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Emerging host cell targets for hepatitis C therapy

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Chronic hepatitis C virus (HCV) infection is a major burden on humanity. The current HCV therapy has limited efficacy, and there is pressing need for new and more effective therapies. Host cell factors that are required for HCV infection, replication and/or pathogenesis represent potential therapeutic targets. Of particular interest are cellular receptors that mediate HCV entry, factors that facilitate HCV replication and assembly, and intracellular pathways involving lipid biosynthesis, oxidative stress and innate immune response. A crucial challenge now is to manipulate such cellular targets pharmacologically for chronic HCV treatment, without being limited by side effects.

Introduction

Estimated to infect ~170 million people worldwide, and being the leading cause of chronic liver diseases in many countries, hepatitis C has become a 'silent pandemic' around the globe. Hepatitis C is caused by hepatitis C virus (HCV) infection and frequently leads to cirrhosis and hepatocellular carcinoma in long-term chronically infected patients [1]. Without an effective vaccine for HCV, our weapons for fighting HCV infection are limited to antiviral therapies. The current best standard of care for hepatitis C therapy entails a concoction of a pegylated interferon (IFN) with an extended half-life, and ribavirin, both of which are nonspecific antiviral drugs targeting cellular factors. Although this combination therapy has led to a dramatic improvement in hepatitis C treatment outcome, it is still fraught with limited efficacy, resistance problems, poor tolerability, high cost of manufacture and inconvenient route of administration, underscoring the need for new and more effective therapies [2,3].

HCV is an enveloped, positive-stranded RNA virus and a member of the *Flaviviridae* family, which includes three genera: *Flavivirus, Pestivirus and Hepacivirus*. The single-stranded RNA genome of HCV codes for a single open reading frame, resulting in the translation of a single polyprotein of ~3010 amino acids, which is subsequently processed by cellular and viral proteases into the structural and non-structural proteins [4] (Figure 1). The flanking 5′ and 3′ untranslated regions (UTRs) of the viral RNA genome contain important *cis* acting signals for the initiation of viral RNA translation and replication. Despite the progress in our current understanding of the function and regulation of HCV gene products, the development of effective anti-HCV therapeutics has been slowed down owing in part to the dearth of a robust cell culture infection system and a suitable animal model [5]. Given their essential roles in the process of HCV replication, as well as their 'drugabilty', the viral NS3-4A serine protease and NS5B RNA-dependent RNA polymerase are currently the most intensively pursued anti-HCV targets for drug development. However, because of the error-prone nature of the RNA-dependent RNA polymerase of RNA viruses, potential drug resistance is still a major concern for any direct antiviral drugs against HCV [6,7].

Viral versus cellular targets for antiviral therapy

Many of the current antiviral drugs are designed to function as specific viral inhibitors, by inhibiting virus-encoded enzymes essential for viral replication. Despite the tremendous success of these viral inhibitor drugs, such as the control of AIDS progression in patients by a combination of HIV reverse transcriptase and protease inhibitors, they suffer from several major drawbacks (Table 1). First, being designed to target and inhibit specific viral

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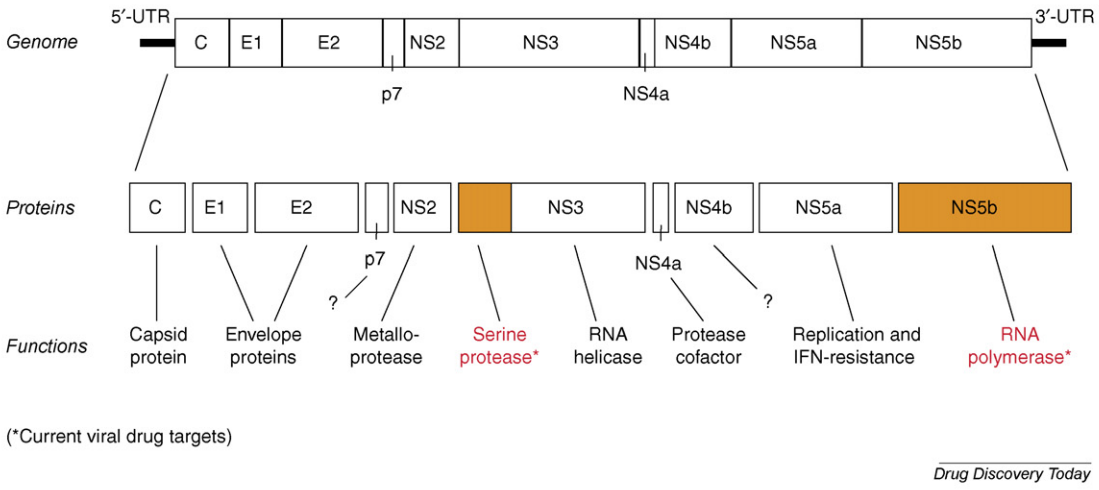

