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To cite this version:

Simonetta Bandiera, C Billie Bian, Yujin Hoshida, Thomas F. Baumert, Mirjam B. Zeisel. Chronic hepatitis C virus infection and pathogenesis of hepatocellular carcinoma. Current Opinion in Virology, 2016, 20, pp.99-105. 10.1016/j.coviro.2016.09.010 . hal-02374336

HAL Id: hal-02374336

https://hal.science/hal-02374336v1

Submitted on 14 Jan 2020

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HHS Public Access

Author manuscript

Curr Opin Virol. Author manuscript; available in PMC 2017 October 11.

Published in final edited form as:

Curr Opin Virol. 2016 October ; 20: 99–105. doi:10.1016/j.coviro.2016.09.010.

Chronic hepatitis C virus infection and pathogenesis of hepatocellular carcinoma

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Abstract

Hepatitis C virus (HCV) infection is one of the major causes of advanced liver disease and hepatocellular carcinoma (HCC) worldwide. While the knowledge about the molecular virology of HCV infection has markedly advanced, the molecular mechanisms of disease progression leading to fibrosis, cirrhosis and HCC are still unclear. Accumulating experimental and clinical studies indicate that HCV may drive hepatocarcinogenesis directly via its proteins or transcripts, and/or indirectly through induction of chronic liver inflammation. Despite the possibility to eradicate HCV infection through direct-acting antiviral treatment, the risk of HCC persists although specific biomarkers to estimate this risk are still missing. Thus, a better understanding of HCV-induced HCC and more physiological liver disease models are required to prevent cancer development.

Keywords

Hepatitis C virus; hepatocellular carcinoma; fibrosis; cancer hallmarks; direct-acting antiviral-based therapies

Introduction

Hepatitis C virus (HCV) is single-strand RNA virus from the *Flaviviridae* family targeting hepatocytes. Chronic HCV infection induces immune dysfunctions such as impaired T-cell

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Declaration of interest

The authors do not have any conflict of interest and did not receive writing assistance.

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functions and inefficient antibody responses, metabolic disorders such as hepatic steatosis, iron accumulation, and insulin resistance often associated with type 2 diabetes. More importantly, HCV is one of the major etiologies of chronic hepatitis and progressive liver fibrosis that lead to development of lethal complications, i.e., cirrhosis and hepatocellular carcinoma (HCC), the second leading cause of cancer mortality worldwide and the only and most rapidly increasing cancer death in the U.S. [1,2]. Chronic HCV infection is highly prevalent globally, including developed countries [3]. In the U.S., more than 1 million individuals, representing the "baby boomer" population, are estimated to develop HCV-related liver cirrhosis and/or HCC by 2020. Recently developed direct-acting antivirals (DAAs) for HCV effectively cure HCV infection, i.e., they enable to achieve sustained virologic response (SVR), but the high costs will limit their wide-spread use [4]. Of note, HCC risk remains high for decades even after SVR, and HCV-related HCC is predicted to increase until 2030 despite improved viral cure by DAAs [5,6].

HCV does not integrate its genetic material into the host genome, and therefore requires continuous replication to maintain chronic infection. Many host factors, playing essential roles in the HCV life cycle and immune evasion, have been identified as candidate targets for antiviral interventions (reviewed in [7]). However, disease pathogenesis that ultimately causes HCC is still unclear. Experimental studies to date have suggested models of viral carcinogenesis unique to HCV [8]. Increasing evidence shows that HCV transmits signals and modulates hepatocyte gene expression following engagement with cellular receptors [9,10]. Moreover, viral proteins have been involved in disrupting signal transduction pathways that affect cell survival, proliferation, and transformation [8]. This suggests that virus-host interactions and signaling during viral infection contribute to cellular transformation and development of HCC directly via HCV proteins or RNA, and/or indirectly through induction of chronic inflammation. Additionally, the genetic background of the host may play a role in HCC pathogenesis. Genetic analyses in HCV-infected patients have unraveled specific mutation or polymorphisms in *MICA/HCP5*, *LEPR* and *IFNL3* loci that are associated with the development of HCC [11–16], indicating that genetic variation may contribute to individual susceptibility for HCV-driven HCC.

Of note, the persisting risk of HCC development even after viral cure suggests that HCV leaves molecular imprinting in the host genome that keeps driving carcinogenesis. Management of post-SVR HCC will be increasingly relevant as more patients achieve SVR by the DAA treatment in clinic. Here, we review several examples of mechanisms that may contribute to HCV-induced HCC and discuss the clinical challenges to prevent HCC development in at-risk patients in the era of DAA-based anti-HCV therapies.

