Published in final edited form as:

Pharmacol Ther. 2011 October ; 132(1): 30–38. doi:10.1016/j.pharmthera.2011.05.005.

The Interaction between HCV and Nuclear Receptor-mediated Pathways

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Abstract

Hepatitis C virus (HCV) is presently the leading indication for liver transplantation in Western countries. Treatment for HCV infection includes a combination of pegylated interferon and ribavirin, which produces highly variable response rates. This reflects the lack of information regarding the roles of host and viral components during viral pathogenesis. Vital processes regulated by the liver, including metabolism, lipid homeostasis, cellular proliferation, and the immune response, are known to be systematically dysregulated as a result of persistent HCV infection. Nuclear receptors and their ligands are recognized as indispensable regulators of liver homeostasis. Pathways mediated by the nuclear receptor superfamily have been shown to be profoundly disrupted during HCV infection, leading to an increased importance in elucidating the exact nature of this complex relationship. Expanded understanding of the role of nuclear receptors in HCV infection may therefore be an essential step in the search for a more universally effective treatment.

Keywords

hepatitis; liver; steatosis; retinoic acid; retinoic acid receptor; autophagy

1. Introduction

Hepatitis C virus (HCV) is a critical global health problem, with over 170 million infected worldwide. After primary infection, 70% of patients develop chronic infection, and 5–20% progress to liver cirrhosis within 20 years ("NIH Consensus Statement on Management of Hepatitis C: 2002," 2002). There are known risk factors that contribute to development of cirrhosis, including alcohol consumption, insulin resistance, and hepatic steatosis; however, the specific molecular mechanisms involved in HCV disease progression are not well understood.

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The nuclear receptor superfamily constitutes a group of ligand-activated transcription factors that play critical roles in such diverse biological processes as xenobiotic metabolism, endobiotic homeostasis, proliferation, differentiation, inflammation, and cell death. It is hypothesized that dysregulation of nuclear receptor-mediated signaling contributes to development of pathological conditions associated with chronic HCV infection. Nuclear receptors have historically been attractive drug targets, and drugs that modulate nuclear receptor activity are among the most prescribed pharmaceuticals on the market (Pearce, 2004). Nuclear receptors have the potential to act as therapeutic targets for HCV-associated pathological changes. This review focuses on the relationship between HCV and nuclear receptors.

1.1 HCV

HCV is an enveloped positive-strand RNA virus, with 6 major genotypes and more than 100 subtypes (Nguyen, 2005). The high degree of variability reflects the lack of fidelity of the viral RNA-dependent RNA polymerase. The viral genome consists of a 5' noncoding region followed by an open reading frame coding structural and nonstructural proteins, followed by a 3' noncoding region that is required for replication. The translation product is a 3,000 amino acid polyprotein that is cleaved by viral and cellular proteases into individual proteins (Joyce, 2010). The primary target of HCV *in vivo* is the hepatocyte. Viral entry involves viral envelope proteins E1 and E2, cell surface receptors CD81, scavenger receptor class B type 1, low-density lipoprotein receptor (LDL-R), and cell surface heparan proteoglycans (Barth, 2006) as well as the more recently identified co-receptors claudin and human occludin (Evans, 2007; Ploss, 2009a). The virus is endocytosed, the envelope disintegrates in the cytoplasm and subsequently translation takes place using both viral and host machinery and the ribosome in the endoplasmic reticulum (ER) to translate the viral RNA into a polyprotein chain. Host and viral proteases cleave the polyprotein into 10 proteins that catalyze viral RNA replication and provide for the assembly of new viral particles (Dubuisson, 2008; Joyce, 2010). Lipid droplets are also essential components of viral replication (Miyanari, 2007). Virus maturation is complete when the encapsulated viral particle is enveloped by a lipid layer as it exits the ER (Pawlotsky, 2007). HCV hijacks host very low-density lipoprotein (VLDL) processing machinery, including microsomal transfer protein and apolipoprotein B, to facilitate viral exit (Gastaminza, 2008). It is clear that HCV is closely associated with cellular lipid homeostasis that is regulated by nuclear receptor-mediated pathways (Figure 1). Thus, the action of nuclear receptors can influence HCV disease progression. Potential mechanisms are discussed below.

In addition to lipid metabolism, HCV disrupts numerous other cellular processes. HCV can hinder the immune response through direct interference with the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, inhibition of antiviral genes such as 2'5' oligoadenylate synthetase and protein kinase RNA-activated, attenuation of interferon sensitizing genes by induction of IL-8, and interference with T-cell response (Burke, 2010; Gale, 2005). Pegylated interferon (IFN) in combination with ribavirin is the current treatment standard for HCV infection. However, there is only a 50% response rate in patients infected with genotype 1 virus, which is the most common genotype in Western populations (Fowell, 2010). Chronic hepatitis C infection typically progresses slowly without symptoms; until recently, many patients typically presented with advanced disease. Disease progression is characterized by hepatic inflammation and often steatosis, leading to fibrosis, cirrhosis, and in some cases, hepatocellular carcinoma (HCC). HCV infection causes chronic hepatic inflammation, which leads to the release of stellate-cell activating cytokine transforming growth factor β (TGF β). Activated hepatic stellate cells (HSCs) promote extracellular matrix deposition and fibrogenesis (Feld, 2006). Virus-mediated insulin resistance can also cause HSC activation; genotypes 1 and 3 are associated with

