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Treating Hepatitis C Infection by Targeting the Host

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Abstract

Over 130 million people worldwide are chronically infected with hepatitis C virus (HCV), which can lead to cirrhosis, liver failure, and hepatocellular carcinoma. While recently-approved HCV NS3-4A protease inhibitors significantly improve treatment response rates, current HCV treatment is still frequently limited by side effects and by the low genetic barrier to viral resistance against direct-acting antiviral agents. A complementary strategy is to target the host cellular factors that support the HCV life cycle. A number of studies, including RNA interference screens, have demonstrated that HCV depends on dozens, if not hundreds, of cellular proteins to complete its life cycle. A better understanding of the interactions between HCV proteins and host factors may help to identify host targets for antiviral therapy. In this review, we highlight some of the host factors that are particularly attractive targets for the treatment of HCV.

Introduction

It is estimated that up to 3% of the world's population is chronically infected with hepatitis C virus (HCV). Around 80% of patients exposed to HCV develop a chronic infection, which is typically asymptomatic. Up to twenty percent of patients with chronic HCV have developed cirrhosis at 20 years of infection, which in turn greatly increases the risk of hepatocellular carcinoma and liver failure. Although the peak incidence of HCV infection in the United States occurred between 1970 and 1990, because of the long interval between initial infection and the development of cirrhosis, the prevalence of HCV cirrhosis is expected to rise in the U.S. for the next decade [1]. Complications of HCV infection now account for the leading indications for liver transplantation worldwide, and HCV-related mortality is continuing to increase [2]. Molecular epidemiology approaches have demonstrated the existence of six major HCV "genotypes" (numbered 1 through 6) that, by definition, differ from one another by greater than 30% at the nucleotide level [3]. Approximately three-quarters of patients with chronic HCV in North America and Europe have genotype 1 infection, with most of the remainder carrying genotypes 2 or 3. These genotypes are clinically relevant as genotype 1 HCV is significantly more resistant to treatment with peginterferon alfa and ribavirin than genotypes 2 and 3, and therefore efforts to develop new therapies against HCV have largely focused on genotype 1 infection.

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While an effective vaccine against HCV would be an invaluable tool to combat and ultimately eradicate HCV infection, complicating the development of vaccines and therapeutics against HCV is its high genetic heterogeneity. In addition to the significant sequence diversity among HCV genotypes, the virus itself mutates readily due to the error-prone viral RNA polymerase. It has been estimated that the HCV RNA polymerase makes one error per 10^4 bases, which in turn means that most HCV genomes contain at least one point mutation. Thus, the HCV-infected individual is in fact a host to a large number of HCV quasispecies, from which mutants that escape neutralizing antibodies and recognition by specific T cells can rapidly emerge. Adding to the challenge is the well-characterized ability of HCV to suppress innate and possibly also adaptive antiviral immune responses. While there has been a great deal of effort invested into developing both prophylactic and therapeutic vaccines that elicit neutralizing antibody and/or T-cell responses, there are no vaccine candidates in advanced clinical development at this time.

Current therapy for chronic HCV infection

Until May of 2011, standard therapy for chronic HCV infection was the combination of peginterferon alfa and ribavirin (RBV). However, a 48-week course of peginterferon/RBV leads to a sustained virologic response (SVR), defined as an undetectable HCV RNA 24 weeks after the completion of therapy, in only about 45% of Caucasian patients with genotype 1 HCV infection. The SVR rate with peginterferon/RBV treatment is substantially lower in African-Americans and Hispanics, as well as in patients with HIV coinfection. In contrast, patients with genotype 2/3 infection have up to an 80% rate of SVR with peginterferon/RBV therapy.

In addition to the relatively low rates of SVR in patients with genotype 1 infection, peginterferon and RBV are often poorly tolerated due to side effects that include influenza-like symptoms, cytopenias, and neuropsychiatric symptoms. This has led to the ongoing search for new therapeutic approaches that can increase the rates of SVR and potentially also be better-tolerated. The main approach so far has been to develop direct-acting antivirals (DAAs) that directly target viral proteins. The viral targets that have received the most attention are the NS3-4A serine protease, the NS5B RNA dependent RNA polymerase, and more recently the viral nonstructural protein NS5A.

