Invited Review

A Hepatitis C Virus-Host Interaction Involved in Viral Replication: toward the Identification of Antiviral Targets

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SUMMARY: Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease. The current standard therapy for hepatitis C patients, which is based on a combination of pegylated interferons and ribavirin, results in viral clearance in about 50% of the treated individuals. Clinical trials of a variety of specific anti-HCV drugs, including several which target virus-encoded enzymes, are on-going, and some of these studies have reported impressive reductions of HCV levels in patients. However, the development of antivirals with diverse mechanisms of action is still required to eliminate this life-threatening virus. Besides specific viral proteins, targeting host cellular factors that are key to efficient viral replication could lead to the development of novel treatment strategies. Therapies against host factors are generally considered to present a low risk of generating drug-resistant viruses. The current understanding of anti-HCV drugs in clinical development and of virus-host interactions implicated in the regulation of HCV replication is summarized.

1. Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Indeed, this virus is responsible for almost 75% of all cases of HCC in Japan (1,2). HCV is primarily transmitted by blood-borne routes, such as blood transfusion, shared needles, and blood products. A recent WHO estimate suggests that a minimum of 2–3% of the world's population is chronically infected with HCV (3). Despite the fact that HCV is targeted by innate, cellular, and humoral immune mechanisms, it can establish persistent infection in a majority of the infected population. Unfortunately, a protective vaccine is not yet available, and therapeutic options are still limited.

2. Treatment

Successful treatment against hepatitis C is characterized by a "sustained virological response" (SVR), as defined by undetectable viral RNA in a sensitive quantitative assay 6 months after the end of therapy. Treatment of chronic HCV infection is currently based on interferon (IFN)-alpha due to its potent antiviral and immunomodulatory properties. The current standard protocol for such treatment involves weekly injections of pegylated IFN-alpha together with twice-daily oral ribavirin. Ribavirin, a member of the nucleoside antimetabolite drugs, is a pleiotropic agent that may act against multiple targets, although the exact mechanism of its role in hepatitis C therapy remains elusive. IFN/ribavirin treatment often has serious side effects, including depression, flu-like symptoms, and hemolytic anemia (4). Furthermore, there are wide differences in the response to the treatment depending on the characteristics of the virus, such as viral loads and viral genotypes, as well as host genetic variations. Only around 50% of patients treated are estimated to achieve SVR (5). Few treatment options are currently available for non-responders infected with HCV genotype 1, which is considered to be the most resistant to IFN therapy. It is noteworthy that IFN and ribavirin are not specific antiviral agents designed for HCV, which means that new approaches to improve response rates are needed. The development of novel and more effective antiviral drugs to treat liver diseases with HCV infection is therefore a major challenge.

3. Drugs in current clinical development

HCV is a positive-strand RNA virus classified in the Hepacivirus genus within the Flaviviridae family. Its ap-
proximately 9.6-kb genome contains an open reading frame encoding a polyprotein precursor of approximately 3,000 amino acids (aa) flanked by untranslated regions (UTRs) at both ends. The 5′ UTR, which is ~341 nucleotides (nts) long, contains an internal ribosomal entry site (IRES), which is essential for cap-independent translation of viral RNA and from which four highly structured domains (domains I–IV) are produced (6–8). The 3′ UTR varies in length between 200 and 235 nts, including a short variable region, a poly(U/C) tract with an average length of 80 nts, and a virtually invariant 98-nt X-tail region (9–11). This X region forms three stable stem-loop structures and, as a result, the HCV genome probably ends with a double-strand stem structure. It appears that the 3′ X region and the poly(U/C) tract are crucial for RNA replication, whereas the remainder of the 3′ UTR plays a role in enhancement of replication. The genome is translated into a single polypeptide of about 3,000 aa in which the structural proteins Core, E1, and E2 reside in the N-terminal region. A crucial function of Core involved in assembly of the viral nucleocapsid. The aa sequence of this protein is well conserved among different HCV strains in comparison with other HCV proteins. The non-structural (NS) proteins NS3–NS5B are considered to assemble into a membrane-associated HCV RNA replicase complex, with NS3 possessing the enzymatic activities of serine protease and RNA helicase and NS4A serving as a cofactor for NS3 protease. NS4B plays a role in the remodeling of host cell membranes, probably to generate the site for replicase assembly. NS5A has been considered to play an important but undefined role in viral RNA replication. Recently, we and other research groups reported that NS5A is also a key molecule for regulating the early phase of HCV particle formation by interacting with Core protein (12–14). NS5B functions as the RNA-dependent RNA polymerase (RdRp).

