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MOLECULAR VIROLOGY OF HCV

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Abstract

Recently, impressive progress has been made in understanding the molecular biology of the hepatitis C virus (HCV). The replication system revolutionized the study of HCV RNA replication and facilitated drug discovery. The new HCV glycoprotein functional assay system enables the verification of HCV receptor candidates and The study of the cell entry mechanism. Recently, recombinant infectious HCV can be produced in cell culture, making all steps in the life cycle of the virus, including entry and release of virus particles, suitable for routine testing. In this review, we summarize the latest developments and discuss future research directions.

Keywords: *hepatitis C virus, polymerase, helicase, protease, viral proteins.*

Introduction

The hepatitis C virus (HCV) is a member of the Flaviviridae family of viruses, which are associated with both human and animal diseases. The Flaviviridae family comprises at least three distinct genera: pestiviruses, which cause disease in cattle and pigs; flaviviruses, which are the most important cause of diseases such as dengue fever, yellow fever and hepaciviruses, whose sole member is HCV (1).

HCV genomic organization

HCV is a single-stranded, positive-sense RNA virus with a genome of approximately 9,500 nucleotides coding for a polypeptide with a length of about 3,000 amino acids (2). This polyprotein precursor is co-and posttranslationally processed by cellular and viral proteases to yield the mature structural and nonstructural proteins C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B (3). The N-terminal part of this polyprotein encodes three to four structural proteins, and the rest are the nonstructural proteins. There are short nontranslated regions (NTRs) at each end of the genome (5'-NTR, 3'-NTR) which are required for replication of the genome, a process that has recently been found to additionally require a cis-acting replication element in the coding sequence of NS5B (4). Translation of viral proteins is dependent on an internal ribosomal entry site (IRES) in the 5'-NTR, which comprises a complex RNA structure element that interacts directly with the 40S ribosomal subunit during translation initiation (5). It also contains RNA elements implicated in the genome replication (6).

The 3'-NTR is 200 to 235 nucleotides long and can be divided into three regions; first from the 5'-end is a region of variable sequence of length from 27 to 70 nucleotides, followed by a poly uracil/ uracil cytosine stretch, and finally a very conserved and structured 98 nt X-region at the end of the 3`-NTR (7). The role of the variable region (VR) is not clear. It has been shown that VR is not required for viral replication (7). The poly-U/UC region, is essential for replication in vivo, it interacts in vitro with several cellular proteins (polypyrimidine-tract-binding-protein) (PTB) which perhaps, regulate viral replication (8). The very conserved X-region interacts specifically with recombinant HCV RNA polymerase and PTB in vitro. It is also required for viral replication (9).

Molecular aspects of viral proteins

Structural proteins

The structural proteins of HCV, the capsid protein (C) and the envelope glycoprotein's E1 and E2, are the components of the viral particle (10).

Core protein

Core protein plays a major role in HCV-induced viral hepatitis (11). The HCV core protein is a highly basic, RNA-binding protein which presumably forms the viral nucleocapsid. The core protein has been reported to interact with numerous cellular proteins and to affect host cell functions such as gene transcription, lipid metabolism, apoptosis and various signalling pathways (11). Further, it has been associated with the induction of steatosis and HCC (10).

There are at least two forms of HCV core protein, one being the initial product of 191 amino acids and the other generated by additional cleavage at it's C-terminus between amino acid 174 and 191, with the latter being likely a component of native viral particles (12).

HCV core protein is localized predominantly in the cytoplasm, with a minor fraction with a different higher-order structure being localized in the nucleus. HCV core protein exhibits versatile functions: it enhances or suppresses apoptosis, depending on the apoptosis-inducing stimuli and the cell type used (12,13).

HCV core protein has also been shown to cooperate with the ras oncogene to transform rodent cells into a tumorigenic phenotype (13) and transgenic mice expressing HCV core protein in the liver tend to develop HCC (13). HCV core protein regulates transcription, either negatively or positively, of certain cellular and viral genes, such as c-myc, c-fos, p53, and hepatitis B virus (14), probably through differential activation of transcription factors and other intracellular signalling pathways (13,14).

Thus, HCV core protein influences a variety of cellular functions, such as gene expression and protein synthesis, and is likely responsible for certain phenotypic changes of HCV-infected cells. The relevance of those different forms in natural infection has not been established. The core protein has been extensively studied and appears to play

multiple roles in various cellular signaling pathways, and potentially in oncogenesis (15).

Envelope glycoproteins

The next two proteins are the envelope glycoprotein's E1 and E2. They are believed to associate as a non-covalent heterodimer (16) and are exposed on the virion surface. E2 mediates viral binding to the cells, by a decrease of infectivity by incubation of the virus with anti-E2 antibodies but the HCV receptor has not yet been identified (16).

