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Hepatitis C virus – host interactions, replication, and viral assembly

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Abstract

As a relatively simple virus, hepatitis C virus (HCV) depends extensively on its host to infect, replicate and disseminate. HCV has evolved host interactions that result in a restricted tropism, both in terms of cell type and species. Efforts into identifying and validating HCV-host interactions have been hampered by a limited number of infectious virus clones and cell lines that support HCV infection. Despite these limitations, consensus HCV-host interactions have emerged that help define the entry, replication, assembly, and tropism of HCV. This has had important implications in expanding our *in vitro* and *in vivo* systems to study HCV replication and pathogenesis. Additionally, a number of these host factors are being targeted for therapeutic development. In this review, we focus on medically relevant pro-viral host factors, their role in HCV biology, and their importance in expanding our model systems.

Introduction

HCV is a major cause of chronic hepatitis leading to liver cirrhosis and hepatocellular carcinoma [1]. An estimated 130 million people worldwide are persistently infected with HCV [2]. The available therapy, a combination of pegylated interferon-α (pegIFN-α) and ribavarin, is efficacious in only 50% of the infected individuals and associated with side effects [3,4]. Direct acting antivirals (DAAs), such as boceprevir and telaprevir, which inhibit the viral NS3/4A protease, will cure ~70% of patients when combined with pegIFN-α/ribavirin, although they are associated with side effects [5,6]. In addition to DAAs, a number of drugs targeting HCV host factors are in development. These may have utility in reducing resistance in combination with DAAs.

Expanding technologies, such as RNA interference (RNAi) screens, have identified literally hundreds of putative HCV cofactors that expand beyond the possible focus of this review. Additionally, HCV infection of Huh7 cell derivatives produces numerous cellular changes in signaling and metabolism that we unfortunately omit due to space considerations. We will instead highlight consensus host cofactors and their roles in expanding HCV model systems and therapeutic options.

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HCV Entry

HCV entry into hepatocytes is a complex process that engages several cellular proteins such as the low density lipoprotein receptor (LDLR) [7,8], the tetraspanin CD81[9–11], the scavenger receptor class B type I (SR-BI) [12,13] and the tight junction (TJ) proteins, claudin-1 (CLDN1) [14] and occludin (OCLN) [15,16]. The *in vitro* data so far suggest that these host factors are used in a sequential manner, with virus particles attaching to hepatic cells via a lower affinity LDLR interaction and a higher affinity binding to SR-BI. Soluble E2 proteins, but not HCV particles, bind CD81 [9,10], suggesting that HCV-SRBI interactions alter HCV virion conformation [17,18], thus enabling E2-CD81 binding and subsequent priming of E2 for pH-dependent fusion [19*]. Following engagement of CD81, signaling events are necessary for recruitment of CLDN1. The receptor tyrosine kinases, epidermal growth factor receptor (EGFR) and ephrin receptor A2 (EphA2) modulate CD81-CLDN1 association, likely by influencing tyrosine kinase signaling [20**]. Following CD81-CLDN1 binding, the HCV-receptor complex is proposed to interact with OCLN and internalize at cellular TJs. HCV internalizes via clathrin-mediated endocytosis and uncoats from acidified endosomes [21,22]. This has led to a model, with similarities to group B coxsackieviruses [23], of initial binding of HCV to SR-BI/CD81 co-receptors followed by signaling and migration to TJs where TJ co-receptors mediate late stages of virion internalization. Numerous steps of this model remain untested for HCV.

In addition to direct virus interactions, cellular receptors that modulate lipid/cholesterol uptake influence HCV entry. Mutations in SR-BI that influence cholesterol uptake, but not E2 binding inhibit HCV entry [24] as does inhibition of the cholesterol uptake receptor Niemann Pick C1 like 1 (NPC1L1) [25*]. Inhibitors of EGFR (erlotinib), EphA2 (dasatinib) and NPC1L1 (ezetimibe) [26] are already licensed molecules and shown to inhibit HCV entry in vitro [20,25]. Additionally, there is a small molecule inhibitor of SR-BI (ITX 5061) which is the most advanced HCV entry inhibitor in clinical trials [27].

