biomarkers of HCC pathogenesis. The development of HCC in HCV-infected patients requires up to 30 years from primary infection.[73] However, the course of HBV-related carcinogenesis is less predictable, as in some patients HCC can even precede cirrhosis, in particular with chronic HBV infection in endemic areas.[74]

In conclusion, the most effective tool for HCC prevention is avoiding risk factors such as viral infection. An effective vaccine has been available for prevention of new infection with HBV; however, to date, no vaccine against HCV infection has been approved.[72]

Importantly, the changes in signalling pathways and gene expression, which are induced by viral proteins in hepatocytes, are often mutated in HCC.[75] Therefore, virally triggered epigenetic modification of tumour suppressor genes can allow the constitutive expression of oncogenes in early tumorigenesis and mutation in these same oncogenes result in higher constitutive expression that supports tumour survival and growth.[76] Thus, inflammation-induced oncogene expression is an early event in HCC.[34] As malignant cell clones expand, they acquire heritable epigenetic changes that result in a permanent change in phenotype.[76] Molecular characterization of these changes will be a fertile ground for the identification of candidate biomarkers and targets for therapeutic intervention. Moreover, future investigations of Trp metabolism and its links with the AhR pathway will be instrumental for the development of therapeutic approaches to break the active immune tolerance towards viral antigens, cure chronic viral infections and prevent hepatic conditions such as cirrhosis and cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Liang Y, Li WW, Yang BW, Tao ZH, Sun HC, Wang L et al. Aryl hydrocarbon receptor nuclear translocator is associated with tumor growth and progression of hepatocellular carcinoma. Int J Cancer 2012; 130: 1745–1754.
2 De Giorgi V, Monaco A, Worchech A, Tornesello M, Izzo F, Buonaguro L et al. Gene profiling, biomarkers and pathways characterizing HCV-related hepatocellular carcinoma. J Transl Med 2009; 7: 85.
3 Tsunedomi R, Iizuka N, Hamamoto Y, Uchimura S, Miyamoto T, Tamesa T et al. Patterns of expression of cytochrome P450 genes in progression of hepatitis C virus-associated hepatocellular carcinoma. Int J Oncol 2005; 27: 661–667.

4 Bock KW, Kohle C. Ah receptor- and TCDD-mediated liver tumor promotion: clonal selection and expansion of cells evading growth arrest and apoptosis. *Biochem Pharmacol* 2005; **69**: 1403–1408.

5 Opitz CA, Litzenburger UM, Sahm F, Ott M, Tritschler I, Trump S *et al.* An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* 2011; **478**: 197–203.

6 Tian J, Feng Y, Fu H, Xie HQ, Jiang JX, Zhao B. The aryl hydrocarbon receptor: a key bridging molecule of external and internal chemical signals. *Environ Sci Technol* 2015; **49**: 9518–9531.

7 Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC *et al.* The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* 2008; **453**: 106–109.

8 Vacchelli E, Aranda F, Eggermont A, Sautes-Fridman C, Tartour E, Kennedy EP *et al.* Trial watch: IDO inhibitors in cancer therapy. *Oncoimmunology* 2014; **3**: e957994.

9 Munn DH, Mellor AL. Indoleamine 2,3-dioxygenase and tumor-induced tolerance. *J Clin Invest* 2007; **117**: 1147–1154.

10 Larrea E, Riezu-Boj JI, Gil-Guerrero L, Casares N, Aldabe R, Sarobe P *et al.* Upregulation of indoleamine 2,3-dioxygenase in hepatitis C virus infection. *J Virol* 2007; **81**: 3662–3666.

11 Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* 2005; **5**: 215–229.

12 Schmidt SK, Muller A, Heseler K, Woite C, Spekker K, MacKenzie CR *et al.* Antimicrobial and immunoregulatory properties of human tryptophan 2,3-dioxygenase. *Eur J Immunol* 2009; **39**: 2755–2764.

13 Pilotte L, Larrieu P, Stroobant V, Colau D, Dolusic E, Frederick R *et al.* Reversal of tumoral immune resistance by inhibition of tryptophan 2,3-dioxygenase. *Proc Natl Acad Sci USA* 2012; **109**: 2497–2502.