insulin resistance and fat accumulation, respectively (Douglas, 2009). HCV proteins can also induce apoptosis via increased oxidative stress (Abdalla, 2005; Okuda, 2002). Eventually, regenerating hepatocytes become surrounded by fibrotic tissue and form the nodules characteristic of cirrhosis. Thus, HCV creates a hepatic microenvironment favorable to the development of HCC, while also acting through other pro-oncogenic pathways.

Research has continually been hampered by the lack of a suitable infectious model, as the natural species tropism of HCV is limited to humans and chimpanzees (Ploss, 2009b). Recently, there has been a great deal of progress, including the establishment of HCV replicons, an infectious culture model, and small animal models expressing the HCV core protein (Brass, 2007). There have also been recent developments in human liver chimeric mice capable of propagating HCV (Bissig, 2010; Mercer, 2001; Washburn, 2011). While these models have been extremely helpful, there are still areas of concern. Viral titer may be different in rodent and human models, and there is a question of the cytopathicity of the core protein to the mouse hepatocyte (Chayama, 2011; Pasquinelli, 1997). There is an urgent need to develop additional models to study the pathogenesis of HCV.

1.2 Nuclear Receptors

Nuclear receptors are classified into three families based on their DNA- and ligand-binding properties. The most well characterized group is the endocrine receptors, including the glucocorticoid receptor. The second class is made up of the orphan nuclear receptors, which are structurally related, but lack known natural ligands. The last group and the focus of this review are the adopted orphan nuclear receptors, originally part of the orphan nuclear receptor class until the discovery of their physiological ligands. Members of this group include the lipid-responsive peroxisome proliferator activated receptors (PPARs), the oxysterol receptor liver X receptor (LXR), the bile acid receptor farnesoid X receptor (FXR), the xenobiotic receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR), the retinoic acid receptor (RAR), and the retinoid x receptor (RXR) (K. Wang, 2008). These receptors are structurally conserved, each containing an N-terminal regulatory domain that functions independently of ligand, a DNA-binding domain, a hinge domain to determine intracellular localization, a ligand binding domain and a C-terminal domain containing a variable sequence unique to the individual nuclear receptor (Lee, 2007). Please see recent reviews for the roles of these receptors (Bushue, 2010; Glass, 2010; Gyamfi, 2010; McKenna, 2009; K. Wang, 2008).

1.3 Nuclear Receptors and HCV

Nuclear receptors are master regulators of lipid homeostasis, inflammation, and endobiotic metabolism, processes intricately involved in HCV infection and progression. HCV-mediated dysregulation of these processes influences the replicative efficiency of the virus (Chang, 2007; Fujita, 2006; Morbitzer, 2005; Paziienza, 2010; Watashi, 2003). Because nuclear receptors regulate processes essential to the progression of chronic hepatitis C (Figure 2), it is important to understand the interaction between HCV and nuclear receptor-mediated signaling pathways (Blackham, 2010; Woodhouse, 2010). A comprehensive understanding of this relationship will elucidate the potential of nuclear receptors as prospective drug targets.

2. PPARs

PPARs play a crucial role in the maintenance of hepatic lipid and glucose homeostasis, the inflammatory process, cell differentiation, and proliferation. Because of this diversity, PPARs have been the subject of intense scrutiny. PPARs are natural targets for HCV-related

study because of their ubiquitous nature in the liver and involvement in processes known to be dysregulated by HCV.

2.1 PPAR α

PPAR α is the major regulator of the hepatic fatty acid metabolism pathway. Defects in PPAR α signaling can lead to reduced energy metabolism and increased lipid accumulation, eventually resulting in steatosis and steatohepatitis. PPAR α exerts direct transcriptional control over genes involved in fatty acid transport, uptake, and oxidation. Its downstream targets include carnitine palmitoyl transferase 1 (CPT-1), acyl-CoA oxidase (AOX), and fatty acid transporters CD36, fatty acid transport protein (FATP), and liver fatty acid binding protein (LFABP) (Motojima, 1998; Poirier, 2001; Reddy, 2001). Closely related is its mediation of the inflammatory response, as fatty acid derivatives such as leukotriene B₄ capable of inducing inflammation are degraded through PPAR α -activated pathways. PPAR α $-/-$ mice display prolonged inflammatory response (Devchand, 1996), and activation of PPAR α leads to decreased levels of acute phase response proteins C-reactive protein, fibrinogen, and serum amyloid A (Gervois, 2004; Kleemann, 2003; Maison, 2002) as well as adhesion molecules (Marx, 1999).

