Structural Proteins of HCV and Biological Functions

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Abstract Hepatitis C virus (HCV) is a major causative agent of liver disorders and a major risk factor for hepatocellular carcinoma. The induction of hepatocellular carcinoma by HCV is thought to involve not only chronic inflammation, but also the biological activity of HCV components. Structural proteins of HCV are composed of the core protein and two envelope proteins, E1 and E2. The HCV core protein has been reported to exhibit multiple biological functions involved in lipid synthesis, iron metabolism, insulin response, oxidative stress and cell growth, and to thereby contribute to the development of carcinogenesis and metabolic disorders. Moreover, several reports suggest that envelope proteins also play an important role in viral entry as well as HCV-related pathogenic events. However, the mechanism by which the structural proteins induce hepatitis C-related disorders has not been fully understood. This review focuses on the current status of biological responses mediated by HCV structural proteins.

Keywords HCV • Structural proteins • Core protein • Envelope protein • Oxidative stress • Insulin resistance • Lipid metabolism • Mitochondria

Hepatitis C virus (HCV) possesses a genome consisting of a single positive strand RNA with a nucleotide length of 9.6 kb, which encodes a single polyprotein. This polyprotein is matured by processing dependent on host and viral proteases, resulting in structural and nonstructural proteins (Grakoui et al. 1993a, b; Harada et al. 1991; Hijikata et al. 1991). Structural proteins consisting of the core protein and two envelope proteins E1 and E2 occupy one third of the N-terminal region of the polyprotein, while the remaining viral proteins consist of the viroporin p7 and nonstructural proteins which form a replication complex with host factors (Grakoui et al. 1993c). The structural proteins and host lipid components are employed for

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formation of the viral particle (for review see (Moriishi and Matsuura 2012). The nucleocapsid consisting of mature core proteins and a viral genome is surrounded by an envelope composed of host lipids and viral envelope proteins.

The HCV core protein and envelope proteins are released from the viral polyprotein by host proteases. HCV structural proteins may provide a host for severe liver disorders over several decades of persistent infection. The HCV core protein is involved in formation of the viral particle as well as the induction of liver disorders, including metabolic diseases. In addition, it is more important that the core protein could induce hepatocellular carcinoma in mice regardless of other HCV viral proteins (Moriya et al. 1998). Accumulating evidence supports the notion that envelope proteins induce a stress response during persistent infection to lead to liver disorders. This review summarizes the biological functions of HCV structural proteins in the development of HCV-related disorders.

1 Maturation of HCV Structural Proteins for Assembly of Viral Particles

1.1 Processing and Modification of HCV Core Protein

Hepatitis C virus (HCV) belongs to the genus Hepacivirus of the *Flaviviridae* family. The *Flaviviridae* family is composed of four genera, Flavivirus, Pestivirus, Pegivirus and Hepacivirus. The viral genomic structures and the composition of viral proteins differ among these genera. The capsid, or core, protein is encoded in the 5′-regions of the viral genomes of three of the four genera, with the exception being Pegivirus. Pegivirus does not have a capsid protein, suggesting that unknown viral or host proteins may be involved in formation of the viral particle. The structure and processing of the capsid protein are variable in genera of the *Flaviviridae* family. The structural proteins of HCV were detected as processed proteins at the first time in mammal and insect cells (Matsuura et al. 1992), contributing to identification of their cleavage sites. The capsid proteins of HCV and GBV-B, which are classified into the genus Hepacivirus, are cleaved by signal peptide peptidase (SPP), following signal peptidase-dependent processing (McLauchlan et al. 2002; Targett-Adams et al. 2006), while the capsid protein of classical swine fever virus (CSFV), which belongs to the genus Pestivirus, is cleaved by SPP (Heimann et al. 2006). The C-terminal end of the mature HCV core protein expressed in insect cells was reported to be Phe177 or Leu179 (Hussy et al. 1996; Ogino et al. 2004). The C-terminal residue of the mature HCV core protein that was expressed in a human cell line was identified as Phe177 by mass spectrometry (Okamoto et al. 2008). Non-primate hepaciviruses have recently been identified in dogs, horses, rodents and bats (Burbelo et al. 2012; Drexler et al. 2013; Kapoor et al. 2011, 2013; Lyons et al. 2012; Tanaka et al. 2014). The C-terminal hydrophobic membrane-anchoring region of HCV core protein shows high

homology to the core protein of equine hepacivirus, which is the most closely related homologue of HCV among non-primate hepaciviruses (Burbelo et al. 2012; Kapoor et al. 2013; Lyons et al. 2012). We recently reported that the core protein of equine hepacivirus was cleaved by SPP and then localized on the lipid droplets and partially on lipid-raft like membranes in a manner similar to HCV core protein (Tanaka et al. 2014). The secondary structures and cis-acting elements of the equine hepacivirus genome also exhibit characteristics similar to those of the HCV genome (Tanaka et al. 2014). The mechanism of the viral propagation may thus be conserved between equine hepacivirus and HCV.

A hydrophocity/hydrophilicity plot suggests that the core protein consists of three domains, domain 1 (2–118), 2 (119–174), and 3 (175–191) (Hope and McLauchlan 2000; McLauchlan 2000). The helix-loop-helix structure located in domain 2 is critical for association of the core protein with lipid droplets and shares common features with the core proteins of GBV-B (Hope et al. 2002). Three hydrophobic amino acid residues, Leu139, Val140, and Leu144, in domain 2 exhibit hydrophobic peaks within domain 2 and are responsible for SPP-dependent cleavage, membrane anchoring and virus production (Okamoto et al. 2004, 2008). Furthermore, comparative analysis between the JFH1 and Jc1 strains suggests that the efficiency of virus assembly is determined by the binding ability of domain 2 to lipid droplets (Shavinskaya et al. 2007). Cysteine residue 172 of HCV core protein is palmitoylated. Palmitoylation of the core protein is responsible for the virus production but not for SPP-dependent processing or LD localization of the core protein (Majeau et al. 2009). These results suggest that the hydrophobicity of domains 2 and 3 is critical for intracellular localization and SPP cleavage of the core protein and viral production.

Recently, herpesviruses and other pathogens have been reported to employ SPP for their life cycles and pathogenesis. Human cytomegalovirus protein US2 promoted dislocation of the class I major histocompatibility complex (MHC) heavy chain from the endoplasmic reticulum (ER) by direct interaction with SPP, resulting in proteasome-dependent degradation of the MHC class I heavy chain (Loureiro et al. 2006). Herpes simplex virus-1 exploited SPP by binding to the viral glycoprotein gK for its own replication (Allen et al. 2014). The human malaria *Plasmodium falciparum* expresses its own SPP on the cell surface. The malaria SPP recognizes Band3 in the red blood cells for invasion (Li et al. 2008). SPP inhibitors, L-685,458, NITD731 and LY411.575, were shown to block the growth of *P. falciparum* and the rodent malarial parasite *P. berghei* (Li et al. 2009c; Harbut et al. 2012; Parvanova et al. 2009). SPP or SPP-like proteases may be employed by other pathogens for their propagation and will be target molecules for the development of therapeutic compounds against several pathogens.

1.2 Structure of Envelope Proteins for the Viral Entry and Assembly

HCV envelope proteins E1 and E2 are cleaved from the polyprotein by signal peptidase (Hijikata et al. 1991). Both E1 and E2, each of which consists of a large ectodomain with a C-terminal transmembrane region, are classified into a group of type I membrane proteins and are reported to form non-covalent heterodimers (Deleersnyder et al. 1997). The envelope proteins are highly modified post-translationally at 6 and 11 potential sites for N-glycosylation (Goffard and Dubuisson 2003; Zhang et al. 2004), some of which are responsible for infectivity (Goffard et al. 2005). The core domain (the ectodomain E2 lacking HVR1) of the HCV E2 protein shares some basic characteristics with other class II fusion proteins, such as an immunoglobulin-like fold consisting of a β-sheet structure (Kong et al. 2013; Khan et al. 2014). However, the precise function of E1 and E2 in membrane fusion has not yet been fully clarified. Two hydrophobic regions spanning from 504 to 522 and from 604 to 624 in E2 are predicted to be potential fusion peptides (Khan et al. 2014; Lavillette et al. 2007; Krey et al. 2010), while the region spanning from 262 to 290 in E1 is reported to be important for membrane fusion (Li et al. 2009b). E2 should be responsible for the HCV entry step in cooperation with E1, but the mechanism underlying this step remains unclear.

The synthesized viral genome is wrapped with the core proteins to form a nucleocapsid on lipid droplets close to the ER, on which the viral genome is synthesized (Miyanari et al. 2007). A nucleocapsid egresses with envelope proteins into the ER membrane in close proximity to the lipid droplets. HCV particles in the patients' sera have been reported to exhibit densities of 1.03–1.25 g/ml (Thomssen et al. 1992, 1993). HCV particles with a density of lower than 1.06 g/ml are infectious to chimpanzees, while those with a higher density exhibit lower infectivity (Bradley et al. 1991; Hijikata et al. 1993). HCV particles interacting with lipoproteins in the sera of patients (Andre et al. 2002) were prepared from the fractions with very low to low buoyant densities (1.03–1.25 g/ml), and have been designated lipo-viro-particles (LVP) (Andre et al. 2002; Nielsen et al. 2006). LVP are composed of HCV particle components and very low-density lipoproteins (VLDL), including apolipoprotein B (ApoB) and apolipoprotein E (ApoE) (Andre et al. 2002). The HCV entry process on the surface of hepatocytes has been reported to be carried out by using entry factors including LDLR, CD81, scavenger receptor class B type I (SR-BI), and the tight junction proteins claudin-1 and occludin (Bartosch et al. 2003; Evans et al. 2007; Pileri et al. 1998; Ploss et al. 2009). Lectin receptors including DC-SIGN, L-SIGN, and langerin may be responsible for the invasive step from sinusoidal endothelial cells (Lozach et al. 2003; Pohlmann et al. 2003; Gardner et al. 2003; Chen et al. 2014). Envelope proteins with Man8/9 N-glycans exhibit higher binding to lectin receptors (DC-SIGN, L-SIGN and langerin) than to non-lectin receptors (CD81, SRBI, claudin-1, and occludin) in the presence of calcium ions, while HCV envelope proteins with Man5 N-glycans bound to non-lectin receptors at a higher affinity than lectin receptors (Chen

et al. 2014). The HCV viral particle may be captured by lectin receptors on sinusoidal endothelial cells at a high affinity followed by infection to hepatocytes via non-lectin receptors. HCV envelope proteins interact with ApoE and ApoB in ER (Boyer et al. 2014). Intracellular and extracellular infectious particles also associate with ApoE and ApoB (Boyer et al. 2014). ApoE, but not E2, on the surface of LVP mediates the SR-BI-dependent entry step via the lipid transfer activity of SR-BI, although the HVR1 of E2 affects this step (Dao Thi et al. 2012). These results suggest that E2 HVR1 enhances the SR-BI-ApoE interaction for HCV entry. Further study will be required to clarify the mechanism by which HCV utilizes SR-BI for its entry step.