Viral factors directly driving hepatocarcinogenesis

The strong and reproducible association of HCV genotype 3 with development of steatosis and HCC, genotype 1b with more frequent progression to HCC, and HCV core gene variants with post-SVR HCC suggests that specific viral factors influence or determine progression of liver disease [17–19]. The viral genome encodes for three structural (core, E1, E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B). Several *in vivo* studies in transgenic mouse models reported direct induction of liver disease by the

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expression of viral proteins (reviewed in [20]). Although none of these models could faithfully recapitulate the full features of human disease, some of the phenotypes were consistent with epidemiological data from HCV-infected patients. Interestingly, these studies highlighted that HCV RNA and proteins can perturb hepatocellular homeostasis by driving several major cancer hallmarks (Figure 1).

First, metabolic reprogramming including disturbance of lipid metabolism and mitochondrial dysfunction were shown to play an important role in HCV-driven HCC (Figure 1). Indeed, chronic HCV infection enhances mitochondrial liver injury together with oxidative stress in human as well as experimental models [21]. Several studies highlight a role of the HCV core protein in steatosis and HCC nodule development as well as in insulin resistance, which is accompanied with intrahepatic lipid accumulation [20,22]. The alteration of lipid metabolism is induced by an HCV core-mediated impairment of lipid β-oxidation, which is associated with a reduced activity of the mitochondrial electron transport chain [20]. Recently, HCV core protein was also shown to contribute to mitochondrial damage by impairing mitophagy [23]. The resulting oxidative stress is regarded as a key trigger of HCC initiation and development (Figure 1). Imbalance of the oxidant/antioxidant state in the liver was shown to induce HCC in HCV core transgenic mice in the absence of inflammation [24]. Moreover, generation of reactive oxygen species (ROS) in the course of HCV infection was associated with genomic instability, a hallmark of cancer cells [20]. Indeed, accumulation of genetic mutations as well as chromosomal alterations crucially drive the development of HCC in patients [25]. By inducing a β-Catenin-dependent upregulation of c-Myc via NS5A, HCV was shown to perturb ROS production in association with enhanced DNA damage and aberrant cell-cycle arrest (Figure 1) [26]. In addition, increased telomerase (TERT) activity, a characteristic of transforming or transformation-prone cells, was observed in HCV core-transfected primary human hepatocytes that acquired an immortalized phenotype [27]. In line with this observation, somatic mutations in the *TERT* promoter that enhance TERT expression were shown to be among the earliest and most prevalent neoplastic event in HCC associated with all major etiologies including HCV [28].

Another major hallmark of cancer that is directly affected by HCV is evasion from cell death and senescence (Figure 1). Although HCV proteins were reported to have both pro-apoptotic and anti-apoptotic properties [8], HCV is likely involved in evasion from apoptosis *in vivo*. A number of studies indicate that Fas-mediated apoptosis is directly inhibited by different HCV proteins [20,29–31]. Given that the Fas system accounts for T cell-mediated cytotoxicity, suppression of cell death is not only a mechanism of sustained cell proliferation but also one strategy that enables HCV to escape immune surveillance by T cells and thus to establish persistent infection [32].

Finally, recent evidence indicates that viral proteins impact on epithelial mesenchymal transition (EMT) pathway, which promotes fibrogenesis, tumor development and metastases (Figure 1). HCV NS5A was shown to activate Twist2, a transcriptional regulator of EMT, and to cooperate with Ras oncogene to enhance tumor cell invasiveness in xenograft mouse models [33]. Furthermore, expression of HCV core in transgenic mice enhances intrahepatic TGF-β signaling, a key regulator of EMT driving the activation of human stellate cells

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(HSCs) [34]. Further studies showed that induction of EMT by HCV core is mediated by at least two mechanisms: i) the inhibition of E-cadherin expression by a complex comprising HCV core, Snail and the histone deacetylases HDAC1/HDAC2 [35]; ii) the HCV core-induced epigenetic silencing of *SFRP1* via DNA methylation and histone modifications that in turn activates Wnt/β-catenin signaling [36]. Yet the clinical relevance of these recent findings remains to be determined.