Two NS3-4A protease inhibitors, boceprevir and telaprevir, have recently obtained regulatory approval for the treatment of genotype 1 chronic HCV infection. Phase 3 clinical trials of these agents in combination with peginterferon/RBV demonstrated a significant improvement in SVR rates in treatment-naïve individuals from ~45% for peginterferon/RBV alone to 67–75% for protease inhibitor-containing regimens [4, 5]. Another important benefit is that many treatment-naïve patients who receive protease inhibitor-containing regimens can be treated with shorter courses of therapy (24–28 weeks) compared to peginterferon/RBV alone (48 weeks).

However, there are important limitations to these protease inhibitors. First, they cannot be given as monotherapy due to the rapid emergence of viral resistance. Indeed, telaprevir monotherapy in a phase 1b clinical trial led to the emergence of viral resistance in many patients within only 14 days of treatment [6]. To reduce the rates of viral resistance, boceprevir and telaprevir must be given in combination with peginterferon and ribavirin. Patients who are intolerant of either peginterferon or ribavirin, as a result, will not be able to receive protease inhibitor therapy. Second, the protease inhibitors are associated with significant side effects of their own, in addition to the considerable side effects of peginterferon and RBV. Third, boceprevir and telaprevir are not currently approved for the treatment of other HCV genotypes, and are therefore not options for patients with non-

genotype 1 infections. Furthermore, first-generation HCV protease inhibitors have a low genetic barrier for resistance in that several single amino acid substitutions (e.g. V36A/M, T54A/S, R155K/T, and A156V/T) confer resistance without imposing a large viral fitness cost. Notably, viral resistance mutations in 9 of the 12 patients from this telaprevir monotherapy clinical trial who developed on-treatment virologic breakthrough were found to persist three to seven months after telaprevir had been discontinued [7].

While the SVR rates for protease-inhibitor containing regimens are excellent for treatment-naïve genotype 1 patients, they are substantially worse for patients who had previously been nonresponders to peginterferon/RBV treatment. For example, patients who had been previous null responders to prior peginterferon/RBV therapy (less than 2 log₁₀ decline in HCV RNA after 12 weeks of treatment) had only a 29–33% rate of SVR with telaprevir/peginterferon/RBV triple therapy [8]; in this trial prior null responder patients with cirrhosis had only a 14% rate of SVR with triple therapy. Moreover, the majority of virologic failures and relapses were associated with the emergence of telaprevir-resistant viral genomes.

Improving SVR rates in difficult-to-treat populations and moving beyond interferon-based regimens will most likely require combination therapy with DAAs (e.g. protease inhibitor plus a NS5B polymerase inhibitor and/or an NS5A inhibitor), which has been supported by early-phase clinical trials [9]. If DAA combination therapy proves to be well tolerated, it is likely that this approach will eventually be able to treat the great majority of patients with chronic HCV infection.

An alternative or complementary approach to DAA development is to target host factors that support the viral life cycle. Like all viruses, HCV is an obligate parasite in that it requires a host cell for its own replication. Indeed, it is becoming increasingly clear that HCV exploits dozens, if not hundreds, of human (host) proteins for its own purposes. Identifying these host cofactors of the viral life cycle greatly expands the number of potential targets for antiviral drug development. In addition, targeting host cofactors may impose a higher genetic barrier for viral resistance than targeting viral proteins; this will be discussed in more detail below. Finally, a strategy of targeting host proteins may enable the development of therapeutics that can be broadly effective against all HCV genotypes.