2 Biological Functions of Structural Proteins

2.1 Modulation of Lipid Metabolism by HCV Core Protein

Liver steatosis is frequently found in persistent HCV infection and results in accumulation of triglyceride and fatty acids in hepatocytes (see Negro 2010). However, the involvement of HCV infection in the development of fatty liver has not yet been clarified completely. Several reports support the notion that HCV core protein contributes to the accumulation of lipid droplets and hepatic steatosis in transgenic mice and cultural cells (Barba et al. 1997; Hope and McLauchlan 2000; Moriya et al. 1997). The lipid profiling of a core transgenic mouse of genotype 1b showed a similar composition to that of a hepatitis C patient (Koike et al. 2010; Miyoshi et al. 2011). Syntheses of triglycerides and fatty acids are transcriptionally regulated by the sterol regulatory element-binding proteins (SREBPs) (Horton et al. 2002). An HCV cell culture system derived from the genotype 3a strain showed that lipid accumulation was enhanced in cells infected with HCV genotype 3a compared to those infected with the genotype 2a strain JFH-1 (Kim et al. 2014). Patients infected with HCV genotype 3a exhibited progression of steatosis at a significantly higher rate than those with genotype 1a or 1b (Adinolfi et al. 2011; Mihm et al. 1997). Expression of the HCV core protein derived from genotype 3a induced lipid accumulation in lipid-free cultured cells at a higher level than expression of other genotype core proteins (Abid et al. 2005). The HCV core protein of genotype 3a stimulated activity of the fatty acid synthetase promoter at a significantly higher level than that of genotype 1b (Abid et al. 2005).

HCV infection or expression of the genotype 3a core protein was found to enhance the cleavage of SREBPs, leading to posttranslational activation of SREBPs (Waris et al. 2007). The recent report by Bose et al. suggested that the forkhead box transcription factor FoxO1 was activated by the HCV core protein or infection followed by activation of srebp-1c promoter activity, leading to the accumulation of lipids (Bose et al. 2014). However, controversial results were reported from hepatitis C patients. McPherson reported that SREBP-1c was not involved in

HCV-related steatosis (McPherson et al. 2008), whereas Lima-Cabello et al. reported that LXRα, SREBP-1c and -2, and fatty acid synthetase were overexpressed in the livers of HCV patients with steatosis (Lima-Cabello et al. 2011), suggesting that LXRα transcriptionally upregulates SREBP-1c expression followed by fatty acid synthetase expression. It has been reported that most of the genes under the control of SREBPs were upregulated during the early stage of HCV infection in the livers of chimpanzees (Bigger et al. 2004). Our previous data indicated that the core protein potentiates the binding ability of the LXRα-RXRα complex to the *srebp-1c* promoter in cultured cells and in the livers of core-transgenic mice (Moriishi et al. 2007). Upregulation of *srebp-1c* promoter activity may be associated with direct interaction between the core protein and RXRα (Tsutsumi et al. 2002b). Cholesterol and ApoB were significantly reduced in patients with severe hepatitis C or core-transgenic mice (Perlemuter et al. 2002). The microsomal triglyceride transfer protein (MTP) positively regulates the formation and secretion of very low-density lipoproteins. In core-transgenic mice, MTP-specific activity is significantly decreased (Perlemuter et al. 2002), resulting in accumulation of lipids in the liver. The gene related to the synthesis and secretion of lipids may be regulated by HCV infection or the core protein at a transcriptional and/or post-translational step.

Peroxisome proliferator activated receptors are nuclear receptors that transcriptionally regulate metabolic signaling (Halilbasic et al. 2013). PPARα regulates the genes encoding enzymes associated with peroxisomal microsomal and mitochondrial γ oxidation (Halilbasic et al. 2013). PPARα is expressed in the liver and downregulated in the HCV-infected liver and the core-expressing HepG2 cells (Dharancy et al. 2005). PPARα was decreased in mice infected with adenovirus expressing the HCV core protein (Yamaguchi et al. 2005). In an earlier study, severe liver steatosis was induced in core-transgenic mice (Moriya et al. 1997). HCV replication transcriptionally induced the expression of miR-27 in cell culture and an in vivo mouse model (Singaravelu et al. 2014). Both the HCV core protein and NS4B promote the expression of miR-27 through a PI3-K-dependent pathway. Transfection of miR-27 enhances the size and volume of lipid droplets in cultured cells and also impairs PPARα signaling. PPARα transcriptionally increases the genes regulating mitochondrial and peroxisomal fatty acid oxidation (Desvergne and Wahli 1999). An increase in miR-27 in infected cells also downregulates ANGPTL3, which is an inhibitor of lipoprotein lipase responsible for fatty acid uptake (Mattijssen and Kersten 2012). These data suggest that induction of miR-27 by HCV infection downregulates fatty acid oxidation via impairment of PPARα signaling and up-regulates fatty acid uptake via inhibition of ANGPTL3 expression, leading to development of liver steatosis. Unexpectedly, PPARα-knockout core-transgenic mice did not show steatosis (Tanaka et al. 2008). Furthermore, PPARα expression was required for induction of hepatocellular carcinoma by HCV core protein (Tanaka et al. 2008). Therefore, the HCV core protein may require a small amount of PPARα for the development of liver disorders and may maintain PPARα at a steady level.

PPARγ is involved in adipocyte differentiation and energy storage by adipocytes mediating an anabolic energy state (Halilbasic et al. 2013). In addition, PPARγ plays an important role in the development of liver steatosis (Gavrilova et al. 2003; Yu et al. 2003). The HCV core protein has been shown to potentiate PPARγ activity and transcriptionally upregulate SREBP1 activity, resulting in lipid accumulation. Furthermore, HCV core protein expression induced leptin receptor activation in hepatic stellate cells and contributed to transcriptional upregulation of MMP-1, PAPRγ and SREBP-1c, leading to promotion of hepatic fibrogenesis (Wu et al. 2013). PPARγ may thus be involved in HCV core-induced liver steatosis, in cooperation with PPARα.

2.2 *Regulation of Iron Metabolism by HCV Core Protein*

Iron overload has been reported as a common hallmark of chronic hepatitis C infection (Bonkovsky 2002; Boucher et al. 1997; Di Bisceglie et al. 1992). Accumulation of iron in the liver by HCV infection promotes liver inflammation and interferon resistance due to inhibition of the JAK-STAT pathway by oxidative stress (Olynyk et al. 1995; Bassett et al. 1999; Nishina et al. 2008; Fujita et al. 2007). Iron is involved in induction of reactive oxygen species (ROS). Iron Fe^{2+} reacts with hydrogen peroxide (H_2O_2) to yield Fe^{3+}, hydroxyl radical ($^{\cdot}OH$), and hydroxide ion ($OH-$) (Fenton reaction) (Graf et al. 1984). Hydroxyl radical reacts with lipids, resulting in lipid peroxidation (Okada 1996). Iron concentration in the liver is regulated by an import protein transferrin receptor and an export protein ferroportin (Pantopoulos et al. 2012). Imported iron atoms are enclosed with ferritin in cells and stored as iron-ferritin complexes (Ganz and Nemeth 2012; Liu and Theil 2005). Another iron-regulating protein, hepcidin, which is encoded on the gene HAMP, is a short peptide inducing internalization and degradation of ferroportin and regulates plasma iron concentration and iron metabolism in the liver (Ganz and Nemeth 2012; Nemeth et al. 2004, 2006). Expression of hepcidin is stimulated by iron overload and inflammation, and is suppressed by anemia and hypoxia (Nemeth and Ganz 2006). BMP6 is produced and secreted by various cell types and is a main regulator of hepcidin expression (Andriopoulos et al. 2009). BMP6 binds and stimulates dimers of BMP-RI/II to cooperate with the coreceptor hemojuvelin (Andriopoulos et al. 2009; Meynard et al. 2009; Xia et al. 2008), leading to downstream signaling including dimerization of Smad4 with Smad1/5/8 (Wang et al. 2005). Smad dimers transcriptionally induce expression of hepcidin. Screening of a whole genome using an siRNA library revealed that hepcidin-knockdown reduced HCV replication significantly (Tai et al. 2009), suggesting that hepcidin expression is required for HCV replication and control of iron metabolism. Hepcidin was shown to be transcriptionally enhanced by the HCV core protein through Smad4, STAT3 and CK2 (Foka et al. 2014). Knockdown of hepcidin impaired HCV replication in a replicon cell line (Bartolomei et al. 2011). Iron upregulates HCV replication by enhancement of IRES-dependent translation

and expression of eIF3 and La (Cho et al. 2008; Theurl et al. 2004; Wang et al. 2012). However, knockdown of hepcidin suppressed IRES- as well as CAP-dependent translation (Tai et al. 2009). Hepcidin may contribute to translational regulations of the viral proteins and host proteins by accumulation of iron in HCV-infected cells.