14 Bessede A, Gargaro M, Pallotta MT, Matino D, Servillo G, Brunacci C *et al.* Aryl hydrocarbon receptor control of a disease tolerance defence pathway. *Nature* 2014; **511**: 184–190.

15 Platten M, von Knebel Doeberitz N, Oezen I, Wick W, Ochs K. Cancer immunotherapy by targeting IDO1/TDO and their downstream effectors. *Front Immunol* 2014; **5**: 673.

16 Mehraj V, Routy JP. Tryptophan catabolism in chronic viral infections: handling uninvited guests. *Int J Tryptophan Res* 2015; **8**: 41–48.

17 Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N *et al.* Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nat Med* 2003; **9**: 1269–1274.

18 Okamoto A, Nikaido T, Ochiai K, Takakura S, Saito M, Aoki Y *et al.* Indoleamine 2,3-dioxygenase serves as a marker of poor prognosis in gene expression profiles of serous ovarian cancer cells. *Clin Cancer Res* 2005; **11**: 6030–6039.

19 Platten M, Wick W, Van den Eynde BJ. Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion. *Cancer Res* 2012; **72**: 5435–5440.

20 Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860–867.

21 Muller AJ, Sharma MD, Chandler PR, Duhadaway JB, Everhart ME, Johnson BA 3rd *et al.* Chronic inflammation that facilitates tumor progression creates local immune suppression by inducing indoleamine 2,3 dioxygenase. *Proc Natl Acad Sci USA* 2008; **105**: 17073–17078.

22 El-Serag HB. Hepatocellular carcinoma: an epidemiologic view. *J Clin Gastroenterol* 2002; **35**(5 Suppl 2): S72–S78.

23 Luke C, Price T, Roder D. Epidemiology of cancer of the liver and intrahepatic bile ducts in an Australian population. *Asian Pac J Cancer Prev* 2010; **11**: 1479–1485.

24 El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. *Ann Intern Med* 2003; **139**: 817–823.

25 Hassan MM, Frome A, Patt YZ, El-Serag HB. Rising prevalence of hepatitis C virus infection among patients recently diagnosed with hepatocellular carcinoma in the United States. *J Clin Gastroenterol* 2002; **35**: 266–269.

26 McGivern DR, Lemon SM. Virus-specific mechanisms of carcinogenesis in hepatitis C virus associated liver cancer. *Oncogene* 2011; **30**: 1969–1983.

27 Nakamoto Y, Guidotti LG, Kuhlen CV, Fowler P, Chisari FV. Immune pathogenesis of hepatocellular carcinoma. *J Exp Med* 1998; **188**: 341–350.

28 Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM *et al.* Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* 2002; **122**: 366–375.

29 Bartsch H, Nair J. Oxidative stress and lipid peroxidation-derived DNA-lesions in inflammation driven carcinogenesis. *Cancer Detect Prev* 2004; **28**: 385–391.

30 Lok AS, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM *et al.* Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009; **136**: 138–148.

31 Yeoman AD, Al-Chalabi T, Karani JB, Quaglia A, Devlin J, Mieli-Vergani G *et al.* Evaluation of risk factors in the development of hepatocellular carcinoma in autoimmune hepatitis: implications for follow-up and screening. *Hepatology* 2008; **48**: 863–870.

32 Teufel A, Weinmann A, Centner C, Piendl A, Lohse AW, Galle PR *et al.* Hepatocellular carcinoma in patients with autoimmune hepatitis. *World J Gastroenterol* 2009; **15**: 578–582.

33 Yeh MM, Daniel HD, Torbenson M. Hepatitis C-associated hepatocellular carcinomas in non-cirrhotic livers. *Mod Pathol* 2010; **23**: 276–283.

34 Arzumanyan A, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer* 2013; **13**: 123–135.

35 Gupta PB, Chaffer CL, Weinberg RA. Cancer stem cells: mirage or reality? *Nat Med* 2009; **15**: 1010–1012.

36 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646–674.

37 Banerjee A, Ray RB, Ray R. Oncogenic potential of hepatitis C virus proteins. *Viruses* 2010; **2**: 2108–2133.

38 Kwon YC, Bose SK, Steele R, Meyer K, Di Bisceglie AM, Ray RB *et al.* Promotion of cancer stem-like cell properties in hepatitis C virus-infected hepatocytes. *J Virol* 2015; **89**: 11549–11556.

