Liver Injury and Disease Pathogenesis in Chronic Hepatitis C

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Abstract Chronic hepatitis C virus (HCV) infection is a leading cause of liver-specific morbidity and mortality in humans, including progressive liver fibrosis, cirrhosis, and hepatocellular carcinoma. It has also been associated with altered function in other organs, including those of the endocrine, hematopoietic, and nervous systems. Disease results from both direct regulation of cellular metabolism and signaling pathways by viral proteins as well as indirect consequences of the host response to HCV infection, including inflammatory responses stemming from immune recognition of the virus. Recent in vitro studies have begun to reveal molecular mechanisms responsible for virus-induced changes in cell metabolism and cellular kinase cascades that culminate in pathologic consequences in the liver, such as steatosis, insulin resistance, and carcinogenesis. Here we discuss how these findings may be relevant to disease pathogenesis in patients, and suggest future directions in the field.

Contents

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1 Chronic HCV Infection and Liver Disease

Persistent HCV infection is typically associated with chronic inflammatory changes within the liver. These reflect an unresolved wound healing response that includes abnormal production of extracellular matrix proteins and progressive fibrosis, the pathogenesis of which is only partially understood. As the disease advances, over a period of years to decades, the liver architecture is disrupted and liver function is inexorably diminished. Fibrotic changes are generally confined to the portal tracts early in the disease process, but with progression there is extension to the centrilobular area with "bridging" between adjacent portal tracts. In a significant proportion of infected patients, the process culminates in frank cirrhosis, in which the basic architecture of the liver is disrupted by fibrotic tissue interspersed with nodules of regenerating hepatocytes. Cirrhosis often develops silently, becoming symptomatic only at a late stage in its development, and it is inherently procarcinogenic. Individuals with hepatitis C-associated cirrhosis are thus at particularly high risk for developing hepatocellular carcinoma (HCC).

To a large extent, these pathologic consequences of HCV infection are likely to result from an active, but ultimately ineffective immune response to the virus that causes liver injury, but fails to eliminate the infection (see chapter "Adaptive Immune Responses in Hepatitis C Virus Infection" by Neumann-Haefelin and Thimme, this volume). This is consistent with the fact that very sensitive multiphoton microscopy methods detect viral antigen in only a small minority (~5–20 %) of hepatocytes (Liang et al. 2009). However, there is continuing controversy about the relative importance of such indirect, immune-mediated mechanisms versus more direct effects stemming from the expression of viral proteins in the development of fibrosis as well as liver cancer. Oxidative stress is an important aspect of HCV pathogenesis, as described in greater detail below, and may result directly from the expression of viral proteins as well as from inflammation related to immune recognition of the virus. Similarly, alterations in intrahepatic lipid metabolism, steatosis, and insulin resistance have been attributed to both virus-specific and nonspecific causes. A paucity of animal models of HCV-related disease continues to fuel this uncertainty, which is unlikely to be resolved until better animal models become available (the current status of animal models is described in the chapter "Cell Culture Systems for Hepatitis C Virus" by Steinmann and Pietschmann, this volume).

2 Fibrosis and Chronic HCV Infection

Chronic inflammation drives fibrogenesis, a process in which there is increased deposition of extracellular matrix proteins leading to fibrotic scarring and ultimately cirrhosis. The perisinusoidal hepatic stellate cell (HSC) plays a central role in this. Fundamental to wound healing within the liver, quiescent HSC that are resident within the liver undergo transformation to become proliferative, contractile myofibroblasts (MFs) [reviewed in Hernandez-Gea and Friedman (2011)]. MFs are

the dominant source of extracellular matrix within the liver, and while they may arise from other cell types, they are derived primarily from HSCs. The activation of HSCs and their transformation to MFs is intimately linked to the immune response to the virus, and both positively and negatively regulated via multiple growth factors, cytokines, and chemokines, including in particular transforming growth factor β (TGF-β) and platelet-derived growth factor (PDGF), tumor necrosis factor alpha (TNF-α), and monocyte chemotactic protein-1 (MCP-1 or CCL2), all of which are thought to act positively to enhance fibrogenesis (Hernandez-Gea and Friedman 2011). Interferon-γ, on the other hand, produced both by NK cells and virus-specific T cells, may negatively regulate fibrosis by inducing phosphorylation of STAT1. Oxidative stress is potent stimulus to HSC activation and is generated in large part by the inflammatory immune response (Brenner 2009). During chronic infection, intrahepatic α/β or γ/δ T, NK, and NKT lymphocytes sustain inflammation and may thus contribute to the continued progression of fibrosis.

However, the expression of specific viral proteins may play a role in HSC activation and initiation of fibrosis, acting directly on quiescent HSC to induce their transformation to MFs. CD81 expressed by HSCs may be bound by the E2 envelope glycoprotein, inducing matrix metalloproteinase 2 that degrades the normal extracellular matrix (Mazzocca et al. 2005). There is also indirect evidence that both structural and nonstructural proteins of HCV may stimulate HSC proliferation and/or increased production of chemokines and cellular adhesion molecules (Bataller et al. 2004). In vitro studies also suggest that the expression of TGF-β and other pro-fibrotic signals may be induced in hepatocytes by HCV infection (Schulze-Krebs et al. 2005). A key question that remains unanswered is whether hepatocytes are the only cell type infected by HCV in the liver, or whether HCV has the potential to infect HSCs or immune cells infiltrating the liver.

The transcription factor NF-κB plays a central role in regulating both the inflammatory response to HCV within the liver, as well as linking inflammation to fibrosis and carcinogenesis (Luedde and Schwabe 2011; Sun and Karin 2008). NF-κB may be activated when HCV infection is sensed by pathogen recognition receptors of the innate immune system, including retinoic acid-inducible gene I (RIG-I) and Toll-like receptor 3 (TLR3), both of which trigger signaling pathways that lead to activation of NF-κB as well as interferon regulatory factor 3 (IRF-3) (Saito et al. 2008; Wang et al. 2009c) (see chapter "Innate immune responses to Hepatitis C Virus" by Schoggins and Rice, this volume). Other studies implicate HCV-induced endoplasmic reticulum stress (Waris et al. 2002), or suggest that the expression of HCV-encoded proteins may directly activate NF-κB signaling (Dolganiuc et al. 2004; Sato et al. 2006; Waris et al. 2003). Similarly, both core and NS3 have been reported to stimulate IL-1 receptor-associated kinase (IRAK) activity in different types of cells, while phosphorylation of p38 and activation of extracellular regulated kinase (ERK) and c-jun N-terminal kinase (JNK) may result from TLR2-mediated sensing of infection (Dolganiuc et al. 2004). All of these responses are likely to promote a fibrogenic response in the continuing presence of infection. However, many of these studies need to be interpreted with caution as their conclusions are based on in vitro systems in which viral protein expression may be many fold higher than it is in the liver in chronic hepatitis C (Liang et al. 2009).

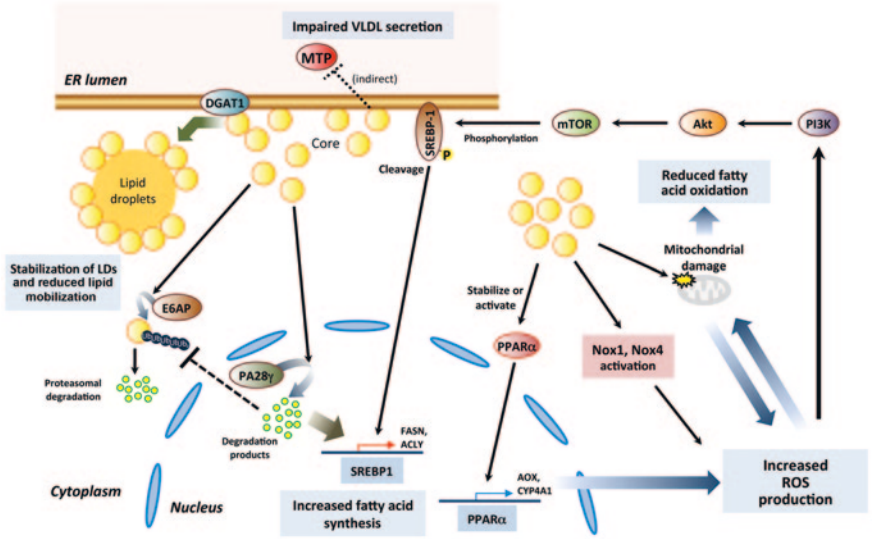

Fig. 1 Potential mechanisms underlying steatosis induced by the HCV core protein. Steatosis results from enhanced lipogenesis, increased stability of lipid droplets (LDs), reduced lipoprotein secretion, and altered mitochondrial function. Core protein localizes to lipid droplets in a DGAT1-dependent manner, slows turnover of LDs, and causes their accumulation in the cytoplasm. Increased transactivation of SREBP-1 by core protein occurs through PA28γ-dependent fashion in nucleus and ROS-activated phosphorylation-dependent activation of SREBP-1 through the PI3K-Akt-mTOR pathway. PA28γ also suppresses the ubiquitin (Ub)-dependent proteasomal degradation of core protein by E6AP, maintaining a high abundance of core protein in the cytoplasm. Oxidative stress induced by core occurs through (1) mitochondrial damage resulting from its interactions with the mitochondrial outer membrane, (2) activation of NADPH oxidase in TGF-β1-dependent or independent manner, or (3) persistent activation of PPARα, which enhances the expression of lipid oxidation enzymes. Reduced fatty acid oxidation resulting from mitochondrial injury also contributes to increased fatty acid accumulation

3 Steatosis in Chronic Hepatitis C

Hepatic steatosis (fatty liver) is frequently associated with HCV infection. This is characterized by the cytoplasmic accumulation of lipid droplets (LDs), mainly composed of triglyceride and cholesteryl ester, and results from multiple inter-related effects of the virus on cellular metabolism as depicted in Fig. 1. Steatosis is more frequently observed in patients with chronic hepatitis C than hepatitis B (Altlparmak et al. 2005; Machado et al. 2011; Persico et al. 2009), and a number of studies suggest that it correlates with both the progression of hepatic fibrosis and development of HCC (Adinolfi et al. 2001; Leandro et al. 2006; Ohata et al. 2003; Pekow et al. 2007). The composition of triglycerides in the liver is uniquely and significantly enriched in carbon monounsaturated (C18:1) fatty acids in chronic hepatitis C (Moriya et al. 2001b). This is distinct from what occurs in obese patients and

suggests that it may be induced via a virus-specific mechanism. A similar conclusion is suggested by a uniquely high prevalence of steatosis in patients infected with genotype 3 HCV, in whom the degree of steatosis correlates well with viral load and sustained antiviral responses to therapy frequently coincide with resolution of the fatty liver (Adinolfi et al. 2001; Poynard et al. 2003; Rubbia-Brandt et al. 2001). A direct viral cause of steatosis is also supported by cell culture studies showing increased LD synthesis following HCV infection (Barba et al. 1997; Hope and McLauchlan 2000), and studies in chimpanzees showing virus induction of lipogenesis (Su et al. 2002). Rates of sustained virological response (SVR) to interferon-based therapy are lower in patients with severe steatosis (Patton et al. 2004; Westin et al. 2007), making it particularly important to understand the underlying pathogenetic mechanisms.

