

Cholesterol uptake and hepatitis C virus entry

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COMMENTARY ON:

Identification of the Niemann–Pick C1-like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor. Sainz B Jr, Barretto N, Martin DN, Hiraga N, Imamura M, Hussain S, Marsh KA, Yu X, Chayama K, Alrefai WA, Uprichard SL. *Nat Med* 2012 January 8;18(2):281–285. doi: 10.1038/nm.2581. Copyright (2012). Abstract reprinted by permission of McMillan Publisher Ltd.

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Abstract: Hepatitis C virus (HCV) is a leading cause of liver disease worldwide. With ~170 million individuals infected and current interferon-based treatment having toxic side effects and marginal efficacy, more effective antivirals are crucially needed. Although HCV protease inhibitors were just approved by the US Food and Drug Administration (FDA), optimal HCV therapy, analogous to HIV therapy, will probably require a combination of antivirals targeting multiple aspects of the viral lifecycle. Viral entry represents a potential multifaceted target for antiviral intervention; however, to date, FDA-approved inhibitors of HCV cell entry are unavailable. Here we show that the cellular Niemann–Pick C1-like 1 (NPC1L1) cholesterol uptake receptor is an HCV entry factor amenable to therapeutic intervention. Specifically, NPC1L1 expression is necessary for HCV infection, as silencing or antibody-mediated blocking of NPC1L1 impairs cell culture-derived HCV (HCVcc) infection initiation. In addition, the clinically available FDA-approved NPC1L1 antagonist ezetimibe potently blocks HCV uptake in vitro via a virion cholesterol-dependent step before virion–cell membrane fusion. Moreover, ezetimibe inhibits infection by all major HCV genotypes in vitro and in vivo delays the establishment of HCV genotype 1b infection in mice with human liver grafts. Thus,

we have not only identified NPC1L1 as an HCV cell entry factor but also discovered a new antiviral target and potential therapeutic agent.

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Although clinical licensing of protease inhibitors in combination with pegylated interferon-alpha and ribavirin has markedly improved the treatment outcome, viral resistance and adverse effects still remain important challenges. HCV entry is the first step of virus–hepatocyte interaction and is not only required to initiate infection, but is also necessary for spread and maintenance of infection. Host entry factors are attractive complementary antiviral targets since they may increase the genetic barrier to viral resistance. HCV particles in patient sera circulate in complexes with host lipoproteins as lipoviral particles, which are enriched in triglycerides, cholesterol, and several apolipoproteins [1,2]. HCV uptake into hepatocytes requires a well characterized set of cellular binding and entry factors including glycosaminoglycans (GAG), scavenger receptor BI (SR-BI), CD81, claudin-1 (CLDN1), occludin (OCLN), and receptor tyrosine kinases (RTKs) (Fig. 1) (reviewed in [3]). CD81-mediated HCV entry is furthermore dependent on membrane cholesterol content and fusion of HCV pseudoparticles (HCVpp) with liposomes is enhanced by cholesterol enrichment of the target membrane [4,5]. Also, production of HCV particles is dependent on cholesterol metabolism using the very-low-density lipoprotein (VLDL) assembly factors microsomal transfer protein, apolipoprotein B, and apolipoprotein E [6]. Together, these data indicate that the HCV life cycle is intrinsically tied to hepatocyte cholesterol metabolism.

In this context, a recent letter in *Nature Medicine* published by Susan Uprichard's laboratory at the University of Illinois–Chicago provides additional evidence for this entanglement [7]. In this study, the authors identified the Niemann–Pick C1-like 1 (NPC1L1) cholesterol absorption receptor as a novel HCV entry factor and a potential target for therapeutic intervention. The authors demonstrated that NPC1L1 expression is necessary for cell culture-derived HCV (HCVcc) cell entry, using both RNAi to knockdown NPC1L1 and a blocking antibody which binds to the cholesterol transporting large extracellular loop 1 of NPC1L1. Using the clinically licensed NPC1L1 antagonist, ezetimibe, the authors inhibited

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Abbreviations: HCV, hepatitis C virus; FDA, US Food and Drug Administration; NPC1L1, Niemann–Pick C1-like 1; HCVcc, cell culture-derived HCV; HCVpp, HCV pseudoparticles; GAG, glycosaminoglycans; SR-BI, scavenger receptor BI; CLDN1, claudin-1; OCLN, occludin; RTKs, receptor tyrosine kinases; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor.



