Hepatitis C virus cell entry, endothelial-mesenchymal transition and hepatocellular carcinoma

or

The multifaceted role of E-Cadherin in hepatitis C virus infection and pathogenesis

Che C. Colpitts[1,2], Joachim Lupberger[1,2] and Thomas F. Baumert[1,2,3,*]

[1]Inserm, U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, 67000 Strasbourg, France; [2]Université de Strasbourg, 67000 Strasbourg, France; [3]Institut Hospitalo-Universitaire, Pôle Hépato-digestif, Hopitaux Universitaires de Strasbourg, 67000 Strasbourg, France.

***Corresponding author**: Prof. Thomas F. Baumert, MD; Inserm U1110, 3 Rue Koeberlé, 67000 Strasbourg, France; Phone: ++33 3 68 85 37 03, Fax: ++33 3 68 85 37 24, e-mail: thomas.baumert@unistra.fr

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Hepatitis C virus (HCV) is a major global cause of end-stage liver disease, including cirrhosis and hepatocellular carcinoma (HCC). Although direct-acting antivirals (DAAs) can cure the majority of infected patients, their high cost prevents access to treatment for the majority of patients worldwide. Moreover, not all patient groups respond to therapy and some patients fail therapy as a result of DAA resistance (1). There is accumulating evidence that viral cure does not eliminate the risk for progressive liver disease once fibrosis has been established (2). Indeed, HCC risk persists following viral cure, particularly among elderly patients (2). The mechanisms of HCV-induced hepatocarcinogenesis as well as its progression to advanced and metastatic disease, however, are still only partially understood. Most importantly, efficient strategies to treat HCV-associated HCC are limited and urgently needed. In this issue of PNAS, Li and colleagues uncover E-cadherin (E-Cad), a major adherens junction protein, as a novel HCV host factor that not only further advances our understanding of the first steps of HCV infection but also identifies tight junctions as a pathogenic link between viral cell entry and hepatocellular carcinoma (3).

HCV infects hepatocytes via a complex, multi-step process requiring an expanding list of host factors, including cluster of differentiation 81 (CD81), scavenger receptor class B type I (SR-BI) and the tight junction proteins claudin-1 (CLDN1) and occludin (OCLN) (4). However, the HCV entry pathway is still not fully understood. In particular, the precise mechanisms that regulate the cellular entry factors and orchestrate the multi-step entry process still need to be elucidated. In this study, the authors elucidate a novel regulatory mechanism for a post-binding HCV entry step, thus contributing another piece to the overall HCV entry puzzle and providing a new link between a viral entry step and the pathogenesis of hepatocellular cancer. Using classical gene silencing and antibody blocking approaches, the authors

establish a role for E-Cad in the entry of all major genotypes of HCV (3). E-Cad, as a regulator of tight junction assembly and composition, is important for the proper localization of essential HCV entry factors, tight junction proteins CLDN1 and OCLN.

Mechanistically, Li *et al.* show that E-Cad regulates the surface distribution of CLDN1 and OCLN to modulate HCV entry at a post-binding step (3). Although the molecular details of this process remain to be determined, the authors show that the expression and localization of zonula occludens 1 (ZO-1), a tight junction protein which links the cadherin complex to actin as an intermediate step in tight junction formation, was not affected by loss of E-Cad (3). Given the observed colocalization between E-Cad and CLDN1/OCLN (3), E-Cad may have a direct effect on the proper localization of these proteins on the cell surface. Another possibility is that E-Cad regulates the composition of tight junctions via signaling molecules such as the small GTPase Rac and atypical protein kinase C (aPKC) (5). Rac is thought to mediate E-Cad-induced activation of aPKC at sites of tight junction assembly (5), thus enabling proper assembly and maintenance of tight junctions. However, the potential role of these molecules in the context of HCV-induced modulation of tight junction integrity still needs to be investigated.

Following HCV infection, E-Cad is downregulated by HCV core protein-mediated hypermethylation and repression of E-Cad gene expression (6, 7). For the virus, E-Cad downregulation is likely beneficial to prevent superinfection of cells (8) and facilitate dissemination of infection. However, aberrant expression of E-Cad is a major contributor to epithelial to mesenchymal transition (EMT) (9), a hallmark of both fibrosis and cancer metastasis. Indeed, Li and colleagues demonstrate that the loss of epithelial marker E-Cad following HCV infection corresponds with increased

expression of major mesenchymal markers such as vimentin (VIM), fibronectin (FN1) and N-cadherin (3). Therefore, HCV-induced downregulation of its entry factor E-Cad potentially contributes to virus-induced liver disease and progression of HCC by inducing EMT. Although HCV core protein had been previously shown to induce the repression of E-Cad gene expression (7), Li and colleagues show here that activation of TGF-β following HCV infection also contributes to the loss of E-Cad and induction of EMT (3). TGF-β has been identified as a key regulator of EMT, particularly in the context of HCC (10).