Human studies show impaired PPAR α activity and decreased CPT-1 expression in the livers of chronic hepatitis C patients (Cheng, 2005; Dharancy, 2005). CPT-1 is a key β -oxidation gene involved in the transport of long-chain fatty acids across the mitochondrial membrane. Consequently, CPT-1 and PPAR α mRNA and protein levels are significantly decreased in steatotic hepatitis C-infected livers compared with non-steatotic livers (Yasui, 2010). It is likely that HCV core protein is the culprit, as it contains RNA binding domains capable of suppressing the transcriptional activity of PPAR α (Dharancy, 2005). This type of interaction has been shown for other viral protein-nuclear receptor partners (RXR α -HCV, HNF4 α -HBV, RXR α -HBV) (H. Tang, 2001; Tsutsumi, 2002).

Conversely, studies in HCV core transgenic mice found expression of core protein is associated with PPAR α activation (Tanaka, 2008a; Tanaka, 2008b). The core serves as a co-activator and nuclear stabilizer of PPAR α , and may transactivate PPAR α through ERK1/2 activation and p38 MAPK phosphorylation (Tanaka, 2008b). Although seemingly counterintuitive, PPAR α up regulates genes involved in the generation of reactive oxygen species (ROS) through activation and induction of AOX and cytochrome P450 4A1. This causes increased β and ω oxidation, respectively, which can damage the mitochondrial membrane, impairing β oxidation and leading to fatty acid accumulation in hepatocytes (Tanaka, 2008a; Tanaka, 2008b). PPAR α also increases expression of fatty acid transporters, promoting fatty acid influx and leading to further PPAR α activation by acting as PPAR ligands (Tanaka, 2008a; Tanaka, 2008b). This helps explain the role of PPAR α in HCV-induced steatosis in the animal model. Human liver, however, expresses less than 1/10 the PPAR α mRNA and functional DNA binding capacity of mouse liver, suggesting that PPAR α signaling may be significantly more robust in mice (Gonzalez, 2008). This may explain species differences in the carcinogenic properties of PPAR α . An animal model that over expresses HCV core protein in humanized PPAR α mice (Q. Yang, 2008) may help to address the species difference.

PPAR α also has anti-inflammatory effects; activation results in decreased levels of intercellular adhesion molecules (I-CAMs) and vascular cell adhesion molecules (V-CAMs), which decreases inflammatory cell infiltration into the mouse liver (Ip, 2004). PPAR α down regulation observed in humans may also exacerbate HCV-induced inflammation (Dharancy, 2005; Li, 2010). For example, the HCV core protein negatively regulates the inhibitory effect of PPAR α in nuclear factor kappa B (NF κ B) activity (Li, 2010) and thus activates NF κ B. PPAR α -mediated pathways also play a role in liver fibrosis. PPAR α activation

reduces hepatic fibrosis in a rat model by enhancing catalase expression and activity, resulting in reduced oxidative stress and less HSC activation, and thus reduced liver injury (Toyama, 2004).

PPAR α is also involved in the development of HCV-related HCC in animal models. PPAR α +/+HCV core transgenic mice develop HCC at a rate of about 30% higher than PPAR α +/-HCV core or PPAR α -/-HCV core transgenic mice (Koike, 2009; Tanaka, 2008b). This may be due to the involvement of PPAR α in ROS generation that subsequently leads to increases in oxidative DNA damage, predisposing hepatocytes to malignant transformation. PPAR α activation in mice also leads to increases in cell division by altering cyclin and cyclin-dependent kinase (CDK) expression without subsequent increases in apoptosis (Tanaka, 2008b). Though activation of PPAR α in rodents causes hepatomegaly and liver cancer, carcinogenic effects of PPAR α have not been noted in humans.

Conflicting evidence has also emerged concerning the role of PPAR α in HCV replication upon study of its ligands. It has been shown that both PPAR α agonists and antagonists inhibit HCV replication (Nishimura-Sakurai, 2010; Rakic, 2006). Bezafibrate, a PPAR α activator, is widely used to treat hyperlipidemia by reducing serum LDL, VLDL, and triglycerides. Fibrates may decrease HCV RNA titers in patients who were previously unresponsive to IFN therapy (Fujita, 2006; Fujita, 2004). This effect is attributable to its reduction of HCV RNA bound to LDL. It is also possible that PPAR α -mediated suppression of NF κ B is involved in HCV repression (Fujita, 2006). The repressive effects of an agonist are logical due to the anti-inflammatory as well as anti-lipogenic properties of PPAR α . However, PPAR α antagonists also make sense given the environment needed for viral replication. HCV replication takes place in membranous ER-derived complexes that associate with lipid droplets. HCV core induces changes in lipid metabolism (Adinolfi, 2001; Kapadia, 2005) as well as the formation and redistribution these droplets (Boulant, 2008; Miyanari, 2007). PPAR α antagonist 2-chloro-5-nitro-N-(pyridyl) benzamide causes hyperlipidemia and consequent disruption in the membranous structures and in the composition of lipid droplets (notably an increase in triglyceride content) in Huh7 cells. This causes changes in the localization of HCV RNA and disruption of the replication complex (Lyn, 2009; Rakic, 2006). It is also likely that the dysregulation of critical lipid metabolizing and transfer genes regulated by PPAR α is involved in the observed repression. The relationship between HCV and PPARs observed in different models is summarized in Table 1.