FIGURE 1

HCV is a *Flavivirus* family member and has a positive-sense, ssRNA genome that serves as the basis for both genome replication and protein translation. The virus genome encodes three structural and seven non-structural proteins. Among the viral proteins are several enzymes involved in either viral protein processing or RNA replication. The NS3–4A protease and NS5B polymerase are current viral drug targets pursued by numerous drug discovery programs.

enzymes, many of these drugs can only be used to treat a specific viral species, or even only certain subtypes of a viral species. This narrow spectrum of action, although reducing the potential for toxicity associated with targeting nonspecific enzymes, greatly limits the usage and market potential of each of these drugs. In addition, many viruses are able to mutate and evolve at high rates owing to their low-fidelity replication mode and, thus, frequently generate drug-resistant, mutant viral species, further limiting the usefulness of viral inhibitors. Resistance to viral inhibitors is a particularly serious problem for the medically important RNA viruses, such as HIV, HCV and influenza virus, which demonstrate even higher mutation rates than do DNA viruses. Indeed, the rapid development of resistance against specific viral inhibitors necessitates the use of combination therapy comprising different antiviral drugs with different targets or mechanisms, and the search for new generations of inhibitor drugs, which is reminiscent of our experience with antibacterial drugs. It is also important to emphasize that many clinically important viruses are small RNA viruses, their genomes encoding no more than 10–12 genes, and only a few function as enzymes and are thus considered to be 'drugable' by small-molecule compounds without substantial investment in pharmaceutical medicinal chemistry [8].

TABLE 1

Pros and cons of viral versus cellular targets

Viral targets	Cellular targets
√[a] Virus-specific	× Lack of specificity; side effects
× Limited action spectrum	√ Broad action spectrum
× Limited market	√ Broad market
× Viral target mutation	√ Lack of cellular target mutation
× Limited number of targets	√ Large number of targets
× Requiring novel inhibitors	√ Existing drugs; indication switch

[a] √, pro; ×, con.

As intracellular obligatory parasitic life forms, the physiology, and often the pathogenicity, of viruses is strictly dependent on host cell factors. Viral proteins and genomes specifically interact with and recruit certain cellular molecules or pathways to facilitate viral genome replication and protein translation, assembly, as well as generation of infectious viral particles. Viral infection also frequently activates the host immune response system and leads to mobilization of cellular defenses against the invader. Thus, viruses have also evolved to use various mechanisms to evade and antagonize host antiviral immune responses, including both intracellular innate immunity and adaptive immunity [9,10]. As a result, the virus replicates, spreads and establishes a persistent infection.

Given that the virus is dependent on the host and interacts with the host defense system, it is reasonable to argue that host genes, pathways or processes that are required for virus infection, replication and/or pathogenesis or induction of antiviral response might be used as targets for antiviral strategies. This concept offers some advantages compared with the viral inhibitor approach. For example, host cells offer a wealth of proven, drugable targets, such as cell surface receptors, protein kinases, nuclear receptors and proteasomes. Perhaps most importantly, by targeting common cellular pathways that are required for the life cycle of different viruses, we might be able to develop 'broad-spectrum', 'silver-bullet' antiviral drugs capable of treating multiple viral diseases [10]. In theory, antiviral drugs targeting cellular pathways should limit the emergence of drug resistance because the human genes encoding targeted cellular proteins would not normally mutate in response to therapy [11]. However, for those pathways that are vital to fundamental cellular processes, modulation rather than ablation of the enzymes that are involved is likely to be a necessity to minimize the potential for severe side effects. Indeed, if such drugs were able to subdue the overt pathogenesis of viral infection into a less virulent subclinical form, this might enable the host immune system to gain the upper hand in clearing the infection.

Current and emerging host cell targets for hepatitis C therapy

Interferons

Several current antiviral drugs do target cellular functions and are used to treat different viral infections. Well-known examples are the recombinant forms of IFN, a class of antiviral cytokines secreted by host cells following viral infection. The IFN monotherapy for hepatitis C was introduced in 1990. The current standard forms of IFN for treating HCV patients are pegylated-IFNs, which demonstrate increased stability and efficacy compared with unmodified IFNs. Several novel forms of IFN are currently under development [12–16] (Table 2). Despite the proven antiviral efficacy of IFNs, many viruses, including HCV, have evolved an assortment of mechanisms to interfere with their antiviral activity, which might contribute to the failure of IFN-based therapies in patients [10,17,18].