The HCV life cycle

The hepatitis C virus belongs to the Flaviviridae family, which includes dengue and yellow fever viruses. It is a positive-sense, single-stranded enveloped RNA virus approximately 9 600 nucleotides in length encoding a continuous open reading frame flanked by non-translated regions (NTRs) at the 5' and 3' ends. The 5' NTR forms a highly structured RNA element that contains an internal ribosomal entry site for initiation of cap-independent translation of the viral RNA.

The life cycle of HCV in the human hepatocyte is depicted in Figure 1. HCV entry is initiated by attachment of viral E1/E2 heterodimers to the hepatocyte cell surface. While the exact sequence of events between viral attachment and viral internalization has not been fully elucidated, the cellular receptors and coreceptors for HCV are believed to include glycosaminoglycans [10], CD81 [11], scavenger receptor type B class I (SR-BI) [12], claudin-1 (CLDN1) [13], and occludin (OCLN) [14]. Heparan sulfate glycosaminoglycans may represent the first interaction site for hepatitis C virions on the hepatocyte cell surface. Both CD81, a member of the tetraspanin superfamily, and SR-BI, which binds a variety of lipoproteins, both interact with the viral E2 envelope protein through extracellular loop domains. Kinetic studies suggest that SR-BI and CD81 act at a similar time point in viral entry [15]. Intriguingly, both CLDN1 and OCLN localize to tight junctions of polarized hepatocytes. Neither of these proteins has been shown to directly interact with HCV, and

both appear to act at a late post-binding step in entry. Following clathrin-mediated endocytosis, the virus undergoes pH-dependent membrane fusion to release its RNA genome [16], which is then translated as a single polyprotein at the cellular endoplasmic reticulum. This polyprotein is cleaved by cellular proteases and virally encoded proteases (NS2 and NS3-4A) at the endoplasmic reticulum into the ten individual viral proteins.

After release of the viral nonstructural proteins, an altered membrane compartment is formed [17]. These virus-induced membrane alterations have been called “membranous webs”; at the electron microscopic level, they are composed of clusters of double-membrane vesicles and more complex membrane structures. Webs contain viral nonstructural proteins, viral RNA, and host proteins. It is here that viral RNA replication is believed to occur. Interestingly, all known single-stranded, positive-sense RNA viruses studied to date induce the formation of internal cellular membranes for their own replication [18]. While expression of the HCV nonstructural protein NS4B is sufficient to induce the formation of membrane structures similar to membranous webs [17], genetic studies have suggested that other viral proteins such as NS5A may also play important roles in web formation [19].

At the membranous web, the positive-strand RNA genome serves as a template for the NS5B RNA polymerase to generate a negative-strand intermediate, which in turn is used to produce multiple progeny positive-sense genomes. The physical location of virion assembly within the infected hepatocyte is not entirely clear. The HCV core protein, which binds to HCV RNA and forms the viral capsid, associates with cellular lipid droplets [20], and the secretion of infectious HCV is correlated with core-LD association [21, 22]. However, it is not clear whether virion assembly and budding occur at the LD surface itself, or whether it occurs from membranes in close proximity to the LD.

There is abundant evidence that the secretion of assembled virions is closely linked to the very low density lipoprotein (VLDL) secretion pathway [23, 24]. While the buoyant density of circulating HCV particles from infected patients is heterogeneous, the specific infectivity of HCV particles is much higher in the low-density fractions. Moreover, HCV appears to associate with host lipoproteins (ApoE and/or ApoB), which has led to the concept that the infectious HCV particle is intimately associated with circulating lipoproteins.

To date, dozens of host factors have been identified as cofactors of viral entry, replication, assembly, and secretion (Table 1). The majority of these cofactors are not likely to be good candidates for antiviral drug development due to the low likelihood of being able to identify selective small molecule inhibitors of their function (poor “druggability”) or because of the relatively weak effect of some of these cofactors on HCV infection. We will, in the following sections, discuss several host factors that are either in active development as targets for HCV therapy (cyclophilins), that have small molecule inhibitors already approved or in development for other indications (receptor tyrosine kinases and diacylglycerol O-acyltransferase 1), or that are attractive candidates for therapeutic development due to their potential “druggability” and critical importance in the replication of HCV and potentially of other viruses (phosphatidylinositol 4-kinases).