2.3 Quality Control of HCV Structural Proteins

The core protein is modified with ubiquitin by host enzymes. The host E3 ligase E6AP catalyzes ubiquitination of the core protein to suppress viral production (Shirakura et al. 2007). The poly-ubiquitinated core protein is degraded in the cytosol in a proteasome-dependent manner. HCV core protein is also degraded in a ubiquitin-independent PA28γ-dependent pathway, leading to upregulation of the viral production by suppression of cytosolic ubiquitin-dependent degradation of the core protein (Moriishi et al. 2003, 2007). The host mechanisms of protein degradation may regulate HCV production and control the quality of the core protein for viral propagation. Although qualitative limitations of HCV envelope proteins have been regulated by an ER-associated degradation (ERAD) system (Saeed et al. 2011), inhibition of ER enhanced the viral production (Saeed et al. 2011), suggesting that unfolded envelope proteins positively regulate the HCV production.

The unfolded protein response (UPR) is carried out by three pathways, an IRE1α, a PERK and an ATF6-dependent pathway (Gardner et al. 2013). The luminal domains of the PERK, ATF6 and IRE1α proteins interact with the ER resident chaperone BiP (Bertolotti et al. 2000). BiP renders PERK, ATF6 and IRE1α inactive without accumulation of unfolded protein (Bertolotti et al. 2000), while the accumulation of unfolded proteins stimulates release of BiP from PERK, ATF6 or IRE1α, leading to the induction of genes related to protein folding, cell survival, autophagy and so on (Bertolotti et al. 2000). UPR stimulates expression of both MAP1LC3B and ATG5 by ATF4 and CHOP, which are induced by activation of PARK and ATF6 (Rouschop et al. 2010; Wang et al. 2014). ATF4 also activates transcription of CHOP (Kojima et al. 2003). The HCV core protein was recently shown to activate both the PERK and ATP6 pathways, but not the IRE1α pathway, to stimulate expression of MAP1LC3B, ATG12 and ATG5 (Wang et al. 2014), suggesting that autophagy is induced by the upregulation of ATG proteins through the UPR of HCV core protein. Expression of HCV envelop proteins induced the expression of CHOP through PERK and IRE1α pathways (Chan and Egan 2005). CHOP stimulates IP3R through Ero1α activation, followed by accumulation of Ca^{2+} in mitochondria (Li et al. 2009a). UPR-induced accumulation of Ca^{2+} in mitochondria may be associated with ROS production in HCV infected cells, as described later.

2.4 Effect of HCV Infection on Mitochondria

The HCV core protein can enhance the production of ROS by damaging the mitochondrial electron transport system, and thereby contribute to the emergence of hepatocellular carcinoma (Moriya et al. 2001; Nunez et al. 2004; Okuda et al. 2002), suggesting that accumulation of lipids advances the occurrence of hepatocellular carcinoma by enhancing ROS production. Expression of HCV polyproteins in cultured sarcoma cells promoted the production of ROS and nitrogen species and inhibited complex I activity, resulting in activation of mitochondrial calcium uptake (Piccoli et al. 2007). The HCV core protein is localized in the lipid droplets, ER and mitochondria (Okuda et al. 2002) and could induce ROS, leading to accumulation of lipid peroxidation products and enhancement of antioxidant gene expression (Okuda et al. 2002). Upregulation of lipid peroxidation was observed in core-transgenic mice but not in wild type mice following treatment with CCl_4 (Okuda et al. 2002). The mitochondria of transgenic mice expressing HCV polyprotein exhibited enhancement of glutathione oxidation, decrease in NADPH contents, impairment of complex I activity and promotion of ROS production (Korenaga et al. 2005). Glutathione oxidation and ROS upregulation were also found in isolated mitochondria in the presence of recombinant core protein (Korenaga et al. 2005). Ca^{2+} uptake was increased by the recombinant core protein in isolated mitochondria (Korenaga et al. 2005). HCV core protein induced ER stress via an unfolded protein response and then potentiated production of ER chaperone proteins and release of Ca^{2+} from the ER store (Benali-Furet et al. 2005; Bergqvist et al. 2003). In addition, the HCV core protein was found to enhance mitochondrial Ca^{2+} uptake via the Ca^{2+} uniporter, which is localized in the mitochondrial inner membrane (Li et al. 2007). Furthermore, HCV core protein interacted with the mitochondria chaperone prohibitin to upregulate prohibitin stability in cultured cells and the transgenic mouse liver (Tsutsumi et al. 2009). HCV core protein inhibited the interaction between prohibitin and COX, resulting in the impairment of COX activity (Tsutsumi et al. 2009). These reports suggest that HCV core protein induces ER stress and Ca^{2+} release from the ER and then stimulates mitochondrial Ca^{2+} uptake to upregulate ROS production. In addition, the HCV core protein may impair COX activity by both sequestering prohibitin and decreasing glutathione, leading to further enhancement of ROS production.

2.5 Insulin Resistance

Epidemiological studies have clearly established an association between type 2 diabetes mellitus and HCV infection (Cavaghan et al. 2000; Kahn 1998). Type 2 diabetes is a complex disease characterized by the high-level production of hepatic glucose due to insulin resistance, resulting in glucose tolerance hyperglycemia (Cavaghan et al. 2000; Kahn 1998). Insulin is ordinarily produced at a

sufficient level in type 2 diabetes mellitus patients; however, the glucose level cannot be decreased due to a disorder in insulin signaling. Insulin receptor is a tyrosine kinase composed of two subunits (Draznin 2006; Youngren 2007). Binding of insulin activates insulin receptor, which triggers Tyr phosphorylation of insulin receptor substrate 1 (IRS1) (Draznin 2006; Youngren 2007). The phosphorylated IRS1 and IRS2 positively regulate PI3K, which phosphorylates phosphatidylinositol 4, 5-bisphophate into phosphatidylinositol-3,4,5-triphosphate (PIP3). PDK1 and PDK2 are recruited by the resulting PIP3 with Akt and then phosphorylate Akt at Thr308 and Ser 473 (Burgering and Coffer 1995; Taniguchi et al. 2006; Alessi et al. 1996; Manning and Cantley 2007), resulting in activation of Akt. Phosphorylated Akt itself phosphorylates a glucose transporter, GLUT-4, contributing to translocation of GLUT-4 to the plasma membrane for upregulation of glucose uptake (Taniguchi et al. 2006; Thirone et al. 2006).

Elevation of TNFα production is one of the risk factors for insulin resistance (Gurav 2012). TNFα can indirectly mediate phosphorylation of IRS1at multiple sites through the activation of several Ser kinases, including JNK, IKKβ and ERK (Gao et al. 2003; Solinas and Karin 2010). TNFα stimulates activation of MEKK1, ASK1 and TAK1, which phosphorylate MKK7 for activation (Nakajima et al. 2006). In the same study, phosphorylated MKK7 was able to phosphorylate JNK (Nakajima et al. 2006). JNK1 has been shown to interact with IRS1 through the region spanning from the residues 555–898 (Aguirre et al. 2000). Phosphorylation of IRS1 Ser307 was detected in cultured cells treated with a JNK agonist, resulting in a decrease in Tyr phosphorylation of IRS1(Aguirre et al. 2000). Rui et al. suggested that a TNFα-dependent, JNK-independent mechanism may also be associated with phosphorylation of IRS1 Ser307 (Rui et al. 2001). Insulin was shown to stimulate the PI3K pathway to enhance phosphorylation of IRS1 Ser307 (Aguirre et al. 2002; Rui et al. 2001). Insulin-stimulated JNK phosphorylates IRS1 S307 may be a negative feedback pathway of insulin signaling (Lee et al. 2003). Phosphorylation of IRS1 Ser307 by JNK impairs the binding ability of IRS1 to insulin receptor (Aguirre et al. 2002), while phosphorylation of IRS1 Ser302 by JNK may also be involved in the negative feedback of insulin signaling (Werner et al. 2004). An increase in TNFα and a decrease in Tyr phosphorylation of IRS1 were observed in both the livers of HCV core gene transgenic mice and hepatitis C patients (Shintani et al. 2004; Miyamoto et al. 2007). Furthermore, JNK and its downstream factor AP-1 have been shown to be activated in core gene transgenic mice (Tsutsumi et al. 2002a). The HCV core protein also activated JNK and enhanced phosphorylation of IRS1 Ser312, leading to a decrease in Tyr phosphorylation of IRS1 and inhibition of insulin signaling (Banerjee et al. 2008). HCV core protein may activate JNK through upregulation of TNFα production, leading to insulin resistance through Ser phosphorylation of IRS1.