HCV-induced inflammatory responses indirectly driving hepatocarcinogenesis

HCV infection can induce chronic hepatic inflammation with varying activity, which causes progressive liver fibrogenesis and leads to development of cirrhosis (Figure 1). Clinically, the majority of HCV-related HCC tumors develop in livers with cirrhosis established after decades of chronic inflammation, underscoring the key role of virus-induced inflammatory responses, besides the viral materials themselves, in HCC pathogenesis. Several inflammatory pathways have been implicated in HCC. First, the sensing of HCV infection by pathogen recognition receptors of the innate immune system activates the NF-κB signaling and downstream proinflammatory chemokines and cytokines including type III interferon (IFN), which is associated with HCC development (Figure 1) [37–39]. Ectopic lymphoid structure aggregated near the portal tract was reported as a niche of HCC initiation associated with striking NF-κB activation in a subset of HCV-infected human livers [40]. Approximately in 70 % of chronic hepatitis C (CHC) patients the immune response fails to eradicate the virus due to impaired T cell and antibody responses, and little antiviral efficacy of IFN-stimulated genes (ISGs) [41]. The adaptive immune response mediated by cytotoxic T cells has been suggested to contribute to liver injury by triggering repeated cycles of hepatocyte death and regeneration/proliferation. The inflammatory response also exacerbates oxidative stress in the liver (Figure 1). Cytokines, ROS and apoptotic signals contribute to HSC activation, which triggers aberrant deposition of extracellular matrix proteins and progressive fibrosis (Figure 1). As such, the functional liver parenchyma is progressively replaced by non-functional fibrotic tissue. Overall, this pattern of chronic inflammation, unresolved wound healing response and increased hepatocellular proliferation in CHC is thought to generate an environment highly permissive for hepatocarcinogenesis.

Despite the growing knowledge, many open questions remain unanswered. The molecular bases of the interplay between the innate and adaptive immune responses in the course of CHC and their relevance for HCC development are still largely unclear [41]. IFN pathway activation is one of the key components of the host responses to HCV, although cell types secreting IFN as well as types of secreted IFN stimulating specific subset of ISGs are still elusive. This is partly because of the lack of a robust immunocompetent *in vivo* HCV infection model that mirrors the cell circuits of HCC development as well as the crosstalk between parenchymal and non-parenchymal cell types driving disease progression under physiological condition as in human [20]. Transgenic mouse models coupled with epidemiological data in patients have provided important insights into mechanistic investigation. This approach was successful in unraveling a pathway of hepatocarcinogenesis driven by the pro-inflammatory cytokine lymphotoxin (LT) α and β [42]. By using

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transgenic mice for either LTs expression or NF-κB signaling components, Haybeack et al. discovered that LTs overexpression induces chronic hepatitis and HCC by altering NF-κB signaling in both hepatocytes and lymphocytes. These observations were corroborated by the enhanced LT expression in clinical liver specimens from virus-induced chronic hepatitis and HCC as compared to healthy liver [42]. More recently, HCV NS5B was shown to promote pro-inflammatory LTβ signaling in liver cells [43]. Likewise, two recent studies casted new light on novel mechanisms of HSC activation and liver fibrogenesis in CHC. The first involves the acetylation of HMGB1 by extracellular osteopontin (OPN), a stress sensor protein that is enhanced in liver disease and elevated in serum of patients who are at risk of HCC development [44]. Acetylated HMGB1 interacts with HDAC1/HDAC2 to promote collagen-I expression by HSCs and increase its histological deposition [45]. The second mechanism relies on the upregulation of the Gas6/Axl pathway in HSC leading to activation of these cells and liver fibrogenesis upon carbon tetrachloride-induced injury in mice [46]. Importantly, the clinical relevance of both mechanisms was evidenced by the correlation between the severity of liver injury and increased expression of OPN/HMGB1 or Gas6/Axl, respectively, in HCV-infected patients [45,46]. However, additional clinical cohort studies may be required to corroborate the involvement of these processes in HCV pathogenesis.