HCV entry and host receptor tyrosine kinases

As described above, HCV entry requires several host factors including CD81, SR-BI, claudin-1, and occludin. A recent study asked whether host kinases might also modulate viral entry. Using a functional siRNA screen of the human kinome, Lupberger et al. identified 58 host kinases that appear to support viral entry [25]. Among these kinases were EGFR and EphA2, which were studied further because of the availability of clinically approved kinase inhibitors such as erlotinib and dasatinib. They found that silencing of either kinase inhibited the entry of HCV pseudoparticles derived from genotypes 1–4 and

that this could be rescued by restoration of kinase expression, both in hepatoma cell lines and also in primary human hepatocytes. The EGFR ligands EGF and TGF- α both enhanced HCV entry. While silencing of either EGFR or EphA2 did not affect expression of the known HCV entry factors, kinetic experiments indicated that EGFR appears to act at a postbinding step of viral entry, possibly by promoting the formation of CD81-claudin 1 complexes as suggested by Förster resonance energy transfer assays, and/or by promoting membrane fusion. Finally, erlotinib was evaluated in a chimeric urokinase plasminogen activator-severe combined immunodeficiency (uPA-SCID) mouse model of HCV infection. In this model, uPA-SCID mice develop subacute liver failure due to the liver-specific expression of the toxic uPA transgene; human hepatocytes are transplanted into and engraft within the livers of uPA-SCID mice, and these chimeric mice support HCV infection [26]. Erlotinib treatment significantly delayed the kinetics of HCV infection and decreased steady-state HCV RNA levels by more than 90%.

Since erlotinib and dasatinib are already approved for cancer treatment in humans, the translation of Lupberger et al's findings to clinical practice could potentially be relatively straightforward. For example, it would be of great interest to learn whether these drugs suppress HCV replication in humans being treated for cancer who also have chronic HCV infection. Proof-of-principle studies in such populations, if promising, might then lead to studies of these agents in patients with chronic HCV infection who are not being treated for cancer. Alternatively, as HCV is the leading cause of hepatocellular carcinoma in Western nations, it would be interesting to ask whether tyrosine kinase inhibitors under development for HCC therapy also have anti-HCV effects in patients with HCC and chronic HCV..

HCV replication and cyclophilins

Cyclophilins are a family of enzymes with peptidyl-prolyl isomerase activity, and the immunosuppressant cyclosporin A (CsA) inhibits T-cell activation by forming a complex with cyclophilins and calcineurin. CsA was identified in a drug compound screen as an inhibitor of HCV replication [27]. While it was initially reported that cyclophilin B supports HCV replication by interacting with the NS5B RNA polymerase subsequent studies have rather convincingly supported a role for cyclophilin A (CyPA) in HCV replication [28–30].

The precise mechanism by which CyPA supports HCV replication has not been fully defined and may involve interactions with multiple viral proteins. While cyclophilins were first reported to bind to the viral RNA polymerase NS5B [31, 32], viral resistance mutations that confer resistance to CsA map primarily to NS5A, with some mapping to NS3 and to NS5B [29, 33, 34]. More recently, CyPA has been shown to interact with NS5A, and this interaction is disrupted by cyclophilin inhibitors [35–37]. Finally, a possible role for NS2 as an additional viral target of CsA has been suggested, based on evidence that HCV genomes lacking NS2 are much less sensitive to CsA than the full-length genome [30].