HCV Replication

As do all other positive-strand RNA viruses, HCV replicates its genome in association with virally-induced membrane structures. RNAi screens have uncovered numerous host cofactors of HCV replication, with the most consistently identified being phosphatidylinositol 4-kinase III α (PI4K-IIIα) [28–35**]. This enzyme phosphorylates phosphatidylinositol (PI) in the 4 position of the inositol ring to generate PI4P. During HCV infection, PI4K-IIIα interacts with the viral NS5A protein resulting in increased local levels of PI4P. PI4K-IIIα kinase activity is required for the fidelity of membranous web formation, as silencing PI4K-IIIα results in an aggregation of double membrane vesicles and HCV replication complexes [35**,36*,37]. The exact function of PI4K-IIIα, PI4P, or further modifications of PI4P in HCV replication are unclear. AL-9, a member of the 4-anilino quinazoline-containing kinase inhibitor family, was recently shown to inhibit HCV replication in vitro by direct inhibition of PI4K-IIIα [38*]. Additionally, resistance mutations to a class of NS5A DAAs map to NS5A domain I, which also interacts with PI4K-IIIα [34**], suggesting that these drugs may impact NS5A activation of PI4K-IIIα [39].

The liver-specific micro-RNA 122 (miR-122), which regulates cholesterol biosynthesis, binds to two closely spaced sites on the 5’ noncoding region of the HCV genome and facilitates viral replication [40–42]. miR-122 enhances HCV translational initiation [43,44], however, this is not sufficient to explain the full effect of miR-122 on HCV replication [45]. Recent data suggest that miR-122 binding to the HCV genome has a protective and stabilizing role. Indeed, miR-122 binds HCV RNA with 3’ overhanging nucleotides that

seem to mask the 5' terminal sequences from nucleolytic degradation or innate immunity cytoplasmic sensors of viral RNA [46*]. miR-122 binds HCV RNA in association with Argonaute 2 (Ago2) protein resulting in slower decay of the viral genome and protection from the cellular exonuclease decay machinery [47*]. In support of the stability hypothesis, HCV deleted in part of the miR-122 binding sites can be partially rescued by recombination with stable viral or cellular RNA structures [48*]. Miravirsen (SPC3649/Santaris Pharma) is a locked nucleic –acid modified oligonucleotide complimentary to miR-122, currently in Phase II clinical trial. When administered in chronically infected chimpanzees, this inhibitor resulted in long-lasting suppression of HCV viremia with no evidence of viral resistance and minimal side effects [49**]. Additionally, miravirsen is capable of potently antagonizing multiple HCV genotypes in vitro [48*].

Cyclophilin A (CypA) is a cellular chaperone with peptidyl-prolyl cis-trans isomerase (PPiase) activity that functions in protein folding and trafficking and plays an essential role in HCV replication and particle production [50–53]. CypA may play a role in the correct folding of several viral proteins, given that its PPiase activity is required for HCV replication *in vitro* [53,54]. Initially, CypA was found to associate with the viral NS5B replicase and modulate its RNA binding and/or synthesis capability [55–57]. More recently, CypA has been shown to bind specific motifs in domain II and III of the viral NS5A protein catalyzing cis/trans isomerization and thus stimulating the RNA binding activity and dimerization of NS5A [58, 59*–62]. Additionally, CypA may affect HCV polypeptide processing at the NS5A-B junction [52]. The original CypA inhibitor was cyclosporine A (CsA), an immunosuppressive drug used routinely in organ transplantation [63]. Chemical modification of CsA resulted in non-immunosuppressive analogs of the inhibitor that potently suppress HCV replication in cell culture and have clinical efficacy in HCV patients [64–66*]. The most promising of the cyclophilin inhibitors is alisporivir (Debio-025/ Novartis) which is currently in Phase III clinical trials. Debio-025 given in combination with pegIFN-α/ribavirin has superior efficacy compared to standard of care treatment [52].

