Anti–Hepatitis C Virus Drugs in Development

Esperance A. K. Schaefer Raymond T. Chung

Massachusetts General Hospital, Department of Medicine, Gastrointestinal Unit, Boston, Massachusetts

Development of robust cell culture models for hepatitis C viral infection has greatly increased our understanding of this virus and its life cycle. This knowledge has led to the development of many drugs that target specific elements of viral replication, including viral proteins and host factors required for replication. The NS3/4A serine protease inhibitors were the first of these to be used in the clinic, and reagents that target other elements of the viral lifecycle are in advanced stages of clinical development. These include new NS3/4A protease inhibitors, NS5B RNA-dependent RNA polymerase inhibitors, NS5A inhibitors, and host-directed antivirals, such as cyclophilin inhibitors. Alternative interferons with possibly improved tolerability, specifically interferon-λ1 (interleukin-29), are also under development. These new reagents against hepatitis C virus should lead to highly effective, well-tolerated, and likely interferon-sparing therapies in the next several years.

Keywords: Protease Inhibitors; NS5B Inhibitors; NS5A Inhibitors; Host Targeted Anti-Virals.

Chronic infection with hepatitis C virus (HCV) affects >170 million individuals—approximately 3% of the world population—and is responsible for approximately 350,000 deaths every year.[1] The virus is cleared spontaneously in only about 20% of individuals[2,3]; chronic infection frequently progresses to cirrhosis, end-stage liver disease, hepatocellular carcinoma, and death. Until recently, the standard of care for all genotypes of HCV was the combination of pegylated-interferon (PEG-IFN) and ribavirin (RBV). The goal of therapy is a sustained virologic response (SVR), defined as the persistent absence of HCV RNA 24 weeks after the completion of therapy. An SVR is considered to be a cure of the disease—the virus remains undetectable in 99% of treated patients who achieve an SVR.[4] The combination of PEG-IFN and RBV lead to an SVR in only 45%–50% of patients with HCV genotype 1 infection.[5] However, in recent years, a number of new drugs against HCV have emerged. Direct-acting antivirals (DAAs) are specifically designed to inhibit viral targets, whereas host-targeted antivirals block host factors that are important for the viral lifecyle. Additionally, alternative interferons could be effective antiviral agents, without the side effects of IFN alfa.

Development of DAAs and host-targeted antivirals came rapidly on the heels of in vitro models of viral replication and the viral lifecycle,[6,7] which improved our understanding of viral structure, entry, replication, and host factors required for the propagation of infectious virus. The promise of the cell culture systems has come to fruition during the past several years. The first DAAs, telaprevir and boceprevir, were approved by the US Food and Drug Administration and the European Medicines Agency in 2011 for the treatment of chronic HCV genotype 1 infection. Numerous additional agents and viral targets are in various stages of clinical and preclinical testing and could revolutionize the way HCV is treated in the years to come.

Viral Structure, Lifecycle, and Genetic Variability

The new antiviral agents target several steps in the viral lifecycle. HCV is a small, positive-sense, single-stranded RNA virus with a 9.6-kb genome. The virus circulates as a highly lipidated molecule that closely resembles host lipoproteins.[8] Once inside the cell, the viral genome is exposed and translated into a polypeptide of ~3000 amino acids (Figure 1). This polypeptide is cleaved by a combination of host and viral proteases into 10 viral proteins. The viral protease NS3/4A is required for the cleavage of downstream, nonstructural proteins, including the NS5A protein and the NS5B RNA-dependent RNA

Abbreviations used in this paper: DAA, direct-acting antivirals; EVR, early virologic response; HCV, hepatitis C virus; IL, interleukin; miRNA-122, microRNA-122; NI, nucleos(t)ide inhibitor; NNI, non-nucleoside inhibitor; PEG-IFN, pegylated-interferon; RBV, ribavirin; RdRp, RNA-dependent-RNA polymerase; SOC, standard of care; SVR, sustained virologic response; SVR12, SVR 12 weeks after therapy.

