Hepatitis C Virus: Molecular Pathways and Treatments

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Hepatitis C Virus (HCV) is a major cause of worldwide morbidity and mortality. The World Health Organization estimates around 150 million of the world’s population being chronically infected \([1]\). An estimated 2.7-3.9 million of the United States population is infected with chronic HCV. Approximately, two thirds of those infected do not know their diagnosis \([2,3]\). A hallmark of HCV is its propensity to establish persistence, with 75-85 % of the infected individuals unable to eliminate the virus without therapy \([4]\). HCV has also been implicated with HCV-associated metabolic syndrome, hypobetalipoproteinemia and insulin resistance \([5]\). As newer molecular targets for HCV treatment are being researched, a comprehensive review of the HCV genome and its lifecycle may serve as an essential tool in its study.

### HCV Genotype and Epidemiology

The Flaviviridae families of viruses are divided into three genera, namely the Flavivirus, Pestivirus, and the Hepacivirus. The HCV belongs to Hepacivirus genus. There are six major genotypes and each differs by at least 30% of their nucleotide sequence. Each genotype in turn contains many subtypes and those are alphabetically designated as a, b, c, etc. Each subtype can have as much as 20% nucleotide sequence diversity. The lack of proof reading capacity in the viral RNA polymerase, the short half-life of the virus (few hours) and an estimated 1012 virion production per day are the basis for the genetic diversity of the virus. The genotypes 1, 2 and 3 have the biggest geographical distribution. HCV genotypes 1a and 1b are the most common genotypes, accounting for approximately 60% of worldwide HCV infections \([6]\). HCV type 1a infections predominate Northern Europe and Americas, and type 1b predominate Southern and Eastern Europe and Japan \([7]\). HCV type 4 is found mostly in the Middle East and Central African countries \([7]\). HCV type 5 has been primarily isolated from South Africa, and genotypes 3 and 6-11 are widely distributed across Asia \([6,7]\).

The determination of specific HCV genotype may determine the type and duration of therapy, thereby assisting in predicting a response to antiviral treatment and outcomes \([8]\).

### Genome Structure

HCV is about 9.6 kb positive strands RNA virus with a radius of about 25 nm. The density of the HCV virion is around 1.24 g/cm3 in Cesium Chloride (CsCl), with a sedimentation coefficient of 200 S in sucrose gradients. A lighter fraction of the virion (density of 1.04 to 1.06 g/cm3) is due to its association with serum beta-lipoprotein. The denser fraction of the virion (density of around 1.17 g/ml in sucrose) is involved in the formation of noninfectious immune complexes \([9]\). The nucleocapsid of the virus has a density of 1.25 g/cm3 in sucrose \([9]\). HCV lacks efficient proofreading ability during replication, making the virion highly mutable. This behavior of the virus brings up the notion that HCV probably persists as a collection of viral quasi-species \([10]\).
(ORF). It codes for a polypeptide with about 3000 amino acids depending on the HCV genotype. The 5' UTR is not capped, and it folds into a complex secondary RNA structure. This structure is formed with a portion of the core-coding domain, an internal ribosome entry site (IRES - that mediates direct binding of ribosomal subunits), and cellular factors which lead to translation. Recent research showed that abundant liver-specific microRNA (miRNA), miR-122, bound to the HCV 5' UTR and enhanced viral RNA replication. This finding uncovered a new avenue for possible antiviral intervention by antagonizing the function of miR-122. This 5' UTR contains 341 Nucleotide (nt) located upstream of the ORF translation initiation codon, the most conserved region of the genome [11]. The 3' UTR is shorter and less structured than the 5' UTR. The 3' UTR contains approximately 225 nt. It is organized in three sections from 5' to 3'; these three sections include a variable region of 30–40 nucleotide, a long poly(U)-poly(U/UC) tract and a highly conserved 3'-terminal stretch [12]. This end is essential for viral replication (Figure 1).

HCV Proteins

The HCV ORF contains 9024 to 9111 nucleotides. However, the number of nucleotides is dependent on the genotype. The ORF encodes at least eleven proteins, including three structural proteins, a small protein p7 ion channel, six Non-Structural (NS) proteins and the Frameshift 'F' protein. The structural proteins consist of core 'C' proteins and envelop 'E' proteins. The function of P7 protein is undetermined. The non-structural proteins consist of NS2, NS3, NS4A, NS4B, NS5A and NS5B types.