HCV Assembly and Release

The molecular events that take place during assembly of infectious HCV particles and the interplay between viral and host factors, are just being uncovered. It is hypothesized that HCV assembly is initiated in close proximity to intracellular lipid droplet (LD) structures on the surface of which the viral core (capsid) protein accumulates [67,68]. In addition to viral factors, several host factors likely participate in HCV particle assembly and envelopment, including the clathrin adaptor AP2M1 [69] and group IVA phospholipase A2 (PLA2G4A) [70*]. Components of the very-low-density lipoprotein (VLDL) synthesis and secretion pathway such as microsomal triglyceride transfer protein [71], apolipoprotein B (apoB) [72] and apolipoprotein E (apoE) have been implicated in HCV assembly [73–75]. Of these, substantial evidence supports a role for apoE as an HCV infectivity factor. HCV infectious virions purified either from cell culture supernatants [73] or infected patients can be specifically immuno-precipitated by anti-apoE monoclonal antibodies [76]. Furthermore, apoE was found to be part of affinity purified infectiousHCV particles [77*]. Live cell imaging of single HCV particles indicates that mature infectious particles containing apoE are transported along the secretory pathway [78**]. It has been suggested that apoE may promote HCV infectivity during entry by virtue of its interaction with LDLR [8] or heparan sulfate [79].

Another host factor implicated in HCV assembly is diacylglycerol acyltransferase-1 (DGAT1) [80*]. DGAT1 induces LD formation in cells and its interaction with viral core protein was identified to be essential in proper localization of core around LDs. Of note,

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DGAT1 inhibitors currently in clinical trials for obesity-related diseases could be potential candidates against HCV [81].

HCV tropism

HCV has a restricted tropism, both in terms of species and cell type. Only humans and chimpanzees are naturally infected, with the primary target cell being the hepatocyte. Extra-hepatic target sites, such as B lymphocytes, neuro-epithelioma cells and endothelial cells of the blood-brain barrier, can support virus entry but not a productive infection by cell culture derived HCV [82–84]. While host restriction factors could influence HCV tropism *in vivo*, the experimental data point to a primary contribution by pro-viral host factors [85]. The approach of screening for cDNAs that enable HCV pseudoparticle infection of non-permissive cells identified the HCV entry factors CLDN1 and OCDN [14,15]. OCDN in conjunction with CD81 define the species barrier to entry [15]. Expression of human OCDN and CD81 in mice, either by adenoviral delivery or transgenic approaches allows infection of mice, albeit without subsequent replication [86**]. This validates the significance of the entry factors in HCV infection, in addition to being an important step in developing a small animal model for HCV.

The main host factors for HCV replication appear to be conserved in multiple cell types and species. HCV JFH1 replicons can replicate in non-hepatic cells, including HeLa and 293T cells, albeit at lower levels than the hepatic Huh-7 cells [87,88]. One host factor that can enhance the tropism of HCV is miR-122. Ectopic expression of miR-122 in HepG2, Hec1b, 293T-CLDN, Hep3B and mouse fibroblasts enhances HCV replication [89–93]. Another tropism enhancing host cofactor is apoE. Over-expression of either mouse or human apoE isoforms increases the production of infectious HCV in mouse cells [92,94] and may be used as a tool to expand the cell types that support a complete infectious HCV cycle, in addition to improving the current small animal models. Notably, expression of the four entry receptor molecules in combination with miR-122 and apoE is sufficient to reconstitute the entire HCV life cycle in 293T cells [95*].

New *in vitro* models to validate and identify HCV-host interactions

A major limitation of studying HCV-host interactions is the limited number of HCV strains and cell types used to characterize them. There are seven known HCV phylogenetic groups (genotypes) with a nucleotide sequence diversity of around 30–35% [96], however viral infectious clones are available only for genotypes 1a, 1b, 2a, 3a and 4a [97–99]. Of these, only genotype 2a JFH1 and adapted genotypes 2a J6, 2b J8, and 1a H77S complete the viral life cycle in cell culture. An advance is the generation of chimeric HCV containing the JFH1 nonstructural genes fused to structural genes from all 7 HCV genotypes [100]. This will enable cross-genotypic comparisons of virus-host interactions with the HCV core, E1, E2, p7 and NS2; for example, infection with all 7 HCV genotype chimeras can be neutralized with antibodies to CD81 and SR-BI [100].