Regulation of E-Cad expression and EMT induction is complex, and additional mechanisms are likely to be involved. For example, it was recently shown that HCV core protein interacted with the Snail transcription factor to enhance its binding to the E-Cad promoter, resulting in decreased E-Cad gene expression (6). Interestingly, Snail downregulated the expression of other tight junction components (namely CLDN1 and OCLN) independently of its effect on E-Cad expression (11). HCV core protein-mediated downregulation of CLDN1 and OCLN has previously been observed following HCV infection (12) and was proposed as a mechanism to prevent superinfection. Given that E-Cad, CLDN1 and OCLN are downregulated following HCV infection, whereas other HCV entry factors such as CD81 and SR-BI were not (8, 12), it may be that superinfection exclusion is at least partially regulated at the level of tight junctions. Overall, these findings underscore the key role of these cellular compartments for HCV infection.

What are clinical implications of these findings for therapeutic approaches and disease biology? Junctional proteins have emerged as key viral cell entry factors and therapeutic targets. Recently, a monoclonal antibody targeting the tight junction protein CLDN1 was able to prevent HCV infection and cure chronic HCV in

monotherapy in human liver chimeric mice, without apparent adverse effects (13). It would be most interesting, therefore, to determine if compounds directed against E-Cad show similar activities *in vivo*. If E-Cad indeed regulates the composition of tight junctions, a similar if not more potent ability to counteract HCV infection would be expected.

The interplay between E-Cad, tight junctions and hepatocellular carcinoma is critical for the understanding of the pathogenesis virus-induced cancer and may provide novel therapeutic perspectives. Interestingly, reduced CLDN1 expression, which was shown by Li *et al*. to occur following E-Cad downregulation (3), has been correlated with malignancy of hepatocellular carcinoma (14). Accumulating evidence indicates that E-Cad downregulation is a critical event leading to EMT, an important mechanism for metastasis of HCC (10). Furthermore, mislocalization of the tight junction protein CLDN1 was associated with colon carcinoma and metastasis (15), suggesting a role role for tight junctions in regulating metastasis.

The study by Li *et al.* opens perspectives to investigate the potential physiological effects of virus-driven E-Cad downregulation. Given the observed alterations in the proper localization of tight junction proteins (3), impaired tight junction function would be expected in the event of E-Cad downregulation. For example, is tight junction permeability altered following repression of E-Cad gene expression? Moreover, within the tissue, do HCV-infected cells exhibit more motility due to the absence of proper tight junction formation? Furthermore, it will be interesting to study the role of E-Cad downregulation and EMT in the context of disease pathogenesis in hepatocytes prior to induction of HCC. Although EMT plays a key role in HCC progression and metastasis (10), it is not thought to be directly involved in the induction of HCC. However, the aberrant expression of E-Cad may

contribute to hepatocarcinogenesis and HCC progression by alternative mechanisms. It is also conceivable that HCV infection of tumor cells, albeit at limited levels (16), could induce or promote EMT and potentially increase metastasis or invasion of HCC within the liver. In this context, it is of interest to note that the authors show that SB-431542, a small-molecule inhibitor that specifically targets TGF-β, partially reversed the HCV-triggered downregulation of E-Cad and thereby abrogated subsequent induction of EMT (3). These findings warrant further study of E-Cad-HCV interactions as a therapeutic target for virus-induced HCC.

Finally, the mechanisms described by Li *et al.* may also be relevant for the pathogenesis of other viral infections, particularly those associated with carcinogenesis. Many viruses use tight junction proteins to gain entry into the cell, whereas others alter tight junction morphology to facilitate their dissemination (17). For example, West Nile virus and dengue virus promote the degradation of tight junction proteins and alter the integrity of tight junctions, respectively (17). Other viruses directly target E-Cad expression. The hepatitis B virus X protein, much like HCV core protein, represses E-Cad expression to induce EMT (18), which may similarly promote to the development of HCC. Repression of E-Cad expression has also been linked to Epstein-Barr virus-associated gastric carcinoma (19) and has been observed in human papillomavirus infection (20). Thus, E-Cad downregulation appears to have broad implications for the pathogenesis of disease biology in the infection of several human cancer-associated viruses.