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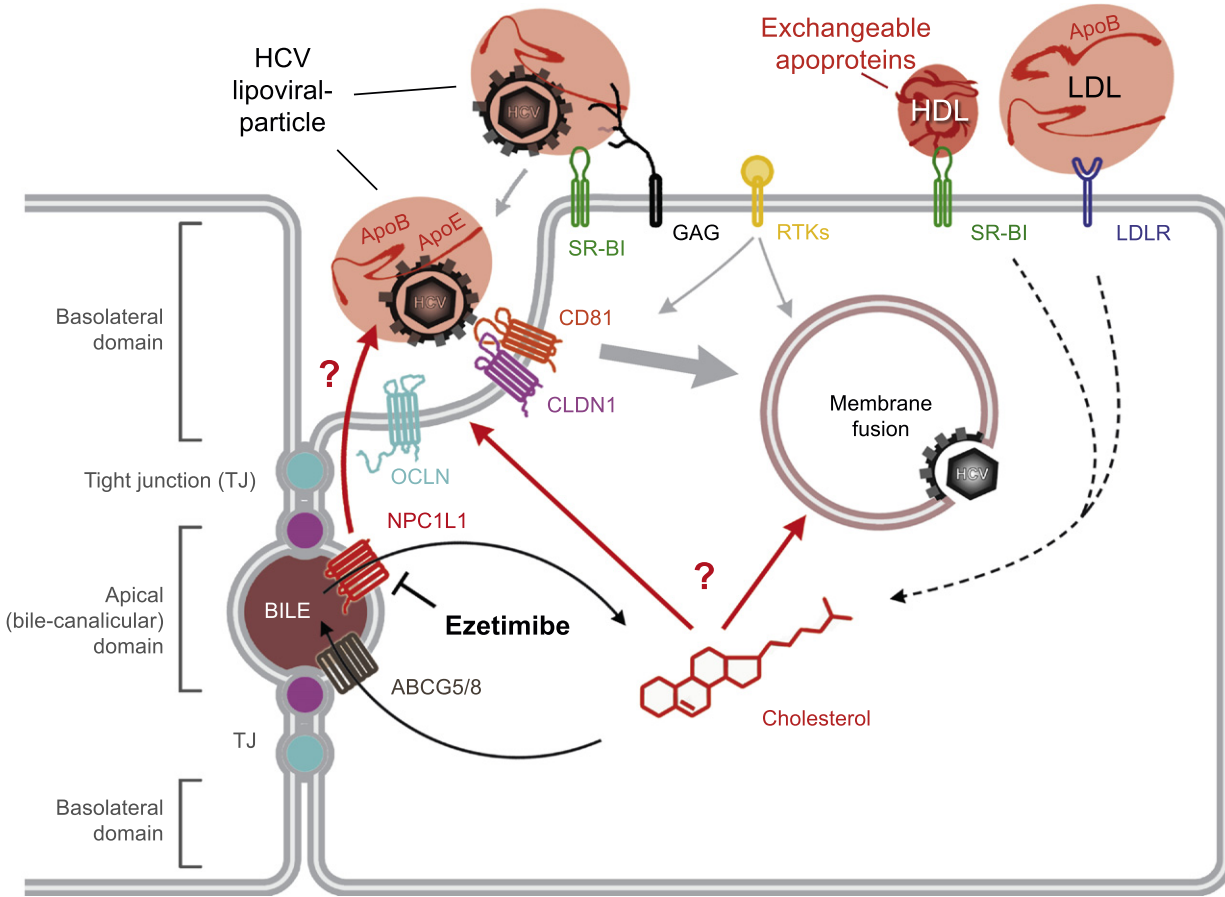