2.2 PPAR γ

PPAR γ , by contrast, is the major regulator of hepatic lipogenesis and adipocyte differentiation. PPAR γ mediates the development of steatosis in mouse models by up regulating critical genes in the lipogenic pathway including fatty acid synthase (FAS), acyl-coA carboxylase (ACC), and stearyl-coA dehydrogenase-1 (SCD-1) (Gavrilova, 2003; S. Yu, 2003). It also shares control of fatty acid transporters CD36 and FATP with PPAR α (Motojima, 1998). As with PPAR α , there is some controversy about the interaction between PPAR γ and HCV. Several cell line studies using the human HCC cell line Huh7 have linked HCV (specifically viral protein NS5A) with increased transcriptional activity of PPAR γ (K. Kim, 2009; K. H. Kim, 2007) as well as increased recruitment of PPAR γ coactivator 1 α (PGC1 α) to the peroxisome proliferator response element (K. Kim, 2009). Increased expression of PPAR γ mRNA has also been observed in human livers infected with HCV, with the highest levels in patients with HCV-associated steatosis (Lima-Cabello, 2010). PPAR γ -mediated up regulation of lipogenic genes is a relatively simple mechanism for HCV-related steatosis. However, HCV genotype 3a-mediated down regulation of PPAR γ in Huh7 cells has also been observed, leading to induction of suppressor of cytokine signaling 7 (SOCS-7), which is normally repressed by PPAR γ , and helping the virus inhibit cytokine

signaling and escape the immune system (Pazienza, 2010). SOCS-7 also plays a role in the development of insulin resistance, a well-known result of chronic HCV infection, by degrading insulin receptor substrate 1 in Huh7 cells (Pazienza, 2007; Pazienza, 2010).

PPAR γ activation attenuates stellate cell activation in mice and rats (L. Yang, 2006; J. Yu, 2010). PPAR γ is expressed in quiescent HSCs, and this expression is absent in active cells (Marra, 2000). Reintroducing PPAR γ into HCV-activated HSCs causes a return of the activated HSC to quiescence (L. Yang, 2006; J. Yu, 2010). A possible mechanism is PPAR γ -mediated inhibition of TGF β , which causes HSC activation (Zhao, 2006). These activities demonstrate PPAR γ 's role in hepatic fibrosis.

PPAR γ ligands thiazolidinediones (TZDs) are in widespread use due to their insulin-sensitizing actions. Pioglitazone, a member of this class, has been used in clinical trials in combination with traditional HCV treatment with some success in improving viral response (Khatab, 2010; Overbeck, 2008). Since increasing levels of insulin resistance are associated with reduced response rates to peg-IFN plus ribavirin, increases in insulin sensitivity may be helping nonresponsive patients in these studies. TZDs also increase adiponectin levels, which are inversely correlated with HCV-induced steatosis (Petit, 2005). Adiponectin is a serum protein secreted by hepatocytes, and its levels correlate inversely with BMI, abdominal fat, and insulin resistance. Adiponectin is involved in increased insulin sensitivity by decreasing triglyceride content in the muscle and liver of mice (Yamauchi, 2001).

3. FXR

FXR is highly expressed in the liver and intestine due to its role in lipid, cholesterol, and bile acid (BA) homeostasis (Gadaleta, 2010). BAs are the products of cholesterol metabolism; they are synthesized solely by the liver, secreted into the small intestine via the gallbladder, and then reabsorbed and passed into enterohepatic circulation via the portal vein (Gadaleta, 2010). The primary BAs (cholate [CA] and chenodeoxycholate [CDCA]) are secreted into the intestinal tract, where they are broken down into secondary BAs (lithocholate [LCA] and deoxycholate [DCA]) (Gadaleta, 2010; Scholtes, 2008). Both primary and secondary BAs are natural ligands for FXR (Gadaleta, 2010). When FXR is activated by BAs, it is translocated to the nucleus and typically dimerizes with RXR (Gadaleta, 2010). FXR also dimerizes with the retinoic acid receptor (RAR) (Vavassori, 2009). The action of FXR is to regulate the expression of cholesterol 7 α -hydroxylase (CYP7A1), which is the rate-limiting enzyme in cholesterol metabolism and subsequent BA formation (Gadaleta, 2010; Vavassori, 2009). FXR controls the synthesis of BAs via a negative feedback loop mediated through small heterodimer partner (SHP) that inhibits further conversion of cholesterol to BAs when cellular levels of BAs are high (Gadaleta, 2010). In addition to regulation of BA synthesis, FXR activation affects BA re-absorption, conjugation, detoxification, and transport (Gadaleta, 2010).

