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HCV infection, IFN response and the coding and non-coding host cell genome

Elena Carnero, Puri Fortes*

Center for Applied Medical Research (CIMA) and Navarra Institute for Health Research (IdiSNA), Department of Gene Therapy and Hepatology, University of Navarra, Pamplona, Spain

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ABSTRACT

HCV is an ideal model to study how the infected cell is altered to allow the establishment of a chronic infection. After infection, the transcriptome of the cell changes in response to the virus or to the antiviral pathways induced by infection. The cell has evolved to sense HCV soon after infection and to activate antiviral pathways. In turn, HCV has evolved to block the antiviral pathways induced by the cell and, at the same time, to use some for its own benefit. In this review, we summarize the proviral and antiviral factors induced in HCV infected cells. These factors can be proteins and microRNAs, but also long noncoding RNAs (lncRNAs) that are induced by infection. Interestingly, several of the lncRNAs upregulated after HCV infection have oncogenic functions, suggesting that upregulation of lncRNAs could explain, at least in part, the increased rate of liver tumors observed in HCV-infected patients. Other lncRNAs induced by HCV infection may regulate the expression of coding genes required for replication or control genes involved in the cellular antiviral response. Given the evolutionary pressure imposed by viral infections and that lncRNAs are specially targeted by evolution, we believe that the study of proviral and antiviral lncRNAs may lead to unexpected discoveries that may have a strong impact on basic science and translational research.

1. HCV

Hepatitis C (HCV) is a hepatotropic virus that affects ~3% of the world's population (150–200 million people)(Mohd Hanafiah et al., 2013). Prevalence is especially high in Asia and the North of Africa. Viral infection, commonly due to transfusion of infected blood or other unsafe medical procedures, causes an acute infection with mild symptoms that resolves spontaneously in about 20% of the cases. The remaining 80–85% develop a chronic infection that may remain undiagnosed for decades. This occurs because chronic infection is often asymptomatic, but the liver injury caused by the virus may progress to liver steatosis, fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Around 20% of people chronically infected with HCV develop cirrhosis over 30 years. Once cirrhosis is established, patients have a 20-fold greater risk of developing HCC, at a rate of ~3% per year (Ghany et al., 2009). Thus, 27% of the liver cirrhosis and 25% of HCC worldwide are caused by a primary infection with HCV. More than 350,000 people die per year from HCV-related liver diseases (Webster et al., 2015).

While there are several diagnostic tests for HCV, including ELISA to detect antibodies against HCV proteins or PCR to quantify HCV genomes in serum, it is believed that most infected people (50–90% in the United States and Canada) remain undiagnosed (Galbraith et al., 2015). There is no vaccine for prevention of HCV infection. Until very recently, the treatment of choice has been a combination of pegylated interferon (IFN) alpha with the antiviral nucleoside analogue ribavirin (McHutchison et al., 1998). This therapy is effective in 50–80% of patients, depending on the HCV genotype. However, this treatment is expensive and often associated with side effects that force discontinuation of the therapy. Recently, several inhibitors that target the viral NS3 protease (boceprevir and telaprevir), the RNA dependent RNA polymerase NS5B (sofosbuvir) or NS5A (ledipasvir and dataclasvir) have been approved for the treatment of HCV (Poordad and Dieterich, 2012). The combination of sofosbuvir with NS5A inhibitors has shown sustained viral response rates between 94% and 100%, raising the real possibility of a cure for HCV infection. Unfortunately, the extremely high cost of this therapy prevents most HCV infected patients from using this treatment. Further, caution should be taken before the cure for HCV can be claimed. Given the high prevalence of the infection and

* Corresponding author at: CIMA. Pio XII 55. Pamplona 31008. Spain. Fax: +34 948194717.
E-mail address: pfortes@unav.es (P. Fortes).

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the ability of HCV to generate escape mutants, research into HCV should not stop.

1.1. The HCV viral particle

HCV is a member of the Flaviviridae family with a single-stranded RNA genome of 9.6 Kb and positive polarity. Similar to other RNA viruses, HCV has an error-prone RNA-dependent RNA polymerase that produces a highly variable progeny. This has allowed an original HCV ancestor to evolve into the seven genotypes and more than 80 subtypes of HCV currently described, according to the nucleotide variation found among different HCV isolates (Jackowiak et al., 2014). The genotypes are found in different proportion in different parts of the world, with genotype 1a and 1b being the most common in the United States and Europe (Messina et al., 2015). Further, due to the high mutation rate, each infected individual harbors a collection of distinct but related HCV genomes generated by replication, known as quasi-species (Martell et al., 1992).

