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Inhibition of the replicative cycle of hepatitis C virus

Richard Bethell, George Kukolj and Peter W. White

It is estimated that 170 million people globally are infected with the hepatitis C virus (HCV). Chronic HCV infection can result in the development of liver cirrhosis and hepatocellular carcinoma, and therefore represents a substantial public health problem. Current treatment for patients infected with HCV is the combination of pegylated interferon-γ and ribavirin, a treatment that can achieve a sustained virological response, that is, a long-term clearance of detectable virus from the plasma. However, both drugs have poor safety profiles, resulting in their contraindication in many patients, and have limited effectiveness, especially against HCV genotype 1. As a result, there has been considerable interest over the past 15 years in identifying specific inhibitors of HCV replication that could be used either as an adjunct to current therapy or in place of it. This poster summarizes the replicative cycle of HCV and the main targets for specific antiviral agents that are currently being developed.

E2
El
Core
RNA
Viral collection or contact
Viral receptor engagement
Cytoplasm
El or E2 receptor binding

Viral entry targets

Cell surface contact. The heterodimeric E1 and E2 proteins are expressed on a virus envelope that is associated with low and very low density lipoprotein (vLDL)[1]. Initial host cell contact is mediated by interaction with the low density lipoprotein receptor and/or glycosaminoglycans. E2 also binds to DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) and L-SIGN (liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin), which is expressed on hepatic endothelial cells and may augment infection[1]. The asialoglycoprotein receptor (ASGP-R) may also interact with HCV structural proteins.

High-affinity receptors and entry. The virus subsequently binds to high-affinity receptors, including scavenger receptor class B type I (SR-BI) and the tetraspanin protein CD81, through the ecto-domain of E2. It then binds to claudin 1 (CLDN1) and occludin[1,2], both of which are expressed at tight junctions of polarized hepatocytes. Internalization of the virus proceeds through clathrin-dependent endocytosis, and viral membrane fusion takes place in the acidified endosome. This releases the viral core into the host cytosol, where uncoating and disassembly of the virus capsid releases the RNA genome.

HCV morphogenesis targets

HCV assembly starts with recruitment of the core protein and NS5A to the surface of lipid droplets, followed by delivery of HCV RNA from the HCV replication complex to the nascent viral particle. The subsequent intracellular assembly of HCV particles resembles vLDL formation: it takes place at the endoplasmic reticulum and requires apolipoprotein B and the microsomal triglyceride transfer protein (MTP), and leads to the formation of particles with a range of buoyant densities[6,7]. Those particles with a low buoyant density are secreted through the secretory system and circulate in association with triglycerides and lipoproteins B and E, whereas those with higher buoyant densities appear to be targeted for intracellular degradation[7]. Other HCV proteins, such as p7, NS2 and NS3, participate in virus assembly, and could form additional targets for therapeutic intervention[8,9].

Endocytosis
Fusion
Viral core release
Endoplasmic reticulum
Polymerase inhibitors NS5B
NS3 Helicase inhibitors
3′
5′
RNA replication
RNA capture
Core
Lipid droplet
Transport from ER
Viral release
Translation
Processing
NS3
NS3 protease inhibitors
Non-structural protein
Viral budding and particle formation
Nucleus

Other possible drug targets

All proteins encoded in the small HCV genome are essential for viral propagation. Small molecules that directly or indirectly inhibit NS4A and NS5A function are in development, although no direct interactions of the molecules with these proteins have been demonstrated yet. Small molecules that bind to the internal ribosome entry site (IRES), p7 and NS4B have also been reported, but none has yet reached development; development of antisense RNAs specific for the IRES has not yet led to a proof of principle in clinical studies. In addition, a number of host-encoded targets have been identified, of which cyclophilin A is the most advanced. Non-immunosuppressive analogues of cyclosporin A that form complexes with cyclophilin A and do not inhibit calcineurin have clinical antiviral activity against HCV[10].