Figure 1. The proposed role of E-Cad in HCV entry and EMT. **(A)** An updated model of HCV entry. E-Cad, by regulating the localization of tight junction proteins CLDN1 and OCLN, facilitates HCV entry at a post-binding step. **(B)** E-Cad expression is

downregulated during HCV infection, which alters the subcellular localization and expression of CLDN1 and OCLN. HCV infection activates TGF-β, which contributes to the induction of EMT and an increase in EMT marker expression, including VIM, FN1 and N-Cad. GAGs, glycosaminoglycans; LDLR/VLDLR, low-density lipoprotein receptor/very low density lipoprotein receptor; SR-BI, scavenger receptor class B type I; TfR, transferrin receptor; RTKs, receptor tyrosine kinases; E-Cad, E-cadherin; HRas, Harvey rat sarcoma viral oncogene homolog; CD81, cluster of differentiation 81; CLDN1, claudin-1; OCLN, occludin; NPC1L1, Niemann-Pick C1-like protein 1; SRFBP1, serum response factor binding protein 1; TGF-β; transforming growth factor β; VIM, vimentin; FN1, fibronectin; N-Cad; N-cadherin.

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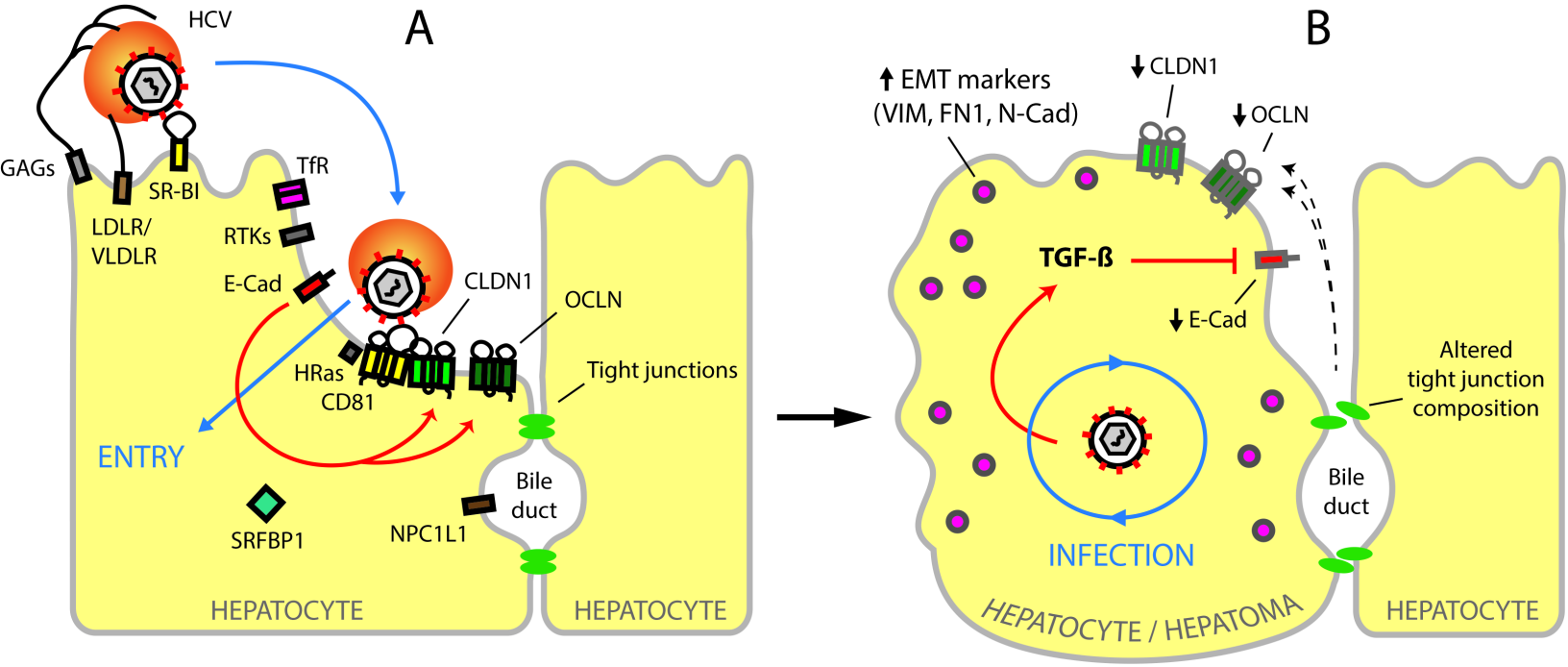
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CLDN1
OCLN
TGF-ß
E-Cad
Altered
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