39 Jin YM, Yun C, Park C, Wang HJ, Cho H. 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.

40 Carreno V, Bartolome J, Castillo I, Quiroga JA. Occult hepatitis B virus and hepatitis C virus infections. *Rev Med Virol* 2008; **18**: 139–157.

41 Moffett JR, Namboodiri MA. Tryptophan and the immune response. *Immunol Cell Biol* 2003; **81**: 247–265.

42 Thackray SJ, Mowat CG, Chapman SK. Exploring the mechanism of tryptophan 2,3-dioxygenase. *Biochem Soc Trans* 2008; **36**(Pt 6): 1120–1123.

43 Piconese S, Timperi E, Pacella I, Schinzari V, Tripodo C, Rossi M *et al.* Human OX40 tunes the function of regulatory T cells in tumor and nontumor areas of hepatitis C virus-infected liver tissue. *Hepatology* 2014; **60**: 1494–1507.

44 Ouaguia L, Mrizak D, Renaud S, Moralès O, Delhem N. Control of the inflammatory response mechanisms mediated by natural and induced regulatory T-cells in HCV-, HTLV-1-, and EBV-associated cancers. *Mediators Inflamm* 2014; **2014**: 564296.

45 Ouaguia L, Morales O, Mrizak D, Ghazal K, Boleslawski E, Auriault C *et al.* Overexpression of regulatory T cells Type 1 (Tr1) specific markers in a patient with HCV-induced hepatocellular carcinoma. *ISRN Hepatol* 2013; **2013**: 928485.

46 Hauben E, Gregori S, Draghici E, Migliavacca B, Olivieri S, Woisetschlager M *et al.* Activation of the aryl hydrocarbon receptor promotes allograft-specific tolerance through direct and dendritic cell-mediated effects on regulatory T cells. *Blood* 2008; **112**: 1214–1222.

47 Mascanfroni ID, Takenaka MC, Yeste A, Patel B, Wu Y, Kenison JE *et al.* Metabolic control of type 1 regulatory T cell differentiation by AHR and HIF1-alpha. *Nat Med* 2015; **21**: 638–646.

48 Choi SH, Chung AR, Kang W, Park JY, Lee MS, Hwang SW *et al.* Silencing of hypoxia-inducible factor-1beta induces anti-tumor effects in hepatoma cell lines under tumor hypoxia. *PLoS ONE* 2014; **9**: e103304.

49 Nevo U, Hauben E. Ecoimmunity: immune tolerance by symmetric co-evolution. *Evol Dev* 2007; **9**: 632–642.

50 Goossens N, Hoshida Y. Hepatitis C virus-induced hepatocellular carcinoma. *Clin Mol Hepatol* 2015; **21**: 105–114.

51 Cozzi A, Zignego AL, Carpendo R, Biagiotti T, Aldinucci A, Monti M *et al.* Low serum tryptophan levels, reduced macrophage IDO activity and high frequency of psychopathology in HCV patients. *J Viral Hepat* 2006; **13**: 402–408.

52 Soliman H, Mediavilla-Varela M, Antonia S. Indoleamine 2,3-dioxygenase: is it an immune suppressor? *Cancer J* 2010; **16**: 354–359.

53 Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J Exp Med* 1999; **189**: 1363–1372.

54 Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol* 2010; **185**: 3190–3198.

55 Molano A, Illarionov PA, Besra GS, Putterman C, Porcelli SA. Modulation of invariant natural killer T cell cytokine responses by indoleamine 2,3-dioxygenase. *Immunol Lett* 2008; **117**: 81–90.

56 Hwu P, Du MX, Lapointe R, Do M, Taylor MW, Young HA. Indoleamine 2,3-dioxygenase production by human dendritic cells results in the inhibition of T cell proliferation. *J Immunol* 2000; **164**: 3596–3599.

57 Mellor AL, Baban B, Chandler P, Marshall B, Jhaver K, Hansen A *et al.* Cutting edge: induced indoleamine 2,3 dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion. *J Immunol* 2003; **171**: 1652–1655.