Evidence of the involvement of suppressor of cytokine signaling (SOCS) proteins in HCV-associated insulin resistance has been accumulating. SOCS-1 and SOCS-3 show relatively high homology and share similar functions. Both SOCS-1 and SOCS-3 can bind to insulin receptors irrespective of their phosphorylation status and impair Try phosphorylation of IRS-1 (Ueki et al. 2004). Further, SOCS-1

and SOCS-3 were shown to promote the degradation of IRS-1/2 via the ubiquitin-proteasome pathway (Rui et al. 2002). SOCS-1, but not SOCS-3, was decreased in the livers of core gene transgenic mice and hepatitis C patients, as well as in HepG2 cells expressing HCV core protein (Miyoshi et al. 2005). Expression of SOCS-3 was promoted after IFN treatment in HCV-infected chimpanzees, whereas the human liver showed variable responses to different treatments (Huang et al. 2007). SOCS-3 expression was significantly promoted in peripheral lymphocytes prepared from genotype 1b-infected IFN-non-responders (Persico et al. 2007). HCV core protein stimulated the expression of SOCS-3 and then enhanced ubiquitination of IRS1 and IRS2, leading to a decrease in IRS1/2 in a proteasome-dependent pathway (Kawaguchi et al. 2004). Induction of SOCS3 expression by the HCV core protein may be associated with the core protein mutations of Met70 and Leu91 (Funaoka et al. 2011), which are statistical predictors of low response to IFN/ribavirin therapy (Akuta et al. 2005, 2010). The regulation of IRS1 by HCV core protein may be accomplished by a genotype-specific pathway. Ubiquitin-dependent degradation of IRS1 was observed by the HCV core protein of genotypes 1b and 3a (Pazienza et al. 2007). In addition, IRS1 was decreased transcriptionally by downregulation of PPARγ and post-translationally by upregulation of SOCS-7 (Pazienza et al. 2007), while the core protein of genotype 1b activated mTOR, which suppresses IRS1 by Ser/Thr phosphorylation (Pazienza et al. 2007). E2 transcriptionally promoted the expression of SOCS3 and ubiquitination-dependent downregulation of IRS1, resulting in the impairment of Akt and GSK3 (Hsieh et al. 2012). The HCV core protein may regulate SOCS proteins cooperating with E2 under infectious conditions.

2.6 *Involvement of Envelope Proteins in Biological Functions*

Envelope proteins may control tight junction and facilitate secondary invasion of HCV after primary infection. Occludin, claudin-1 and ZO-1, which are tight junction proteins, are localized in the baso-lateral membrane position of Huh7, while these tight junction proteins were defused in Huh7 harboring a full-genomic but not a sub-genomic HCV replicon (Benedicto et al. 2008). Exogenous expression of HCV structural proteins, but not core alone, resulted in the translocation of tight junction proteins irrespective of the viral replication (Benedicto et al. 2008). HCV envelope proteins may facilitate subsequent virus infection by disruption of tight junction.

HCV envelope proteins may regulate ROS production and cell death. HCV infection was shown to stimulate production of ROS and NO and to reduce mitochondrial transmembrane potential (Machida et al. 2006), leading to double-stranded DNA breaks and apoptosis. Although expression of core, E1, or NS3 could induce ROS production in cultured cells (Machida et al. 2006), regulation of

apoptosis by E2 is controversial. Chiou et al. reported that E2 induces apoptosis by cytoplasmic release of cytochrome c, upregulation of Bax and downregulation of Bcl-2 followed by activations of caspases-3, 8, and 9 (Chiou et al. 2006), whereas apoptosis induced by the death ligand TRAIL was suppressed by the expression of E2 (Lee et al. 2005). The expression of E2 may be capable of supporting the HCV replication by inhibiting apoptosis (Lee et al. 2005).

Phosphorylation and activation of STAT1 were enhanced by the expression of both HCV E2 and HIV gp120 (Balasubramanian et al. 2006). Lyn kinase, p38MAP kinase and protein kinase C δ are responsible for STAT1 phosphorylation (Balasubramanian et al. 2006). An increase in STAT1 might contribute to apoptosis in the hepatocytes of patients co-infected with HCV and HIV. HCV infection down-regulated the amounts of miR181c, which targets homeobox A1 (HOXA1), by modulating C/EBP-β (Mukherjee et al. 2014). In the same study, HOXA1 expression was potentiated in HCV-infected cells (Mukherjee et al. 2014). In addition, miR-181c was shown to bind directly to the E1 or NS5A gene (Mukherjee et al. 2014). Finally, HOXA1 promotes cell growth through upregulation of STAT3 and STAT5 (Mohankumar et al. 2007). These data suggest that the transcriptional and posttranscriptional down-regulation of miR-181c by HCV infection might contribute to activation of HOXA1 followed by upregulation of STA3 and STAT5.

3 Conclusions

The structural proteins of HCV are basically employed for formation of a viral particle like structural proteins of other enveloped viruses. The HCV core proteins is processed by host proteases, and then associated with lipid droplets and intracellular compartments for formation of nucleocapsid, while HCV glycoproteins, E1 and E2, are localized in ER membrane in close proximity to the lipid droplets. Both envelope proteins are classified into a group of type I membrane proteins, and are reported to form non-covalent heterodimers. The recent report of structural analysis revealed that HCV E2 protein is classified into the family of class II fusion protein. The envelope proteins play an important role in an entry step cooperating with several host entry factors, lectin and lipoproteins. In this text, we also summarized the biological functions of HCV structural proteins (Fig. 1). To date, it has not been fully clarified how HCV can cause hepatocellular carcinoma in humans. Persistent inflammation over a long period of time is expected to be associated with the development of hepatocellular carcinoma, due to both genomic alterations and biological functions of the HCV proteins. HCV core protein upregulates uptake of free fatty acids and the transcriptional activities of SREBPs, and down-regulates MTP function and β-oxidation, leading to liver steatosis. In addition, the structural proteins induce accumulation of mitochondrial Ca^{2+} and iron via ER stress and functions of hepcidin and Ca^{2+} uniporter, resulting in an increase in ROS. Oxidative stress induced by HCV infection may be one of the causative agents related to the

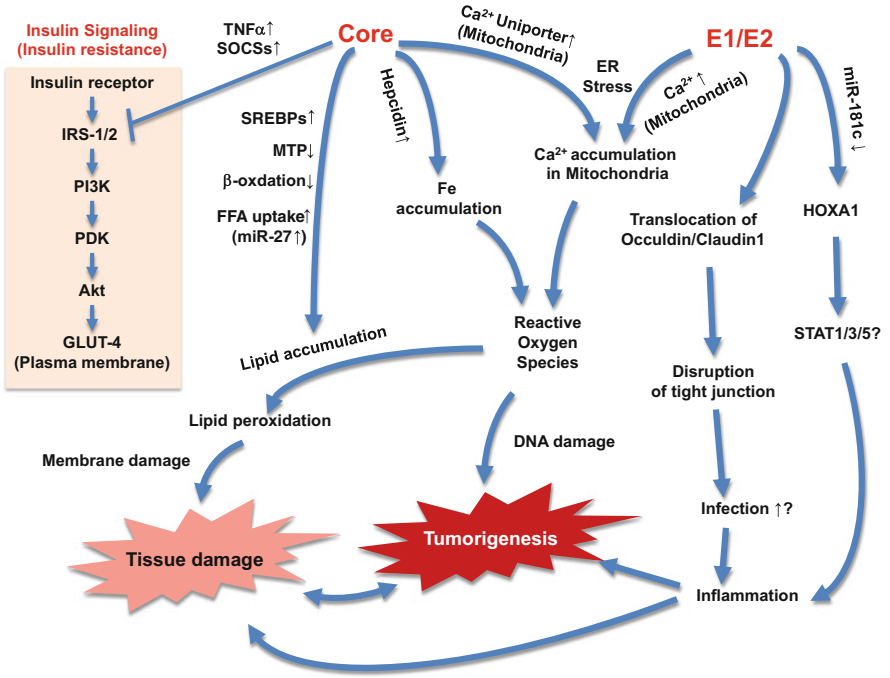

Fig. 1 Schematic diagram of the biological functions of HCV structural proteins. The biological and pathological actions induced by HCV structural proteins are summarized following the text

genetic alterations, including DNA double-strand breaks. In addition, retrotransposition targeting specific genes is predicted to be one of the potential causative agents of hepatocellular carcinoma in hepatitis B and C patients (Shukla et al. 2013). However, the mechanism by which HCV-related retrotransposition is induced has not been fully understood. Further study will be required to understand how carcinogenesis is related to hepatitis viruses and to develop antiviral agents for the eradication of these viruses in humans.

References

Abid K, Pazienza V, de Gottardi A, Rubbia-Brandt L, Conne B, Pugnale P, Rossi C, Mangia A, Negro F (2005) An in vitro model of hepatitis C virus genotype 3a-associated triglycerides accumulation. J Hepatol 42(5):744–751

Adinolfi LE, Restivo L, Zampino R, Lonardo A, Loria P (2011) Metabolic alterations and chronic hepatitis C: treatment strategies. Expert Opin Pharmacother 12(14):2215–2234

Aguirre V, Uchida T, Yenush L, Davis R, White MF (2000) The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). J Biol Chem 275(12):9047–9054

Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, White MF (2002) Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J Biol Chem 277(2):1531–1537

Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H (2005) Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48 (6):372–380

Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Chayama K, Nakamura Y, Kumada H (2010) Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology 52(2):421–429

Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA (1996) Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J 15(23):6541–6551

Allen SJ, Mott KR, Matsuura Y, Moriishi K, Kousoulas KG, Ghiasi H (2014) Binding of HSV-1 glycoprotein K (gK) to signal peptide peptidase (SPP) is required for virus infectivity. PLoS One 9(1):e85360

Andre P, Komurian-Pradel F, Deforges S, Perret M, Berland JL, Sodoyer M, Pol S, Brechot C, Paranhos-Baccala G, Lotteau V (2002) Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. J Virol 76(14):6919–6928

Andriopoulos B Jr, Corradini E, Xia Y, Faasse SA, Chen S, Grgurevic L, Knutson MD, Pietrangelo A, Vukicevic S, Lin HY, Babitt JL (2009) BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. Nat Genet 41(4):482–487

Balasubramanian A, Ganju RK, Groopman JE (2006) Signal transducer and activator of transcription factor 1 mediates apoptosis induced by hepatitis C virus and HIV envelope proteins in hepatocytes. J Infect Dis 194(5):670–681

Banerjee S, Saito K, Ait-Goughoulte M, Meyer K, Ray RB, Ray R (2008) Hepatitis C virus core protein upregulates serine phosphorylation of insulin receptor substrate-1 and impairs the downstream akt/protein kinase B signaling pathway for insulin resistance. J Virol 82 (6):2606–2612