0016-5085/$36.00
doi:10.1053/j.gastro.2012.02.015

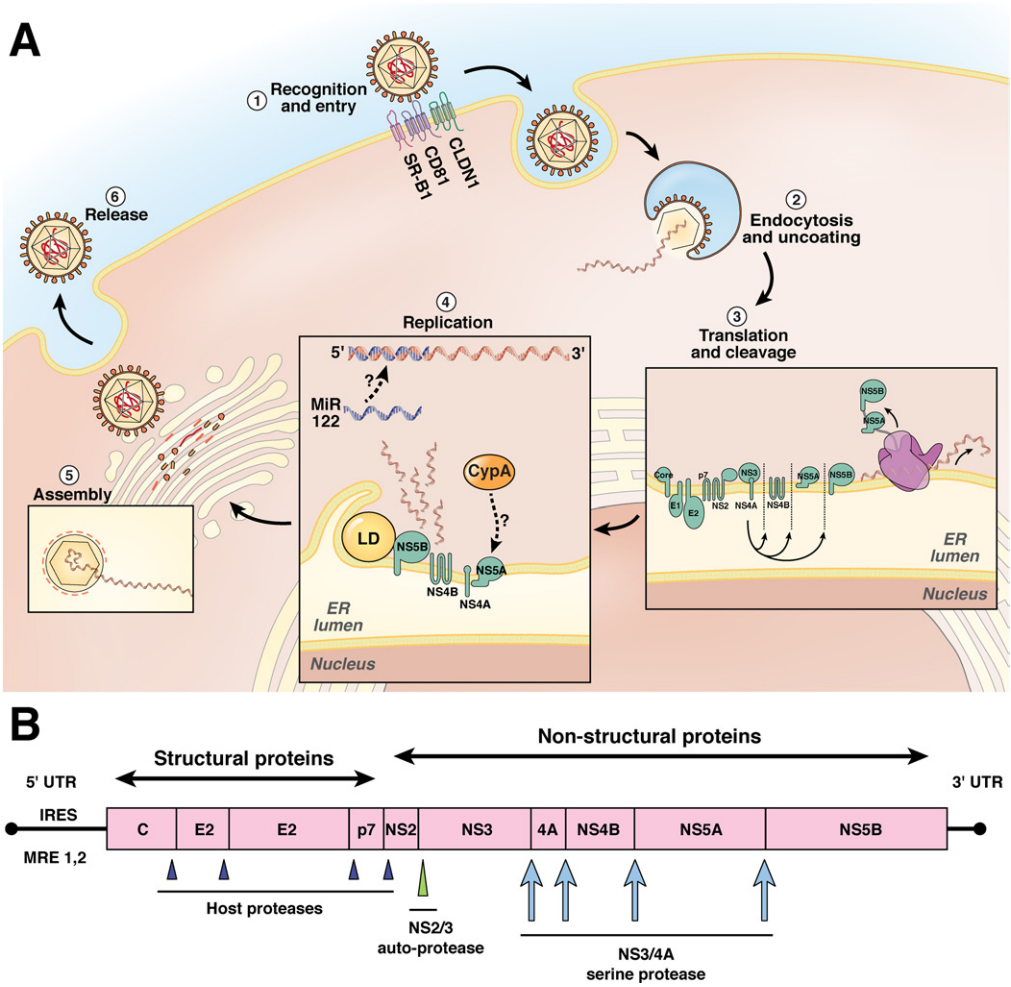

Figure 1. HCV viral lifecycle, HCV polypeptide structure, and cleavage sites. (*A*) The HCV viral lifecycle. The virus circulates as a highly lipidated lipoviral particle (LVP). The LVP requires several cells surface receptors for entry (*step 1*) into the hepatocyte, including scavenger receptor class B1 (SR-B1), CD-81, claudin (CLDN1), and occludin (*not pictured*). Once internalized, the viral genome is uncoated, revealing the naked viral RNA and viral nucleocapsid. The viral RNA is translated by host ribosomes into the viral polypeptide (*step 3*), which is then cleaved by a combination of host and viral proteases into the 10 viral proteins. Replication occurs at an endoplasmic reticulum membrane–derived replication complex (the membranous web), which includes the lipid droplet (LD) and nonstructural viral proteins NS4A–NS5B (*step 4*). Viral replication is also dependent on the participation of key host factors, which include miR-122 and cyclophilin A (CypA). The newly synthesized viral RNA is assembled into new LVP by the Golgi apparatus and subsequently released by the cell (*steps 5 and 6*). (*B*) HCV viral genome. The viral genome is a positive-sense, single-stranded RNA genome. The 5′ untranslated region (UTR) contains 2 important domains. The internal ribosome entry site (IRES) directs translation in a cap-independent manner. The 5′ UTR also contains 2 recognition sites by miR-122 that are critical for viral replication. After translation, a single viral polypeptide is generated. The structural proteins are cleaved by host proteases. The NS2/3 autoprotease cleaves the NS2–NS3 junction. The NS3/4A protease initially serves as an autoprotease and separates NS3–NS4A, but then subsequently cleaves the remaining nonstructural proteins.

polymerase (RdRp). The nonstructural proteins contribute to the formation of the viral replication complex and are required for viral propagation. Viral replication also requires many host factors,[9–12] such as proteins involved in lipid metabolism and microRNA-122 (miR-122), which can be targeted to impair viral replication.

HCV has a large amount of genetic variation—an important factor to consider in drug development. There are 6 known major genotypes and >100 subtypes of HCV. The virus produces approximately 10 infectious virions each day in an infected adult,[12] and the RdRp NS5B has no proofreading ability, producing mutations at a rate of 10^{-4} per nucleotide.[13] The virus therefore circulates in an individual as a quasi-species of numerous genetic variants. Statistical modeling experiments predict that, given the small size of the viral genome and high rate of replication, viruses with a change at every single nucleotide and every combination of 2 nucleotides in the genome have been produced in an individual before therapy.[14] Sequence analysis of virus isolated from treatment-naïve patients found that almost 9% harbor viral variants that have mutations that make them resistant to antiviral drugs.[15] Although DAAs have promise as targeted therapies, there is risk for rapid development of resistance.

DAAs

NS3/4A Protease Inhibitors

Inhibitors of the NS3/4A protease are attractive targets for drug development because they inhibit cleav-