The *in vitro* studies validating host cell factors important for HCV replication were carried out predominantly in a single human hepatoma cell line, Huh-7 and other sublines termed Huh-7.5 [101], Huh-7.5.1 [102] and Huh-7 Lunet cells [103]. Human hepatocytes possess a unique complex polarity that is finely keened for uptake of nutrients at the basolateral surface (sinusoidal pole) and secretion of bile at the apical surface (bile canaliculus). The role of TJs in HCV entry is not clear yet, since these cell structures do not seem to be accessible to virions *in vivo* and entry into non-polarized Huh-7.5 cells does not preferentially occur at inter-cellular junctions, likely because these cells do not form TJs under normal culturing conditions [21]. Some progress has been made in developing polarized cell models for HCV entry, such as the Caco-2 colorectal adenocarcinoma cells

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that develop columnar polarity and the HepG2 hepatoma cells that develop complex hepatic polarity [104,105]. HepG2-CD81 cells expressing miR-122 have particular promise [89]. It is also likely that culturing conditions of existing HCV cell culture models can impact polarity. Recently, it shown that Huh-7 hepatocytes embedded in matrigel 3-dimensional cultures do polarize and support the entire HCV life cycle thus creating a system to validate host cell factors with respect to cell polarity [106].

Primary hepatocytes from patients can be productively infected with HCV; however, there is high variability between patients and access to fresh hepatocytes is limited [107,108]. An alternative approach is the development of induced pluripotent stem (iPS) cells. Multiple groups have successfully developed iPS cell culture systems that can be differentiated into human hepatocyte-like cells and infected with HCV [109*–111*]. Interestingly, the stage of cell differentiation susceptible to HCV infection correlates with expression of miR-122 and PI4K-IIIα [111]. Thus far, these cultures have (at least) two intriguing properties: (i) they produce an inflammatory response to infections and (ii) they can be infected with HCV1a and 1b patient isolates, suggesting that they may support the replication of a broad range of HCV genotypes [109*,111*].

Conclusions

Despite significant experimental hurdles, numerous *bona fide* HCV-host interactions have been defined. The newly developed model systems can begin to address questions that remain for these host factors. For instance, polarized hepatocytes are crucial to understanding HCV entry and trafficking. These model systems will also be important in defining the significance and function of the hundreds of less characterized putative host cofactors of HCV infection. Are there requirements conserved for multiple HCV genotypes in multiple cell lines? Are the HCV-induced changes in Huh7 cell physiology conserved in other hepatocyte models? How does the inflammatory signaling in response to HCV infection of iPS cells alter HCV replication and spread? Can driving over-expression of apoE in current HCV mouse models produce a robust small animal model? Finally, what will be the impact of therapeutics targeting host factors? Given the number of DAAs in advanced clinical trials, host targets are unlikely to be a primary therapeutic in the short term. However, they may emerge as useful tools in an arsenal to treat difficult populations. In any case, HCV should prove to be an important test model for targeting the host in emerging viral infections.

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Highlights

- ➢ Consensus HCV-host interactions have been identified at all stages of the viral life cycle.
- ➢ Many of these interactions are targets of drug development.
- ➢ These interactions define HCV tropism and are being used to expand our model systems.

