Hepatitis C virus: virology and life cycle

Chang Wook Kim¹ and Kyong-Mi Chang²,³

¹Department of Internal Medicine, The Catholic University of Korea College of Medicine, Seoul, Korea; ²GI/Hepatology Research Center, Philadelphia VA Medical Center; ³Department of Internal Medicine, University of Pennsylvania, Philadelphia, PA, USA

Hepatitis C virus (HCV) is a positive sense, single-stranded RNA virus in the Flaviviridae family. It causes acute hepatitis with a high propensity for chronic infection. Chronic HCV infection can progress to severe liver disease including cirrhosis and hepatocellular carcinoma. In the last decade, our basic understanding of HCV virology and life cycle has advanced greatly with the development of HCV cell culture and replication systems. Our ability to treat HCV infection has also been improved with the combined use of interferon, ribavirin and small molecule inhibitors of the virally encoded NS3/4A protease, although better therapeutic options are needed with greater antiviral efficacy and less toxicity. In this article, we review various aspects of HCV life cycle including viral attachment, entry, fusion, viral RNA translation, posttranslational processing, HCV replication, viral assembly and release. Each of these steps provides potential targets for novel antiviral therapeutics to cure HCV infection and prevent the adverse consequences of progressive liver disease.

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INTRODUCTION

Hepatitis C virus (HCV) is a hepatotropic RNA virus of the genus Hepacivirus in the Flaviviridae family, originally cloned in 1989 as the causative agent of non-A, non-B hepatitis.¹,² It causes acute and chronic hepatitis in humans and chimpanzees with a high propensity for chronicity. If untreated, chronic hepatitis C can progress to cirrhosis and hepatocellular carcinoma in a subset of patients.³ Until recently, the standard of care for patients with chronic hepatitis C involved dual therapy with pegylated interferon (IFN) alpha and ribavirin (PEG IFN/riba) in most countries. Dual PEG IFN/riba therapy achieved sustained virological response (SVR) in only 50% of patients infected with the more common HCV genotype 1 compared to 80% SVR rate in patients infected with HCV genotype 2 or 3.⁴ Moreover, combined PEG IFN/riba therapy is costly and prolonged (e.g. 24-48 weeks) with numerous adverse effects that are difficult to tolerate. In 2011, two inhibitors of the virally encoded NS3/4A protease became available as a part of standard therapy in some countries, especially against HCV genotype 1. Triple therapy combining one of these first-generation protease inhibitors with PEG IFN/riba therapy has improved SVR rate from around 50% to 70% in some clinical trial cohorts.⁵-⁸ However, this new regimen has limited efficacy in certain special populations (e.g. cirrhotic patients, transplant recipients, primary non-responders and hemodialysis patients) due to underlying IFN resistance, emergence of protease inhibitor resistance mutations and/or increased drug toxicity. Thus, there are ongoing efforts towards better therapeutic options with shorter treatment duration..."
with less toxicity and drug resistance—preferably as IFN-free, all oral combination regimens.

The recent HCV therapeutic development has been greatly enhanced by basic understanding of HCV virology and life cycle, through studies using HCV cell culture systems and replication assays. In this article, we review various steps in HCV life cycle that also serve as relevant targets for potential novel therapeutics, including viral attachment, entry, fusion, viral RNA translation, posttranslational processing, HCV replication, viral assembly and release (Fig. 1).

### HCV genome and its products

HCV is a positive-sense, single-stranded enveloped RNA virus approximately 9600 nucleotides in length. Approximately $10^{12}$ viral particles are generated daily in chronically HCV-infected patients. Due to the highly error prone RNA polymerase, HCV also displays remarkable genetic diversity and propensity for selection of immune evasion or drug resistance mutations. There are 6 major HCV genotypes (numbered 1-6) that vary by over 30% in nucleotide sequence from one another. The HCV genome has one continuous open reading frame flanked by nontranslated regions (NTRs) at 5' and 3' ends. The HCV 5'NTR contains 341 nucleotides located upstream of the coding region and is composed of 4 domains (numbered I to IV) with highly structured RNA elements including numerous stem loops and a pseudoknot. The 5' NTR also contains the internal ribosome entry site (IRES), that initiates the cap-independent translation of HCV genome into a single polyprotein by recruiting both viral proteins and cellular proteins such as eukaryotic initiation factors (eIF) 2 and 3.

The HCV open reading frame contains 9024 to 9111 nucleotides depending on the genotype. It encodes a single polyprotein that is cleaved by host and viral proteases into 10 individual viral proteins with various characteristics.

#### Structural proteins

HCV core is the viral nucleocapsid protein with numerous functionalities involving RNA binding, immune modulation, cell signaling, oncogenic potential and autophagy. HCV core protein also associates with the lipid droplets where HCV assembly also takes place. HCV E1/E2 are glycosylated envelope glycoproteins that surround the viral particles. HCV envelope is targeted by virus neutralizing antibody selection pressure with high degree of sequence variation that may render antibody responses ineffective and contributes to HCV persistence.

The small ion channel protein p7 is downstream of the envelope region and is required for viral assembly and release.

#### Nonstructural proteins

NS2 is the viral autoprotease that plays a key role in viral assembly, mediating the cleavage between NS2 and NS3. NS3 encodes the N-terminal HCV serine protease and C-terminal RNA helicase-NTPase. NS3 protease play a critical role in HCV processing by cleaving downstream of NS3 at 4 sites (between NS3/4A, NS4A/4B, NS4B/NS5A, NS5A/NS5B). It also cleaves the TLR3 adaptor protein TRIF and mitochondrial antiviral signaling protein MAVS, thereby blocking the cellular type I IFN induction pathway. NS3 is one of the key targets for HCV antiviral drug development. NS4A forms a stable complex with NS3 and is a cofactor for NS3 protease. The role of NS4B is not well understood, although it is known to induce the membranous web formation. NS5A is a dimeric zinc-binding metalloprotein which binds the viral RNA and various host factors in close proximity to HCV core and lipid droplets. Inhibitors of HCV NS5A showed antiviral effect in patients and are in rapid clinical development. Finally, NS5B is the RNA-dependent RNA polymerase (RdRp) which is also being actively targeted for antiviral drug development.

Collectively, these proteins also contribute to various aspects of HCV life cycle, including viral attachment, entry and fusion, HCV RNA translation, posttranslational processing, HCV replication, virus assembly and release.