Target	Function	Attractive features	Challenges
IRES (340 bases)	Initiation of cap-independent translation	Highly conserved and structured	Specificity of non-oligonucleotide inhibitors has not been demonstrated
Core (191 amino acids)	Viral nucleocapsid and RNA packaging	Good sequence conservation across isolates	Protein is poorly structured prior to assembly; core–core interaction avidity can reduce potency of any inhibitors *in vivo*
p7 (63 amino acids)	Ion channel or porin	Class targeted for other viruses; biochemical inhibition of p7 recently demonstrated	Low sequence conservation
NS2 (217 amino acids)	Cysteine autoprotease	Crystal structure of C-terminal cysteine protease domain solved; cysteine proteases have been targeted successfully	Development of autoproteolysis assays is challenging; small molecule inhibition of activity not shown
NS4A (54 amino acids)	Part of NS3 protease structure; membrane anchoring of replication complex	One class of clinically validated inhibitors selects for resistance mutations in NS4A, suggesting that this may be a 'druggable' target	No structural information known, except that the central portion binds to NS3. Direct binding of ligands has not been demonstrated
NS4B (261 amino acids)	Possibly, formation of membranous web structure and assembly of replication complex and/or NTPase	ATPases and GTPases have previously been targeted successfully; NS4B inhibitors reported recently	Hydrophobic and poorly structured protein a challenge for biochemical analysis
NS5A (447 amino acids)	Possibly, regulation of RNA replication and assembly; multiple viral–viral and viral–host protein interactions	N-terminal half is well folded; crystal structure published. Many viral replication inhibitors select for resistance in NS5A and strong clinical validation achieved for at least one	C-terminal region not well structured *in vitro*; direct binding of ligands to protein has not been demonstrated

RNA replication targets

NS3 protease helicase. The amino-terminal third of the NS3 protein possesses serine protease activity that is essential for viral replication. This is the target of BILN 2061, the first small molecule inhibitor of HCV to be tested in humans[3]. Two other protease inhibitors, telaprevir and boceprevir, are in Phase III clinical trials and may become the first anti-HCV drugs, whereas several other protease inhibitors are in earlier-stage human trials[4]. The carboxy-terminal two-thirds of NS3 constitute the helicase domain, which is also essential for the replication of viral RNA. The helicase domain might stimulate the activity of NS5B polymerase, resolve RNA secondary structure immediately prior to replication by the polymerase and/or separate newly synthesized double-stranded RNA into separate positive and negative strands. No helicase inhibitors are currently reported in clinical trials or advanced stages of preclinical development, although this is an active area of research.

NS5B polymerase. The NS5B RNA-dependent RNA polymerase catalyses viral RNA synthesis and genome replication. It shares a common fold with other nucleic acid polymerases with characteristic thumb, finger and palm domains[5]. A well-conserved active site for nucleotide and metal cofactor binding allows for selective incorporation of nucleoside analogues, a promising class of anti-HCV compounds. Several pockets form binding sites for allosteric inhibitors that interfere with conformational changes required during the initiation of RNA synthesis.
Two loops (λ1 and λ2) bridge the fingers and the top surface of the thumb domain and might regulate RNA binding. Benzimidazole and indole-based inhibitors prevent the association of the N-terminal finger loop with the thumb domain by binding to thumb pocket 1, which is situated at the top of the thumb domain. Thiophenecarboxylic acid and dihydropyrone-based inhibitors bind to thumb pocket 2 at the base of the thumb domain, proximal to thumb pocket 1, and induce conformational changes that prevent the initiation of RNA synthesis. Two overlapping pockets, palm site 1 and palm site 2, are located near the enzyme active site. Palm site 1 binds benzothiadiazine and acylpyrrolidine analogues, whereas benzofuran-based derivatives bind to palm site 2.

Active site
Thumb site 1
Thumb site 2
Palm site 1
Palm site 2

The HCV genome and polyprotein

Envelope genes
Non-structural (viral replication) genes
5′ UTR, Core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B, 3′ UTR
Host protease cleavage sites
NS2/3 protease cleavage site
NS3 protease cleavage sites

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Supplementary information S1 | **Further reading**

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