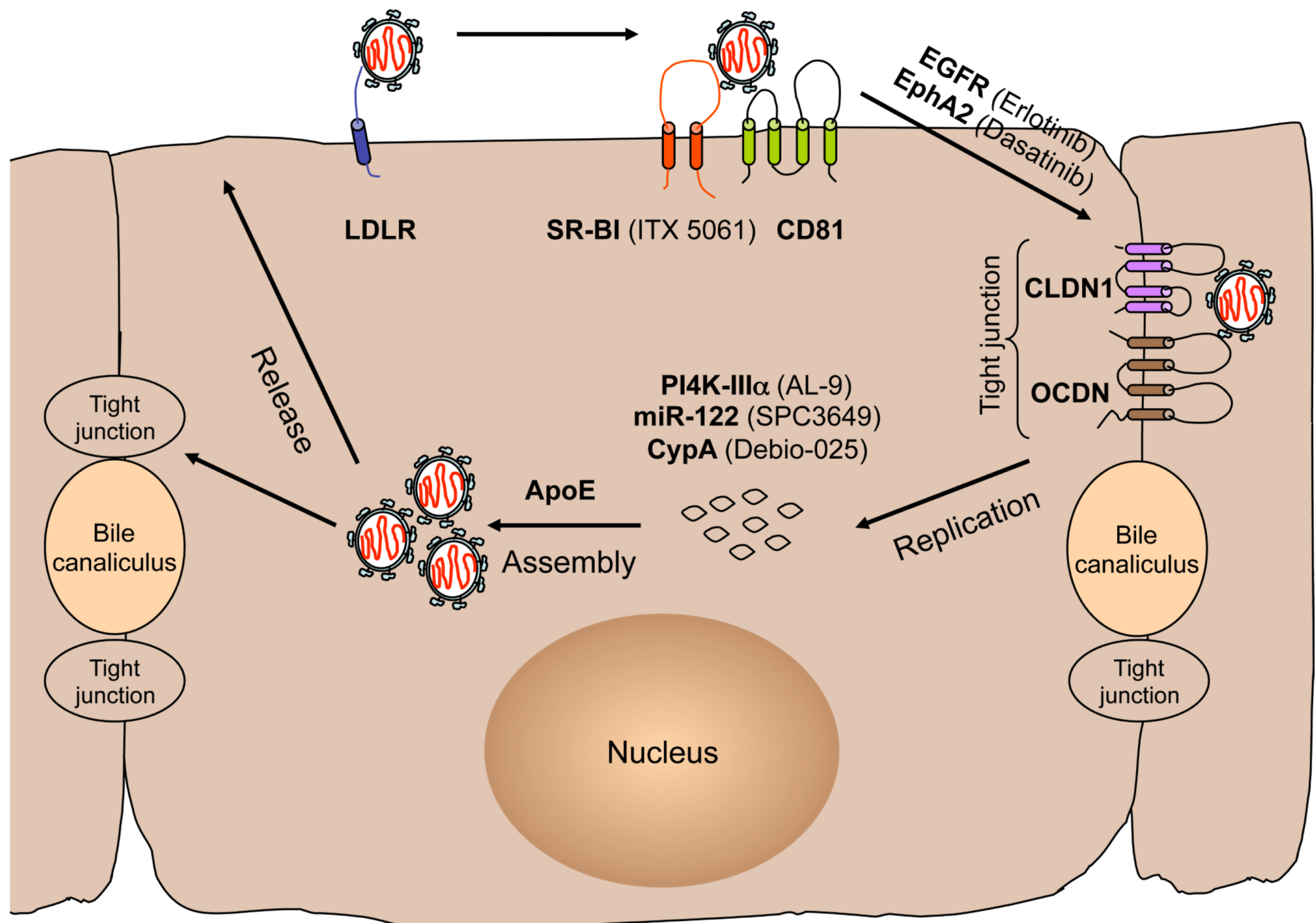

Figure 1. Model of hepatitis C virus life cycle in polarized hepatocytes

Pro-viral host factors are highlighted in bold and their respective inhibitors in parenthesis. Abbreviations: LDLR, lipoprotein receptor; SR-BI, scavenger receptor type B1; EGFR, epidermal growth factor receptor; EphA2, ephrin receptor A2; CLDN-1, claudin-1; OCDN, occludin; PI4K-IIIa, phosphatidylinositol 4-kinase III α; miR-122, microRNA-122; CypA, cyclophilin A; ApoE, apolipoprotein E.

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