Both in patients as well as chimpanzees with HCV infection, there is increased hepatic expression of sterol regulatory element-binding protein-1 (SREBP-1) and enhanced transcription of SREBP-1-related genes involved in lipogenesis (Fujino et al. 2010; Su et al. 2002). It is likely that HCV proteins directly alter lipid metabolism in infected cells, as the intrahepatic expression of full-length genotype 1b HCV polyprotein induces steatosis in transgenic mice (Alonzi et al. 2004; Lerat et al. 2002). In at least one of these transgenic lineages, FL-N/35 mice (Lerat et al. 2002), the level of HCV protein expression was comparable to that in human subjects, in whom HCV proteins are typically very difficult to detect by immunoblotting or histochemical approaches (Liang et al. 2009). The development of steatosis and an increased frequency of HCC in FL-N/35 mice, in the absence of an immune response or inflammation, provides compelling evidence that HCV proteins expressed in the liver may play key roles in disease pathogenesis. Not all strains of mice appear to be equally susceptible to these effects of HCV protein expression, however, implying a potentially important role for host genetics.

HCV proteins could promote steatosis by increasing lipogenesis and fatty acid uptake, or decreasing fatty acid oxidation and lipoprotein secretion (Fig. 1). FL-N/35 transgenic mice have lower plasma triglyceride levels than control mice due to impaired microsomal triglyceride transfer protein (MTP) activity, and they demonstrate enhanced cleavage of SREBP-1c mediated by site-1 protease leading to increased expression of SREBP-1 regulated genes, such as ATP citrate-lyase, fatty acid synthase, and hepatic stearoyl-CoA desaturase 1 (Lerat et al. 2009). HCV infection also activates SREBPs in cell culture by stimulating its transcription and phosphorylation in addition to proteolytic cleavage (Waris et al. 2007).

Among HCV proteins, the core protein stands out as potentially important in the pathogenesis of hepatic steatosis and HCC in transgenic mice. A number of studies have confirmed an association between core expression and steatosis in transgenic mice (Lerat et al. 2002; Moriishi et al. 2007; Moriya et al. 1998; Naas et al. 2005; Tanaka et al. 2008), while others have found no such phenotype in transgenic mice expressing HCV structural proteins including core (Honda et al. 1999; Kawamura et al. 2006; Kawamura et al. 1997). This may reflect the influence of different genetic backgrounds in these transgenic mice, the level of transgene expression, or possibly the choice of promoters used to drive expression of transgenes. Importantly, however, the ectopic expression of core by itself

in cultured cells can recapitulate steatogenic effects observed in transgenic mice models, including MTP inhibition (Perlemuter et al. 2002; Yamaguchi et al. 2005) and activation of SREBP-1 (Moriishi et al. 2007). These observations underscore the importance of core as a principal regulator of HCV-associated steatosis. Consistent with this, Waris et al. (2007) demonstrated greater stimulation of SREBP-1 and SREBP-2 activity with genotype 3 core protein than the genotype 1 protein (Fig. 1), which correlates with the strong association of genotype 3 with steatosis in patients mentioned above.

Moriishi et al. (2003, 2007, 2010) have proposed that cellular pathways involved in degradation of the core protein might be important with respect to lipogenesis (Fig. 1). Core is normally degraded through interactions with E6-associated protein (E6AP), a HECT domain E3 ubiquitin ligase, or PA28γ, a proteasome activator, in the cytoplasm and nucleus, respectively (Moriishi et al. 2003; Shirakura et al. 2007; Suzuki et al. 2009). Significantly, transgenic mice expressing the core protein, but defective for PA28γ expression, do not exhibit increased SREBP-1 transactivation and are protected from hepatic steatosis (Moriishi et al. 2007). Core protein is normally found primarily within the cytoplasm, but it is most abundant in the nuclei of hepatocytes in the PA28$\gamma^{-/-}$ knockout mice. Consistent with this, core is found in the nucleus in cell cultures treated with a proteasome inhibitor, MG-132. This suggests that core is normally transported to the nucleus where it undergoes rapid PA28γ-dependent degradation (Moriishi et al. 2003). The predominant nuclear localization of core in PA28$\gamma^{-/-}$ liver may reflect a requirement for PA28γ to activate the trypsin-like peptidase of the 20S proteasome (Li and Rechsteiner 2001). These results suggest that core may need to be processed in a PA28γ-dependent manner to stimulate lipogenesis. However, PA28γ appears to have a contrary, protective role in E6AP-mediated degradation of core, as knockdown of PA28γ lessens the abundance of core within the cytoplasm (Moriishi et al. 2010). Thus, it is possible that the absence of cytoplasmic core in the liver of PA28$\gamma^{-/-}$ mice may result from accelerated ubiquitin-mediated degradation of cytoplasmic core protein (Fig. 1).

Other work suggests that the steatogenic effect of core requires diacylglycerol acyltransferase 1 (DGAT1). Core is localized on the surface of cytoplasmic lipid droplets (Barba et al. 1997; Hope and McLauchlan 2000), which have proven to be important in the assembly of infectious virus (Masaki et al. 2008; Miyanari et al. 2007) (see chapter "Virion Assembly and Release" by Lindenbach, this volume). Core protein transport to the lipid droplet and production of infectious virus is dependent upon the activity of DGAT1, but not DGAT2 (Herker et al. 2010). Consistent with this, in murine embryo fibroblasts lacking DGAT1, core protein does not associate with lipid droplets (Harris et al. 2011). Interestingly, the translocation of core to lipid droplets results in an increase in their stability, as indicated by delayed turnover of triglyceride and cholesteryl ester in the presence of core (Harris et al. 2011). The stabilizing effect of core on lipid droplets might contribute to enhanced cellular abundance of lipids (Fig. 1), as core transgenic mice lacking DGAT1 do not develop steatosis. While DGAT1 mediates core-induced steatosis, the over-expression of DGAT2, but not DGAT1, independently induces hepatic steatosis in mice (Monetti et al. 2007). There is no known

cross-talk between PA28γ and DGAT1, but PA28γ-mediated inhibition of E6AP-dependent degradation of core could facilitate core interactions with DGAT1 in the cytoplasm, thereby allowing core to translocate to lipid droplets (Fig. 1).

However, the interpretation of these data demands caution, as these studies rely heavily on protein over-expression. The abundance of core may thus be higher in these studies than it is normally in vivo, and both its localization and function could be affected by the absence of other viral proteins, such as NS3 or NS5A, with which it normally interacts (Masaki et al. 2008; Miyanari et al. 2007; Mousseau et al. 2011; Shi et al. 2002). Also, the potential involvement of other viral proteins should not be disregarded, as NS2 and NS4B are also capable of inducing accumulation of lipid droplets in cell culture (Oem et al. 2008; Waris et al. 2007). Steatosis is likely to result from effects mediated by multiple HCV proteins.

4 Insulin Resistance and HCV Infection

Insulin resistance and type 2 diabetes mellitus are associated with chronic hepatitis C to a greater extent than other chronic liver diseases, including chronic hepatitis B (Hui et al. 2003; Mason et al. 1999). The severity of insulin resistance correlates with poor response to interferon-based therapies and an increased risk of fibrosis (D'Souza et al. 2005; Muzzi et al. 2005; Ratziu et al. 2003) as well as HCC (Veldt et al. 2008; Wang et al. 2009b). Insulin resistance also has been implicated causally in steatosis and fibrosis (Fartoux et al. 2005). Importantly, insulin resistance and type 2 diabetes mellitus appear to occur as a consequence of the infection, because they may resolve with successful antiviral therapy (Arase et al. 2009; Kawaguchi et al. 2007; Simo et al. 2006).

Potential points of interaction of the virus with insulin signaling pathways have been sought in clinical studies, transgenic mice, and cell culture (Fig. 2). These studies have focused mainly on the insulin receptor, insulin receptor substrate 1 (IRS-1), and downstream phosphoinositol-3 kinase (PI3K)-Akt signaling. In patients with chronic hepatitis C, insulin receptor and IRS-1 expression were increased several fold compared with control subjects, but the receptor-IRS-1 interaction, IRS-1 phosphorylation, downstream PI3K activity, and phosphorylated Akt levels were significantly decreased (Aytug et al. 2003). Core transgenic mice also exhibit insulin resistance with significantly increased basal serum insulin levels and a several-fold increase in islet area (Shintani et al. 2004). In these animals, a loss of IRS-1 phosphorylation was restored by administrating anti-TNFα antibody. TNFα initiates signaling cascades that lead to insulin resistance within the liver by activating JNK kinases (Xu et al. 2008) and S6K1 through IKKβ (Zhang et al. 2008), and induces serine phosphorylation of IRS-1 that interferes with insulin-induced tyrosine phosphorylation (Kanety et al. 1995) (Fig. 2). Importantly, core protein expression triggers TNFα promoter activity in cell culture in a PA28γ-dependent manner in association with impaired insulin signaling