Barba G, Harper F, Harada T, Kohara M, Goulinet S, Matsuura Y, Eder G, Schaff Z, Chapman MJ, Miyamura T, Brechot C (1997) Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc Natl Acad Sci U S A 94(4):1200–1205

Bartolomei G, Cevik RE, Marcello A (2011) Modulation of hepatitis C virus replication by iron and hepcidin in Huh7 hepatocytes. J Gen Virol 92(Pt 9):2072–2081

Bartosch B, Vitelli A, Granier C, Goujon C, Dubuisson J, Pascale S, Scarselli E, Cortese R, Nicosia A, Cosset FL (2003) Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor. J Biol Chem 278 (43):41624–41630

Bassett SE, Di Bisceglie AM, Bacon BR, Sharp RM, Govindarajan S, Hubbard GB, Brasky KM, Lanford RE (1999) Effects of iron loading on pathogenicity in hepatitis C virus-infected chimpanzees. Hepatology 29(6):1884–1892

Benali-Furet NL, Chami M, Houel L, De Giorgi F, Vernejoul F, Lagorce D, Buscail L, Bartenschlager R, Ichas F, Rizzuto R, Paterlini-Brechot P (2005) Hepatitis C virus core triggers apoptosis in liver cells by inducing ER stress and ER calcium depletion. Oncogene 24 (31):4921–4933

Benedicto I, Molina-Jimenez F, Barreiro O, Maldonado-Rodriguez A, Prieto J, Moreno-Otero R, Aldabe R, Lopez-Cabrera M, Majano PL (2008) Hepatitis C virus envelope components alter localization of hepatocyte tight junction-associated proteins and promote occludin retention in the endoplasmic reticulum. Hepatology 48(4):1044–1053

Bergqvist A, Sundstrom S, Dimberg LY, Gylfe E, Masucci MG (2003) The hepatitis C virus core protein modulates T cell responses by inducing spontaneous and altering T-cell receptor-triggered Ca2+ oscillations. J Biol Chem 278(21):18877–18883

Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D (2000) Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol 2(6):326–332

Bigger CB, Guerra B, Brasky KM, Hubbard G, Beard MR, Luxon BA, Lemon SM, Lanford RE (2004) Intrahepatic gene expression during chronic hepatitis C virus infection in chimpanzees. J Virol 78(24):13779–13792

Bonkovsky HL (2002) Iron as a comorbid factor in chronic viral hepatitis. Am J Gastroenterol 97 (1):1–4

Bose SK, Kim H, Meyer K, Wolins N, Davidson NO, Ray R (2014) Forkhead box transcription factor regulation and lipid accumulation by hepatitis C virus. J Virol 88(8):4195–4203

Boucher E, Bourienne A, Adams P, Turlin B, Brissot P, Deugnier Y (1997) Liver iron concentration and distribution in chronic hepatitis C before and after interferon treatment. Gut 41 (1):115–120

Boyer A, Dumans A, Beaumont E, Etienne L, Roingeard P, Meunier JC (2014) The association of hepatitis C virus glycoproteins with apolipoproteins E and B early in assembly is conserved in lipoviral particles. J Biol Chem 289(27):18904–18913

Bradley D, McCaustland K, Krawczynski K, Spelbring J, Humphrey C, Cook EH (1991) Hepatitis C virus: buoyant density of the factor VIII-derived isolate in sucrose. J Med Virol 34 (3):206–208

Burbelo PD, Dubovi EJ, Simmonds P, Medina JL, Henriquez JA, Mishra N, Wagner J, Tokarz R, Cullen JM, Iadarola MJ, Rice CM, Lipkin WI, Kapoor A (2012) Serology-enabled discovery of genetically diverse hepaciviruses in a new host. J Virol 86(11):6171–6178

Burgering BM, Coffer PJ (1995) Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. Nature 376(6541):599–602

Cavaghan MK, Ehrmann DA, Polonsky KS (2000) Interactions between insulin resistance and insulin secretion in the development of glucose intolerance. J Clin Invest 106(3):329–333

Chan SW, Egan PA (2005) Hepatitis C virus envelope proteins regulate CHOP via induction of the unfolded protein response. FASEB J Off Publ Fed Am Soc Exp Biol 19(11):1510–1512

Chen PC, Chuang PK, Chen CH, Chan YT, Chen JR, Lin SW, Ma C, Hsu TL, Wong CH (2014) Role of N-linked glycans in the interactions of recombinant HCV envelope glycoproteins with cellular receptors. ACS Chem Biol 9(7):1437–1443

Chiou HL, Hsieh YS, Hsieh MR, Chen TY (2006) HCV E2 may induce apoptosis of Huh-7 cells via a mitochondrial-related caspase pathway. Biochem Biophys Res Commun 345(1):453–458

Cho H, Lee HC, Jang SK, Kim YK (2008) Iron increases translation initiation directed by internal ribosome entry site of hepatitis C virus. Virus Genes 37(2):154–160

Dao Thi VL, Granier C, Zeisel MB, Guerin M, Mancip J, Granio O, Penin F, Lavillette D, Bartenschlager R, Baumert TF, Cosset FL, Dreux M (2012) Characterization of hepatitis C virus particle subpopulations reveals multiple usage of the scavenger receptor BI for entry steps. J Biol Chem 287(37):31242–31257

Deleersnyder V, Pillez A, Wychowski C, Blight K, Xu J, Hahn YS, Rice CM, Dubuisson J (1997) Formation of native hepatitis C virus glycoprotein complexes. J Virol 71(1):697–704

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 20(5):649–688

Dharancy S, Malapel M, Perlemuter G, Roskams T, Cheng Y, Dubuquoy L, Podevin P, Conti F, Canva V, Philippe D, Gambiez L, Mathurin P, Paris JC, Schoonjans K, Calmus Y, Pol S, Auwerx J, Desreumaux P (2005) Impaired expression of the peroxisome proliferator-activated receptor alpha during hepatitis C virus infection. Gastroenterology 128(2):334–342

Di Bisceglie AM, Axiotis CA, Hoofnagle JH, Bacon BR (1992) Measurements of iron status in patients with chronic hepatitis. Gastroenterology 102(6):2108–2113

Draznin B (2006) Molecular mechanisms of insulin resistance: serine phosphorylation of insulin receptor substrate-1 and increased expression of p85alpha: the two sides of a coin. Diabetes 55 (8):2392–2397

Drexler JF, Corman VM, Muller MA, Lukashev AN, Gmyl A, Coutard B, Adam A, Ritz D, Leijten LM, van Riel D, Kallies R, Klose SM, Gloza-Rausch F, Binger T, Annan A, Adu-Sarkodie Y, Oppong S, Bourgarel M, Rupp D, Hoffmann B, Schlegel M, Kummerer BM, Kruger DH, Schmidt-Chanasit J, Setien AA, Cottontail VM, Hemachudha T, Wacharapluesadee S, Osterrieder K, Bartenschlager R, Matthee S, Beer M, Kuiken T, Reusken C, Leroy EM, Ulrich RG, Drosten C (2013) Evidence for novel hepaciviruses in rodents. PLoS Pathog 9(6): e1003438

Evans MJ, von Hahn T, Tscherne DM, Syder AJ, Panis M, Wolk B, Hatziioannou T, McKeating JA, Bieniasz PD, Rice CM (2007) Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. Nature 446(7137):801–805

Foka P, Dimitriadis A, Kyratzopoulou E, Giannimaras DA, Sarno S, Simos G, Georgopoulou U, Mamalaki A (2014) A complex signaling network involving protein kinase CK2 is required for hepatitis C virus core protein-mediated modulation of the iron-regulatory hepcidin gene expression. Cell Mol Life Sci CMLS 71(21):4243–4258

Fujita N, Sugimoto R, Urawa N, Araki J, Mifuji R, Yamamoto M, Horiike S, Tanaka H, Iwasa M, Kobayashi Y, Adachi Y, Kaito M (2007) Hepatic iron accumulation is associated with disease progression and resistance to interferon/ribavirin combination therapy in chronic hepatitis C. J Gastroenterol Hepatol 22(11):1886–1893

Funaoka Y, Sakamoto N, Suda G, Itsui Y, Nakagawa M, Kakinuma S, Watanabe T, Mishima K, Ueyama M, Onozuka I, Nitta S, Kitazume A, Kiyohashi K, Murakawa M, Azuma S, Tsuchiya K, Watanabe M (2011) Analysis of interferon signaling by infectious hepatitis C virus clones with substitutions of core amino acids 70 and 91. J Virol 85(12):5986–5994

Ganz T, Nemeth E (2012) Hepcidin and iron homeostasis. Biochim Biophys Acta 1823 (9):1434–1443

Gao Z, Zuberi A, Quon MJ, Dong Z, Ye J (2003) Aspirin inhibits serine phosphorylation of insulin receptor substrate 1 in tumor necrosis factor-treated cells through targeting multiple serine kinases. J Biol Chem 278(27):24944–24950

Gardner JP, Durso RJ, Arrigale RR, Donovan GP, Maddon PJ, Dragic T, Olson WC (2003) L-SIGN (CD 209L) is a liver-specific capture receptor for hepatitis C virus. Proc Natl Acad Sci U S A 100(8):4498–4503

Gardner BM, Pincus D, Gotthardt K, Gallagher CM, Walter P (2013) Endoplasmic reticulum stress sensing in the unfolded protein response. Cold Spring Harb Perspect Biol 5(3):a013169

Gavrilova O, Haluzik M, Matsusue K, Cutson JJ, Johnson L, Dietz KR, Nicol CJ, Vinson C, Gonzalez FJ, Reitman ML (2003) Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. J Biol Chem 278(36):34268–34276

Goffard A, Dubuisson J (2003) Glycosylation of hepatitis C virus envelope proteins. Biochimie 85 (3–4):295–301

