VIRAL HEPATITIS

HCV compartmentalization in HCC: driver, passenger or both?

Jacinta A. Holmes and Raymond T. Chung

Hepatocellular carcinoma is associated with HCV infection but the underlying interplay between virus and tumour remains to be elucidated. Now, Harouaka *et al.* report that in patients with HCV-related cirrhosis, HCV replication is restricted within liver tissue originating from hepatocellular carcinoma, with an associated increase in the diversity and complexity of the HCV quasispecies.

Refers to Harouaka, D. *et al.* Diminished viral replication and compartmentalization of hepatitis C virus in hepatocellular carcinoma tissue. *Proc. Natl Acad. Sci. USA* **113**, 1375–1380 (2016)

The incidence of hepatocellular carcinoma (HCC) has been sharply rising globally with the number of incident cases tripling over the past 15 years, largely driven by HCV infection[1]. Chronic inflammation from HCV infection results in progressive liver damage and fibrosis, leading to the complications of cirrhosis, HCC and end-stage liver failure[2]. HCC still carries a poor prognosis, and is one of only a few cancers with a rising mortality. Early diagnosis of HCC is critical to improve long-term survival, as early-stage disease can be amenable to curative therapies. However, it is currently difficult to predict which patients infected with HCV will develop HCC, and therefore all those with advanced fibrosis are enrolled in screening programmes for HCC. In their new paper[3], Harouaka and colleagues report that HCV replication is severely restricted within HCC tissue compared with the surrounding nontumorous liver, and that the genetic diversity (genetic distance between viral variants) and complexity (number of viral variants) of HCV also differs substantially between tumorous and nontumorous tissue. Taken together, these novel findings suggest there is compartmentalization of the virus in cirrhotic livers from HCV-infected patients with HCC (FIG. 1).

The precise role of HCV in HCC pathogenesis has not been completely defined, although both direct and indirect roles have been identified[4]. HCV exists as quasispecies of viral variants in liver and serum that are strongly influenced by host immune pressure[5]. This pressure allows the quasispecies to evolve over time, developing escape mechanisms that permit viral persistence with the hypothesis that, as the disease progresses, HCV diversity decreases due to less immune pressure. Thus, there has been much interest in evaluating HCV diversity, complexity and replication in both serum and liver tissue from patients infected with HCV to gain further understanding of HCV-related HCC pathogenesis. However, efforts have been limited by the lack of adequate experimental models and access to high-quality samples from multiple areas of the liver from the same patient.

In the latest study, Harouaka *et al.*[3] showed that the contrast in HCV replication between tumorous and nontumorous tissue was not related to changes in microRNA-122 expression (a well-described host factor required for HCV replication[6]) and, moreover, circulating HCV RNA levels were found to be comparable between patients with ($n = 8$) and without ($n = 4$) HCC. Differences in HCV quasispecies diversity were not observed in areas of nontumorous tissue adjacent to the HCC, the distant peripheral non-HCC tissue, or between the right and left lobes of the non-HCC cirrhotic control livers. Arbitrary variability of HCV RNA levels was, therefore, excluded as an explanation for the observed findings. The authors then assessed whether changes in quasispecies could be attributed to natural selection by assessing the proportion of synonymous (non-coding) and nonsynonymous (coding) viral RNA substitutions. The high frequency of synonymous substitutions suggested that the changes in the viral variants observed were not a result of selection. When the degree of HCV RNA replication restriction was further analysed, patients with HCC could be divided into those with either a <2 log or >2 log reduction in HCV RNA levels between the nontumorous and tumorous tissue. The degree of change in the HCV quasispecies diversity

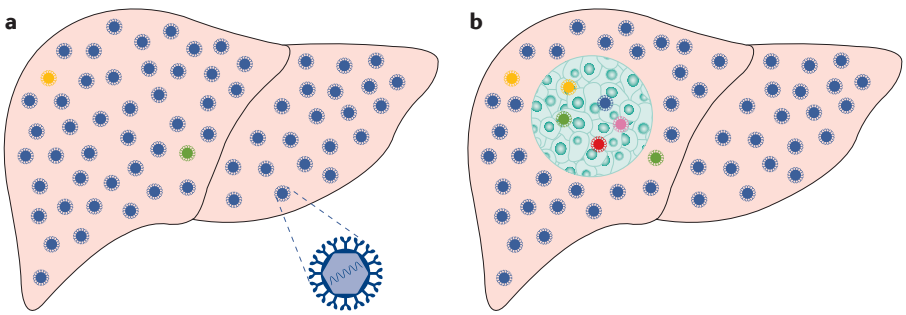

Figure 1 | **HCV compartmentalization in hepatocellular carcinoma. a** | Changes in HCV replication and quasispecies in the context of HCV related cirrhosis and **b** | following the development of hepatocellular carcinoma. HCV replication was found to be restricted within tumorous liver tissue, with a greater diversity and complexity of the HCV quasispecies (part **b**).

was significantly greater in those with more HCV replication restriction (>2 log versus <2 log HCV RNA reduction, $P = 0.0257$). Interestingly, when malignant cell proliferation was assessed by staining for the enzyme MIB1 in a subset of the HCC samples, those with a >2 log reduction in HCV RNA level demonstrated a greater degree of malignant hepatocyte proliferation than those with a <2 log reduction. These data suggest that there is a higher rate of tumour cell proliferation in patients who have increased HCV quasispecies diversity and restriction of HCV replication, which might have implications for HCC pathogenesis.