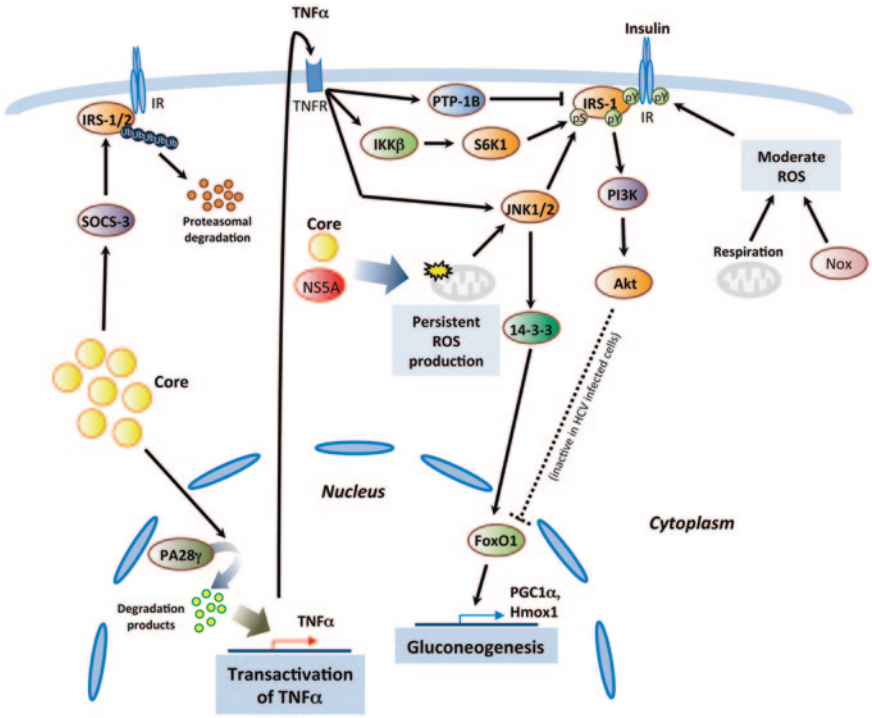

Fig. 2 Potential HCV interactions with insulin signaling. Insulin signaling normally occurs through the insulin receptor (IR)-IRS-PI3K-Akt pathway which leads to inhibition of FoxO1-mediated transcription by facilitating cytosolic translocation of the transcription factor from the nucleus following its phosphorylation at Ser319. Core protein may interfere with IRS-1/2 signaling by (1) enhancing SOCS-3 mediated proteasomal degradation, (2) upregulation of TNFα synthesis in PA28γ-dependent fashion, or (3) inducing persistent production of ROS due to mitochondrial injury, resulting in activation of JNK. JNK in turn phosphorylates 14-3-3, a cytoplasmic binding partner of FoxO1, facilitating its movement to the nucleus and subsequent activation of FoXO1-mediated transcription leading to gluconeogenesis. Thus, in vitro studies suggest that FoxO1 transcription is regulated primarily by an Akt-independent pathway in the context of HCV infection. TNFα negatively regulates tyrosine phosphorylation of IRS-1 by increasing PTP-1B expression and JNK1/2 and IKKβ-mediated S6K1 activity, which phosphorylates IRS-1 (pS) and thus interferes with tyrosine phosphorylation (pY)-mediated signal transduction. Tyrosine phosphorylation of IR is normally maintained by modest levels of endogenous ROS produced by mitochondria or NADPH oxidases (Nox), which oxidizes the IR beta-chain of IR and induces its autophosphorylation. Dysfunction of mitochondria associated with HCV infection may disable these normal regulatory functions

(Miyamoto et al. 2007). This finding may be of clinical relevance as TNFα levels are frequently elevated in patients with chronic hepatitis C (Polyak et al. 2001).

Oxidative stress (see Sect. 5) correlates strongly with the homeostasis model assessment-insulin resistance (HOMA-IR) score (Mitsuyoshi et al. 2008), and it is possible that insulin resistance might result from altered redox state regulation of the phosphorylation of kinases involved in insulin signaling (Bonnard et al. 2008; Yu et al. 2006). Numerous studies suggest that reactive oxygen species (ROS) generated

from mitochondria have an important influence on insulin signaling (Fig. 2). Insulin activates downstream signaling cascades by inducing tyrosine phosphorylation of its receptor, a process which requires modest elevation of ROS. H_2O_2 produced by mitochondrial respiration or NADPH oxidases are considered the major sources of endogenous ROS required for this (Storozhevykh et al. 2007). However, chronic exposure to high levels of ROS can result in insulin resistance (Anderson et al. 2009; Houstiset al. 2006), probably by inducing sustained autophosphorylation of the insulin receptor and activation of stress-response kinases, such as JNK, protein kinase C (PKC), and IKK, which can inactivate IRS-1 and -2 through serine phosphorylation (Lowell and Shulman 2005). Overproduction of ROS, leading to insulin resistance, can be triggered by mitochondrial damage due directly to expression of HCV proteins as well as mitochondrial hyperpolarization caused by chronic exposure to high glucose (Yu et al. 2006). Conversely, insulin resistance can trigger mitochondrial dysfunction by hyperactivating the transcription factor FoxO1, that in turn transactivates expression of Hmox1, thereby disrupting the respiratory chain and ATP production in mitochondria (Cheng et al. 2009). These studies suggest a very complex cause and effect relationship between insulin resistance and mitochondrial dysfunction centering on FoxO1.

FoxO1 regulates the expression of multiple genes involved in gluconeogenesis and lipid metabolism. It is regulated by IRS-1/2 signaling through Akt, which phosphorylates FoxO1, leading to its export from the nucleus (Dong et al. 2008). NS5A and, to a lesser extent, NS4A have been suggested to induce sustained activation of FoxO1-mediated transcription by inducing increases in its nuclear abundance (Deng et al. 2011) (Fig. 2). While Akt is activated in HCV-infected cells (and might be expected to suppress FoxO1 transcription), NS5A expression induces a state of oxidative stress that activates the stress-related kinase JNK. JNK in turn acts to phosphorylate 14-3-3, a cytoplasmic FoxO1 binding partner. This results in the dissociation of FoxO1 from 14-3-3, and the movement of FoxO1 to the nucleus where it can mediate transcription (Fig. 2). Thus, increases in gluconeogenesis that have been observed in cells over-expressing NS5A (or infected with HCV) can be blocked both by JNK inhibitors and ROS scavengers (Deng et al. 2011). Core protein, another potent ROS inducer, also activates JNK and induces sustained activation of FoxO1 transcription (Banerjee et al. 2010; Banerjee et al. 2008; Okuda et al. 2002). However, it is not clear whether either NS5A or core is expressed in sufficient abundance within hepatocytes in vivo to directly mediate such changes in FoxO1 transcription. Nonetheless, these observations explain how increased ROS production can be a primary cause of insulin resistance. HCV infection induces ROS production in cultured cells by causing mitochondrial injury (Deng et al. 2008), and, as discussed below, oxidative stress and increased ROS abundance are typically present within the chronically infected liver.

Degradation of IRS-1 leading to depletion of glucose transporter type 4 (GLUT4) expression may exacerbate increased gluconeogenesis (Bose et al. 2012). Other mechanisms may also be involved in HCV-associated insulin resistance, including possibly suppressor of cytokine signaling 3 (SOCS-3)-mediated ubiquitination and degradation of IRS-1 and -2 (Kawaguchi et al. 2004), or

dephosphorylation of Akt by protein phosphatase 2A (PP2A), which is overexpressed in the liver of HCV transgenic mice as well as HCV-infected patients (Bernsmeier et al. 2008) (Fig. 2). In addition, a recent study has demonstrated cross-talk between insulin signaling and interferon pathways. IRS-2 depletion increases tyrosine phosphatase PTP-1B activity, thereby negatively regulating JAK-STAT1 signaling due to loss of STAT1 phosphorylation (Garcia-Ruiz et al. 2012). This might be relevant to the increased rates of failure of interferon therapy in patients with insulin resistance, as IRS-1/2 abundance is frequently reduced.

5 HCV-Induced Oxidative Stress

Oxidative stress is more frequently associated with HCV than HBV infection (Fujita et al. 2008), and is accompanied by glutathione depletion (Barbaro et al. 1999b), increased oxidative DNA damage (Cardin et al. 2001; Fujita et al. 2007) and lipid peroxidation (Farinati et al. 1995), both in the liver and blood cells. Increased oxidative stress is thought to occur as a consequence of chronic inflammation, alteration of cellular oxidative enzymes, depletion of antioxidants and/or viral protein expression. Especially core and some nonstructural proteins are potent inducers of ROS in vitro (Garcia-Mediavilla et al. 2005; Gong et al. 2001; Korenaga et al. 2005; Li et al. 2002; Okuda et al. 2002; Thoren et al. 2004) and in transgenic mice (Machida et al. 2006; Moriya et al. 2001a; Tanaka et al. 2008; Wang et al. 2009a).

A fraction of the core protein localizes to the mitochondrial outer membrane, where it interacts with prohibition (Tsutsumi et al. 2009) and suppresses function of electron transport complex I, leading to increased Ca^{2+} influx and subsequent ROS production (Korenaga et al. 2005; Li et al. 2007). Breakdown of the mitochondrial outer membrane caused by core expression may be dependent upon activation of PPARα, followed by transactivation of genes involved in lipid oxidation, such as acyl-CoA oxidase and CYP4A1, as PPARα$^{-/-}$ core transgenic mice do not exhibit such abnormalities (Tanaka et al. 2008). Persistent activation of PPARα induced by core protein culminates in inhibition of the β-oxidation of lipids, and may contribute to steatosis in core transgenic mice.

In addition to mitochondrial ROS production, cellular oxidative enzymes, NADPH oxidases (Nox), are induced during HCV infection and may act as important sources of ROS, generating superoxides by catalyzing oxidation of NADPH (de Mochel et al. 2010). Nox4 contributes to TGF-β1-dependent ROS production in HCV-infected cell cultures (Boudreau et al. 2009). The transactivation of TGF-β1 by core protein may involve NFκB and/or the MAPK pathway (Lin et al. 2010; Taniguchi et al. 2004), although there are conflicting opinions as to whether TGF-β1 activation is a cause or effect of ROS production mediated by Nox4. This is potentially relevant to fibrosis, as TGF-β promotes the transformation of HSC to MF. Another study has shown that while Nox1 and Nox4 are markedly induced in cell culture as well as liver tissue from chronic hepatitis C patients, only Nox4 has the ability to translocate to the nucleus where it causes accumulation of nitrotyrosine, a marker for peroxynitrite production

(de Mochel et al. 2010). Immune cells involved in the inflammatory response to HCV infection also contribute to the development of oxidative stress.

A direct link between oxidative stress and steatosis has been suggested by Waris et al. (2007), who demonstrated that lipogenesis could be initiated by oxidative stress via the induction of PI3K-Akt signaling leading to activation of SREBP-1 and SREBP-2. Antioxidants, PI3K inhibitors, and calcium signaling inhibitors all suppressed HCV-induced transactivation and phosphorylation of SREBPs, consistent with a role for PI3K-Akt signaling and downstream involvement of mammalian target of rapamycin (mTORC1) (Laplante and Sabatini 2009; Wang and Sul 1998). This pathway is likely to contribute to insulin resistance associated with HCV infection as discussed above. Importantly, fatty liver is more prone to oxidation than normal tissue because of the enhanced accumulation of polyunsaturated fatty acids that are susceptible to peroxidation. Lipid peroxidation can rapidly spread via a chain reaction once initiated through interaction with ROS (Cheeseman and Slater 1993). Steatogenic effects initiated by oxidation may be amplified through such a mechanism. Lipid peroxidation is commonly detected in liver from patients with chronic hepatitis C (Barbaro et al. 1999a; Farinati et al. 1995), but significantly higher levels of protein adducts with lipid peroxidation products, malondialdehyde, 4-hydroxy-2-nonenal, and 4-hydroxy-2-hexanal, are found in patients with steatosis (Kitase et al. 2005). Multiple manifestations of injury associated with chronic hepatitis C might thus stem from persistent unresolved oxidative stress.

6 Hepatitis C-Associated Cancer

6.1 Hepatocellular Carcinoma

In the United States, HCC rates have risen faster than any other cancer over the past few decades, primarily as a result of chronic HCV infection (El-Serag and Rudolph 2007). While a lack of long-term studies makes it difficult to estimate the overall risk of HCC development in HCV-infected persons, in those patients who have progressed to cirrhosis, the annual risk for cancer development is 1–4 %. The mechanisms by which HCV promotes liver cancer are not well understood and, as with fibrosis and disturbances in lipid metabolism discussed above, the lack of a small animal model of chronic HCV infection has hampered progress in this direction.