Structural proteins

a) Core 'C' Protein: The HCV core protein 'C' is a basic RNA-binding protein. This core forms the viral capsid. This C protein consists of three domains: an N-terminal hydrophilic domain of 120 amino acids, a C-terminal hydrophobic domain of about 50 amino acid, and a single peptide of 20 amino acids [13]. These core proteins are either membrane-bound or membrane-free, and exist as either a dimeric or multimeric form [14]. They interact with cellular proteins and are important in the viral lifecycle [15]. The HCV core proteins regulate the activity of cellular genes (c-myc and c-fos), and have apoptotic and anti-apoptotic functions. These cellular regulatory activities control the transcription of viral promoters [16].

b) Envelope 'E' Glycoproteins: The envelope glycoproteins consist of E1 and E2, an essential component of the HCV virion that is vital for viral entry and fusion. Two areas of hydrophobic amino acids, which are separated by a short polar regions of fully conserved, charged residues, make up the transmembrane domains of E1 and E2. These envelope glycoproteins are localized in endoplasmic reticulum compartment, and have multiple functions. These functions include membrane anchoring, endoplasmic reticulum localization and heterodimer assembly [17].

c) Frameshift 'F' Protein: The F protein or Alternate Reading Frame Protein (ARFP) is created due to a ribosomal frame-shift at the N-terminal core-encoding region of the viral polypeptide. The clinical significance of this protein is evident in recent studies, which have identified antibodies to F protein in chronically HCV infected patients. This may suggest the production of the F protein during the infective phase of the virus [18].

Non-structural proteins

a) p7: p7 ion channel is a membrane polypeptide made up of 63 amino acids. p7 transmembrane domains are arranged in α-helices that are connected by a cytoplasmic loop. Though p7 is not required for RNA replication in vitro, it seems to determine the productivity of infection in vivo. Mutations of the cytoplasmic loop in chimpanzees have shown to suppress infectivity by affecting intra-liver transfection of HCV cDNA [19].

b) NS2: NS2 is a non-glycosylated transmembrane protein. It is a catalytic short-lived protein that loses its protease activity after self-cleavage from NS3. It is essential for the complete replication of the virus. A phosphorylation dependent degradation of NS2 occurs by means of protein kinase casein kinase 2 [20].

c) NS3-NS4A: NS3 protein contains a serine protease domain at its N-terminal (1/3rd region) and a helicase/NTPase domain at its C-terminal (2/3rd region). NS4A is a cofactor for the NS3's protease activity [21]. NS3–4A serine protease cleaves and thereby inactivates two crucial adaptor proteins in innate immune sensing which might have a crucial implication on the pathogenesis and persistence of HCV infection. Just as in HIV treatment, the NS3-4A serine protease has emerged as a prime target for the design of specific inhibitors as antiviral agents. NS4A interacts with NS4B protease inhibitors - Boceprevir and Teleprevir have been approved for the treatment of genotype 1 HCV infections [22]. Other PI in development includes ABT-450 and TMC 435. The NS3 helicase works on unwinding of the double stranded RNA for replication.

d) NS4B: NS4B is an integral membrane protein. Its functions have been elucidated as membrane anchoring for the replication complexes. Endoplasmic Reticulum (ER) derived membrane localization. It serves as a scaffold for the HCV replication complex. It has a possible role in HCV carcinogenesis, impairment of ER function, and regulation of both viral and host translation [23]. The membrane functions of NS4B are carried out by least four trans-membrane domains and an N-terminal amphipathic helix. A small molecule drug ACH-806 is in phase 1b/2 clinical trial, as an NS4B antagonist for HCV treatment [24].

e) NS5A: NS5A is a phosphorylated zinc-metalloprotein. It plays an important role in regulation of cellular pathways, membrane localization, transcriptional activation, and assembly of the replication complex [25]. Although NS5A's role in replication is not entirely clear, it seems associated with lipid rafts. Lipid rafts are derived from intracellular membranes that bind to the C-terminal region of a vesicle-associated membrane-associated protein, crucial for the formation of the HCV replication complex [25]. It is hypothesized that it forms a two-dimensional array on intracellular membranes; thereby creating a 'basic railway' that would allow the sliding of RNA. It also might function as a molecular switch between replication and assembly of the virus. NS5A also plays a role in interferon resistance by inhibiting RNA-activated Protein Kinase. NS5A inhibitors are currently in phase II and III stages of clinical trial [26].

f) NS5B: NS5B is an RNA dependent RNA polymerase (RdRNP) that is responsible for the replication of the virus. The first step is the formation of the negative strand complementary RNA which will be the template for the subsequent positive strand HCV RNA replication. The conformational structure is such that it creates a completely enclosed replication site. Nucleoside and non-nucleoside NS5B inhibitors are in various stages of clinical trials [27].

HCV Lifecycle

The precise mechanisms of HCV replication are still poorly understood, however studies predict its replication processes to be
similar to other positive-strand RNA viruses. The HCV virus interacts with a number of cell surface receptor molecules such as CD81 and LDL receptors to enter the cell [9]. The initial attachment and penetration processes lead to local pH changes. These pH changes in effect lead to cellular and viral endosomes fusion and conformational changes to the envelope proteins [9]. The NNSB RNA-dependent RNA polymerase now catalyzes a two-step process of HCV replication. The initial step involves intermediate complementary negative-strand synthesis carried out by positive-strand genomic RNA template. A negative-strand RNA now serves as a template to produce numerous progeny strands. These progeny strands are positively charged and will eventually be used for polyprotein translation, synthesis of viral RNA replication. Mol Cell 18: 425-434.

References

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