Goffard A, Callens N, Bartosch B, Wychowski C, Cosset FL, Montpellier C, Dubuisson J (2005) Role of N-linked glycans in the functions of hepatitis C virus envelope glycoproteins. J Virol 79(13):8400–8409

Graf E, Mahoney JR, Bryant RG, Eaton JW (1984) Iron-catalyzed hydroxyl radical formation. Stringent requirement for free iron coordination site. J Biol Chem 259(6):3620–3624

Grakoui A, McCourt DW, Wychowski C, Feinstone SM, Rice CM (1993a) Characterization of the hepatitis C virus-encoded serine proteinase: determination of proteinase-dependent polyprotein cleavage sites. J Virol 67(5):2832–2843

Grakoui A, McCourt DW, Wychowski C, Feinstone SM, Rice CM (1993b) A second hepatitis C virus-encoded proteinase. Proc Natl Acad Sci U S A 90(22):10583–10587

Grakoui A, Wychowski C, Lin C, Feinstone SM, Rice CM (1993c) Expression and identification of hepatitis C virus polyprotein cleavage products. J Virol 67(3):1385–1395

Gurav AN (2012) Periodontitis and insulin resistance: casual or causal relationship? Diabetes Metab J 36(6):404–411

Halilbasic E, Baghdasaryan A, Trauner M (2013) Nuclear receptors as drug targets in cholestatic liver diseases. Clin Liver Dis 17(2):161–189

Harada S, Watanabe Y, Takeuchi K, Suzuki T, Katayama T, Takebe Y, Saito I, Miyamura T (1991) Expression of processed core protein of hepatitis C virus in mammalian cells. J Virol 65 (6):3015–3021

Harbut MB, Patel BA, Yeung BK, McNamara CW, Bright AT, Ballard J, Supek F, Golde TE, Winzeler EA, Diagana TT, Greenbaum DC (2012) Targeting the ERAD pathway via inhibition of signal peptide peptidase for antiparasitic therapeutic design. Proc Natl Acad Sci U S A 109 (52):21486–21491

Heimann M, Roman-Sosa G, Martoglio B, Thiel HJ, Rumenapf T (2006) Core protein of pestiviruses is processed at the C terminus by signal peptide peptidase. J Virol 80 (4):1915–1921

Hijikata M, Kato N, Ootsuyama Y, Nakagawa M, Shimotohno K (1991) Gene mapping of the putative structural region of the hepatitis C virus genome by in vitro processing analysis. Proc Natl Acad Sci U S A 88(13):5547–5551

Hijikata M, Shimizu YK, Kato H, Iwamoto A, Shih JW, Alter HJ, Purcell RH, Yoshikura H (1993) Equilibrium centrifugation studies of hepatitis C virus: evidence for circulating immune complexes. J Virol 67(4):1953–1958

Hope RG, McLauchlan J (2000) Sequence motifs required for lipid droplet association and protein stability are unique to the hepatitis C virus core protein. J Gen Virol 81(Pt 8):1913–1925

Hope RG, Murphy DJ, McLauchlan J (2002) The domains required to direct core proteins of hepatitis C virus and GB virus-B to lipid droplets share common features with plant oleosin proteins. J Biol Chem 277(6):4261–4270

Horton JD, Goldstein JL, Brown MS (2002) SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 109(9):1125–1131

Hsieh MJ, Lan KP, Liu HY, Zhang XZ, Lin YF, Chen TY, Chiou HL (2012) Hepatitis C virus E2 protein involve in insulin resistance through an impairment of Akt/PKB and GSK3beta signaling in hepatocytes. BMC Gastroenterol 12:74

Huang Y, Feld JJ, Sapp RK, Nanda S, Lin JH, Blatt LM, Fried MW, Murthy K, Liang TJ (2007) Defective hepatic response to interferon and activation of suppressor of cytokine signaling 3 in chronic hepatitis C. Gastroenterology 132(2):733–744

Hussy P, Langen H, Mous J, Jacobsen H (1996) Hepatitis C virus core protein: carboxy-terminal boundaries of two processed species suggest cleavage by a signal peptide peptidase. Virology 224(1):93–104

Kahn BB (1998) Type 2 diabetes: when insulin secretion fails to compensate for insulin resistance. Cell 92(5):593–596

Kapoor A, Simmonds P, Gerold G, Qaisar N, Jain K, Henriquez JA, Firth C, Hirschberg DL, Rice CM, Shields S, Lipkin WI (2011) Characterization of a canine homolog of hepatitis C virus. Proc Natl Acad Sci U S A 108(28):11608–11613

Kapoor A, Simmonds P, Scheel TK, Hjelle B, Cullen JM, Burbelo PD, Chauhan LV, Duraisamy R, Sanchez Leon M, Jain K, Vandegrift KJ, Calisher CH, Rice CM, Lipkin WI (2013) Identification of rodent homologs of hepatitis C virus and pegiviruses. mBio 4(2):e00216-00213

Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, Taniguchi E, Kumemura H, Hanada S, Maeyama M, Baba S, Koga H, Kumashiro R, Ueno T, Ogata H, Yoshimura A, Sata M (2004) Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol 165(5):1499–1508

Khan AG, Whidby J, Miller MT, Scarborough H, Zatorski AV, Cygan A, Price AA, Yost SA, Bohannon CD, Jacob J, Grakoui A, Marcotrigiano J (2014) Structure of the core ectodomain of the hepatitis C virus envelope glycoprotein 2. Nature 509(7500):381–384

Kim S, Date T, Yokokawa H, Kono T, Aizaki H, Maurel P, Gondeau C, Wakita T (2014) Development of hepatitis C virus genotype 3a cell culture system. Hepatology in press. doi:10.1002/hep.27197

Koike K, Tsutsumi T, Yotsuyanagi H, Moriya K (2010) Lipid metabolism and liver disease in hepatitis C viral infection. Oncology 78(Suppl 1):24–30

Kojima E, Takeuchi A, Haneda M, Yagi A, Hasegawa T, Yamaki K, Takeda K, Akira S, Shimokata K, Isobe K (2003) The function of GADD34 is a recovery from a shutoff of protein synthesis induced by ER stress: elucidation by GADD34-deficient mice. FASEB J Off Publ Fed Am Soc Exp Biol 17(11):1573–1575

Kong L, Giang E, Nieusma T, Kadam RU, Cogburn KE, Hua Y, Dai X, Stanfield RL, Burton DR, Ward AB, Wilson IA, Law M (2013) Hepatitis C virus E2 envelope glycoprotein core structure. Science 342(6162):1090–1094

Korenaga M, Wang T, Li Y, Showalter LA, Chan T, Sun J, Weinman SA (2005) Hepatitis C virus core protein inhibits mitochondrial electron transport and increases reactive oxygen species (ROS) production. J Biol Chem 280(45):37481–37488

Krey T, d'Alayer J, Kikuti CM, Saulnier A, Damier-Piolle L, Petitpas I, Johansson DX, Tawar RG, Baron B, Robert B, England P, Persson MA, Martin A, Rey FA (2010) The disulfide bonds in glycoprotein E2 of hepatitis C virus reveal the tertiary organization of the molecule. PLoS Pathog 6(2):e1000762

Lavillette D, Pecheur EI, Donot P, Fresquet J, Molle J, Corbau R, Dreux M, Penin F, Cosset FL (2007) Characterization of fusion determinants points to the involvement of three discrete regions of both E1 and E2 glycoproteins in the membrane fusion process of hepatitis C virus. J Virol 81(16):8752–8765

Lee YH, Giraud J, Davis RJ, White MF (2003) c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. J Biol Chem 278(5):2896–2902

Lee SH, Kim YK, Kim CS, Seol SK, Kim J, Cho S, Song YL, Bartenschlager R, Jang SK (2005) E2 of hepatitis C virus inhibits apoptosis. J Immunol 175(12):8226–8235

Li Y, Boehning DF, Qian T, Popov VL, Weinman SA (2007) Hepatitis C virus core protein increases mitochondrial ROS production by stimulation of Ca2+ uniporter activity. FASEB J Off Publ Fed Am Soc Exp Biol 21(10):2474–2485

Li X, Chen H, Oh SS, Chishti AH (2008) A Presenilin-like protease associated with Plasmodium falciparum micronemes is involved in erythrocyte invasion. Mol Biochem Parasitol 158 (1):22–31

Li G, Mongillo M, Chin KT, Harding H, Ron D, Marks AR, Tabas I (2009a) Role of ERO1-alpha-mediated stimulation of inositol 1,4,5-triphosphate receptor activity in endoplasmic reticulum stress-induced apoptosis. J Cell Biol 186(6):783–792

Li HF, Huang CH, Ai LS, Chuang CK, Chen SS (2009b) Mutagenesis of the fusion peptide-like domain of hepatitis C virus E1 glycoprotein: involvement in cell fusion and virus entry. J Biomed Sci 16:89

Li X, Chen H, Bahamontes-Rosa N, Kun JF, Traore B, Crompton PD, Chishti AH (2009c) Plasmodium falciparum signal peptide peptidase is a promising drug target against blood stage malaria. Biochem Biophys Res Commun 380(3):454–459

Lima-Cabello E, Garcia-Mediavilla MV, Miquilena-Colina ME, Vargas-Castrillon J, Lozano-Rodriguez T, Fernandez-Bermejo M, Olcoz JL, Gonzalez-Gallego J, Garcia-Monzon C, Sanchez-Campos S (2011) Enhanced expression of pro-inflammatory mediators and liver X-receptor-regulated lipogenic genes in non-alcoholic fatty liver disease and hepatitis C. Clin Sci (Lond) 120(6):239–250

Liu X, Theil EC (2005) Ferritins: dynamic management of biological iron and oxygen chemistry. Acc Chem Res 38(3):167–175