Ribavirin and IMPDH inhibitors

Ribavirin is a small-molecule nucleoside analogue that acts as a pleiotropic antiviral agent and is considered to be a cellular target-directed antiviral drug. Ribavirin is known to inhibit inosine-5′-monophosphate dehydrogenase (IMPDH), a cellular enzyme that catalyzes a crucial step in the biosynthesis of guanine nucleotides and presumably reduces the cellular nucleotide pool preferentially required for viral replication. Ribavirin has also been shown to modulate the Th1–Th2 immune response balance and might modify immunosuppression. Although ribavirin treatment alone does not produce an antiviral effect in HCV patients, it potentiates the long-term response to IFN by greatly reducing post-therapy relapse. Today, combination therapy comprising a pegylated IFN compound and ribavirin gives clinicians the ability to achieve sustained clearance of HCV and subsequent improvements in liver disease in >50% of their treated patients. Not surprisingly, several ribavirin analogs are currently in clinical development [12,14,15,19] (Table 2). Of note, viramidine has been touted for its liver-targeting properties and has been advanced into clinical trials.

Recent advances in our understanding of the molecular interplay between HCV and the host cell has begun to reveal an expanding list of host factors as potential therapeutic targets, notably cellular receptors and/or coreceptors that mediate HCV entry, host cell proteins that facilitate HCV replication through functional interactions with the viral RNA and/or replication complex (RC), and disruption of host-cell glycosylation machinery for viral morphogenesis and secretion (Figure 2). Other possibilities include intracellular pathways involving the innate immune response, lipid biosynthesis and oxidative stress. Some of the following cellular functions are better understood, and clinical candidates based on them are already being tested in hepatitis C patients. For the others, the underlying biological mechanisms of their functions remain poorly understood, and further research is required to validate these potential targets.

TLR-mediated innate immune pathways

Toll-like-receptors (TLRs) are the molecular sentinels of cells; they detect the presence of invading microorganisms by recognizing molecular structures characteristic of such pathogens as bacteria, viruses and parasites [20,21]. TLRs are expressed by cells of the immune system, and, following activation, they initiate an acute inflammatory response by inducing the expression of antimicro-

