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International Hepatology

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5 **Cholesterol uptake and hepatitis C virus entry**
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44 **Abbreviations**
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46 HCV: Hepatitis C virus; FDA: US Food and Drug Administration; NPC1L1: Niemann-Pick
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48 C1-like 1; HCVcc: cell culture-derived HCV; HCVpp: HCV pseudoparticles; GAG:
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50 glycosaminoglycans; SR-BI: scavenger receptor BI; CLDN1: claudin-1; OCLN: occludin;
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52 RTKs: receptor tyrosine kinases; VLDL: very-low-density lipoprotein; HDL: high-density
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54 lipoprotein; LDL: low-density lipoprotein; LDLR: low-density lipoprotein receptor.
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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” by Bruno Sainz Jr, Naina Barretto, Danyelle N. Martin, Nobuhiko Hiraga, Michio Imamura, Snawar Hussain, Katherine A. Marsh, Xuemei Yu, Kazuaki Chayama, Waddah A. Alrefai & Susan L. Uprichard, published in Nature Medicine 2012, epub Jan 08. Abstract reprinted with permission of Nature Medicine.

<http://www.ncbi.nlm.nih.gov/pubmed/22231557>

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 amendable 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.

COMMENTARY

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2 Although clinical licensing of protease inhibitors in combination with pegylated interferon-
3 alpha and ribavirin has markedly improved the treatment outcome, viral resistance and
4 adverse effects still remain important challenges. HCV entry is the first step of virus-
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6 hepatocyte interaction and is not only required to initiate infection, but is also necessary for
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8 spread and maintenance of infection. Host entry factors are attractive complementary antiviral
9 targets since they may increase the genetic barrier to viral resistance. HCV particles in patient
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11 sera circulate in complexes with host lipoproteins as lipoviral particles, which are enriched
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13 with triglycerides, cholesterol, and several apolipoproteins [1, 2]. HCV uptake into
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15 hepatocytes requires a well characterized set of cellular binding and entry factors including
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17 glycosaminoglycans (GAG), scavenger receptor BI (SR-BI), CD81, claudin-1 (CLDN1),
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19 occludin (OCLN), and receptor tyrosine kinases (RTKs) (Fig. 1) (reviewed in [3]). CD81-
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21 mediated HCV entry is furthermore dependent on membrane cholesterol content and fusion of
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23 HCV pseudoparticles (HCVpp) with liposomes is enhanced by cholesterol enrichment of the
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25 target membrane [4, 5]. Also, production of HCV particles is dependent on cholesterol
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27 metabolism using the very-low-density lipoprotein (VLDL) assembly factors microsomal
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29 transfer protein, apolipoprotein B, and apolipoprotein E [6]. Together, these data indicate that
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31 the HCV life cycle is intrinsically tied to hepatocyte cholesterol metabolism.
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46 In this context, a recent letter in Nature Medicine published by Susan Uprichard's
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48 laboratory at the University of Illinois-Chicago provides additional evidence for this
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50 entanglement [7]. In this study, the authors identified the Niemann-Pick C1-like 1 (NPC1L1)
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52 cholesterol absorption receptor as a novel HCV entry factor and a potential target for
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54 therapeutic intervention. The authors demonstrated that NPC1L1 expression is necessary for
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56 cell culture-derived HCV (HCVcc) cell entry, using both RNAi to knockdown NPC1L1 and a
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58 blocking antibody which binds to the cholesterol transporting large extracellular loop 1 of
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1 NPC1L1. Using the clinically licensed NPC1L1 antagonist, ezetimibe, the authors inhibited
2 HCV infection of all major HCV genotypes *in vitro*. Comparing the effects of ezetimibe on
3 HCV particles with varying cholesterol content, the study provides evidence for a cholesterol-
4 dependent function of NPC1L1 on HCV entry, probably in a postbinding step. Furthermore,
5 the authors showed efficacy of ezetimibe in an HCV animal model, demonstrating a
6 functional role of therapeutic targeting of NPC1L1 for HCV entry.
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17 The results of this study are relevant for our understanding of the molecular
18 mechanism of HCV entry in the context of cholesterol homeostasis. Based on an experiment
19 comparing the susceptibility of HCVpp and HCVcc entry to ezetimibe, the authors
20 demonstrated that the cholesterol content of viral particles correlate with NPC1L1-mediated
21 infection. Since ezetimibe treatment inhibits entry of HCVcc (high cholesterol content) but
22 not HCVpp (low cholesterol content) the results suggest direct interaction of NPC1L1 with
23 the cholesterol of the viral envelope. But the implications of this study on the molecular
24 mechanisms of cholesterol-dependent HCV entry must also be discussed in context of cell
25 polarity, since Huh7 cells do not fully polarize in cell culture. *In vivo*, the majority of
26 NPC1L1 is localized on the canalicular surface of hepatocytes and reabsorbs free cholesterol
27 excreted into bile by ABCG5/8 transporters (Fig. 1) [8]. This localization is separated by tight
28 junction complexes from the putative site of HCV-cell interaction on the basolateral surface
29 of hepatocytes [3], suggesting that in polarized cells, NPC1L1 may act indirectly by
30 modulating cholesterol levels. Since biliary secretion is the only route of cholesterol
31 catabolism and export, NPC1L1 plays a critical role in whole body cholesterol homeostasis
32 [8]. Ezetimibe inhibits NPC1L1 activity, diminishing absorption of dietary and biliary
33 cholesterol thereby effectively lowering serum cholesterol levels. The use of cholesterol
34 diminishing statin drugs prior to pegylated interferon treatment appears to be associated with
35 a higher sustained virological response [9]. However, the interplay between cholesterol
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1 metabolism and HCV infection is complex since low cholesterol levels are associated with
2 non-response to pegylated interferon treatment, and atorvastatin treatment alone does not alter
3 HCV levels [9]. Thus, it is conceivable that the cholesterol lowering effect of ezetimibe in
4 uPA/SCID mice grafted with human hepatocytes may modulate HCV infection.
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11 Beyond the mechanistic data, this paper offers an interesting clinical impact since
12 ezetimibe has been approved as adjunct to dietary measures during treatment of
13 hypercholesterolaemia. In this study, the authors show data supporting a role of NPC1L1 as
14 an antiviral target since pretreatment with ezetimibe delayed the establishment of HCV
15 infection in mice and resulted in partial protection against HCV infection. Nevertheless, the
16 use of ezetimibe may have certain limitations since the antiviral dose *in vitro* is ~100 fold
17 above the maximal plasma concentration reached in patients. Thus, it should be carefully
18 evaluated whether increasing ezetimibe plasma concentrations also increases liver toxicity as
19 has been reported in some cases [10].
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36 Taken together, the key finding of this study is the discovery that cholesterol
37 metabolism plays an important role for the HCV entry process and that targeting NPC1L1 and
38 cholesterol homeostasis adds a new perspective for novel antiviral strategies targeting HCV
39 infection.
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48 **References**

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Figure legend

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 and colleagues [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 to 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.

FIGURE 1