One of the advantages of the latest study is that multidirectional sampling of HCC tissue (up to five samples from the tumour) and surrounding non-HCC liver tissue (up to 17 samples in total, averaging 13 samples per patient) was performed in HCV-infected patients with cirrhosis and HCC, thereby overcoming the issue of inadequate liver sampling. Paired sera were available allowing comparison of the characteristics of circulating HCV to intrahepatic HCV, and HCV-infected patients with cirrhosis but without HCC were included as controls, with samples taken from the right and left lobes of the liver (four samples per patient). This comprehensive sampling strategy permitted a more global assessment of HCV RNA levels and quasispecies within both HCC tissue and surrounding nontumorous tissue than many previous studies. However, it is still difficult to conclude from data taken at a single time point whether the changes in HCV replication and the HCV quasispecies are driving tumorigenesis, or if these changes are induced by the tumour milieu. Although serial tissue sampling over time or the availability of precancerous lesions would help shed light on this question, both are practically challenging to obtain. Nonetheless, some intriguing data support the concept that perhaps the observed findings are both the cause and effect of HCC.

The interferon-induced double-stranded RNA-activated protein kinase (EIF2AK2, also known as PKR) recognizes double-stranded RNA and phosphorylates eukaryotic translation initiation factor 2 subunit 1 (also known as eukaryotic initiation factor-2 or eIF-2α), which has been shown to block HCV replication by inhibiting initiation of HCV translation[7]. Levels of PKR and eIF-2α are also significantly elevated in HCC liver tissue compared with surrounding non-tumorous tissue ($P = 0.001$)[8], suggesting that the host response to the tumour might induce factors that alter localized immune pressure on HCV. Interestingly, overexpression of PKR has been associated with reduced HCV RNA levels in HCC tissue[9], suggesting that overexpressed PKR remains functionally active in HCC. PKR also upregulated the JNK1 and ERK1/3 pathways, which led to increased HCC cell proliferation in the context of HCV infection. HCV-driven PKR expression might therefore act both to control localized HCV levels as well as to promote tumour growth in response to HCV infection. Such a model is amenable to testing and suggests that PKR could be an interesting therapeutic target.

As the incidence of HCC continues to rapidly increase, largely driven by the ageing HCV population[10], its poor prognosis and extremely limited therapies contribute to the observation that HCC is now the fastest rising cause of global cancer-related mortality[1]. We currently remain unable to predict with precision which patients will develop HCC. Thus, because patients with advanced fibrosis or cirrhosis are at risk, it is still recommended that all of these patients be enrolled in screening programmes for HCC. Although tempting to speculate that local HCV RNA concentrations or quasispecies variations within the liver could foreshadow HCC, the utility of such a tool would be limited given the need for extensive tissue sampling and the complexity of quasispecies analysis. Also, it remains to be seen whether these viral compartment changes are only found in the context of established HCC. As such, identification of alternative biomarkers for future HCC risk would be extremely useful, even in the face of a successful HCV cure now achievable with contemporary antiviral therapy. In this regard, use of gene expression signatures from HCV-infected cirrhotic tissue could be important for prognosis. The study by Harouaka *et al.*[3] sheds light on the events that attend HCV-related tumorigenesis, but further studies are needed to identify possible mediators of these findings.

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1. Lozano, R. *et al.* Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**, 2095–2128 (2012).
2. Shepard, C. W., Finelli, L. & Alter, M. J. Global epidemiology of hepatitis C virus infection. *Lancet Infect. Dis.* **5**, 558–567 (2005).
3. Harouaka, D. *et al.* Diminished viral replication and compartmentalization of hepatitis C virus in hepatocellular carcinoma tissue. *Proc. Natl Acad. Sci. USA* **113**, 1375–1380 (2016).
4. Koike, K. Hepatitis C virus contributes to hepatocarcinogenesis by modulating metabolic and intracellular signaling pathways. *J. Gastroenterol. Hepatol.* **22**, S108–S111 (2007).
5. Merani, S. *et al.* Effect of immune pressure on hepatitis C virus evolution: insights from a single-source outbreak. *Hepatology* **53**, 396–405 (2011).
6. Jopling, C. L., Yi, M., Lancaster, A. M., Lemon, S. M. & Sarnow, P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* **309**, 1577–1581 (2005).
7. Chang, K. S. *et al.* Replication of hepatitis C virus (HCV) RNA in mouse embryonic fibroblasts: protein kinase R (PKR)-dependent and PKR-independent mechanisms for controlling HCV RNA replication and mediating interferon activities. *J. Virol.* **80**, 7364–7374 (2006).
8. Hiasa, Y. *et al.* Protein kinase R is increased and is functional in hepatitis C virus-related hepatocellular carcinoma. *Am. J. Gastroenterol.* **98**, 2528–2534 (2003).
9. Watanabe, T. *et al.* Protein kinase R modulates c-Fos and c-Jun signaling to promote proliferation of hepatocellular carcinoma with hepatitis C virus infection. *PLoS ONE* **8**, e67750 (2013).
10. Davis, G. L., Alter, M. J., El-Serag, H., Poynard, T. & Jennings, L. W. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology* **138**, 513–521.e6 (2010).

Competing interests statement
The authors declare no competing interests.