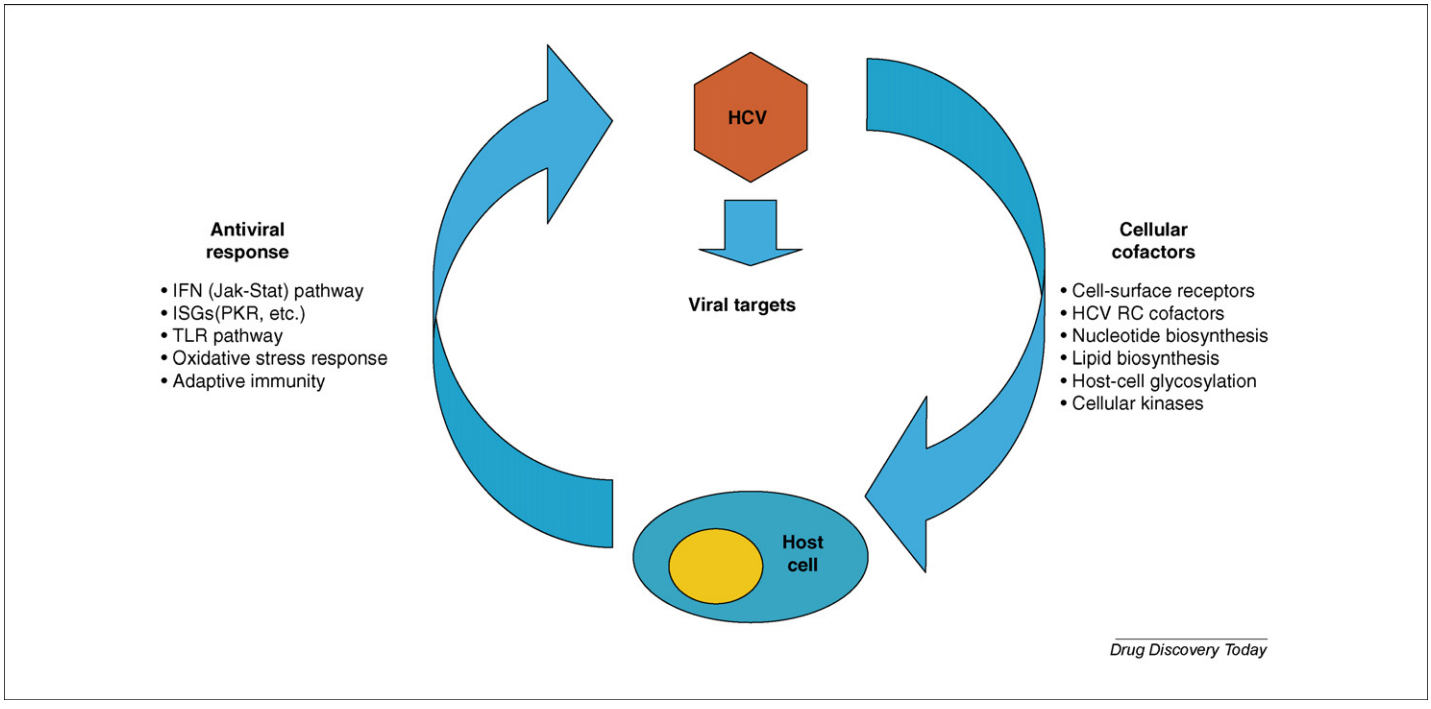

FIGURE 2

HCV uses numerous cellular pathways and cofactors to complete its life cycle. Several key pathways and factors involved in the HCV replication cycle are discussed in the text. At the same time, the host cells use multiple antiviral response pathways to inhibit viral replication. Both cellular cofactors and antiviral pathways might offer novel anti-HCV drug targets. Either disruption of cellular cofactors or stimulation of cellular antiviral pathways might result in the inhibition of HCV replication. A combination of drugs aimed at various targets, both viral and cellular, might be required for optimal anti-HCV therapy. Abbreviation: ISGs, IFN-stimulated genes.

TABLE 2

Current and potential antiviral agents targeting host cell functions

Agent	Target or mechanism	Application or disease	Refs
IFNs (IFN-α-2a, IFN-α-2b, IFN-α-n1, consensus IFN, pegylated IFN-α2b, pegylated IFN-α2a, Albuferon-α, IFN-β1, oral IFN-α, IFN-γ1b, IFN-ω)	IFN receptors and JAK–STAT pathway	HCV, HBV	[12,14–16,19]
Ribavirin	IMPDH inhibitor? Immunomodulation?	HCV	[14,15,19]
Viramidine	IMPDH inhibitor, prodrug of ribavirin	HCV	[16,19]
Merimepodib (VX-497)	IMPDH inhibitor	HCV	[16,19]
Mycophenylate mofetil	IMPDH inhibitor	HCV	[16,19]
Amantadine	Immunomodulation	Influenza A, HCV?	[14,16]
Rimantadine	Immunomodulation	Influenza A	[14,16]
ANA245	TLR7 agonist	HCV	[6,14,19]
ANA975	TLR7 agonist, prodrug of ANA245	HCV	[6,19]
CPG10101	TLR9 agonist	HCV	[6,19]
Imiquimod (R-837)	TLR7 and TLR8 agonist	Anogenital warts	[85,86]
Resiquimod (R848)	TLR7 and TLR8 agonist	HCV? HSV2? Other viral infections, vaccine adjuvant	[85–87]
Thymosin α1 (Zadaxin)	Immunomodulation	HCV, HBV	[88]
Histamine (Ceplene)	H2 receptor, immunomodulation	HCV	[89]
Azathioprine	Immunomodulation	HCV?	[14]
Imino sugar UT-231-B, Celgosivir	ER α-glucosidase inhibitor, immunomodulation	HCV, WHV, BVDV, DV-2, JEV	[63,90,91]
Retinoids?	Upregulation of GI-GPx[a] expression	HCV	[19]
Methyl donors (SAMe, betaine)?	STAT1 methylation?	HCV	[24]
CsA, DEBIO-025, NIM811	?	HCV	[42,43,45–47]
Antioxidants?	?	HCV	[38]
NA255	Serine palmitoyltransferase	HCV	[54]
Cholesterol-lowering drugs?	?	HCV	[56]
PKR activators?	PKR	Various viral infections?	[10,17]
PRK2 inhibitors?	PRK2	HCV	[74]
Prenylation inhibitors; GGTI-286	geranylgeranyltransferase-1	HCV, HIV	[14,57,58,60,92]
Lovastatin	HMG-CoA reductase	HCV, HIV	[57,92]
Gleevec	Abl tyrosine kinase, c-Kit	Vaccinia virus, KSHV	[82,93,94]
EB1089 (vitamin D analog)	Vitamin D receptor	KSHV	[95]
Salubrinal	EIF-2-α	HSV-1	[96]
BMS-279652, BMS-279654, BMS-279655, indomethacin, aspirin	COX-2	HCMV	[81,97]
FTI-277, FTI-2153	Farnesyltransferase	HDV	[98–100]
siRNA	hnRNP-K	HBV	[101]
KU-55933	ATM kinase	HIV	[102]
CNI-1493	Deoxyhypusine synthase	HIV	[103]
Valporic acid	Histone deacetylase	HIV	[104,105]
PP2, SU6656	c-Yes (Src family kinases)	WNV	[106]
mTOR inhibitors (sirolimus, everolimus)	mTOR	CMV	[107]
NF-κB inhibitors?	NF-κB pathway	HIV?	[108]
CI-1033	ErbB inhibitor	Poxvirus?	[109]
Cdk inhibitors?	Cdk	VZV?	[110]