The development of HCC in the setting of chronic HCV infection shares features in common with liver cancer arising due to other causes, such as alcohol, HBV infection, and nonalcoholic fatty liver disease (NAFLD). It is a multistep process, and as with other disease manifestations of HCV infection discussed above, the extent to which direct virus-specific mechanisms versus indirect effects of inflammation and accelerated hepatocellular turnover remain uncertain (Lemon and McGivern 2012). In patients with chronic hepatitis C, cancer typically (but not always) arises in the setting of advanced fibrosis and cirrhosis (Lok et al. 2011). As discussed above, the transcription factor NF-κB is a central regulator of the inflammatory responses

that lead to hepatic fibrogenesis and it is frequently activated in HCV-infected liver (Tai et al. 2000). NF-κB functions in multiple cell types within the liver, and there is abundant evidence that its activation promotes the activation, survival, and pro-fibrogenic inflammatory responses of HSCs and hepatic MFs (Luedde and Schwabe 2011).

HCCs are highly heterogeneous between patients and frequently exhibit aneuploidy, suggesting a role for mechanisms that promote genomic instability (Thorgeirsson and Grisham 2002). ROS present within the infected liver (see above) are likely to contribute to chromosomal damage, and thus may promote the development of cancer. Alcohol ingestion adds to the level of oxidative stress in the liver, potentially explaining synergy between alcohol and HCV infection in hepatic carcinogenesis. As discussed above, steatosis also correlates with an increased risk of cancer in the HCV-infected patient, consistent with an important role for oxidative stress. Thus, the development of HCC is very likely to represent a downstream result of the pathogenetic processes described in the preceding chapters that lead to abnormalities in lipid metabolism, steatosis, inflammation, and fibrosis.

Apoptosis of infected hepatocytes is likely to result from immune responses to the virus (Guidotti and Chisari 2006), and this could be an important factor in the development of cancer. The ability of the liver to regenerate following injury is unusual among adult organs, and HCC of all causes typically arises in an environment of chronic disease in which there are repeated cycles of hepatocyte death and regeneration. Recent studies suggest that chronic hepatocellular injury and compensatory proliferation may itself drive liver carcinogenesis. Knockdown of the pro-apoptotic p53-upregulated modulator of apoptosis (PUMA) reduces the incidence of HCC in mice challenged with the carcinogen diethylnitrosamine (Qiu et al. 2011). This finding might seem paradoxical at first glance, as apoptosis is often considered to be anticarcinogenic and cancer cells are often resistant to apoptosis. However, in the context of chronic liver disease and the early stages of tumorigenesis, apoptotic cell death and stimulation of regenerative cell growth may allow amplification of previously quiescent cells in which chromosomal damage has occurred. NF-κB activation may contribute to the proliferation and survival of such cells (Luedde and Schwabe 2011). Continuous cycling of these events in an environment of oxidative stress over a period of many years may permit accumulation of mutations in some cells, contributing eventually to their escape from normal growth controls and leading to a transformed phenotype. In agreement with this model, cancer has been observed in mice with constitutive genetic stimulation of hepatocyte turnover (Yamaji et al. 2010). In this model, HCV infection (or any other cause of chronic liver injury) is only indirectly associated with carcinogenesis, since the HCC would originate from an uninfected hepatocyte or hepatocyte progenitor.

However, there is evidence for more direct involvement of the virus in hepatocellular carcinogenesis. HCC occurs at high frequency in several HCV transgenic mouse lineages in which the HCV proteins are expressed specifically in the liver, either individually or together as part of the HCV polyprotein. The mouse genetic background seems to be important for the cancer phenotype, and C57BL/6 mice are particularly prone to development of HCC following expression of HCV proteins (Klopstock et al. 2009). This is interesting with respect to the relationship

between steatosis and HCC discussed above, as nontransgenic C57BL/6 mice are particularly prone to develop steatosis as they age (Lerat et al. 2002). Transgenic mouse studies implicate both structural and nonstructural proteins of HCV in carcinogenesis. Mice expressing a high abundance of a genotype 1b core protein developed hepatic adenomas at 12 months of age, and poorly differentiated HCC after 16 months (Moriya et al. 1998). This phenotype was not observed in nontransgenic littermates and was more frequent in males (26–31 %) than females (0–14 %). Steatosis was observed from the age of 3 months in the transgenic mice. In addition, the FL-N/35 mice discussed above, in which there is constitutive, low level, liver-specific expression of the entire polyprotein of a genotype 1b HCV also demonstrate age- and sex-dependent steatosis and development of hepatocellular adenoma and carcinoma (Lerat et al. 2002). Cancer was observed at higher rates in FL-N/35 mice than in other transgenic lineages that expressed only the structural proteins, suggesting a contribution of the nonstructural proteins to carcinogenesis. Consistent with this, a specific role for NS5A is suggested by an increased frequency of HCC in NS5A transgenic mice (Wang et al. 2009a). Importantly, HCV transgenic mice are more susceptible than their nontransgenic littermates to cancer development following a variety of secondary insults, including iron overload (Furutani et al. 2006), diethylnitrosamine exposure (Kamegaya et al. 2005), infection with Helicobacter hepaticus (Fox et al. 2010), aflatoxin (Jeannot et al. 2012), or alcohol (Machida et al. 2009).

A common feature of these transgenic models is that cancer develops in the absence of inflammation or immune recognition of the transgene. This speaks to the importance of viral proteins in HCV-related carcinogenesis, and could result from the presence of oxidative stress in the transgenic liver. A related possibility is that HCV protein expression may render the hepatocyte more susceptible to chromosomal damage and genomic instability through modulation of host cell cycle checkpoints. Such a hypothesis is supported by in vitro studies of HCV, which have revealed multiple interactions of HCV proteins with host proteins that act as tumor suppressors or otherwise control cellular proliferation (McGivern and Lemon 2009). These interactions may specifically impair the ability of infected cells to respond to DNA damage caused by the oxidative stress induced by HCV replication (Korenaga et al. 2005; Okuda et al. 2002), thereby allowing a genetically damaged cell to continue to proliferate.

Mutation or loss of p53, a master regulator of the cell that plays a critical role in the response to chromosomal DNA damage, is a common feature of HCC (Hussain et al. 2007). Various studies suggest that the HCV core protein (Kao et al. 2004; Kwun et al. 2001), NS3 (Ishido and Hotta 1998; Tanaka et al. 2006), and NS5A protein (Majumder et al. 2001; Qadri et al. 2002) interact with p53 in vitro, and may modulate p53 function (Kwun et al. 2001). This could leave the cell more susceptible to DNA damage as a result of oxidative stress or environmental insults. However, although intriguing, the results of these studies should be interpreted with caution since they have generally used protein overexpression systems or cell lines that may not be physiologically relevant.

Another cellular protein commonly mutated in HCC is the retinoblastoma tumor suppressor protein (Rb). Rb abundance is negatively regulated in cultured human

hepatoma cells supporting replication of subgenomic and full-length genotype 1b HCV replicons (Munakata et al. 2005), and also in cells infected with laboratory strains of HCV (McGivern et al. 2009; Munakata et al. 2007). The HCV polymerase, NS5B, associates with Rb through a motif with homology to the LXCXE motif found in cellular and viral Rb-binding proteins (Munakata et al. 2005). Further mechanistic studies demonstrated that the association of Rb with NS5B results in the recruitment of the ubiquitin ligase E6AP, targeting Rb for ubiquitin-dependent proteasomal degradation (Munakata et al. 2007). This targeting of Rb for degradation may be important for HCC development, since loss of Rb is a key event for promoting tumorigenesis (Mayhew et al. 2007; McClendon et al. 2011). Rb is important in regulation of the cell cycle, and required for certain checkpoints, thus its loss as a result of NS5B expression may render the infected hepatocyte more vulnerable to DNA damage. Such a model of HCV-related carcinogenesis does not require continued expression of NS5B for maintenance of the malignant phenotype (McGivern and Lemon 2009).

Other studies suggest that HCV may interfere with cellular pathways controlling differentiation. In the case of the Wingless/Int (Wnt) signaling pathway, NS5A protein causes stabilization of β-catenin either by direct interaction (Milward et al. 2010; Park et al. 2009) or by interaction with the p85 subunit of PI3K to modulate downstream signaling pathways, ultimately leading to β-catenin stabilization (Park et al. 2009; Street et al. 2005). In addition, the production of Hedgehog (Hh) ligands is increased in patients with chronic hepatitis C as well as in cultured hepatoma cells infected with HCV. Aberrant production of Hh ligands may impact Hh-responsive cell populations in the liver that expand during fibrosis and cirrhosis, including MFs and epithelial progenitors (Pereira Tde et al. 2010).

Finally, there is recent evidence that HCV infection can directly promote apoptosis. Infection of Huh-7 hepatoma cells with laboratory strains of HCV such as JFH1 or its derivatives results in a fraction of the cells undergoing apoptosis (Deng et al. 2008; Kannan et al. 2011; Walters et al. 2009), while HCV infection sensitizes Huh-7 cells to TRAIL-dependent apoptosis (Lan et al. 2008). HCV infection of chimeric SCID/Alb-uPA mice bearing human liver tissue results in disturbed lipid metabolism, endoplasmic reticulum and oxidative stress, and increased hepatocellular apoptosis (Joyce et al. 2009), all of which could contribute to the development of cancer.

Whether different genotypes or subtypes of HCV vary in their ability to cause cancer is a difficult question to answer since studies must take into account a number of potentially confounding factors such as duration of infection, age, and sex of the patient. More cancers tend to be associated with genotype 1 strains of HCV but this association may be related to the worldwide prevalence of this genotype. Some studies have found no association between HCV genotype and cancer risk (Ryu et al. 2009; Seto et al. 2010), while others suggest that infection with genotype 1b may confer a greater risk of HCC (Bruno et al. 2007; Raimondi et al. 2009). Specific aminoacid polymorphisms in core in genotype 1b HCV (especially Gln70 and Leu91) have been associated with a higher risk for cancer (Akuta

et al. 2007; Hu et al. 2009; Kobayashi et al. 2010; Nakamoto et al. 2010). The mechanism(s) driving the differences in HCC risk are not clear, but an interesting possibility is that these polymorphisms act to regulate expression of an N-terminally truncated core protein referred to as p8 minicore (Eng et al. 2009). The biological properties of this protein are not well characterized, and it could function to promote carcinogenesis (Ahmad et al. 2011).

Not all patients who develop cirrhosis will go on to develop cancer, and it is very likely that this is controlled at least in part by host genetic variation. Genome-wide association studies have identified polymorphisms that associate with enhanced susceptibility to HCC in patients with chronic HCV (Kumar et al. 2011; Miki et al. 2011), but the underlying mechanisms remain to be elucidated. The identification of host genetic factors may permit more effective surveillance of chronic HCV patients at risk for HCC development. This would be an important advance, since early diagnosis and intervention have a profound impact on patient survival.