BAs play an important role in HCV replication in hepatocytes through modulation of FXR signaling pathways (Chang, 2007; Scholtes, 2008). BAs increase HCV RNA and protein level in GS4.1 cells, which harbor the HCV genotype 1b replicon vector (Chang, 2007). Furthermore, z-guggulsterone, an antagonist of FXR, prevents CDCA-induced HCV RNA expression (Chang, 2007). When IFN α or IFN γ and each bile acid are incubated together, bile acids significantly reduced the anti-HCV effect of IFN. BAs inhibit key innate immune system factors including IFN, protein kinase A (PKA), and STAT1 (Chang, 2007; Chang, 2004). Thus, BAs are factors for IFN non-responsiveness (Chang, 2007). Taken together, BAs and FXR play a role in HCV replication.

Activation of FXR favors viral replication, but FXR activation also has anti-fibrotic and antiinflammatory effects. FXR inhibits HSC activation by decreasing TGF β expression.

Additionally, FXR activation results in down regulation of monocyte chemotactic protein-1, keratinocyte-derived chemokine, and VCAM-1, whose encoded proteins promote infiltration of inflammatory cells (Zhang, 2009). FXR can also induce SHP expression, which suppresses the expression of extracellular matrix genes α -1 collagen, α -smooth muscle actin, and tissue inhibitor of metalloproteinase 1 and 2 (Fiorucci, 2004; Fiorucci, 2005) and thus has anti-fibrotic effects.

4. LXR

LXR has two isoforms, α and β ; LXR β is expressed in all tissue, while LXR α is expressed in the spleen, liver, adipose tissue, intestine, kidney, and lung (Zhao, 2010). For the purpose of this review, we will only discuss LXR α as it relates to liver function. Oxysterols, oxidized derivatives of cholesterol, are synthesized in the liver under conditions of excessive cellular cholesterol levels; these are natural ligands for LXRs (Gadaleta, 2010; Lima-Cabello, 2010). Fittingly, LXR is a fatty acid-activated transcription factor and sterol sensor that regulates cholesterol homeostasis and prevents damage from excessive cholesterol accumulation (Lima-Cabello, 2010). Activation of LXR stimulates reverse cholesterol transport from hepatocytes and biliary cholesterol secretion through up regulation of ABCG5/ABCG8 hepatic canalicular transporter (Gadaleta, 2010). LXR also regulates the expression of steroid regulatory element-binding protein-1c (SREBP-1c), a family member of SREBPs (Moriishi, 2007) that plays a pivotal role in maintaining lipid homeostasis (Lima-Cabello, 2010). Via LXR activation, SREBP-1c is involved in positive transcriptional control of acetyl-CoA carboxylase (ACC), FAS, and SCD-1 (Moriishi, 2007). Increase in the activity of these enzymes causes a greater production of saturated and unsaturated fatty acids and triglycerides (Moriishi, 2007). Therefore, activation of LXR and its subsequent pathways leads to an increase in lipogenesis and thus steatosis (Moriishi, 2007). It has been hypothesized that the HCV core protein-associated increase in the incidence of steatosis is mediated by the SREBP-1c pathway after activation of LXR α /RXR α (Moriishi, 2007). The localization and consequential degradation of the HCV core protein in the hepatocyte nucleus occurs in a proteasome activator PA28 γ /REG γ -dependent manner (Moriishi, 2007). PA28 γ /REG γ regulates the expression of host nuclear receptor protein as well as of the HCV core protein (Moriishi, 2007). PA28 γ $-/-$ /HCV Core $+/+$ mice show nuclear accumulation of HCV core protein. The accumulated HCV core protein in the nucleus disrupts the development of steatosis normally seen in HCV core transgenic mice (Moriishi, 2007). It was found that HCV core protein can enhance the binding of LXR α /RXR α to the LXR response element in the SREBP-1c gene, and this enhancement only occurs in the presence of PA28 γ . Thus, steatosis that occurs as a result of HCV infection may be mediated by the interaction between PA28 γ and nuclear receptor LXR α /RXR α (Moriishi, 2007).

Like FXR, LXR has anti-inflammatory and anti-fibrotic effects. LXR is among the most highly expressed nuclear receptors in HSCs and is involved in the repression of pro-inflammatory gene expression as well as of stellate cell activation. However, in patients with HCV infection and steatosis, LXR down regulates the immune system, including Interleukin-6 and TNF α (Lima-Cabello, 2010), which can lead to viral evasion of the immune system and increased viral propagation. In addition, LXR-null mice are more susceptible to fibrosis, and show increased expression of HSC-activation related genes (Beaven, 2010).

5. RXR and RAR

Retinoid X receptor (RXR) is unique among the adopted orphan nuclear receptors in that it can bind as a homodimer to DNA (Wan, 2000). In addition, RXR dimerizes with RAR, PPARs, LXRs, FXR, CAR, and VDR. RXR has three isoforms (α , β , and γ); 9-cis retinoic

acid (RA) is the ligand for all isoforms (Chambon, 1996). The HCV core protein binds to RXR α and stabilizes the binding of RXR α /PPAR α to the DNA resulting in up regulation of lipid metabolism enzymes including cellular retinol binding protein II (CRBP II) and AOX (Tsutsumi, 2002).