[a] ATM, ataxia telangiectasia mutant; CMV. cytomegalovirus; COX-2, cyclo-oxygenase 2; GI-GPx, gastrointestinal-glutathione peroxidase; HBV, hepatitis B virus; HCMV, human cytomegalovirus; HDV, hepatitis D virus; HSV, herpes simplex virus; KSHV, Kaposi sarcoma-associated herpes virus; NF-κB, nuclear factor κB; VZV, varicella zoster virus; WMV, West Nile virus.

bial genes and proinflammatory cytokines and chemokines, including IFNs. In humans, there are at least ten different known TLRs, each recognizing molecular signatures associated with a specific class of microbial species. For example, TLR3 recognizes double-stranded (ds) RNA viruses by sensing dsRNA released from dying cells; TLR7 and TLR8 detect G/U-rich single-stranded (ss) RNA associated with viruses that enter cells through endocytosis; and TLR9 recognizes unmethylated 2′-deoxyribo(cytidine-phosphate-guanosine) (CpG) DNA sequences present in DNA viruses]. Given the pivotal role of TLR7 and TLR9 in initiating the antiviral innate immune response, they are currently being pursued as drug targets for treating viral diseases.

It was suggested that TLR7 ligands were able to induce anti-HCV immunity not only by IFN induction, but also through an IFN-independent mechanism. In this sense, TLR ligands might cause more robust antiviral effects compared with IFN therapy, particularly in patients who failed IFN therapy owing to either host or viral factors. One such ligand is ANA-245 (7-thia-8-oxoguanosine) (Table 2). Systemic administration of ANA-245 in patients resulted in dose-dependent induction of immunological biomarkers and a statistically significant antiviral effect with relatively few and mild side effects [22]. In addition, TLR7 ligand SM360320 [9-benzyl-8-hydroxy-2-(2-methoxyethoxy)adenine] was shown to reduce HCV RNA levels in Huh-7 cells carrying HCV replicon, at least in part, by induction of type I IFN through TLR7 stimulation [23]. Besides TLR7 agonists, CPG10101 (Table 2), a TLR9 agonist and a CpG-containing oligonucleotide, also generated promising results in HCV patients.

Exploiting STAT1 methylation: next generation of IFN pathway-based therapy?

In addition to boosting IFN-mediated antiviral response by using more potent or stable forms of IFN, we might also be able to target this pathway through regulation of downstream intracellular signaling events. Activation of the transcription factor signal transducer and activator of transcription 1 (STAT1) not only involves phosphorylation by protein kinases, such as Janus family kinases (JAKs), but also involves arginine methylation. Interestingly, HCV causes hypomethylation of STAT1, which might contribute to the inhibition of the IFN-mediated antiviral response. In this regard, treatment of HCV replicon cells (a human hepatoma cell line carrying an artificial RNA construct that contains a selectable marker gene and genes encoding HCV proteins for selectable, autonomously replicating HCV RNAs in cell culture) with nutrients that function as methyl donors such as *S*-adenosylmethionine (SAMe) and betaine (also known as trimethyl glycine) could restore STAT1 methylation and improve IFN-α signaling, resulting in an enhanced antiviral effect of IFN-α in cell culture [24]. It is worth noting that SAMe has already been used to treat alcoholic liver disease by acting as a precursor for glutathione biosynthesis, restoring hepatic reduced glutathione content in the liver [25]. Thus, the addition of SAMe or related drugs to the standard IFN-based therapy might improve IFN-induced antiviral effects and increase virological response in hepatitis C patients.

Cellular receptors for HCV as therapeutic targets

All viruses attach to and enter host cells through specific cell surface receptor molecules, which therefore might serve as excellent drug targets. Because the interactions between viral envelope proteins and their receptors occur in the extracellular space, they could be targeted with peptide- or antibody-based drugs. Such an approach to interfere with virus entry is currently being pursued for HIV therapy, including monoclonal antibodies and small-molecule inhibitors that target HIV binding to its host receptor, CD4, or the chemokine receptor CCR5 [8].