The potent immunosuppressive activity of CsA makes it a poor candidate for anti-HCV drug development. However, nonimmunosuppressive analogs of CsA have been developed that have very low affinities for calcineurin. Alisporivir (DEBIO-025) potently inhibits replication of both genotype 1 and genotype 2 HCV in cell culture models [38], and is able to delay or prevent the development of resistance to NS3-4A protease inhibitors and polymerase inhibitors *in vitro* [39]. Studies of another nonimmunosuppressive cyclophilin inhibitor called NIM811 have shown that the combination of NIM811 and NS5B polymerase inhibitors is synergistic *in vitro*; in addition, NIM811 can suppress the emergence of viral drug resistance mutations against NS3-4A protease inhibitors or NS5B polymerase inhibitors. Finally, the authors observed that it was significantly more difficult to isolate NIM811-resistant HCV replicons *in vitro* than to isolate DAA-resistant replicons,

supporting the hypothesis that host-target therapies may have a higher genetic barrier to resistance compared to DAAs. Similarly, an analysis of alisporivir resistance mutations in 18 treatment-naïve patients with genotype 1–4 infection who received alisporivir monotherapy for 4 weeks [40] was recently reported in abstract form (Tiongyip et al, 6th International Workshop on Hepatitis C, Resistance and New Compounds, Cambridge MA, 2011). In this study, three patients were found to have a D320E resistance mutation in NS5A; this mutation only confers a modest 2–3 fold increase in EC₅₀ to alisporivir *in vitro*. However, it should be pointed out that alisporivir monotherapy was not associated with a large decline in HCV RNA in this study, suggesting that there may have been insufficient antiviral efficacy to select for high-level alisporivir resistance mutations.

The results of a multicenter phase IIb clinical trial of alisporivir in combination with peginterferon/RBV has recently been reported [41]. In this study, standard-of-care peginterferon/RBV was compared with three different durations of alisporivir plus peginterferon/RBV therapy in 288 treatment-naïve patients with genotype 1 HCV infection. The SVR rate in patients who received alisporivir/peginterferon/RBV for 48 weeks was 76%, compared to 55% in the peginterferon/RBV arm. Unfortunately, it was not reported whether patients who failed to clear HCV to alisporivir-based therapy developed mutations that are known to confer resistance to alisporivir *in vitro*. While a phase III trial of alisporivir combined with peginterferon/RBV is currently underway in patients with genotype 1 HCV infection, it would be of greater interest to ask whether alisporivir could reduce the rates of viral resistance to DAAs in the absence of peginterferon/RBV. If so, this would be a significant advance towards the goal of interferon-free regimens for HCV treatment. Another important clinical question is whether alisporivir, in combination with peginterferon/RBV, can improve SVR rates in patients with genotype 2 or 3 infection who have failed previous attempts at treatment with peginterferon/ribavirin alone, as the currently approved HCV protease inhibitors are not approved for the treatment of non-genotype 1 HCV infection.

HCV replication and phosphatidylinositol 4-kinases

A strategy to identify host cofactors of the HCV life cycle is to perform an RNA interference screen against host genes. Silencing of essential host cofactors is expected to block the HCV life cycle, which can be readily detected using genetically modified viruses encoding reporter genes (e.g. luciferase, or a fluorescent protein). A number of the HCV RNAi screens performed to date have identified the phosphatidylinositol 4-kinase PI4KA as a required protein for viral replication [42–48]. There are four mammalian phosphatidylinositol 4-kinases (PI 4-kinases) that catalyze the conversion of phosphatidylinositol (PI) to PI 4-phosphate (PI(4)P), and they are believed to regulate intracellular membrane trafficking events.

PI4KA silencing strongly inhibits replication of both genotype 1 and genotype 2a HCV in cell culture systems, and this can be rescued by expression of wild-type PI4KA [42]. Furthermore, immunofluorescence microscopy has revealed that PI4KA and its product PI(4)P are both enriched on HCV membranous webs [48], suggesting that PI4KA might somehow be involved in web formation and/or maintenance. However, membranous web formation is dependent on ongoing viral RNA replication and polyprotein translation; as a result, studying the mechanisms required for web formation requires nonreplicative systems of HCV web generation. With the use of such nonreplicative systems, we and others have shown that PI4KA plays a role in formation of the HCV membranous web [42, 48]. While two studies have reported that the PI 4-kinase PI4KB also functions as a cofactor for the HCV life cycle [44, 46], others have not [47, 48]; moreover, PI4KB inhibition does not appear to alter HCV membranous web formation [48].