Retinoic acid receptor (RAR) has three isoforms (α , β , and γ), all of which are activated by all-trans RA and 9-cis RA. Heterodimers of RAR and RXR mediate the physiological action of these ligands as they pertain to cell survival, apoptosis, metabolism, and a host of other cellular functions. RAR/RXR acts as a transcriptional repressor in the absence of ligand (RA) (X. H. Tang, 2010). All-trans RA has long been known to have anti-carcinogenic properties and is currently in clinical trials for a number of cancers (Bushue, 2010). RA has been used in combination with traditional HCV treatment with some success in reducing viral load in genotype 1 patients previously nonresponsive to therapy (Bocher, 2008). A study in Huh7 cells found that RA enhances the anti-replicative effect of IFN through up regulation of type I interferon receptor (Hamamoto, 2003). Interestingly, HCV core protein sensitizes cells to RA-mediated apoptosis in human breast cancer MCF-7 cells (Watashi, 2003). This enhancement is accompanied by increased expression of tissue transglutaminase, an important mediator of RA-dependent apoptosis and an RAR target gene (Watashi, 2003). HCV core proteins also activate RAR α -mediated transcription by interacting with Sp110b, a potent transcriptional corepressor of RAR α (Watashi, 2003). HCV core recruits Sp110b from its normal location in the nucleus to the cytoplasm and thus abolishes its ability to repress RAR α . This observation explains HCV core related enhancement of apoptosis induced by RA (Watashi, 2003). RA treatment also induces the expression of gastrointestinal glutathione peroxidase (GI-GPx), an antioxidant enzyme which counteracts cellular stress, in Huh7 cells (Morbitzer, 2005). GI-GPx is normally inhibited by HCV; probably contributing to the oxidative stress common in HCV infected cells (Morbitzer, 2005).

9-cis RA and all-trans RA suppress α -1 collagen expression by activating RXR β and RAR α , which together repress its transcription in transfected rat HSCs. This inhibition is mediated somewhat unusually by binding, along with coactivators steroid receptor coactivator-1 and growth hormone receptor interacting protein-1, to RA response elements in the α -1 collagen promoter (L. Wang, 2002). Activation of these two receptors also decreases oxidative stress (L. Wang, 2007). The combination of 9-cis and PPAR γ agonist rosiglitazone has an additive effect in inhibiting TGF β signaling and HSC proliferation and thus may slow fibrosis progression (Bruck, 2009).

6. Nuclear Receptors, Autophagy and HCV

Autophagy constitutes another important link between nuclear receptors and HCV. Autophagy is a method of self-cannibalization by which the cell degrades various cellular components either because the products of the breakdown are necessary for survival or to free itself from damaged elements (Rabinowitz, 2010). Autophagy is essential for normal tissue homeostasis. Both nuclear receptors and autophagy have regulatory metabolic roles, and it is likely that interaction between the autophagic pathway and nuclear receptors has important implications for metabolism (Rabinowitz, 2010). Indeed, there is emerging evidence that nuclear receptors may be involved in the regulation of autophagy. PXR-null mice have decreased autophagy proteins LCB-I and -II as well as Beclin-1 (K. Wang, 2010). Both PPAR γ activation (Zhou, 2009) and disruption of PPAR γ signaling (Jiang, 2010) induce autophagy, and our unpublished data show that RA can induce autophagy in human liver cancer cells. The relationship between autophagy and HCV is an interesting one. Given that autophagy is involved in the degradation of liver lipid droplets (lipophagy) (Singh, 2009), which are required for HCV replication, and that defects in autophagy contribute to

insulin resistance (L. Yang, 2010), HCV-mediated suppression of autophagy would be logical. However, it has been shown that autophagy machinery is actually required for initiation of HCV replication (Dreux, 2009; Guevin, 2010). There are several possible mechanisms by which autophagy may facilitate viral replication. Autophagy remodels ER membranes and therefore may be involved in the formation of the membranous webs upon which HCV replicates. Autophagic proteins may be required for translation or delivery of the viral genome to the translation site (Dreux, 2009). Other research has shown that HCV indeed induces the formation of autophagosomes but inhibits their fusion with lysosomes and therefore prevents the completion of the autophagic program (Sir, 2008). Thus, it is a possible mechanism for HCV-induced disruption of autophagic signaling and may be involved in virus-mediated insulin resistance. Knockdown of autophagy proteins in immortalized human hepatocytes inhibits HCV replication via up regulation of the interferon signaling pathway, further indicating the possibility of a commensal relationship between HCV and autophagy (Shrivastava, 2011). It is apparent that much more research is necessary to elucidate the connections between nuclear receptors, autophagy, and HCV.