The viral particle is small (40–80 nm in size), enveloped, and contains the genetic material bound to the core protein (Gastaminza et al., 2010; Catanese et al., 2013). The viral genome, a positive stranded RNA, serves as messenger RNA (mRNA). The open reading frame is flanked by highly structured untranslated regions (UTR) that contain several domains important for viral replication. These domains are fairly well conserved compared to the rest of the genome, thus supporting their importance in viral fitness (Pineiro and Martinez-Salas, 2012). The 5′UTR includes an internal ribosome entry site (IRES) that allows the viral RNA to be directly translated in a cap-independent manner to produce the viral polyprotein. The open reading frame encodes a polyprotein cleaved co- and post-transcriptionally by viral and cellular proteases into the three major structural proteins (core, E1 and E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B). The viral particle is formed by the two envelope glycoproteins, E1 and E2, required for cell attachment and entry, and by the core capsid protein. P7, a protein from the viporin family that forms an ion-channel, participates in virus assembly and in the release of infectious virions (Atoom et al., 2014; Moradpour and Penin, 2013). NS2 autoprotease also participates in virus assembly and might affect replication indirectly, as the cleavage at the NS2/NS3 junction seems to be a rate limiting step and a fully processed NS3 protein is required for replication (Moradpour and Penin, 2013). NS3, NS4A, NS4B, NS5A and NS5B form the replicase complex. The viral helicase/protease NS3, has an essential role in the processing of the non-structural proteins and of some host cellular proteins, such as MAVS or TRIF (see below), involved in the antiviral response (Moradpour and Penin, 2013). NS3 helicase activity should play a role in viral RNA unwinding and replication and in viral assembly (Murray et al., 2008). NS4A is a cofactor that tethers NS3 to the endoplasmic reticulum (ER), and increases NS3 stability and protease and helicase activity. NS4B is involved in membrane remodeling and participates in virus packaging. NS5A forms a complex with viral RNA and several cellular and viral proteins. These interactions may be required for the multifunctionality of NS5A, which induces the formation of a membranous web (see below), is involved in replication and in viral assembly and affects the antiviral response (Gale et al., 1997; Pflugheber et al., 2002). NS5B is the RNA dependent RNA polymerase required for replication and HCV assembly (Gouklani et al., 2012).

1.2. The HCV replication cycle

Most HCV virions circulate in the blood as lipo-viro-particles (LVPs) (Fig. 1). LVPs contain the virion associated with very low or low-density lipoproteins (VLDLs and LDLs), high levels of triglycerides, Apolipoprotein (Apo) E, ApoC, ApoB and low levels of cholesterol or phopolipids (Bassendine et al., 2013). HCV virion association with lipids may help evasion from neutralizing antibodies and facilitates the infection of the hepatocytes, which are the primary target cells for infection. The exact mechanism of viral entry is still unclear. LVPs are trapped at the host cell membrane by low-affinity binding to glycosaminoglycans and the LDL Receptor (LDLR) (Barth et al., 2003). Then, the viral envelope proteins E1 and E2 bind different cellular proteins identified as receptors for viral entry such as scavenger receptor class B type 1 (SRB1) and CD81 (Bartosch et al., 2003b). Also required for entry are the tight junction proteins claudin-1 (CLDN1) and occludin (OCLN), the epidermal growth factor receptor (EGFR) and the Niemann-Pick C1-like cholesterol absorption protein (NPC1L1), which may bind to virion-associated cholesterol at a late stage of HCV entry (Evans et al., 2007; Lupberger et al., 2011; Ploss et al., 2009; Sainz et al., 2012). Entry occurs very likely by clathrin-mediated endocytosis (Blanchard et al., 2006). After entry, the virion undergoes uncoating driven by the acidification of the endosome that induces fusion of the viral envelope with the endosome and leads to the release of the viral RNA into the cytoplasm. The viral RNA locates to the rough ER for translation of the viral polyprotein that is then processed to the structural and non-structural proteins.

HCV replicates in association with a specialized membrane structure named membranous web (MW), formed by double-membrane vesicles (Moradpour et al., 2003; Romero-Brey et al., 2012) (Fig. 1). The MW most likely originates from ER membranes by the action of NS4B and NS5A, helped by the remaining components of the replication complex. NS5B, the viral RNA-dependent RNA polymerase, uses the viral genome as a template to produce negative-strand RNA intermediates that are then amplified to large amounts of new positive-strand RNA viral genomes. Nascent genomes can be translated, replicated or packaged into new viral particles.

During viral assembly, HCV structural proteins synchronize with the viral replication complex to form the virion (Fig. 1). The assembly process takes place in close proximity to membrane-bound lipid droplets (LDs), intracellular organelles storing cholesterol and triglycerides. LDs play an essential role in viral packaging and inhibition of the synthesis of their lipid components blocks viral assembly. Core protein and NS5A localize to LDs (Targett-Adams et al., 2008). NS5A, as part of the replication complex, connects virus replication and packaging. NS5A binds the core protein and the newly synthesized RNA, allowing the binding of core protein to the viral RNA to build a nucleocapsid attached to a LD (Masaki et al., 2008). Newly formed nucleocapsids seem to acquire their envelope after budding into the ER. The new virus particles are bound to VLDL or LDL components and are thought to use the secretory pathway to be released as LVPs (Lindenbach and Rice, 2003). Thus, HCV release is intimately associated with the synthesis of VLDLs and the lipid secretion pathway (Bassendine et al., 2013). Once released, LVPs can infect new host cells as described. However, cell-to-cell transmission has also been described as a route for HCV propagation in vivo, involves a similar set of cell receptors and may provide a way to avoid antibody neutralization (Timpe et al., 2008).

1.3. Models to study HCV replication

Research on HCV has expanded dramatically in the last decade, since the development of experimental model systems that sustain the complete virus cycle in cell culture and in small animal models. These systems allow the study of viral replication, the identification and functional analysis of host cell factors required for productive infections and the analysis of the mechanisms employed by the virus to establish chronicity and produce liver disease. Interestingly, these systems can also be used to identify viral or cellular