58 Baban B, Hansen AM, Chandler PR, Manlapat A, Bingaman A, Kahler DJ *et al.* A minor population of splenic dendritic cells expressing CD19 mediates IDO-dependent T cell suppression via type I IFN signaling following B7 ligation. *Int Immunol* 2005; **17**: 909–919.

59 Munn DH, Sharma MD, Hou D, Baban B, Lee JR, Antonia SJ *et al.* Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. *J Clin Invest* 2004; **114**: 280–290.

60 Conrad C, Gilliet M. Plasmacytoid dendritic cells and regulatory T cells in the tumor microenvironment: A dangerous liaison. *Oncoimmunology* 2013; **2**: e23887.

61 Boasso A, Herbeuval JP, Hardy AW, Anderson SA, Dolan MJ, Fuchs D *et al.* HIV inhibits CD4+ T-cell proliferation by inducing indoleamine 2,3-dioxygenase in plasmacytoid dendritic cells. *Blood* 2007; **109**: 3351–3359.

62 Manches O, Fernandez MV, Plumas J, Chaperot L, Bhardwaj N. Activation of the noncanonical NF-kappaB pathway by HIV controls a dendritic cell immunoregulatory phenotype. *Proc Natl Acad Sci USA* 2012; **109**: 14122–14127.

63 Mimura J, Fujii-Kuriyama Y. Functional role of AhR in the expression of toxic effects by TCDD. *Biochim Biophys Acta* 2003; **1619**: 263–268.

64 Puccetti P, Fallarino F, Italiano A, Soubeyran I, MacGrogan G, Debled M *et al.* Accumulation of an endogenous tryptophan-derived metabolite in colorectal and breast cancers. *PLoS ONE* 2015; **10**: e0122046.

65 Stone TW, Darlington LG. The kynurenine pathway as a therapeutic target in cognitive and neurodegenerative disorders. *Br J Pharmacol* 2013; **169**: 1211–1227.

66 Nguyen NT, Kimura A, Nakahama T, Chinen I, Masuda K, Nohara K *et al.* Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc Natl Acad Sci USA* 2010; **107**: 19961–19966.

6

67 Hotchkiss RS, Moldawer LL. Parallels between cancer and infectious disease. *N Engl J Med* 2014; **371**: 380–383.

68 Vaccari M, Boasso A, Fenizia C, Fuchs D, Hryniewicz A, Morgan T *et al.* Fatal pancreatitis in simian immunodeficiency virus SIV(mac251)-infected macaques treated with 2',3'-dideoxyinosine and stavudine following cytotoxic-T-lymphocyte-associated antigen 4 and indoleamine 2,3-dioxygenase blockade. *J Virol* 2012; **86**: 108–113.

69 Sereti I, Estes JD, Thompson WL, Morcock DR, Fischl MA, Croughs T *et al.* Decreases in colonic and systemic inflammation in chronic HIV infection after IL-7 administration. *PLoS Pathog* 2014; **10**: e1003890.

70 Makarova-Rusher OV, Medina-Echeverz J, Duffy AG, Greten TF. The yin and yang of evasion and immune activation in HCC. *J Hepatol* 2015; **62**: 1420–1429.

71 Choudhury N, Nakamura Y. Importance of immunopharmacogenomics in cancer treatment: patient selection and monitoring for immune checkpoint antibodies. *Cancer Sci* 2016; **107**: 107–115.

72 Marra M, Sordelli IM, Lombardi A, Lamberti M, Tarantino L, Giudice A *et al.* Molecular targets and oxidative stress biomarkers in hepatocellular carcinoma: an overview. *J Transl Med* 2011; **9**: 171.

73 Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; **12**(4 Pt 1): 671–675.

74 McMahon BJ. Epidemiology and natural history of hepatitis B. *Semin Liver Dis* 2005; **25**(Suppl 1): 3–8.

75 Feitelson MA. Parallel epigenetic and genetic changes in the pathogenesis of hepatitis virus-associated hepatocellular carcinoma. *Cancer Lett* 2006; **239**: 10–20.

76 Baylin SB, Ohm JE. Epigenetic gene silencing in cancer-a mechanism for early oncogenic pathway addiction? *Nat Rev Cancer* 2006; **6**: 107–116.