The HCV envelope glycoproteins E1 and E2 form a complex thought to be present at the surface of HCV particles, and it is therefore the obvious candidate ligand for cellular receptors. Several putative HCV receptors have been identified based on their interactions with the HCV E1–E2 complex. These molecules include CD81 tetraspanin [26], scavenger receptor class B type I (SR-BI) [27], asialoglycoprotein receptor [28], mannose-binding C-type lectins dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and liver and lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN) [29–32], low-density lipoprotein receptor [33] and heparan sulfate proteoglycans [34]. However, their exact role in HCV entry remains to be ascertained, owing to the lack of an HCV infectious system, which only recently became available [35]. Excitingly, in the new HCV strain infection system, which is based on an HCV clone, JFH-1, isolated from a fulminant hepatitis patient with HCV, infectivity was neutralized by CD81-specific antibodies, supporting an important role for CD81 in HCV cell entry. Because viral envelope proteins are under constant immune pressure and mutate frequently, drugs that target the virus–receptor interaction step might still encounter viral resistance. Furthermore, the development of such inhibitors would require a better understanding of HCV receptor usage, binding sites on the HCV glycoproteins E1 and E2, and conformational changes induced by receptor binding.

Modulating hepatic oxidative stress

Chronic hepatitis C is associated with intrahepatic oxidative stress, with increased levels of reactive oxygen species and reduced levels of antioxidants being reported in the livers of patients. HCV gene expression elevates the levels of reactive oxygen species through calcium signaling, and expression of HCV proteins, including core (C), NS3 and NS5A, have all been shown to induce oxidative stress in cultured cells [36]. Oxidative stress in the liver might have a detrimental effect on IFN therapy because conditions associated with increased oxidative stress were shown to result in suppression of IFN-α-induced activation of STAT in cultured cells [37]. Therefore, high levels of hepatic oxidative stress in patients might directly impair IFN-α signaling and contribute to a poor IFN response. Is it therefore possible to improve viral response to current therapy by alleviating hepatic oxidative stress in HCV patients? A clinical trial on chronic HCV patients using a combination antioxidant therapy showed normalization of liver enzymes in 44% of patients, liver histological improvement in 36% of the patients and a reduction of viral load in 25% of the patients [38]. However, because antioxidants could also modulate the necro-inflammatory events in the liver [39], it is difficult to ascertain if the observed beneficial effects were indeed due to a true direct antiviral effect of antioxidants.

Cyclophilins

Cyclophilins (Cyps) are a family of peptidyl prolyl cis-trans isomerases that catalyze the *cis–trans* interconversion of peptide bonds N-terminal to proline residues, and thus facilitate switches

in protein conformation. The Cyp family includes more than ten subtypes that function in numerous cellular processes, ranging from transcriptional regulation, immune response and protein secretion to mitochondrial function [40,41]. Cyps seem to have a role in HCV replication. It has been shown that the immunosuppressant drug cyclosporin A (CsA), which inhibits Cyp function [40,41], has the ability to suppress HCV RNA replication in replicon cells, and the anti-HCV activity of CsA was independent of its immunosuppressive function [42,43]. Furthermore, CsA was found to exert beneficial effects in the chimpanzee model [44] and in a small pilot study of chronic hepatitis C patients [45]. Importantly, a higher sustained virological response rate was achieved for IFN–CsA combination therapy compared with IFN monotherapy, correlating with the blood CsA concentration [45].

Novel forms of cyclosporin are also being examined as drug candidates for HCV therapy. DEBIO-025 is a non-immunosuppressive cyclosporin that displays more potent anti-HCV activity than does CsA in the HCV replicon cell culture system [46] and more recently in HIV–HCV coinfected patients (http://www.debio.com/e/newsevents/items_e.php?id=263&type=1). Another non-immunosuppressive CsA analog, NIM811, also strongly suppresses HCV replication in the replicon system, and it was found that inhibition of CypB is crucial for this anti-HCV activity [47]. It seems probable that cyclosporin inhibits HCV replication through a Cyp-dependent mechanism – but what is the connection between Cyp and HCV replication machinery? A recent study [48] reported that CypB is crucial for efficient HCV replication in cell cultures, by directly interacting with NS5B to stimulate its RNA-binding activity, suggesting that CypB might be a host cell component of the viral polymerase in the HCV replication machinery and might be a part of the viral RC. However, a new study [49] found that different HCV strains demonstrated different levels of dependence on CypB and thus different sensitivity to CsA and NIM811 treatment. These results raise concerns about the use of Cyp as a universal drug target in HCV patients.