Treatment of HCV infection and prevention of HCC

Rapidly evolving DAA-based anti-HCV therapies now enable more than 90% of SVR rate with all-oral regimens even in the cases hard to cure before [47]. In patients previously treated with older, IFN-based regimens, SVR was significantly associated with reduced but not eliminated future risk of HCC development over a decade [48]. In the retrospective studies, several clinical characteristics such as more advanced liver fibrosis, older age, and male sex among others have been suggested as predisposing factors for post-SVR HCC (Table 1). However, estimation of HCC risk in patients newly achieving an SVR is still infeasible and the mechanisms of carcinogenesis are totally unknown. Given the annual incidence of post-SVR HCC, which is likely below the threshold that rationalizes regular HCC surveillance, HCC risk biomarkers or indices will play a critical role to perform cost-effective and practically feasible HCC surveillance by triaging the patients according to the predicted HCC risk [21]. Also, such biomarkers may provide clues to targets of HCC chemopreventive interventions. It is still unanswered question whether HCC risk after DAA-based or other types of anti-HCV therapies such as viral entry inhibition [49] is comparable to that of IFN-based therapies. Modulation of cellular signaling pathways such as IFN, EGF, mTOR, and retinoid X receptor-α pathways and drugs for metabolic disorder, some of which have been already clinically evaluated, may serve as alternative options of HCC chemoprevention for broader etiologies, including post-SVR HCC [50–56]. Experimental systems that allow mechanistic assessments of the carcinogenic drivers will be critical in identifying and developing rational molecular-targeted HCC chemoprevention therapies.

Acknowledgments

The authors acknowledge grant support of the European Union (ERC-2014-AdG-671231-HEPCIR (T.F.B, Y.H.), H2020-667273-HEPCAR (T.F.B.), EU-ANR ERA-NET Infect-ERA hepBccc (T.F.B.), ANRS (T.F.B., M.B.Z.), the French Cancer Agency (ARC IHU201301187 (T.F.B.)), the IdEx program of the University of Strasbourg (M.B.Z., T.F.B.), the Foundation University of Strasbourg (T.F.B.), NIH/NIDDK R01 DK099558 (Y.H.), and Irma T. Hirschl

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Trust (Y.H.). This work has been published under the framework of the LABEX ANR-10-LAB-28 and benefits from a funding from the state managed by the French National Research Agency as part of the Investments for the future program.

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** of outstanding interest

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Highlights

- The molecular mechanisms of HCV-driven HCC are still elusive.
- HCV perturbs hepatocellular homeostasis by driving several major cancer hallmarks.
- HCV-induced inflammatory responses indirectly drive hepatocarcinogenesis.
- Biomarkers to predict HCC risk in patients after HCV cure are missing.
- HCV may leave a cancer-prone molecular imprinting in the host genome.
- Novel experimental systems are needed to assess HCC drivers mechanistically.