Loureiro J, Lilley BN, Spooner E, Noriega V, Tortorella D, Ploegh HL (2006) Signal peptide peptidase is required for dislocation from the endoplasmic reticulum. Nature 441 (7095):894–897

Lozach PY, Lortat-Jacob H, de Lacroix de Lavalette A, Staropoli I, Foung S, Amara A, Houles C, Fieschi F, Schwartz O, Virelizier JL, Arenzana-Seisdedos F, Altmeyer R (2003) DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2. J Biol Chem 278(22):20358–20366

Lyons S, Kapoor A, Sharp C, Schneider BS, Wolfe ND, Culshaw G, Corcoran B, McGorum BC, Simmonds P (2012) Nonprimate hepaciviruses in domestic horses, United kingdom. Emerg Infect Dis 18(12):1976–1982

Machida K, Cheng KT, Lai CK, Jeng KS, Sung VM, Lai MM (2006) Hepatitis C virus triggers mitochondrial permeability transition with production of reactive oxygen species, leading to DNA damage and STAT3 activation. J Virol 80(14):7199–7207

Majeau N, Fromentin R, Savard C, Duval M, Tremblay MJ, Leclerc D (2009) Palmitoylation of hepatitis C virus core protein is important for virion production. J Biol Chem 284 (49):33915–33925

Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. Cell 129 (7):1261–1274

Matsuura Y, Harada S, Suzuki R, Watanabe Y, Inoue Y, Saito I, Miyamura T (1992) Expression of processed envelope protein of hepatitis C virus in mammalian and insect cells. J Virol 66 (3):1425–1431

Mattijssen F, Kersten S (2012) Regulation of triglyceride metabolism by Angiopoietin-like proteins. Biochim Biophys Acta 1821(5):782–789

McLauchlan J (2000) Properties of the hepatitis C virus core protein: a structural protein that modulates cellular processes. J Viral Hepat 7(1):2–14

McLauchlan J, Lemberg MK, Hope G, Martoglio B (2002) Intramembrane proteolysis promotes trafficking of hepatitis C virus core protein to lipid droplets. EMBO J 21(15):3980–3988

McPherson S, Jonsson JR, Barrie HD, O'Rourke P, Clouston AD, Powell EE (2008) Investigation of the role of SREBP-1c in the pathogenesis of HCV-related steatosis. J Hepatol 49 (6):1046–1054

Meynard D, Kautz L, Darnaud V, Canonne-Hergaux F, Coppin H, Roth MP (2009) Lack of the bone morphogenetic protein BMP6 induces massive iron overload. Nat Genet 41(4):478–481

Mihm S, Fayyazi A, Hartmann H, Ramadori G (1997) Analysis of histopathological manifestations of chronic hepatitis C virus infection with respect to virus genotype. Hepatology 25 (3):735–739

Miyamoto H, Moriishi K, Moriya K, Murata S, Tanaka K, Suzuki T, Miyamura T, Koike K, Matsuura Y (2007) Involvement of the PA28gamma-dependent pathway in insulin resistance induced by hepatitis C virus core protein. J Virol 81(4):1727–1735

Miyanari Y, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, Bartenschlager R, Wakita T, Hijikata M, Shimotohno K (2007) The lipid droplet is an important organelle for hepatitis C virus production. Nat Cell Biol 9(9):1089–1097

Miyoshi H, Fujie H, Shintani Y, Tsutsumi T, Shinzawa S, Makuuchi M, Kokudo N, Matsuura Y, Suzuki T, Miyamura T, Moriya K, Koike K (2005) Hepatitis C virus core protein exerts an inhibitory effect on suppressor of cytokine signaling (SOCS)-1 gene expression. J Hepatol 43 (5):757–763

Miyoshi H, Moriya K, Tsutsumi T, Shinzawa S, Fujie H, Shintani Y, Fujinaga H, Goto K, Todoroki T, Suzuki T, Miyamura T, Matsuura Y, Yotsuyanagi H, Koike K (2011) Pathogenesis of lipid metabolism disorder in hepatitis C: polyunsaturated fatty acids counteract lipid alterations induced by the core protein. J Hepatol 54(3):432–438

Mohankumar KM, Xu XQ, Zhu T, Kannan N, Miller LD, Liu ET, Gluckman PD, Sukumar S, Emerald BS, Lobie PE (2007) HOXA1-stimulated oncogenicity is mediated by selective upregulation of components of the p44/42 MAP kinase pathway in human mammary carcinoma cells. Oncogene 26(27):3998–4008

Moriishi K, Matsuura Y (2012) Exploitation of lipid components by viral and host proteins for hepatitis C virus infection. Front Microbiol 3:54

Moriishi K, Okabayashi T, Nakai K, Moriya K, Koike K, Murata S, Chiba T, Tanaka K, Suzuki R, Suzuki T, Miyamura T, Matsuura Y (2003) Proteasome activator PA28gamma-dependent nuclear retention and degradation of hepatitis C virus core protein. J Virol 77 (19):10237–10249

Moriishi K, Mochizuki R, Moriya K, Miyamoto H, Mori Y, Abe T, Murata S, Tanaka K, Miyamura T, Suzuki T, Koike K, Matsuura Y (2007) Critical role of PA28gamma in hepatitis C virus-associated steatogenesis and hepatocarcinogenesis. Proc Natl Acad Sci U S A 104 (5):1661–1666

Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, Miyamura T, Koike K (1997) Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol 78(Pt 7):1527–1531

Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K (1998) The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med 4(9):1065–1067

Moriya K, Nakagawa K, Santa T, Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Miyazawa T, Ishibashi K, Horie T, Imai K, Todoroki T, Kimura S, Koike K (2001) Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res 61(11):4365–4670

Mukherjee A, Shrivastava S, Bhanja Chowdhury J, Ray R, Ray RB (2014) Transcriptional suppression of miR-181c by hepatitis C virus enhances homeobox A1 expression. J Virol 88 (14):7929–7940

Nakajima A, Komazawa-Sakon S, Takekawa M, Sasazuki T, Yeh WC, Yagita H, Okumura K, Nakano H (2006) An antiapoptotic protein, c-FLIPL, directly binds to MKK7 and inhibits the JNK pathway. EMBO J 25(23):5549–5559

Negro F (2010) Hepatitis C virus-induced steatosis: an overview. Dig Dis 28(1):294–299

Nemeth E, Ganz T (2006) Regulation of iron metabolism by hepcidin. Annu Rev Nutr 26:323–342

Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J (2004) Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306(5704):2090–2093

Nemeth E, Preza GC, Jung CL, Kaplan J, Waring AJ, Ganz T (2006) The N-terminus of hepcidin is essential for its interaction with ferroportin: structure-function study. Blood 107(1):328–333

Nielsen SU, Bassendine MF, Burt AD, Martin C, Pumeechockchai W, Toms GL (2006) Association between hepatitis C virus and very-low-density lipoprotein (VLDL)/LDL analyzed in iodixanol density gradients. J Virol 80(5):2418–2428

Nishina S, Hino K, Korenaga M, Vecchi C, Pietrangelo A, Mizukami Y, Furutani T, Sakai A, Okuda M, Hidaka I, Okita K, Sakaida I (2008) Hepatitis C virus-induced reactive oxygen species raise hepatic iron level in mice by reducing hepcidin transcription. Gastroenterology 134(1):226–238

Nunez O, Fernandez-Martinez A, Majano PL, Apolinario A, Gomez-Gonzalo M, Benedicto I, Lopez-Cabrera M, Bosca L, Clemente G, Garcia-Monzon C, Martin-Sanz P (2004) Increased intrahepatic cyclooxygenase 2, matrix metalloproteinase 2, and matrix metalloproteinase 9 expression is associated with progressive liver disease in chronic hepatitis C virus infection: role of viral core and NS5A proteins. Gut 53(11):1665–1672

Ogino T, Fukuda H, Imajoh-Ohmi S, Kohara M, Nomoto A (2004) Membrane binding properties and terminal residues of the mature hepatitis C virus capsid protein in insect cells. J Virol 78 (21):11766–11777

Okada S (1996) Iron-induced tissue damage and cancer: the role of reactive oxygen species-free radicals. Pathol Int 46(5):311–332

Okamoto K, Moriishi K, Miyamura T, Matsuura Y (2004) Intramembrane proteolysis and endoplasmic reticulum retention of hepatitis C virus core protein. J Virol 78(12):6370–6380

Okamoto K, Mori Y, Komoda Y, Okamoto T, Okochi M, Takeda M, Suzuki T, Moriishi K, Matsuura Y (2008) Intramembrane processing by signal peptide peptidase regulates the

membrane localization of hepatitis C virus core protein and viral propagation. J Virol 82 (17):8349–8361

Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, Weinman SA (2002) Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. Gastroenterology 122(2):366–375

Olynyk JK, Reddy KR, Di Bisceglie AM, Jeffers LJ, Parker TI, Radick JL, Schiff ER, Bacon BR (1995) Hepatic iron concentration as a predictor of response to interferon alfa therapy in chronic hepatitis C. Gastroenterology 108(4):1104–1109

Pantopoulos K, Porwal SK, Tartakoff A, Devireddy L (2012) Mechanisms of mammalian iron homeostasis. Biochemistry 51(29):5705–5724

Parvanova I, Epiphanio S, Fauq A, Golde TE, Prudencio M, Mota MM (2009) A small molecule inhibitor of signal peptide peptidase inhibits Plasmodium development in the liver and decreases malaria severity. PLoS One 4(4):e5078

Pazienza V, Clement S, Pugnale P, Conzelman S, Foti M, Mangia A, Negro F (2007) The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms. Hepatology 45(5):1164–1171

Perlemuter G, Sabile A, Letteron P, Vona G, Topilco A, Chretien Y, Koike K, Pessayre D, Chapman J, Barba G, Brechot C (2002) Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. FASEB J 16(2):185–194