Unfortunately, there are no selective small molecule inhibitors of PI4KA. Wortmannin and LY294002 both inhibit PI4KA, though they also inhibit type I PI 3-kinases with over 10-fold lower IC₅₀s, as well as mTOR. Wortmannin does inhibit HCV replication with an IC₅₀ consistent with PI4KA [42], suggesting that selective PI4KA inhibitors might have anti-HCV activity. Although the catalytic domain of PI4KA shares significant similarity with PI4KB and the type I PI 3-kinases, the successful development of selective inhibitors of the PI 3-kinase isoforms indicates that it should be possible to identify selective PI4KA inhibitors. A concern regarding PI 4-kinase inhibitors is the potential for toxicity. While cultured cells tolerate PI4KA silencing well (at least up to 90% silencing at the mRNA level), the effect of PI 4-kinase inhibition in metazoans is not known.

Interestingly, coxsackievirus and poliovirus, both also positive-sense single-stranded RNA viruses, have been shown to form PI(4)P-enriched viral replication organelles analogous to the HCV membranous web [49]. In contrast to HCV, these viruses appear to generate PI(4)P by recruitment of PI4KB, not PI4KA. It appears, therefore, that several RNA viruses exploit host cellular PI 4-kinases to generate PI(4)P-enriched membranes for viral replication. We are, however, a long way from the clinical use of PI 4-kinase inhibitors in humans. Selective small-molecule PI 4-kinase inhibitors with favorable pharmacokinetics and pharmacodynamics have not been described, and such agents would have to demonstrate good tolerability. On the other hand, the potential attractiveness of targeting PI 4-kinases is the possibility of identifying broadly antiviral therapeutic agents.

Hepatitis C virus particle assembly and secretion

As discussed above, HCV particle assembly is believed to occur either at or in association with lipid droplets. LDs are composed of neutral lipids, mostly triglycerides and cholesteryl esters, surrounded by a phospholipid monolayer believed to originate from the ER. Diacylglycerol O-acyltransferase enzymes (DGAT1 and DGAT2) catalyze the final step of triglyceride synthesis from diacylglycerol and acyl-CoA. Herker and colleagues found that silencing of DGAT1, but not DGAT2, inhibited spread of HCV infection in cell culture [50]. This was found to be due to a block in HCV viral assembly rather than to RNA replication or polyprotein translation. A selective small molecule inhibitor of DGAT1 significantly lowered secreted HCV titers in both human hepatoma and primary human hepatocyte models. Interestingly, DGAT1 knockdown did not change lipoprotein secretion or lipid droplet size or number in hepatoma cells. Rather, DGAT1 inhibition resulted in dissociation of HCV core protein from LDs and its retention in the ER. DGAT1 and core protein could be coimmunoprecipitated when overexpressed in cultured cells, and this interaction was not blocked by DGAT1 inhibitor treatment. The authors hypothesize that DGAT1 is required to localize the HCV core protein to lipid droplets, thus recruiting virion components to LDs for assembly, and thus potentially explaining how DGAT1 inhibitors inhibit secretion of hepatitis C virions. While this mechanism suggests that DGAT1 inhibition may not be able to clear viral replication from infected hepatocytes, it may be useful in combination with agents that directly target viral replication.

Of note, DGAT1 knockout mice are viable, with the interesting phenotype that they are resistant to diet-induced obesity [51]. Pharmacologic DGAT1 inhibitors are currently in clinical trials for the potential treatment of obesity and diabetes [52], with the leading candidates already in phase II trials. It would appear that DGAT1 inhibition is well-tolerated in humans. As discussed above in the case of erlotinib and dasatinib, the development of DGAT1 inhibitors for the treatment of conditions other than HCV will greatly facilitate clinical trials of these agents against HCV in humans.