6. Conclusion and Future Direction

There is sufficient evidence that HCV infection affects nuclear receptor-mediated pathways and leads to problems in hepatic metabolism. It is clear that this area of research warrants greater attention. It is becoming clear that metabolic problems may be at the root of many disease states, including cancer and Alzheimer's. Because of their complexity, these pathways have been passed over in the past in favor of more obvious answers, but they are again coming to the forefront and may provide a way to resolve the myriad of unexplored potential connections that remain between HCV and nuclear receptors. For example, one of the target genes of LXR is the inducible degrader of the LDL receptor (IDOL) (Hong, 2010), a receptor known to be involved in viral entry. HCV core protein can enhance LXR binding to DNA and therefore may enhance the expression of IDOL, but the subsequent effect on viral entry is unknown.

Most of the research to date concerning nuclear receptors and fibrosis has been done in either cell lines or small animal models. Considering the crucial role nuclear receptors play in suppressing fibrosis, it is imperative to confirm these findings in humans. Additionally, most of the above-mentioned studies utilized a high fat diet-induced non-alcoholic fatty liver disease (NAFLD) or chemical (thioacetamide or carbon tetrachloride) model to induce fibrosis. While this provides a solid foundation, research into the interplay between nuclear receptors and HCV-specific fibrosis is necessary. There is a pressing need for studies using human tissue to validate the information generated from cell lines and animal models. Although HCV induces fibrosis through many of the same mechanisms as NAFLD, it also has unique methods of inducing inflammation, oxidative stress, and insulin resistance, all of which contribute to the development and progression of fibrosis (Sheikh, 2008). Preventing or slowing fibrosis progression would be a huge stride towards comprehensive HCV therapy. Progression from mild fibrosis to cirrhosis is an important prognostic step in the development of decompensated chronic hepatitis C. Nuclear receptors play vital roles in the prevention of fibrosis and may prove excellent therapeutic targets.

The relationship between hepatitis C and nuclear receptors is undoubtedly complex, and it is difficult to assemble the often discordant findings into a comprehensive picture. However, what emerges clearly is the profound impact of nuclear receptor-regulated pathways on the critical steps of the viral process. In-depth understanding of the interaction may prove a crucial step in the development of treatment and prevention strategies.

Abbreviations

ACC	acyl-coA carboxylase
AOX	acyl-coA oxidase
BA	bile acid
CPT-1	carnitine palmitoyl transferase 1
CYP7A1	cholesterol 7 α -hydroxylase
ER	endoplasmic reticulum
FAS	fatty acid synthase
FAT/CD36	fatty acid transporter
FATP	fatty acid transport protein
FXR	farnesoid X receptor
GI-GPx	gastrointestinal glutathione peroxidase
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HSC	hepatic stellate cell
I-CAM	intercellular adhesion molecule
IDOL	inducible degrader of the LDL receptor
IFN	interferon
JAK	Janus kinase
LDL-R	low-density lipoprotein receptor
LFABP	liver fatty acid binding protein
LXR	liver X receptor
NAFLD	nonalcoholic fatty liver disease
NFκB	nuclear factor kappa B
PPAR	peroxisome proliferator activated receptor
PXR	pregnane X receptor
RA	retinoic acid
RAR	retinoic acid receptor
ROS	reactive oxygen species
RXR	retinoid X receptor
SCD-1	stearyl-coA dehydrogenase
SHP	small heterodimer partner
SOCS-7	suppressor of cytokine signaling 7
SREBP	steroid regulatory element-binding protein
STAT	signal transducer and activator of transcription
TGFβ	transforming growth factor β

tTGase	tissue transglutaminase
TZD	thiazolidinedione
V-CAM	vascular adhesion molecule
VLDL	very low-density lipoprotein

Acknowledgments

The authors acknowledge Drs. Ivan Damjanov and Maura O'Neil for the pathology photographs. The authors thank Dr. Chuanghong Wu for his commentary and Mr. David Johnson for editing this manuscript. We dedicate this article to the KU Liver Center Tissue Bank (http://www.kumc.edu/livercenter/liver_tissue_bank.html), which is supported by KUMC. More than 1,000 human liver specimens have been deposited in this Bank. The specimens are mostly HCV positive and made available for research use. This work is supported by NIH grant CA 53596.

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Figure 1. Nuclear receptor control of lipid homeostasis in the hepatocyte

PPAR-controlled fatty acid transporters CD36 and FATP import fatty acids into the hepatocyte (Motojima, 1998). These fatty acids can then be stored as triglycerides or undergo ω - or β -oxidation, principally controlled by PPAR α (Reddy, 2001). Inhibition of the PPAR α pathway can lead to steatosis. LXR induces lipogenic transcription factor SREBP-1c, which acts by up regulating lipogenic genes including FAS, ACC, and SCD-1 (Lima-Cabello, 2010). PPAR γ also induces these genes (Gavrilova, 2003; S. Yu, 2003). PXR promotes lipogenesis through inhibition of PPAR α and activation of PPAR γ (Zhou, 2006). FXR inhibits lipogenesis through SHP induction, which blocks LXR. SHP also inhibits CYP7A1, the rate-limiting enzyme for the formation of bile acids (Gadaleta, 2010). ACC, acyl-coA carboxylase; AOX, acyl-coA oxidase ;CPT-1, carnitine palmitoyl transferase 1; CYP4A1, cytochrome P450 4A1; CYP7A1, cholesterol 7 α -hydroxylase; FAS, fatty acid synthase; FATP, fatty acid transport protein; FXR, farnesoid X receptor; LXR, liver X receptor; PPAR, peroxisome proliferator activated receptor; PXR, pregnane X receptor; SCD-1, stearyl-coA dehydrogenase; SHP, small heterodimer partner; SREBP-1c, steroid regulatory element-binding protein.