Persico M, Capasso M, Persico E, Svelto M, Russo R, Spano D, Croce L, La Mura V, Moschella F, Masutti F, Torella R, Tiribelli C, Iolascon A (2007) Suppressor of cytokine signaling 3 (SOCS3) expression and hepatitis C virus-related chronic hepatitis: insulin resistance and response to antiviral therapy. Hepatology 46(4):1009–1015

Piccoli C, Scrima R, Quarato G, D'Aprile A, Ripoli M, Lecce L, Boffoli D, Moradpour D, Capitanio N (2007) Hepatitis C virus protein expression causes calcium-mediated mitochondrial bioenergetic dysfunction and nitro-oxidative stress. Hepatology 46(1):58–65

Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, Abrignani S (1998) Binding of hepatitis C virus to CD81. Science 282 (5390):938–941

Ploss A, Evans MJ, Gaysinskaya VA, Panis M, You H, de Jong YP, Rice CM (2009) Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. Nature 457 (7231):882–886

Pohlmann S, Zhang J, Baribaud F, Chen Z, Leslie GJ, Lin G, Granelli-Piperno A, Doms RW, Rice CM, McKeating JA (2003) Hepatitis C virus glycoproteins interact with DC-SIGN and DC-SIGNR. J Virol 77(7):4070–4080

Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkouls K, Keulers T, Mujcic H, Landuyt W, Voncken JW, Lambin P, van der Kogel AJ, Koritzinsky M, Wouters BG (2010) The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. J Clin Invest 120(1):127–141

Rui L, Aguirre V, Kim JK, Shulman GI, Lee A, Corbould A, Dunaif A, White MF (2001) Insulin/IGF-1 and TNF-alpha stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. J Clin Invest 107(2):181–189

Rui L, Yuan M, Frantz D, Shoelson S, White MF (2002) SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. J Biol Chem 277 (44):42394–42398

Saeed M, Suzuki R, Watanabe N, Masaki T, Tomonaga M, Muhammad A, Kato T, Matsuura Y, Watanabe H, Wakita T, Suzuki T (2011) Role of the endoplasmic reticulum-associated degradation (ERAD) pathway in degradation of hepatitis C virus envelope proteins and production of virus particles. J Biol Chem 286(43):37264–37273

Shavinskaya A, Boulant S, Penin F, McLauchlan J, Bartenschlager R (2007) The lipid droplet binding domain of hepatitis C virus core protein is a major determinant for efficient virus assembly. J Biol Chem 282(51):37158–37169

Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K (2004) Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology 126(3):840–848

Shirakura M, Murakami K, Ichimura T, Suzuki R, Shimoji T, Fukuda K, Abe K, Sato S, Fukasawa M, Yamakawa Y, Nishijima M, Moriishi K, Matsuura Y, Wakita T, Suzuki T, Howley PM, Miyamura T, Shoji I (2007) E6AP ubiquitin ligase mediates ubiquitylation and degradation of hepatitis C virus core protein. J Virol 81(3):1174–1185

Shukla R, Upton KR, Munoz-Lopez M, Gerhardt DJ, Fisher ME, Nguyen T, Brennan PM, Baillie JK, Collino A, Ghisletti S, Sinha S, Iannelli F, Radaelli E, Dos Santos A, Rapoud D, Guettier C, Samuel D, Natoli G, Carninci P, Ciccarelli FD, Garcia-Perez JL, Faivre J, Faulkner GJ (2013) Endogenous retrotransposition activates oncogenic pathways in hepatocellular carcinoma. Cell 153(1):101–111

Singaravelu R, Chen R, Lyn RK, Jones DM, O'Hara S, Rouleau Y, Cheng J, Srinivasan P, Nasheri N, Russell RS, Tyrrell DL, Pezacki JP (2014) Hepatitis C virus induced up-regulation of microRNA-27: a novel mechanism for hepatic steatosis. Hepatology 59 (1):98–108

Solinas G, Karin M (2010) JNK1 and IKKbeta: molecular links between obesity and metabolic dysfunction. FASEB J Off Publ Fed Am Soc Exp Biol 24(8):2596–2611

Tai AW, Benita Y, Peng LF, Kim SS, Sakamoto N, Xavier RJ, Chung RT (2009) A functional genomic screen identifies cellular cofactors of hepatitis C virus replication. Cell Host Microbe 5(3):298–307

Tanaka N, Moriya K, Kiyosawa K, Koike K, Gonzalez FJ, Aoyama T (2008) PPARalpha activation is essential for HCV core protein-induced hepatic steatosis and hepatocellular carcinoma in mice. J Clin Invest 118(2):683–694

Tanaka T, Kasai H, Yamashita A, Okuyama-Dobashi K, Yasumoto J, Maekawa S, Enomoto N, Okamoto T, Matsuura Y, Morimatsu M, Manabe N, Ochiai K, Yamashita K, Moriishi K (2014) Hallmarks of hepatitis C virus in equine hepacivirus. J Virol in press. doi:10.1128/JVI.02280-02214

Taniguchi CM, Emanuelli B, Kahn CR (2006) Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 7(2):85–96

Targett-Adams P, Schaller T, Hope G, Lanford RE, Lemon SM, Martin A, McLauchlan J (2006) Signal peptide peptidase cleavage of GB virus B core protein is required for productive infection in vivo. J Biol Chem 281(39):29221–29227

Theurl I, Zoller H, Obrist P, Datz C, Bachmann F, Elliott RM, Weiss G (2004) Iron regulates hepatitis C virus translation via stimulation of expression of translation initiation factor 3. J Infect Dis 190(4):819–825

Thirone AC, Huang C, Klip A (2006) Tissue-specific roles of IRS proteins in insulin signaling and glucose transport. Trends Endocrinol Metab TEM 17(2):72–78

Thomssen R, Bonk S, Propfe C, Heermann KH, Kochel HG, Uy A (1992) Association of hepatitis C virus in human sera with beta-lipoprotein. Med Microbiol Immunol 181(5):293–300

Thomssen R, Bonk S, Thiele A (1993) Density heterogeneities of hepatitis C virus in human sera due to the binding of beta-lipoproteins and immunoglobulins. Med Microbiol Immunol 182 (6):329–334

Tsutsumi T, Suzuki T, Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Matsuura Y, Kimura S, Koike K, Miyamura T (2002a) Alteration of intrahepatic cytokine expression and AP-1 activation in transgenic mice expressing hepatitis C virus core protein. Virology 304 (2):415–424

Tsutsumi T, Suzuki T, Shimoike T, Suzuki R, Moriya K, Shintani Y, Fujie H, Matsuura Y, Koike K, Miyamura T (2002b) Interaction of hepatitis C virus core protein with retinoid X receptor alpha modulates its transcriptional activity. Hepatology 35(4):937–946

Tsutsumi T, Matsuda M, Aizaki H, Moriya K, Miyoshi H, Fujie H, Shintani Y, Yotsuyanagi H, Miyamura T, Suzuki T, Koike K (2009) Proteomics analysis of mitochondrial proteins reveals

overexpression of a mitochondrial protein chaperon, prohibitin, in cells expressing hepatitis C virus core protein. Hepatology 50(2):378–386

Ueki K, Kondo T, Kahn CR (2004) Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. Mol Cell Biol 24(12):5434–5446

Wang RH, Li C, Xu X, Zheng Y, Xiao C, Zerfas P, Cooperman S, Eckhaus M, Rouault T, Mishra L, Deng CX (2005) A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. Cell Metab 2(6):399–409

Wang Q, Liu Y, An D, Diao H, Xu W, He X, Sun R, Wei L, Li L (2012) Regulation of hepatitis C virus translation initiation by iron: role of eIF3 and La protein. Virus Res 167(2):302–309

Wang J, Kang R, Huang H, Xi X, Wang B, Wang J, Zhao Z (2014) Hepatitis C virus core protein activates autophagy through EIF2AK3 and ATF6 UPR pathway-mediated MAP1LC3B and ATG12 expression. Autophagy 10(5):766–784

Waris G, Felmlee DJ, Negro F, Siddiqui A (2007) Hepatitis C virus induces proteolytic cleavage of sterol regulatory element binding proteins and stimulates their phosphorylation via oxidative stress. J Virol 81(15):8122–8130

Werner ED, Lee J, Hansen L, Yuan M, Shoelson SE (2004) Insulin resistance due to phosphorylation of insulin receptor substrate-1 at serine 302. J Biol Chem 279(34):35298–35305

Wu CF, Lin YL, Huang YT (2013) Hepatitis C virus core protein stimulates fibrogenesis in hepatic stellate cells involving the obese receptor. J Cell Biochem 114(3):541–550

Xia Y, Babitt JL, Sidis Y, Chung RT, Lin HY (2008) Hemojuvelin regulates hepcidin expression via a selective subset of BMP ligands and receptors independently of neogenin. Blood 111 (10):5195–5204

Yamaguchi A, Tazuma S, Nishioka T, Ohishi W, Hyogo H, Nomura S, Chayama K (2005) Hepatitis C virus core protein modulates fatty acid metabolism and thereby causes lipid accumulation in the liver. Dig Dis Sci 50(7):1361–1371

Youngren JF (2007) Regulation of insulin receptor function. Cell Mol Life Sci: CMLS 64 (7–8):873–891

Yu S, Matsusue K, Kashireddy P, Cao WQ, Yeldandi V, Yeldandi AV, Rao MS, Gonzalez FJ, Reddy JK (2003) Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor gamma1 (PPARgamma1) overexpression. J Biol Chem 278(1):498–505

Zhang M, Gaschen B, Blay W, Foley B, Haigwood N, Kuiken C, Korber B (2004) Tracking global patterns of N-linked glycosylation site variation in highly variable viral glycoproteins: HIV, SIV, and HCV envelopes and influenza hemagglutinin. Glycobiology 14(12):1229–1246