6.2 Lymphoproliferative Disorders

Findings from a variety of epidemiologic studies suggest that patients who are seropositive for anti-HCV are at significantly increased risk of developing non-Hodgkin lymphoma (NHL), particularly B cell NHL, compared to controls (Dal Maso and Franceschi 2006; Giordano et al. 2007; Gisbert et al. 2003). This risk is higher in HCV-infected patients with mixed cryoglobulinemia (MC) (see below) and vasculitis (Monti et al. 2005; Saadoun et al. 2005). Recent studies suggest that effective antiviral therapy might be beneficial in the management of HCV-positive low-grade NHL (Gisbert et al. 2005; Hermine et al. 2002), which would be consistent with a direct causative role for the virus, but the pathogenesis of HCV-associated lymphoma remains unclear. One possibility is that persistent antigenic stimulation associated with an infection that persists for decades may lead to antigen-dependent, benign clonal B cell lymphoproliferation, and eventually malignant transformation (Gisbert et al. 2005; Sansonno et al. 1998). The B lymphocyte stimulator (BLyS) receptor-ligand system may be involved in this process. BLyS is a critical survival factor for B cells, promoting their activation and maturation, and its abnormal production allows the survival of autoreactive B cells, thus triggering B cell lymphoproliferative disorders (De Vita et al. 2008). Perhaps relevant to its development, BLyS ligand-receptor activity is increased in HCV-induced clonal B cell proliferation, including NHL and MC (Landau et al. 2009). A second possibility is that HCV may be capable of directly infecting B cells, where it has been proposed to induce a mutator phenotype (Machida et al. 2004). Interestingly, peripheral blood mononuclear cells from patients with chronic HCV infection often show rearrangement of the bcl-2 gene with t(14;18) translocation, a common chromosomal translocation in B cell lymphomas (Kitay-Cohen et al. 2000).

7 Extrahepatic Manifestations of HCV Infection

Although HCV is generally considered to be an hepatotropic virus, various clinical manifestations of persistent infection involve other organ systems, including the endocrine system, lymphatic system, central and peripheral nerve systems, eyes, kidneys, heart, blood vessels, skin, and joints (Sène et al. 2004). In many cases, these may have a considerable influence on the patient's quality of life, approach to treatment, and, in some, risk of death. Mixed cryoglobulinemia (MC), lymphoproliferative disorders (see above), glomerulonephritis (GN), and sicca complex (Sjögren syndrome) are the most representative and substantial extrahepatic manifestations.

7.1 Mixed Cryoglobulinemia and Glomerulonephritis

Cryoglobulins are immunoglobulins that become insoluble below 37 °C and form high-molecular weight aggregates (Dammacco and Sansonno 1997). MC is a well-documented manifestation of HCV infection and may be present in up to 60 % of HCV-infected patients, but clinically evident manifestations develop in only 5–20 % of these patients (Dammacco et al. 1993; Lunel et al. 1994; Pawlotsky et al. 1994). MC associated with HCV infection may present with various manifestations including vasculitis, purpura, arthritis, sicca syndrome, neuropathy, and GN (Ferri et al. 2004). Therefore, patients with MC symptoms should be screened for HCV infection.

HCV-associated vasculitis is immune-complex in origin, and characterized by the intravascular deposition of circulating immunoglobulin (IgG and IgM) complexes containing HCV particles (Agnello and Abel 1997; Agnello et al. 1992; Sansonno et al. 1995; Sansonno et al. 2003). Chronic stimulation of B lymphocytes resulting from persistent HCV infection, or possibly direct infection of B cells as discussed above, may be responsible for oligo/monoclonal expansions of cells producing antibodies, including rheumatoid factor (IgM anti-IgG), that are present in the cryoglobulins (Dammacco et al. 2000; Pal et al. 2006). Antiviral suppression of viral replication results in a significant improvement in cryoglobulinemia and associated vasculitis, supporting a causal relationship between infection and MC (Ferri et al. 1993; Misiani et al. 1994; Saadoun et al. 2006). For patients with severe vasculitis, including GN with renal insufficiency, the anti-CD20 antibody rituximab has been used before initiation of antiviral therapy (Saadoun et al. 2008), and has been reported to reduce signs of vasculitis (Cacoub et al. 2008).

The association between HCV infection and renal insufficiency is also well established (Dalrymple et al. 2007). Immune complexes containing viral RNA have been detected along capillary walls and in subepithelial regions of glomeruli (Okada et al. 1996; Stokes et al. 1997). These immune complexes may activate mesangial TLR3, leading to the amplification of inflammatory cytokine production and contributing to the establishment of glomerulopathy by facilitating intrarenal migration and activation of inflammatory cells through the induction of intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1),

and macrophage colony-stimulating factor (M-CSF) (Merkle et al. 2012; Wörnle et al. 2006). Interferon-based antiviral therapies are effective to some extent in patients with HCV-related GN (Fabrizi et al. 2007), and it is likely that these patients will benefit from direct-acting antivirals as well.

Sicca complex, manifested by dryness of mucous membranes, is also observed in patients with MC vasculitis (Ferri et al. 2004). An association has also been suggested between HCV infection and Sjögren syndrome, an autoimmune disease that involves destruction of the salivary and lachrymal glands (Haddad et al. 1992), but this remains under debate. Peripheral neuropathy and rheumatologic disorders such as polyarthralgia and arthritis have also been reported in patients with HCV infection (Sène et al. 2004). However, these manifestations could be part of the MC syndrome (Ferri et al. 2004).

7.2 Central Nervous System Manifestations

Mild neuropsychiatric disturbances and loss of cognitive function also occur in some patients with chronic hepatitis C in the absence of hepatic encephalopathy due to metabolic causes or frank depression (Forton et al. 2006). While it is difficult to assess the significance of these findings in patients with underlying liver disease in whom a history of substance abuse is common, they may be accompanied by abnormalities in magnetic resonance scans of the brain. Positron emission tomography (PET) scans also suggest the presence of microglial inflammation in some of these patients (Grover et al. 2012). The possibility that virus may directly infect tissues of the central system was initially suggested by differences in quasispecies distribution among viruses sampled from the liver, brain, and serum of patients (Forton et al. 2004). More recent evidence supports the possibility that HCV may infect endothelial cells that constitute the blood–brain barrier, potentially enhancing their permeability and altering their barrier function (Fletcher et al. 2012). Related CNS-derived cell lines express the receptors required for HCV entry and are permissive for HCV replication (Fletcher et al. 2012; Fletcher et al. 2010). This is an emerging area of investigation that may prove important if CNS infection provides a sanctuary for the virus against antiviral therapies.

References

Adinolfi LE, Gambardella M, Andreana A et al (2001) Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 33:1358–1364

Agnello V, Abel G (1997) Localization of hepatitis C virus in cutaneous vasculitic lesions in patients with type II cryoglobulinemia. Arthritis Rheum 40:2007–2015

Agnello V, Chung RT, Kaplan LM (1992) A role for hepatitis C virus infection in type II cryoglobulinemia. N Engl J Med 327:1490–1495

Ahmad J, Eng FJ, Branch AD (2011) HCV and HCC: clinical update and a review of HCC-associated viral mutations in the core gene. Semin Liver Dis 31:347–355
Akuta N, Suzuki F, Kawamura Y et al (2007) Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology 46:1357–1364
Alonzi T, Agrati C, Costabile B et al (2004) Steatosis and intrahepatic lymphocyte recruitment in hepatitis C virus transgenic mice. J Gen Virol 85:1509–1520
Altlparmak E, Koklu S, Yalinkilic M et al (2005) Viral and host causes of fatty liver in chronic hepatitis B. World J Gastroenterol 11:3056–3059
Anderson EJ, Lustig ME, Boyle KE et al (2009) Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. J Clin Invest 119:573–581
Arase Y, Suzuki F, Suzuki Y et al (2009) Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. Hepatology 49:739–744
Aytug S, Reich D, Sapiro LE et al (2003) Impaired IRS-1/PI3-kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. Hepatology 38:1384–1392
Banerjee S, Saito K, Ait-Goughoulte M et al (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 82:2606–2612
Banerjee A, Meyer K, Mazumdar B et al (2010) Hepatitis C virus differentially modulates activation of forkhead transcription factors and insulin-induced metabolic gene expression. J Virol 84:5936–5946
Barba G, Harper F, Harada T et al (1997) Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc Natl Acad Sci USA 94:1200–1205
Barbaro G, Di Lorenzo G, Asti A et al (1999a) Hepatocellular mitochondrial alterations in patients with chronic hepatitis C: ultrastructural and biochemical findings. Am J Gastroenterol 94:2198–2205
Barbaro G, Di Lorenzo G, Ribersani M et al (1999b) Serum ferritin and hepatic glutathione concentrations in chronic hepatitis C patients related to the hepatitis C virus genotype. J Hepatol 30:774–782
Bataller R, Paik YH, Lindquist JN et al (2004) Hepatitis C virus core and nonstructural proteins induce fibrogenic effects in hepatic stellate cells. Gastroenterology 126:529–540
Bernsmeier C, Duong FH, Christen V et al (2008) Virus-induced over-expression of protein phosphatase 2A inhibits insulin signalling in chronic hepatitis C. J Hepatol 49:429–440
Bonnard C, Durand A, Peyrol S et al (2008) Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. J Clin Invest 118:789–800
Bose SK, Shrivastava S, Meyer K et al (2012) Hepatitis C virus activates mTOR/S6K1 signaling pathway in inhibiting IRS-1 function for insulin resistance. J Virol 86:6315–6322
Boudreau HE, Emerson SU, Korzeniowska A et al (2009) Hepatitis C virus (HCV) proteins induce NADPH oxidase 4 expression in a transforming growth factor beta-dependent manner: a new contributor to HCV-induced oxidative stress. J Virol 83:12934–12946
Brenner DA (2009) Molecular pathogenesis of liver fibrosis. Trans Am Clin Climatol Assoc 120:361–368
Bruno S, Crosignani A, Maisonneuve P et al (2007) Hepatitis C virus genotype 1b as a major risk factor associated with hepatocellular carcinoma in patients with cirrhosis: a seventeen-year prospective cohort study. Hepatology 46:1350–1356
Cacoub P, Delluc A, Saadoun D et al (2008) Anti-CD20 monoclonal antibody (rituximab) treatment for cryoglobulinemic vasculitis: where do we stand? Ann Rheum Dis 67:283–287
Cardin R, Saccoccio G, Masutti F et al (2001) DNA oxidative damage in leukocytes correlates with the severity of HCV-related liver disease: validation in an open population study. J Hepatol 34:587–592
Cheeseman KH, Slater TF (1993) An introduction to free radical biochemistry. Br Med Bull 49:481–493