Figure 2. Nuclear receptors and the pathogenesis of HCV

Nuclear receptor-mediated pathways influence the progression of HCV-induced chronic hepatitis. HCV infection can lead to (1) inflammation, (2) steatosis, (3) steatohepatitis, (4) fibrosis, (5) cirrhosis, and (6) HCC. Depending on the model used, conflicting results were obtained. For example, PPAR α can either promote or prohibit viral replication (Lyn, 2009; Nishimura-Sakurai, 2010; Rakic, 2006; Tanaka, 2008a; Tanaka, 2008b). Some of the findings have only been found in animal or cell line models (*). Please see Table 1 for details. Depending on the stage of the disease and the cell type involved, activation of nuclear receptors can either inhibit or promote the pathogenic process. For example, activation of PPAR γ and LXR favors steatosis (Moriishi, 2007; Yasui, 2010) but inhibits fibrosis (Beaven, 2010; Zhao, 2006), and activation of FXR favors viral replication (Chang, 2007) but may have anti-inflammatory and anti-fibrotic effects (Fiorucci, 2004; Fiorucci, 2005; Zhang, 2009). This complicated scheme makes treatment challenging and stage-specific treatment strategies may have to be considered. FXR, farnesoid X receptor; LXR, liver X receptor; PPAR, peroxisome proliferator activated receptor; RXR, retinoid X receptor; SHP, small heterodimer partner.

Table 1

Relationship between HCV and PPARs

Model	Vector	Genotype	Treatment	Effect*	Reference
Cell line					
HepG2	pEF352neo expressing HCV core protein	1b		PPAR α ↓	(Cheng et al., 2005; Dharancy et al., 2005)
	pFK-I389neo/NS3-3'/5.1 subgenomic replicon	1b, 2a	PPAR α agonists clofibrate, fenofibrate	HCV↓ after treatment	(Nishimura-Sakurai et al., 2010)
	pFK-I389neo/NS3-3'/5.1 subgenomic replicon	1b	PPAR α antagonist BA [#]	HCV↓ after treatment	(Rakic et al., 2006)
	pFK-I389neo/NS3-3'/5.1 subgenomic replicon	1a, 3a	PPAR α antagonist BA	HCV↓ after treatment	(Lyn et al., 2009)
Huh7	pFK-I389neo/NS3-3'/5.1 subgenomic replicon	1b	PPAR α antagonist BA	HCV↓ after treatment	(Rakic et al., 2006)
	pRep-Feo replicon	1b		PPAR γ ↑	(Kim et al., 2009)
	pIRES2-EGFP expressing HCV 3a core protein	3a		PPAR γ ↓	(Pazienza et al., 2010)
	pIRES2-EGFP expressing HCV 3a or 1b core protein	1a, 3a		PPAR γ ↓	(Pazienza et al., 2007)
	HCV status (patient sample size)				
	HCV- (40)	1, 2, 3, 4		PPAR α ↓ in HCV+ patients	(Cheng et al., 2005; Dharancy et al., 2005)
	HCV+ (46)				
Human	HCV+ steatosis- (57)	1, 2		PPAR α ↓ in steatosis patients	(Yasui et al., 2010)
	HCV+ steatosis+ (43)				
	HCV+ treatment (6)	1b, 2a	PPAR α agonist bezafibrate	HCV↓ by PPAR α agonist	(Fujita et al., 2004)
	HCV+ no treatment (15)				
	HCV+ treatment (15)	1b, 2a, 2b	PPAR α agonist bezafibrate	HCV↓ by PPAR α agonist	(Fujita et al., 2006)
	Normal (23)	1			
	NAFLD+ HCV- (43)				
	HCV+ (44)				
	HCV+ Peg-IFN α 2b/ribavirin (49)				
	HCV+ Peg-IFN α 2b/ribavirin + treatment (48)	4	PPAR γ agonist pioglitazone	HCV↓ by PPAR γ agonist	(Khattab et al., 2010)
Mouse	Genetic background				
	C57BL/6N HCV core transgenic	1b		PPAR α ↑	(Tanaka et al., 2008b)
	C57BL/6N HCV core transgenic	1b		PPAR α ↑	(Koike, 2009; Tanaka et al., 2008a)

* Effect: Modulation in PPAR-mediated signaling or HCV replication

[#] BA: 2-chloro-5-nitro-N-(pyridyl) benzamide