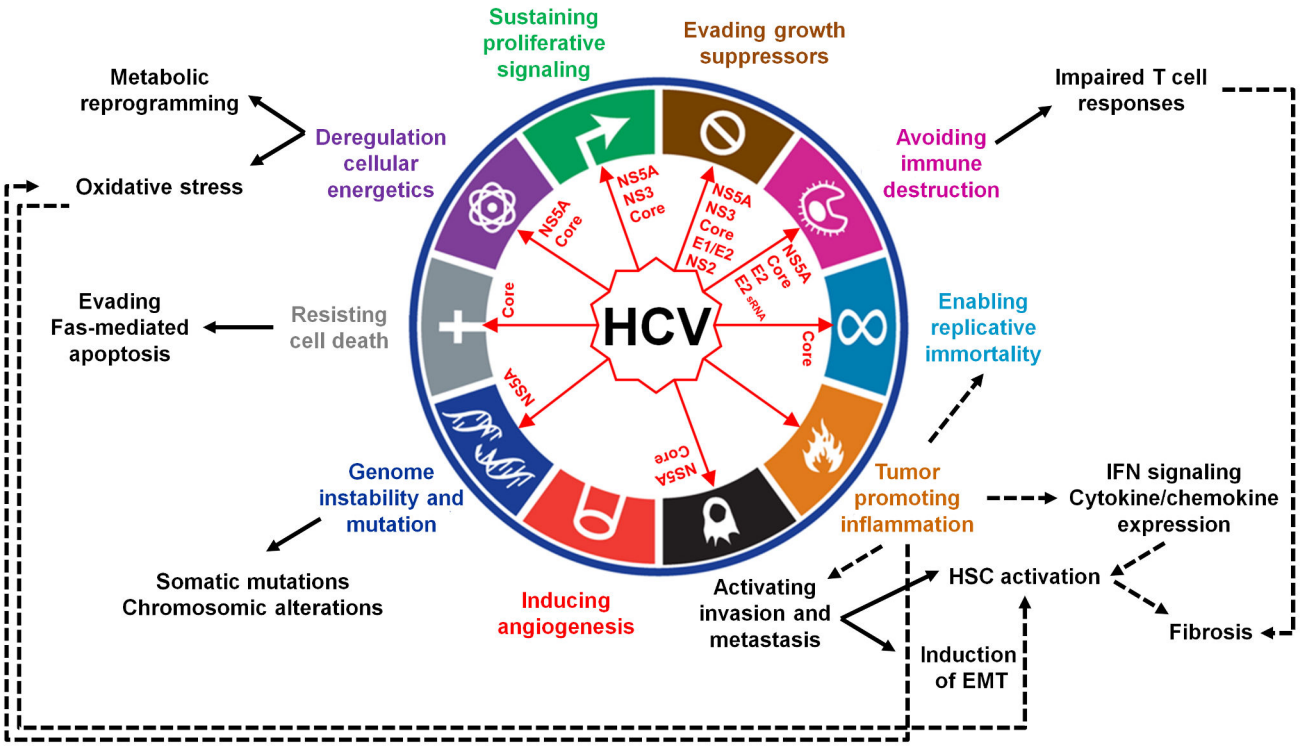

Figure 1. HCV RNA and proteins perturb hepatocellular homeostasis by driving major cancer hallmarks

The diagram (adapted from [8]) shows the HCV-host interactions and signaling upon viral infection that contribute to cellular transformation and development of HCC. The red arrows indicate HCV RNA and proteins exerting a direct effect on a specific hallmark. Black arrows link specific hallmarks to examples of mechanisms of HCV-driven HCC, which were observed in both clinical and *in vivo* experimental models. Regarding the tumor promoting inflammation hallmark (in orange in the diagram), this is activated by the pathogen recognition receptors that sense HCV infection. Dotted lines indicate examples of inflammation-driven carcinogenesis. sRNA, small RNA.

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Table 1

Clinical risk factors for post-SVR HCC development

Type of anti-HCV therapy	No. SVR patients	Follow-up period (y)	Risk factors for HCC development	References
IFN-based	1197	5.9	Age ≥ 50 y, male, F3/4 fibrosis	[57]
IFN-based	1056	4.7	Age ≥ 60 y, AST ≥ 100 U/L, platelets < 150 ×10^9/L	[58]
IFN-based	871	3.4	F3/4 fibrosis, age ≥ 60 y, post-SVR AFP ≥ 20 ng/mL, platelets < 150 ×109/L	[59]
IFN-based	1751	8.1	Diabetes, male, alcohol, age (every 10 y)	[60]
IFN-based	1425	3.3	Post-SVR AFP ≥ 5 ng/mL, Age ≥ 65 y	[61]
IFN-based	562	4.8	F2/3/4 fibrosis, age ≥ 50 y, ethanol ≥ 30 g/d, pre-SVR AFP ≥ 8 ng/mL	[62]
IFN-based	642	4.4	GGT ≥ 75 U/L, age ≥ 65 y, F2/3 fibrosis	[63]
IFN-based	522	7.2	Diabetes, FIB-4 index	[64]
IFN-based	801	5.0	Age ≥ 60 y, post-SVR AFP ≥ 20 ng/mL platelets < 150 ×109/L, F3/4 fibrosis	[15]
IFN-based	83 HCC among 2152 SVR	6.7	No surveillance (for risk of advanced HCC)	[65]
IFN-based	10817	2.8	cirrhosis, age ≥ 65 y, diabetes, HCV genotype 3	[66]
IFN-based	1351	n.a.	Pre/post-SVR AFP ≥ 15 ng/mL, pre/post-SVR APRI ≥ 0.7	[67]
IFN-based	399	7.8	Cirrhosis, diabetes	[68]
IFN-based	24 SVR HCC cases vs. 96 matched controls	n.a.	Compensated cirrhosis, post-SVR albumin ≤ 36 g/L	[69]
IFN-based	376	7.6	Advanced fibrosis/cirrhosis, diabetes, LSM > 12 kPa	[70]
IFN-based	1094	4.2	Age ≥ 60 y, male, F3/4 fibrosis, post-SVR AFP ≥ 10 ng/mL	[71]
IFN-based	598	5.1	Pre/post-SVR APRI ≥ 1.0	[72]

SVR: sustained virologic response, HCC: hepatocellular carcinoma, HCV: hepatitis C virus, y: years, IFN: interferon, AFP: alpha-fetoprotein, GGT: gamma-glutamyl transpeptidase, FIB-4: fibrosis-4, LSM: liver stiffness measurement, APRI: aspartate aminotransferase-to-platelet ratio index, n.a.: not available.