As is the case with other viruses, such as HIV, inhibitors targeting HCV-encoding enzymes that are essential to the viral life cycle have primarily been developed as anti-HCV drugs by pharmaceutical companies worldwide, as described in recent reviews (15–17). Many HCV-specific inhibitors are in preclinical development and several have reached clinical trials. Although NS3-4A protease has received the most attention as a drug target, its unusual structural features, such as shallow active binding site, make it difficult to design small molecule inhibitors. The first HCV protease inhibitor to enter clinical trials, namely BILN-2061 (by Boehringer Ingelheim), showed impressive antiviral activity in chronic hepatitis C patients (18). However, it was later withdrawn due to cardiotoxicity in animals. At present, VX-950 (by Vertex) (19) and SCH503034 (by Schering-Plough) (20), both of which have shown an ability to increase SVR rates in combination with IFN/ribavirin relative to standard therapy, are the most advanced HCV inhibitors. The inhibitors of another major target for anti-HCV drugs, namely NS5B, belong to two categories: nucleoside/nucleotide inhibitors target the catalytic site of the enzyme, whereas non-nucleoside inhibitors target the allosteric sites of the RdRp. Several compounds of both categories are currently in Phase I or II trials. Early clinical data indicate that several inhibitors are effective and well tolerated, although some of them have been discontinued after Phase II trials due to their side-effects.

NS5A, which has no enzymatic activity but appears to be a key player during regulation of the HCV lifecycle, would appear to be another valid target for antivirals. BMS-790052 (by Bristol-Myers Squibb) (21), which is in Phase I trials, was reported to cause an impressive reduction in HCV titers of all the genotypes tested at very low concentrations. Although its antiviral mechanism is still unclear, these promising results provide a proof of concept for targeting the non-enzymatic properties of HCV proteins when developing new chemotherapies.

4. Advances in HCV research concerning viral replication

An improved understanding of the viral lifecycle should contribute to the development of innovative therapeutic and preventive strategies for liver diseases caused by HCV infection. In addition to specific viral proteins, targeting host cellular factors which are vital for efficient viral replication may lead to the development of novel treatment strategies. In general, therapies against host factors are considered to present a low risk of generating drug-resistant viruses. This chapter summarizes the interactions between HCV and host factors that may be possibly involved in viral replication.

As mentioned above, IRES-dependent translation of the viral open reading frame yields a polyprotein precursor that is processed by cellular and viral proteases (22). In addition to proteins involved in the translational machinery, such as eukaryotic initiation factors, several host cell proteins, including polypyrimidine tract binding protein (PTB) (23), La autoantigen (24–26), RNA helicase A, nuclear factors NF90, NF110, NF45 (27), heterogenous nuclear ribonucleoproteins (hnRNPs) (28–30), and FUSE binding protein (31), have been reported to interact with the 5′- or 3′ UTRs of the HCV genome. Of these, hnRNPA1, which is implicated in several RNA metabolic process such as transport cellular RNAs, was found to bind to both 5′- and 3′ UTR regions (30). This protein also interacts with the scaffold protein septin 6 and HCV NS5B in cells, thus suggesting that hnRNPA1 and septin 6 play a key role in viral replication by forming a complex with NS5B and the viral RNA genome.