Human vesicle-associated membrane protein-associated protein A (of 33 kDa) (hVAP-A/hVAP-33): a cellular cofactor for the HCV RC

hVAP-A/hVAP-33 is a widely expressed, endoplasmic reticulum (ER)–Golgi-localized protein involved in intracellular vesicle trafficking. First identified as a host factor interacting with both NS5A and NS5B, two non-structural HCV proteins involved in the viral RC [50], hVAP-A was later found to be crucial for the formation of HCV RC and RNA replication in HCV replicon cells [51]. It is hypothesized that hVAP-A serves as a docking site for assembly or localization of HCV RC on intracellular membranes. Therefore, it might be a promising strategy to target the interactions between hVAP-A and the HCV non-structural proteins with molecules that disrupt these protein interactions. However, blocking protein–protein interactions with small-molecule drugs poses a challenging task for drug makers, often as a result of the large interaction surface and the presence of multiple, weak interaction points [52,53]. Because these protein interactions involve a viral protein partner that can mutate quickly following treatment with drugs designed to block the interactions, drugs targeting these cellular–viral protein interactions can suffer from viral resistance as well.

HCV and lipid biosynthesis pathways – sphingolipids, cholesterols and geranylgeranylation

HCV RNA replication depends on viral RC association with intracellular ER–Golgi-derived membranes, most probably the lipid rafts, which are membrane microdomains enriched with sphingolipids, cholesterols and membrane-bound cellular proteins. A recent study [54] reported that a small-molecule HCV replication inhibitor, NA255, prevented the *de novo* synthesis of sphingolipids by inhibiting serine palmitoyltransferase and thus indirectly disrupted HCV non-structural viral protein assembly on lipid rafts. Interestingly, HCV NS5B has a sphingolipid-binding motif that can interact with sphingomyelin directly. Therefore, it is possible that NA255 functions by preventing the association of NS5B with lipid rafts by inhibiting sphingolipid biosynthesis. NA255 might thus serve as a novel HCV inhibitor by targeting lipid rafts, and inhibition of host cell sphingolipid metabolism could provide a new therapeutic approach for the treatment of HCV infection. Besides disruption of the sphingolipid pathway, depletion of cellular cholesterols has also been shown to reduce HCV RNA replication in HCV replicon cells [55]. Indeed, commercial cholesterol-lowering drugs possess anti-HCV activity *in vitro* [56]. However, the concentrations of the drug required for the *in vitro* effect would be toxic *in vivo* and might limit the utility of such an approach for HCV treatment [57].

However, other reports have demonstrated that inhibition of geranylgeranylation, rather than the synthesis of cholesterol itself, is responsible for inhibiting HCV RNA replication [57,58]. The geranylgeranylation pathway was first implicated in HCV replication by observations that HCV RNA replication was disrupted by treatment with lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase, or with an inhibitor of protein geranylgeranyl transferase I, and both inhibitors induced dissociation of HCV RC [57]. It was suggested that assembly of the viral RC requires geranylgeranylation of one or more host proteins. However, safety might be of issue for the use of broad-spectrum geranylgeranyl transferase I inhibitors as anti-HCV agents because they have been found to be too toxic in preclinical trials, despite the fact that they have demonstrated activity against some tumors [59]. A later study [60] identified FBL2 (F-Box/Leucine-rich repeat protein 2), a geranylgeranylated host cell protein that is required for HCV RNA replication. Hence, it might be 'safer' to inhibit FBL2 selectively, although the exact function of FBL2 during HCV replication requires further characterization.

Disrupting viral assembly with glycosylation inhibitors

Mammalian viruses also rely on the host-cell glycosylation machinery during viral assembly [61]. The key cellular enzymes of the *N*-linked glycosylation pathway are the ER-localized glucosidases, which are potential broad-spectrum antiviral targets [62]. Indeed, derivatives of imino sugars, such as *N*-butyldeoxynojirimycin and *N*-nonyl-deoxynojirimycin, which competitively inhibit ER glucosidases, had potent antiviral activity against woodchuck hepatitis virus (WHV) and bovine viral diarrhea virus (BVDV), an *in vitro* surrogate model of HCV, as well as the flaviviruses dengue virus serotype 2 (DV2) and Japanese encephalitis virus (JEV). Therefore, targeting ER α-glucosidases could be a potential strategy for treating viral infections, although it is not known to what extent these enzymes can be inhibited without

compromising the host cell. On an optimistic note, the glycosidase inhibitor celgosivir is currently being evaluated in clinical trials and has recently shown some beneficial effects in a Phase II trial [63].