Fig. 1. Potential functional role of Niemann-Pick C1-like 1 (NPC1L1) cholesterol absorption receptor within the HCV entry based on the findings of Sainz et al. [7]. The majority of NPC1L1 is localized on the apical canalicular surface of polarized hepatocytes reabsorbing free cholesterol excreted into bile by ABCG5/8 transporters. Cholesterol levels are also maintained by high-density lipoprotein (HDL) and low-density lipoprotein (LDL) metabolism via scavenger receptor BI (SR-BI) and LDL receptor (LDLR). As a bloodborne pathogen, initial HCV-hepatocyte binding to glycosaminoglycans (GAG) is presumed to be localized at the basolateral domain of polarized hepatocytes with entry being mediated by SR-BI, CD81, claudin-1 (CLDN1), occludin (OCLN), and receptor tyrosine kinases (RTKs) that promote CD81-CLDN1 association and membrane fusion. NPC1L1 could promote HCV entry either directly by interaction with the cholesterol of lipoviral particles or indirectly by modulating cholesterol homeostasis and thus membrane composition required for HCV entry and membrane fusion. Ezetimibe is an inhibitor of NPC1L1 function.

HCV infection of all major HCV genotypes *in vitro*. Comparing the effects of ezetimibe on HCV particles with varying cholesterol content, the study provides evidence for a cholesterol-dependent function of NPC1L1 on HCV entry, probably in a post-binding step. Furthermore, the authors showed efficacy of ezetimibe in an HCV animal model, demonstrating a functional role of therapeutic targeting of NPC1L1 for HCV entry.

The results of this study are relevant for our understanding of the molecular mechanism of HCV entry in the context of cholesterol homeostasis. Based on an experiment comparing the susceptibility of HCVpp and HCVcc entry to ezetimibe, the authors demonstrated that the cholesterol content of viral particles correlate with NPC1L1-mediated infection. Since ezetimibe treatment inhibits entry of HCVcc (high cholesterol content) but not HCVpp (low cholesterol content) the results suggest direct interaction of NPC1L1 with the cholesterol of the viral envelope. But the implications of this study on the molecular mechanisms of cholesterol-dependent HCV entry must also be discussed in context of cell polarity, since Huh7 cells do not fully polarize in cell culture. *In vivo*, the majority of NPC1L1 is localized at the canalicular surface of hepatocytes and reabsorbs free cholesterol excreted into bile by ABCG5/8 transporters (Fig. 1) [8]. This localization is sep-

arated by tight junction complexes from the putative site of HCV-cell interaction on the basolateral surface of hepatocytes [3], suggesting that in polarized cells, NPC1L1 may act indirectly by modulating cholesterol levels. Since biliary secretion is the only route of cholesterol catabolism and export, NPC1L1 plays a critical role in whole body cholesterol homeostasis [8]. Ezetimibe inhibits NPC1L1 activity, diminishing absorption of dietary and biliary cholesterol thereby effectively lowering serum cholesterol levels. The use of cholesterol diminishing statin drugs prior to pegylated interferon treatment appears to be associated with a higher sustained virological response [9]. However, the interplay between cholesterol metabolism and HCV infection is complex since low cholesterol levels are associated with non-response to pegylated interferon treatment, and atorvastatin treatment alone does not alter HCV levels [9]. Thus, it is conceivable that the cholesterol lowering effect of ezetimibe in uPA/SCID mice grafted with human hepatocytes may modulate HCV infection.

Beyond the mechanistic data, this paper offers an interesting clinical impact since ezetimibe has been approved as adjunct to dietary measures during treatment of hypercholesterolaemia. In this study, the authors show data supporting a role of NPC1L1 as an antiviral target since pretreatment with ezetimibe delayed

the establishment of HCV infection in mice and resulted in partial protection against HCV infection. Nevertheless, the use of ezetimibe may have certain limitations since the antiviral dose *in vitro* is ~100-fold above the maximal plasma concentration reached in patients. Thus, it should be carefully evaluated whether increasing ezetimibe plasma concentrations also increases liver toxicity as has been reported in some cases [10].

Taken together, the key finding of this study is the discovery that cholesterol metabolism plays an important role for the HCV entry process and that targeting NPC1L1 and cholesterol homeostasis adds a new perspective for novel antiviral strategies targeting HCV infection.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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