Cyclophilins (Cyps) and FKBP are classified as immunophilins that are capable of binding to the immunosuppressants cyclosporine and FK506, respectively. Cyps are present with peptidyl-prolyl cis-trans isomerase (PPI) activity and are involved in de novo protein folding and isomerization of native proteins. Furthermore, there is evidence to suggest that cyclophilin B (CypB) is a positive modulator of HCV RdRp in the viral replication complex (32). CypB interacts specifically with NS5B, thereby increasing the RNA binding property of NS5B. Recent studies demonstrated that cyclophilin A (CypA) functions as a cofactor for HCV infection, with the PPI activity of CypA being shown to play a key role in viral replication (33,34). Another immunophilin, FK506-binding protein 8 (FKBP8), interacts specifically with HCV NS5A and recruits a molecu-
lar chaperone, heat shock protein 90 (Hsp90), to the viral RNA replication complex as a result of an interaction between the carboxylate clump structure of FKBP8 and the C-terminal MEEVD motif of Hsp90. Knockdown of FKBP8 reduced the replication efficiency of the HCV genome in replicon and HCV-infected cells, thereby suggesting that FKBP8 is required for the replication of HCV via formation of the replication complex (35,36). Several studies demonstrated that Hsp90 and chaperones may participate in formation of the HCV replication complex through interaction with NS5A or other HCV proteins (37–40). The chaperone activity of Hsp90 contributes to the refolding of an unfolded protein in an ATP-dependent manner. Geldanamycin and its derivatives, which are known to be as specific Hsp90 inhibitors, are known to lead to degradation of the substrate proteins. Human butyrate-induced transcript 1 (hB-ind1), which possesses a significant homology with the chaperone p23, is also involved in the propagation of HCV through interaction with NS5A and Hsp90.

HCV NS5A has also been shown to interact with other cellular proteins such as vesicle-associated membrane protein (VAMP)-associated protein (VAP) subtypes A (VAP-A) and B (VAP-B) and FBL2 (41–45). VAP-A is detected in a detergent-resistant membrane fraction containing the viral replication complex, whereas the interaction of VAP-A with NS5A is required for efficient replication of HCV genomic RNA (41). VAP-B also participates in HCV replication through the formation of homo- and/or heterodimers with VAP-A (42). VAP-A and VAP-B form hetero- and homodimers through their transmembrane regions and interact with both NS5A and NS5B. VAP-C, the third subtype of VAP, which is an alternative spliced isoform of VAP-B, has recently been shown to act as a negative regulator for HCV propagation and to be partly involved in the determination of the tissue specificity of HCV replication (46). Statins, which decrease the production of mevalonate by inhibiting 3-hydroxy-3-methylglutaryl CoA reductase, have been shown to inhibit HCV RNA replication (44,47). This effect can be reversed by adding geranylgeraniol, thus suggesting that viral replication requires geranylgeranylated proteins. The geranylgeranylated protein, FBL2, which contains an F-box motif and is therefore likely involved in protein degradation, plays a role in HCV RNA replication via its interaction with NS5A (45).

Viral replication requires energy and macromolecule synthesis, and it is the host cells which provide the virus with the metabolic resources necessary for efficient replication. It is therefore highly likely that the interaction of viruses with host-cell metabolic pathways, including energy-generating systems, affects the viral growth cycle.

We have recently demonstrated that creatine kinase B (CKB), a key ATP-generating enzyme, interacts with HCV NS4A and is important for efficient replication of the viral genome (48). Recruitment of CKB to the HCV

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<th>Interacting HCV factor</th>
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<tr>
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<td>Nuclear factors NF90, NF110, NF45</td>
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replication machinery through its interaction with NS4A may have important implications for the maintenance or enhancement of the functional replicase activity in the membrane compartment containing the replication complex, where high-energy phosphorylation groups are required.

5. Future perspectives

Findings regarding the interplay between HCV and host cell factors have been accumulating since development of the cell culture system based on JFH-1 isolate (49–51) and the autonomous viral RNA replication system (52). A variety of host cellular proteins besides those described above have been identified as important cofactors for HCV replication, and are listed in Table 1. Further investigation of the functions of these host factors should improve our understanding of the mechanisms of HCV replication. Moreover, this improved knowledge could lead to identification of their cellular and/or viral targets and thus the development of novel therapeutic approaches for the treatment of hepatitis C.

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Conflict of interest

None to declare.

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