Cheng Z, Guo S, Copps K et al (2009) Foxo1 integrates insulin signaling with mitochondrial function in the liver. Nat Med 15:1307–1311

Dal Maso L, Franceschi S (2006) Hepatitis C virus and risk of lymphoma and other lymphoid neoplasms: a meta-analysis of epidemiologic studies. Cancer Epidemiol Biomarkers Prev 15:2078–2085

Dalrymple LS, Koepsell T, Sampson J et al (2007) Hepatitis C virus infection and the prevalence of renal insufficiency. Clin J Am Soc Nephrol 2:715–721

Dammacco F, Sansonno D (1997) Mixed cryoglobulinemia as a model of systemic vasculitis. Clin Rev Allergy Immunol 15:97–119

Dammacco F, Sansonno D, Cornacchiulo V et al (1993) Hepatitis C virus infection and mixed cryoglobulinemia: a striking association. Int J Clin Lab Res 23:45–49

Dammacco F, Sansonno D, Piccoli C et al (2000) The lymphoid system in hepatitis C virus infection: autoimmunity, mixed cryoglobulinemia, and Overt B-cell malignancy. Semin Liver Dis 20:143–157

de Mochel NS, Seronello S, Wang SH et al (2010) Hepatocyte NAD(P)H oxidases as an endogenous source of reactive oxygen species during hepatitis C virus infection. Hepatology 52:47–59

De Vita S, Quartuccio L, Fabris M (2008) Hepatitis C virus infection, mixed cryoglobulinemia and BLyS upregulation: targeting the infectious trigger, the autoimmune response, or both? Autoimmun Rev 8:95–99

Deng L, Adachi T, Kitayama K et al (2008) Hepatitis C virus infection induces apoptosis through a Bax-triggered, mitochondrion-mediated, caspase 3-dependent pathway. J Virol 82:10375–10385

Deng L, Shoji I, Ogawa W et al (2011) Hepatitis C virus infection promotes hepatic gluconeogenesis through an NS5A-mediated, FoxO1-dependent pathway. J Virol 85:8556–8568

Dolganiuc A, Oak S, Kodys K et al (2004) Hepatitis C core and nonstructural 3 proteins trigger toll-like receptor 2-mediated pathways and inflammatory activation. Gastroenterology 127:1513–1524

Dong XC, Copps KD, Guo S et al (2008) Inactivation of hepatic Foxo1 by insulin signaling is required for adaptive nutrient homeostasis and endocrine growth regulation. Cell Metab 8:65–76

D'Souza R, Sabin CA, Foster GR (2005) Insulin resistance plays a significant role in liver fibrosis in chronic hepatitis C and in the response to antiviral therapy. Am J Gastroenterol 100:1509–1515

El-Serag HB, Rudolph KL (2007) Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 132:2557–2576

Eng FJ, Walewski JL, Klepper AL et al (2009) Internal initiation stimulates production of p8 minicore, a member of a newly discovered family of hepatitis C virus core protein isoforms. J Virol 83:3104–3114

Fabrizi F, Bruchfeld A, Mangano S et al (2007) Interferon therapy for HCV-associated glomerulonephritis: meta-analysis of controlled trials. Int J Artif Organs 30:212–219

Farinati F, Cardin R, De Maria N et al (1995) Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. J Hepatol 22:449–456

Fartoux L, Poujol-Robert A, Guechot J et al (2005) Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. Gut 54:1003–1008

Ferri C, Marzo E, Longombardo G et al (1993) Interferon-alpha in mixed cryoglobulinemia patients: a randomized, crossover-controlled trial. Blood 81:1132–1136

Ferri C, Sebastiani M, Giuggioli D et al (2004) Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. Semin Arthritis Rheum 33:355–374

Fletcher NF, Yang JP, Farquhar MJ et al (2010) Hepatitis C virus infection of neuroepithelioma cell lines. Gastroenterology 139:1365–1374

Fletcher NF, Wilson GK, Murray J et al (2012) Hepatitis C virus infects the endothelial cells of the blood-brain barrier. Gastroenterology 142:634–643

Forton DM, Karayiannis P, Mahmud N et al (2004) Identification of unique hepatitis C virus quasispecies in the central nervous system and comparative analysis of internal translational efficiency of brain, liver, and serum variants. J Virol 78:5170–5183

Forton DM, Taylor-Robinson SD, Thomas HC (2006) Central nervous system changes in hepatitis C virus infection. Eur J Gastroenterol Hepatol 18:333–338
Fox JG, Feng Y, Theve EJ et al (2010) Gut microbes define liver cancer risk in mice exposed to chemical and viral transgenic hepatocarcinogens. Gut 59:88–97
Fujino T, Nakamuta M, Yada R et al (2010) Expression profile of lipid metabolism-associated genes in hepatitis C virus-infected human liver. Hepatol Res 40:923–929
Fujita N, Horiike S, Sugimoto R et al (2007) Hepatic oxidative DNA damage correlates with iron overload in chronic hepatitis C patients. Free Radic Biol Med 42:353–362
Fujita N, Sugimoto R, Ma N et al (2008) Comparison of hepatic oxidative DNA damage in patients with chronic hepatitis B and C. J Viral Hepat 15:498–507
Furutani T, Hino K, Okuda M et al (2006) Hepatic iron overload induces hepatocellular carcinoma in transgenic mice expressing the hepatitis C virus polyprotein. Gastroenterology 130:2087–2098
Garcia-Mediavilla MV, Sanchez-Campos S, Gonzalez-Perez P et al (2005) Differential contribution of hepatitis C virus NS5A and core proteins to the induction of oxidative and nitrosative stress in human hepatocyte-derived cells. J Hepatol 43:606–613
Garcia-Ruiz I, Solis-Munoz P, Gomez-Izquierdo E et al (2012) Protein tyrosine phosphatases are involved in the interferon resistance associated with insulin resistance in HepG2 cells and obese mice. J Biol Chem 287:19564–19573
Giordano TP, Henderson L, Landgren O et al (2007) Risk of non-Hodgkin lymphoma and lymphoproliferative precursor diseases in US veterans with hepatitis C virus. JAMA 297:2010–2017
Gisbert JP, Garcia-Buey L, Pajares JM et al (2003) Prevalence of hepatitis C virus infection in B-cell non-Hodgkin's lymphoma: systematic review and meta-analysis. Gastroenterology 125:1723–1732
Gisbert JP, Garcia-Buey L, Pajares JM et al (2005) Systematic review: regression of lymphoproliferative disorders after treatment for hepatitis C infection. Aliment Pharmacol Ther 21:653–662
Gong G, Waris G, Tanveer R et al (2001) Human hepatitis C virus NS5A protein alters intracellular calcium levels, induces oxidative stress, and activates STAT-3 and NF-kappa B. Proc Natl Acad Sci USA 98:9599–9604
Grover VP, Pavese N, Koh SB et al (2012) Cerebral microglial activation in patients with hepatitis C: in vivo evidence of neuroinflammation. J Viral Hepat 19:e89–e96
Guidotti LG, Chisari FV (2006) Immunobiology and pathogenesis of viral hepatitis. Annu Rev Pathol 1:23–61
Haddad J, Deny P, Munz-Gotheil C et al (1992) Lymphocytic sialadenitis of Sjogren's syndrome associated with chronic hepatitis C virus liver disease. Lancet 339:321–323
Harris C, Herker E, Farese RV Jr et al (2011) Hepatitis C virus core protein decreases lipid droplet turnover: a mechanism for core-induced steatosis. J Biol Chem 286:42615–42625
Herker E, Harris C, Hernandez C et al (2010) Efficient hepatitis C virus particle formation requires diacylglycerol acyltransferase-1. Nat Med 16:1295–1298
Hermine O, Lefrere F, Bronowicki JP et al (2002) Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med 347:89–94
Hernandez-Gea V, Friedman SL (2011) Pathogenesis of liver fibrosis. Annu Rev Pathol 6:425–456
Honda A, Arai Y, Hirota N et al (1999) Hepatitis C virus structural proteins induce liver cell injury in transgenic mice. J Med Virol 59:281–289
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 81:1913–1925
Houstis N, Rosen ED, Lander ES (2006) Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 440:944–948
Hu Z, Muroyama R, Kowatari N et al (2009) Characteristic mutations in hepatitis C virus core gene related to the occurrence of hepatocellular carcinoma. Cancer Sci 100:2465–2468
Hui JM, Sud A, Farrell GC et al (2003) Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. Gastroenterology 125:1695–1704

Hussain SP, Schwank J, Staib F et al (2007) TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. Oncogene 26:2166–2176
Ishido S, Hotta H (1998) Complex formation of the nonstructural protein 3 of hepatitis C virus with the p53 tumor suppressor. FEBS Lett 438:258–262
Jeannot E, Boorman GA, Kosyk O et al (2012) Increased incidence of aflatoxin B1-induced liver tumors in hepatitis virus C transgenic mice. Int J Cancer 130:1347–1356
Joyce MA, Walters KA, Lamb SE et al (2009) HCV induces oxidative and ER stress, and sensitizes infected cells to apoptosis in SCID/Alb-uPA mice. PLoS Pathog 5:e1000291
Kamegaya Y, Hiasa Y, Zukerberg L et al (2005) Hepatitis C virus acts as a tumor accelerator by blocking apoptosis in a mouse model of hepatocarcinogenesis. Hepatology 41:660–667
Kanety H, Feinstein R, Papa MZ et al (1995) Tumor necrosis factor alpha-induced phosphorylation of insulin receptor substrate-1 (IRS-1). Possible mechanism for suppression of insulin-stimulated tyrosine phosphorylation of IRS-1. J Biol Chem 270:23780–23784
Kannan RP, Hensley LL, Evers LE et al (2011) Hepatitis C virus infection causes cell cycle arrest at the level of initiation of mitosis. J Virol 85:7989–8001
Kao CF, Chen SY, Chen JY et al (2004) Modulation of p53 transcription regulatory activity and post-translational modification by hepatitis C virus core protein. Oncogene 23:2472–2483
Kawaguchi T, Yoshida T, Harada M et al (2004) Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol 165:1499–1508
Kawaguchi T, Ide T, Taniguchi E et al (2007) Clearance of HCV improves insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate 1 and 2. Am J Gastroenterol 102:570–576
Kawamura T, Furusaka A, Koziel MJ et al (1997) Transgenic expression of hepatitis C virus structural proteins in the mouse. Hepatology 25:1014–1021
Kawamura H, Govindarajan S, Aswad F et al (2006) HCV core expression in hepatocytes protects against autoimmune liver injury and promotes liver regeneration in mice. Hepatology 44:936–944
Kitase A, Hino K, Furutani T et al (2005) In situ detection of oxidized n-3 polyunsaturated fatty acids in chronic hepatitis C: correlation with hepatic steatosis. J Gastroenterol 40:617–624
Kitay-Cohen Y, Amiel A, Hilzenrat N et al (2000) Bcl-2 rearrangement in patients with chronic hepatitis C associated with essential mixed cryoglobulinemia type II. Blood 96:2910–2912
Klopstock N, Katzenellenbogen M, Pappo O et al (2009) HCV tumor promoting effect is dependent on host genetic background. PLoS ONE 4:e5025
Kobayashi M, Akuta N, Suzuki F et al (2010) Influence of amino-acid polymorphism in the core protein on progression of liver disease in patients infected with hepatitis C virus genotype 1b. J Med Virol 82:41–48
Korenaga M, Wang T, Li Y et al (2005) Hepatitis C virus core protein inhibits mitochondrial electron transport and increases reactive oxygen species (ROS) production. J Biol Chem 280:37481–37488
Kumar V, Kato N, Urabe Y et al (2011) Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. Nat Genet 43:455–458
Kwun HJ, Jung EY, Ahn JY et al (2001) p53-dependent transcriptional repression of p21(waf1) by hepatitis C virus NS3. J Gen Virol 82:2235–2241
Lan L, Gorke S, Rau SJ et al (2008) Hepatitis C virus infection sensitizes human hepatocytes to TRAIL-induced apoptosis in a caspase 9-dependent manner. J Immunol 181:4926–4935
Landau DA, Rosenzwajg M, Saadoun D et al (2009) The B lymphocyte stimulator receptor-ligand system in hepatitis C virus-induced B cell clonal disorders. Ann Rheum Dis 68:337–344
Laplante M, Sabatini DM (2009) An emerging role of mTOR in lipid biosynthesis. Curr Biol 19:R1046–R1052
Leandro G, Mangia A, Hui J et al (2006) Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. Gastroenterology 130:1636–1642
Lemon SM, McGivern DR (2012) Is hepatitis C virus carcinogenic? Gastroenterology 142:1274–1278