Kinase-mediated cell signaling pathways: a treasure trove of drug targets for HCV therapy?

Viruses interact with numerous intracellular signal transduction pathways, including the protein kinase-mediated host antiviral immune response. A well-known cellular protein kinase that mediates the host cell antiviral response is dsRNA-activated protein kinase (PKR), an IFN-induced antiviral kinase. There is evidence that HCV inactivates the IFN-induced antiviral response, at least in part, by using NS5A to inhibit PKR [17,18]. Thus, finding a means to block the NS5A–PKR interaction or increase PKR activity might offer an avenue for a more effective treatment regimen against HCV infection by stimulating the antiviral response while circumventing the problem of virus sequence variability and drug resistance.

Another cellular kinase that directly interacts with the HCV NS5A protein is the phosphatidylinositol 3-kinase (PI3K), and NS5A interaction seems to stimulate the PI3K–Akt (or PKB) pathway, which was predicted to suppress cellular apoptosis, enabling viral persistence and/or malignant transformation [64,65]. Interestingly, it was also suggested that HCV might stimulate the PI3K–AKT pathway indirectly through recruitment of N-Ras, and that activation of the N-Ras–PI3K–AKT–mammalian target of rapamycin (mTOR) pathway might contribute not only to the survival of HCV-infected cells, but also to HCV replication [66], although the mechanism for the latter is not known. Nevertheless, these findings imply that the Ras–Raf–PI3K–AKT pathway is another potential cellular target for developing novel therapeutics for HCV infection.

For many small RNA viruses, their genomes encode proteins that are capable of performing more than one function, perhaps at different stages during the viral life cycle and when necessary, depending on the status of the host cell. Phosphorylation (as well as other post-translational modifications) of a viral protein by a host cell protein kinase(s) to produce different functional 'biological' forms of the same viral protein is one clever way to implement these strategies. Studies using the HCV replicon cell culture system have suggested an important role for NS5A hyperphosphorylation in HCV RNA replication [67–70]. Thus, host cell factors that modulate NS5A hyperphosphorylation might also provide new avenues for therapeutic interventions for HCV infection. Although multiple candidate NS5A kinases have been reported [71], the CKIα protein kinase was recently identified as a major determinant of NS5A hyperphosphorylation [72,73]. Future work will be required to assess whether specific modulation of CK1α will impact HCV replication in animal models.

In addition to NS5A, the NS5B polymerase of HCV has also been suggested to be phosphorylated by host protein kinases. Protein kinase C-related kinase 2 (PRK2) is a cellular kinase reported to interact with and phosphorylate HCV NS5B polymerase on serine residues and, presumably, to regulate NS5B activity, and appears to be important to HCV RNA replication in cell culture [74].

PRK2 kinase might thus serve as a potential cellular target for HCV therapy. Interestingly, recent studies suggest that the C-terminus of a closely related family member, PRK1, is crucially involved in the control of the catalytic kinase activity and activation by lipids [75]. Because this C-terminal segment is the least conserved domain among members of the protein kinase C superfamily, it has been suggested that this region might serve as a promising target for specific pharmaceutical interventions for this family of enzymes.

Conclusions and future directions

Despite the presence of numerous drug candidates in the anti-HCV pipeline, and the commitment of major R&D resources by many pharmaceutical companies, it might still take several years for any new anti-HCV drugs to reach the market [3,7,76]. Although many companies are focusing their efforts on developing viral inhibitors, drug targets in the host are beginning to emerge for hepatitis C therapy. Cellular targets, particularly those in the immunoregulatory pathways, are attractive because they might enable the development of broad-spectrum antiviral drugs with less chance for viral resistance, although these might cause severe side-effects. However, because many of the potential cellular targets were identified from *in vitro* replication systems and thus might be vulnerable to cell culture-adapted artifacts, they must be validated by *in vivo* infection systems. Further, as we continue to examine the effects of acute and chronic HCV infection on cellular signaling, cDNA microarrays and proteomics have begun to provide a comprehensive look at the effects of HCV on cellular gene expression [77–79]. It remains to be seen if this type of analysis, coupled with a bioinformatics approach, would validate existing cellular targets and pathways or identify novel ones for anti-HCV therapeutics. Finally, one should not forget that there are many drug molecules, targeting various cellular factors, on the market. Although they are intended to treat non-viral diseases, some of these compounds might have previously unrecognized antiviral properties and might serve as candidates for further development as next-generation antiviral agents [80–84]. This, in turn, might help to reduce the ever-increasing R&D costs. The challenge is now to modulate such cellular targets effectively, particularly for treatment of chronic virus infections, without being limited by side effects.

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