Another host pathway exploited by HCV for its secretion is the lipoprotein secretion pathway. Very low-density lipoprotein (VLDL) secretion requires microsomal triglyceride transfer protein (MTP) as well as apolipoproteins (apo) B-100 and apoE. Inhibitors of MTP activity block HCV particle secretion from infected cells without altering intracellular viral RNA replication [23, 24, 53]. While agents targeting only viral secretion would not be expected to clear cells of viral infection if used as monotherapy, they might be valuable in the setting of combination therapy, either with DAAs or with drugs targeting other steps of the viral life cycle.

Conclusions

Many cellular proteins are known to be required for all stages of the hepatitis C virus life cycle, with many more yet to be discovered. Currently-approved direct-acting NS3-4A protease inhibitors, while representing a significant advance from peginterferon/RBV alone, are limited by genotype specificity and by the rapid emergence of viral resistance when used without interferon/RBV. Newer DAAs in clinical development appear to have higher barriers for viral resistance, particularly when used in combination, and some have pangenotypic efficacy. Nonetheless, understanding the host cofactors that support HCV infection could lead to novel antiviral strategies with at least two theoretical benefits. First, targeting host factors involved in different aspects of the viral life cycle increases the number of options for combination drug therapy, which is likely to be necessary for successful HCV eradication. Second, the genetic barrier for viral resistance to host-targeted therapies may be greater than for viral-targeted therapies. As proof of principle that targeting the host can be an effective antiviral strategy, nonimmunosuppressive cyclophilin inhibitors such as alisporivir are in clinical trials for chronic HCV infection. In addition, several other host cofactors of the hepatitis C life cycle already have pharmacologic inhibitors in clinical development for other indications (DGAT1) or which have already been approved for cancer therapy (EGFR, EphA2).

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Abbreviations

CsA	cyclosporine A
DAA	direct-acting antiviral
LD	lipid droplet
RBV	ribavirin
SVR	sustained virologic response

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Figure 1.

Many host factors are involved at each step of the HCV life cycle, which starts with virus binding to its specific receptors (CD81, CLDN1, OCLN, and SR-BI). The receptor tyrosine kinases EGF-R and EphA2 also appear to participate in viral entry at a post-binding step. After viral uncoating, the positive-sense HCV RNA is translated and also serves as a template for RNA replication and polypeptide translation. Viral RNA replication occurs on an altered host membrane compartment known as the membranous web; a short list of host factors believed to support web formation and/or RNA replication is shown here. Viral assembly and secretion occur through the secretory pathway with the help of other host factors such as DGAT1, apoB, and/or apoE.

Table 1

A partial list of host factors that support the HCV life cycle

Host Factor	Interacting HCV factor(s)	Putative function(s) in HCV life cycle	References
apoB, apoE	NS5A	Virion assembly and secretion	[24, 54–57]
c-Src	NS5A, NS5B	Participates in formation of NS5A-NS5B protein complex	[58]
CyPA	NS5A, NS5B	Peptidyl-prolyl isomerase, ?isomerization of NS5A	[28, 29, 36]
DGAT1	Core	Recruitment of HCV core to lipid droplets for virion assembly	[50]
EGFR & EphA2	Unknown	Participate in viral entry at a post- binding step	[25]
FBP	3'NTR, NS5A	Regulates replication, possibly through RNA helicase activity	[59]
FKBP8	NS5A	?Regulates replication via interaction with Hsp90	[60]
hB-ind1	NS5A	?Regulates replication by interaction with FKBP8 and Hsp90	[61]
OSBP	NS5A	Particle assembly and/or release	[62, 63]
PI4KA	NS5A	Formation of membranous web	[42–48]
Rab5, Rab7	NS4B	Participate in replication, mechanism unknown	[47, 64]
TBC1D20	NS5A	Regulates HCV replication as a GTPase-activating protein for Rab1	[65, 66]
VAPA, VAPB	NS5A, NS5B	Required for HCV replication, involved in cellular membrane trafficking	[67, 68]