Lerat H, Honda M, Beard MR et al (2002) Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus. Gastroenterology 122:352–365
Lerat H, Kammoun HL, Hainault I et al (2009) Hepatitis C virus proteins induce lipogenesis and defective triglyceride secretion in transgenic mice. J Biol Chem 284:33466–33474
Li J, Rechsteiner M (2001) Molecular dissection of the 11S REG (PA28) proteasome activators. Biochimie 83:373–383
Li K, Prow T, Lemon SM et al (2002) Cellular response to conditional expression of hepatitis C virus core protein in Huh7 cultured human hepatoma cells. Hepatology 35:1237–1246
Li Y, Boehning DF, Qian T et al (2007) Hepatitis C virus core protein increases mitochondrial ROS production by stimulation of Ca2+ uniporter activity. Faseb J 21:2474–2485
Liang Y, Shilagard T, Xiao SY et al (2009) Visualizing hepatitis C virus infections in human liver by two-photon microscopy. Gastroenterology 137:1448–1458
Lin W, Tsai WL, Shao RX et al (2010) Hepatitis C virus regulates transforming growth factor beta1 production through the generation of reactive oxygen species in a nuclear factor kappaB-dependent manner. Gastroenterology 138:2509–2518
Lok AS, Everhart JE, Wright EC et al (2011) Maintenance peginterferon therapy and other factors associated with hepatocellular carcinoma in patients with advanced hepatitis C. Gastroenterology 140:840–849
Lowell BB, Shulman GI (2005) Mitochondrial dysfunction and type 2 diabetes. Science 307:384–387
Luedde T, Schwabe RF (2011) NF-kappaB in the liver–linking injury, fibrosis and hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 8:108–118
Lunel F, Musset L, Cacoub P et al (1994) Cryoglobulinemia in chronic liver diseases: role of hepatitis C virus and liver damage. Gastroenterology 106:1291–1300
Machado MV, Oliveira AG, Cortez-Pinto H (2011) Hepatic steatosis in hepatitis B virus infected patients: meta-analysis of risk factors and comparison with hepatitis C infected patients. J Gastroenterol Hepatol 26:1361–1367
Machida K, Cheng KT, Sung VM et al (2004) Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes. Proc Natl Acad Sci USA 101:4262–4267
Machida K, Cheng KT, Lai CK et al (2006) Hepatitis C virus triggers mitochondrial permeability transition with production of reactive oxygen species, leading to DNA damage and STAT3 activation. J Virol 80:7199–7207
Machida K, Tsukamoto H, Mkrtchyan H et al (2009) Toll-like receptor 4 mediates synergism between alcohol and HCV in hepatic oncogenesis involving stem cell marker Nanog. Proc Natl Acad Sci USA 106:1548–1553
Majumder M, Ghosh AK, Steele R et al (2001) Hepatitis C virus NS5A physically associates with p53 and regulates p21/waf1 gene expression in a p53-dependent manner. J Virol 75:1401–1407
Masaki T, Suzuki R, Murakami K et al (2008) Interaction of hepatitis C virus nonstructural protein 5A with core protein is critical for the production of infectious virus particles. J Virol 82:7964–7976
Mason AL, Lau JY, Hoang N et al (1999) Association of diabetes mellitus and chronic hepatitis C virus infection. Hepatology 29:328–333
Mayhew CN, Carter SL, Fox SR et al (2007) RB loss abrogates cell cycle control and genome integrity to promote liver tumorigenesis. Gastroenterology 133:976–984
Mazzocca A, Sciammetta SC, Carloni V et al (2005) Binding of hepatitis C virus envelope protein E2 to CD81 up-regulates matrix metalloproteinase-2 in human hepatic stellate cells. J Biol Chem 280:11329–11339
McClendon AK, Dean JL, Ertel A et al (2011) RB and p53 cooperate to prevent liver tumorigenesis in response to tissue damage. Gastroenterology 141:1439–1450
McGivern DR, Lemon SM (2009) Tumor suppressors, chromosomal instability, and hepatitis C virus-associated liver cancer. Annu Rev Pathol 4:399–415

McGivern DR, Villanueva RA, Chinnaswamy S et al (2009) Impaired replication of hepatitis C virus containing mutations in a conserved NS5B retinoblastoma protein-binding motif. J Virol 83:7422–7433

Merkle M, Ribeiro A, Köppel S et al (2012) TLR3-dependent immune regulatory functions of human mesangial cells. Cell Mol Immunol 9:334–340

Miki D, Ochi H, Hayes CN et al (2011) Variation in the DEPDC5 locus is associated with progression to hepatocellular carcinoma in chronic hepatitis C virus carriers. Nat Genet 43:797–800

Milward A, Mankouri J, Harris M (2010) Hepatitis C virus NS5A protein interacts with beta-catenin and stimulates its transcriptional activity in a phosphoinositide-3 kinase-dependent fashion. J Gen Virol 91:373–381

Misiani R, Bellavita P, Fenili D et al (1994) Interferon alfa-2a therapy in cryoglobulinemia associated with hepatitis C virus. N Engl J Med 330:751–756

Mitsuyoshi H, Itoh Y, Sumida Y et al (2008) Evidence of oxidative stress as a cofactor in the development of insulin resistance in patients with chronic hepatitis C. Hepatol Res 38:348–353

Miyamoto H, Moriishi K, Moriya K et al (2007) Involvement of the PA28gamma-dependent pathway in insulin resistance induced by hepatitis C virus core protein. J Virol 81:1727–1735

Miyanari Y, Atsuzawa K, Usuda N et al (2007) The lipid droplet is an important organelle for hepatitis C virus production. Nat Cell Biol 9:1089–1097

Monetti M, Levin MC, Watt MJ et al (2007) Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. Cell Metab 6:69–78

Monti G, Pioltelli P, Saccardo F et al (2005) Incidence and characteristics of non-Hodgkin lymphomas in a multicenter case file of patients with hepatitis C virus-related symptomatic mixed cryoglobulinemias. Arch Intern Med 165:101–105

Moriishi K, Okabayashi T, Nakai K et al (2003) Proteasome activator PA28gamma-dependent nuclear retention and degradation of hepatitis C virus core protein. J Virol 77:10237–10249

Moriishi K, Mochizuki R, Moriya K et al (2007) Critical role of PA28gamma in hepatitis C virus-associated steatogenesis and hepatocarcinogenesis. Proc Natl Acad Sci USA 104:1661–1666

Moriishi K, Shoji I, Mori Y et al (2010) Involvement of PA28gamma in the propagation of hepatitis C virus. Hepatology 52:411–420

Moriya K, Fujie H, Shintani Y et al (1998) The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med 4:1065–1067

Moriya K, Nakagawa K, Santa T et al (2001a) Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res 61:4365–4370

Moriya K, Todoroki T, Tsutsumi T et al (2001b) Increase in the concentration of carbon 18 monounsaturated fatty acids in the liver with hepatitis C: analysis in transgenic mice and humans. Biochem Biophys Res Commun 281:1207–1212

Mousseau G, Kota S, Takahashi V et al (2011) Dimerization-driven interaction of hepatitis C virus core protein with NS3 helicase. J Gen Virol 92:101–111

Munakata T, Nakamura M, Liang Y et al (2005) Down-regulation of the retinoblastoma tumor suppressor by the hepatitis C virus NS5B RNA-dependent RNA polymerase. Proc Natl Acad Sci USA 102:18159–18164

Munakata T, Liang Y, Kim S et al (2007) Hepatitis C virus induces E6AP-dependent degradation of the retinoblastoma protein. PLoS Pathog 3:1335–1347

Muzzi A, Leandro G, Rubbia-Brandt L et al (2005) Insulin resistance is associated with liver fibrosis in non-diabetic chronic hepatitis C patients. J Hepatol 42:41–46

Naas T, Ghorbani M, Alvarez-Maya I et al (2005) Characterization of liver histopathology in a transgenic mouse model expressing genotype 1a hepatitis C virus core and envelope proteins 1 and 2. J Gen Virol 86:2185–2196

Nakamoto S, Imazeki F, Fukai K et al (2010) Association between mutations in the core region of hepatitis C virus genotype 1 and hepatocellular carcinoma development. J Hepatol 52:72–78

Oem JK, Jackel-Cram C, Li YP et al (2008) Activation of sterol regulatory element-binding protein 1c and fatty acid synthase transcription by hepatitis C virus non-structural protein 2. J Gen Virol 89:1225–1230

Ohata K, Hamasaki K, Toriyama K et al (2003) Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. Cancer 97:3036–3043

Okada K, Takishita Y, Shimomura H et al (1996) Detection of hepatitis C virus core protein in the glomeruli of patients with membranous glomerulonephritis. Clin Nephrol 45:71–76

Okuda M, Li K, Beard MR et al (2002) Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. Gastroenterology 122:366–375

Pal S, Sullivan DG, Kim S et al (2006) Productive replication of hepatitis C virus in perihepatic lymph nodes in vivo: implications of HCV lymphotropism. Gastroenterology 130:1107–1116

Park CY, Choi SH, Kang SM et al (2009) Nonstructural 5A protein activates beta-catenin signaling cascades: implication of hepatitis C virus-induced liver pathogenesis. J Hepatol 51:853–864