Among the potential candidates, CD81 (a tetraspanin expressed on hepatocytes and B lymphocytes that thought to function as a cellular receptor or coreceptor for the virus) a biquitous molecule, binds to E2 but does not mediate viral entry. The other candidate is the lipodensity lipoprotein receptor (LDLR) (17). Since circulating HCV particles in sera of the patients are associated with lipids and lipoproteins, the nonspecific uptake of the virus through the LDLR is possible. E1 and E2 contain both an endoplasmic reticulum (ER) retentionsignal, which limits their intracellular localization (17).

Cell-surface expression of E1 and E2 is very limited, which may explain why the infected cells can escape from the immune recognition (18). E1 and core can interact with each other, suggesting that the viral nucleocapsid is enveloped through this interaction. Beside its structural role, E2 has been shown to modulate the interferon-alpha (IFN-α) response (18).

Alternative reading frame protein

The synthesis of a protein encoded by an alternative reading frame within the core region was reported by several groups. It was designated ARFP (alternative reading frame protein) or F (frameshift) protein and comprises up to 160 amino acids. The ARFP/F protein is dispensable for HCV RNA replication. Whether it is expressed during natural HCV infection has still to be clarified (19).

P7

P7 is a 63-amino acid polypeptide located at the junction between the structural and nonstructural region. It is unknown whether P7 is packaged into viral particles. It is composed of two transmembrane domains and has recently been reported to form hexamers with ion channel activity (20). It is believed that p7 could be important for viral assembly because the corresponding protein of the related bovine viral diarrhea virus (BVDV) is essential for the production of infectious progeny virus but not for RNA replication (20).

Non-structural proteins

The nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) have various functions involved in viral RNA replication or proteolytic processing of the polyprotein (21). NS2 and NS3 are the two viral proteases responsible for the cleavage of all the NS proteins (21).

NS2-3 auto protease

The NS2/3 junction is cleaved by a remarkable autoprotease consisting of NS2 and the N-terminal third of NS3. Although NS2-3 protease activity is required for the replication in vivo, it is dispensable for replication of subgenomic replicons in vitro. It is unclear whether NS2 fulfills any further functions after separation from NS3 (22).

NS3-4A

NS3 is a multifunctional protein because it harbors a serine protease located in the N-terminal one-third that is responsible for the downstream cleavage in the nonstructural region and a NTPase/RNA helicase domain in the C-terminal two-thirds. NS4A, a 54 amino acid polypeptide, targets NS3 to intracellular membranes and is required as a cofactor for the NS3 serine protease. The crystal structure of the NS3-4A complex revealed that NS4A is an integral component of the enzyme core (23). Surprisingly, the NS3 serine protease recently turned out to influence the innate cellular host defense by inhibition of RIG-I and TLR3 signalling (23,24).

This observation renders the NS3 protease particularly attractive as an antiviral target (24). Serine protease inhibitors have emerged as extremely efficient antiviral components (23). The enzymatic activity of the NS3 NTPase/helicase activity is indispensable for RNA replication. Putative functions during replication could be to unwind replicative double strand RNA intermediates, to eliminate RNA secondary structures or to separate the genome from nucleic acid binding proteins (23,24).

The advances in the understanding of the molecular mechanisms of this enzyme could enable a specific inhibition as a novel antiviral strategy (25).

NS4B

Due to it's very hydrophobic properties, NS4B belongs to the difficult-to-study HCV proteins that are poorly understood. So far, it is known that NS4B is a 27-kDa integral membrane protein that localizes to an ER derived membranous compartment (26). Interestingly, the expression of NS4B induces a specific membrane alteration, designated as membranous web that serves as a scaffold for the formation of the viral replication complex (26).

NS5A

NS5A is a phosphorylated zinc metalloprotein of unknown function (27). Little efforts has been devoted to a rigorous biochemical characterization of this protein and Limited proteolysis experiments recently allowed the definition of three protein domains within the cytosolic domain (27).

More recently, the three-dimensional structure of the N-terminal domain I could be resolved by crystallography. It 'claw like' structure which believed to provide an RNA binding site that could be involved in regulated genome targeting within the replication complex (27).

NS5B

The key enzyme of the replicase that promotes synthesis of new RNA genomes is the NS5B RNA dependent RNA polymerase (RdRp). NS5B is a tail anchored protein, characterized by a transmembrane domain at the C-terminus of the protein responsible for posttranslational membrane targeting (28).

Replication proceeds via synthesis of a complementary minus-strand RNA using the genome as a template and the subsequent synthesis of genomic plus strand RNA from this minus-strand RNA intermediate. As central component of the HCV replicase, NS5B has emerged as a major target for antiviral intervention (28).

Zn-II activate the activity of antiviral agent against different viruses such as SARS-CoV-2, Influenza A virus subtype H5N1 and H9N2 (29-33)

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