Patton HM, Patel K, Behling C et al (2004) The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients. J Hepatol 40:484–490

Pawlotsky JM, Ben Yahia M, Andre C et al (1994) Immunological disorders in C virus chronic active hepatitis: a prospective case-control study. Hepatology 19:841–848

Pekow JR, Bhan AK, Zheng H et al (2007) Hepatic steatosis is associated with increased frequency of hepatocellular carcinoma in patients with hepatitis C-related cirrhosis. Cancer 109:2490–2496

Pereira Tde A, Witek RP, Syn WK et al (2010) Viral factors induce Hedgehog pathway activation in humans with viral hepatitis, cirrhosis, and hepatocellular carcinoma. Lab Invest 90:1690–1703

Perlemuter G, Sabile A, Letteron P et al (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 16:185–194

Persico M, Masarone M, La Mura V et al (2009) Clinical expression of insulin resistance in hepatitis C and B virus-related chronic hepatitis: differences and similarities. World J Gastroenterol 15:462–466

Polyak SJ, Khabar KS, Rezeiq M et al (2001) Elevated levels of interleukin-8 in serum are associated with hepatitis C virus infection and resistance to interferon therapy. J Virol 75:6209–6211

Poynard T, Ratziu V, McHutchison J et al (2003) Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. Hepatology 38:75–85

Qadri I, Iwahashi M, Simon F (2002) Hepatitis C virus NS5A protein binds TBP and p53, inhibiting their DNA binding and p53 interactions with TBP and ERCC3. Biochim Biophys Acta 1592:193–204

Qiu W, Wang X, Leibowitz B et al (2011) PUMA-mediated apoptosis drives chemical hepatocarcinogenesis in mice. Hepatology 54:1249–1258

Raimondi S, Bruno S, Mondelli MU et al (2009) Hepatitis C virus genotype 1b as a risk factor for hepatocellular carcinoma development: a meta-analysis. J Hepatol 50:1142–1154

Ratziu V, Munteanu M, Charlotte F et al (2003) Fibrogenic impact of high serum glucose in chronic hepatitis C. J Hepatol 39:1049–1055

Rubbia-Brandt L, Leandro G, Spahr L et al (2001) Liver steatosis in chronic hepatitis C: a morphological sign suggesting infection with HCV genotype 3. Histopathology 39:119–124

Ryu SH, Fan X, Xu Y et al (2009) Lack of association between genotypes and subtypes of HCV and occurrence of hepatocellular carcinoma in Egypt. J Med Virol 81:844–847

Saadoun D, Suarez F, Lefrere F et al (2005) Splenic lymphoma with villous lymphocytes, associated with type II cryoglobulinemia and HCV infection: a new entity? Blood 105:74–76

Saadoun D, Resche-Rigon M, Thibault V et al (2006) Antiviral therapy for hepatitis C virus–associated mixed cryoglobulinemia vasculitis: a long-term followup study. Arthritis Rheum 54:3696–3706

Saadoun D, Delluc A, Piette JC et al (2008) Treatment of hepatitis C-associated mixed cryoglobulinemia vasculitis. Curr Opin Rheumatol 20:23–28

Saito T, Owen DM, Jiang F et al (2008) Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. Nature 454:523–527

Sansonno D, Cornacchiulo V, Iacobelli AR et al (1995) Localization of hepatitis C virus antigens in liver and skin tissues of chronic hepatitis C virus-infected patients with mixed cryoglobulinemia. Hepatology 21:305–312

Sansonno D, De Vita S, Iacobelli AR et al (1998) Clonal analysis of intrahepatic B cells from HCV-infected patients with and without mixed cryoglobulinemia. J Immunol 160:3594–3601

Sansonno D, Lauletta G, Nisi L et al (2003) Non-enveloped HCV core protein as constitutive antigen of cold-precipitable immune complexes in type II mixed cryoglobulinaemia. Clin Exp Immunol 133:275–282

Sato Y, Kato J, Takimoto R et al (2006) Hepatitis C virus core protein promotes proliferation of human hepatoma cells through enhancement of transforming growth factor alpha expression via activation of nuclear factor-kappaB. Gut 55:1801–1808

Schulze-Krebs A, Preimel D, Popov Y et al (2005) Hepatitis C virus-replicating hepatocytes induce fibrogenic activation of hepatic stellate cells. Gastroenterology 129:246–258

Sène D, Limal N, Cacoub P (2004) Hepatitis C virus-associated extrahepatic manifestations: a review. Metab Brain Dis 19:357–381

Seto WK, Lai CL, Fung J et al (2010) Natural history of chronic hepatitis C: genotype 1 versus genotype 6. J Hepatol 53:444–448

Shi ST, Polyak SJ, Tu H et al (2002) Hepatitis C virus NS5A colocalizes with the core protein on lipid droplets and interacts with apolipoproteins. Virology 292:198–210

Shintani Y, Fujie H, Miyoshi H et al (2004) Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology 126:840–848

Shirakura M, Murakami K, Ichimura T et al (2007) E6AP ubiquitin ligase mediates ubiquitylation and degradation of hepatitis C virus core protein. J Virol 81:1174–1185

Simo R, Lecube A, Genesca J et al (2006) Sustained virological response correlates with reduction in the incidence of glucose abnormalities in patients with chronic hepatitis C virus infection. Diabetes Care 29:2462–2466

Stokes MB, Chawla H, Brody RI et al (1997) Immune complex glomerulonephritis in patients coinfected with human immunodeficiency virus and hepatitis C virus. Am J Kidney Dis 29:514–525

Storozhevykh TP, Senilova YE, Persiyantseva NA et al (2007) Mitochondrial respiratory chain is involved in insulin-stimulated hydrogen peroxide production and plays an integral role in insulin receptor autophosphorylation in neurons. BMC Neurosci 8:84

Street A, Macdonald A, McCormick C et al (2005) Hepatitis C virus NS5A-mediated activation of phosphoinositide 3-kinase results in stabilization of cellular beta-catenin and stimulation of beta-catenin-responsive transcription. J Virol 79:5006–5016

Su AI, Pezacki JP, Wodicka L et al (2002) Genomic analysis of the host response to hepatitis C virus infection. Proc Natl Acad Sci USA 99:15669–15674

Sun B, Karin M (2008) NF-kappaB signaling, liver disease and hepatoprotective agents. Oncogene 27:6228–6244

Suzuki R, Moriishi K, Fukuda K et al (2009) Proteasomal turnover of hepatitis C virus core protein is regulated by two distinct mechanisms: a ubiquitin-dependent mechanism and a ubiquitin-independent but PA28gamma-dependent mechanism. J Virol 83:2389–2392

Tai DI, Tsai SL, Chen YM et al (2000) Activation of nuclear factor kappaB in hepatitis C virus infection: implications for pathogenesis and hepatocarcinogenesis. Hepatology 31:656–664

Tanaka M, Nagano-Fujii M, Deng L et al (2006) Single-point mutations of hepatitis C virus NS3 that impair p53 interaction and anti-apoptotic activity of NS3. Biochem Biophys Res Commun 340:792–799

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

Taniguchi H, Kato N, Otsuka M et al (2004) Hepatitis C virus core protein upregulates transforming growth factor-beta 1 transcription. J Med Virol 72:52–59

Thoren F, Romero A, Lindh M et al (2004) A hepatitis C virus-encoded, nonstructural protein (NS3) triggers dysfunction and apoptosis in lymphocytes: role of NADPH oxidase-derived oxygen radicals. J Leukoc Biol 76:1180–1186

Thorgeirsson SS, Grisham JW (2002) Molecular pathogenesis of human hepatocellular carcinoma. Nat Genet 31:339–346

Tsutsumi T, Matsuda M, Aizaki H et al (2009) Proteomics analysis of mitochondrial proteins reveals overexpression of a mitochondrial protein chaperon, prohibitin, in cells expressing hepatitis C virus core protein. Hepatology 50:378–386

Veldt BJ, Chen W, Heathcote EJ et al (2008) Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. Hepatology 47:1856–1862

Walters KA, Syder AJ, Lederer SL et al (2009) Genomic analysis reveals a potential role for cell cycle perturbation in HCV-mediated apoptosis of cultured hepatocytes. PLoS Pathog 5:e1000269

Wang D, Sul HS (1998) Insulin stimulation of the fatty acid synthase promoter is mediated by the phosphatidylinositol 3-kinase pathway. Involvement of protein kinase B/Akt. J Biol Chem 273:25420–25426

Wang AG, Lee DS, Moon HB et al (2009a) Non-structural 5A protein of hepatitis C virus induces a range of liver pathology in transgenic mice. J Pathol 219:253–262

Wang CS, Yao WJ, Chang TT et al (2009b) The impact of type 2 diabetes on the development of hepatocellular carcinoma in different viral hepatitis statuses. Cancer Epidemiol Biomarkers Prev 18:2054–2060

Wang N, Liang Y, Devaraj S et al (2009c) Toll-like receptor 3 mediates establishment of an antiviral state against hepatitis C virus in hepatoma cells. J Virol 83:9824–9834

Waris G, Tardif KD, Siddiqui A (2002) Endoplasmic reticulum (ER) stress: hepatitis C virus induces an ER-nucleus signal transduction pathway and activates NF-kappaB and STAT-3. Biochem Pharmacol 64:1425–1430

Waris G, Livolsi A, Imbert V et al (2003) Hepatitis C virus NS5A and subgenomic replicon activate NF-kappaB via tyrosine phosphorylation of IkappaBalpha and its degradation by calpain protease. J Biol Chem 278:40778–40787

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

Westin J, Lagging M, Dhillon AP et al (2007) Impact of hepatic steatosis on viral kinetics and treatment outcome during antiviral treatment of chronic HCV infection. J Viral Hepat 14:29–35

Wörnle M, Schmid H, Banas B et al (2006) Novel role of toll-like receptor 3 in hepatitis C-associated glomerulonephritis. Am J Pathol 168:370–385

Xu J, Kim HT, Ma Y et al (2008) Trauma and hemorrhage-induced acute hepatic insulin resistance: dominant role of tumor necrosis factor-alpha. Endocrinology 149:2369–2382

Yamaguchi A, Tazuma S, Nishioka T et al (2005) Hepatitis C virus core protein modulates fatty acid metabolism and thereby causes lipid accumulation in the liver. Dig Dis Sci 50:1361–1371

Yamaji S, Zhang M, Zhang J et al (2010) Hepatocyte-specific deletion of DDB1 induces liver regeneration and tumorigenesis. Proc Natl Acad Sci USA 107:22237–22242

Yu T, Robotham JL, Yoon Y (2006) Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. Proc Natl Acad Sci USA 103:2653–2658

Zhang J, Gao Z, Yin J et al (2008) S6K directly phosphorylates IRS-1 on Ser-270 to promote insulin resistance in response to TNF-(alpha) signaling through IKK2. J Biol Chem 283:35375–35382