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MINI-REVIEW

The Lipid Conundrum: Unveiling Liver Steatosis in Chronic Hepatitis C

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Abstract

Liver steatosis, a defining feature of chronic hepatitis C (CHC) patients, plays a crucial role in the pathogenesis of this disease. The intricate process involves disrupted lipid dynamics, characterized by increased lipid uptake, decreased lipid secretion, enhanced lipid synthesis, and impaired lipid degradation caused by hepatitic C virus (HCV) infection. Notably, the regulation of low density lipoprotein receptor (LDL-R) levels and peroxisome proliferator-activated receptor (PPAR) α activity directly influences lipid uptake by the liver and is modulated by HCV. Furthermore, HCV infection hampers the secretion of lipids through microsomal triglyceride transfer protein (MTTP) and the formation of very low density lipoprotein receptor (VLDL). Key components of lipid synthesis, including sterol regulatory element-binding protein (SREBP)-1, SREBP-2, SREBP-1c, fatty acid synthase (FASN), HMG CoA reductase (HMGCR), liver X receptor (LXR), acetyl-CoA carboxylase 1 (ACC1), hepatic CB(1) receptors, and retinoid X receptor (RXR) α, are upregulated, driving the progression of liver steatosis. In contrast, the degradation of lipids within the liver is diminished in CHC patients. Notably, the heterogeneity of HCV core genes across different genotypes strongly influences the induction of liver steatosis, providing compelling evidence for their impact. Furthermore, emerging evidence suggests a significant interplay between steatosis and the HCV life cycle, unraveling a mechanistic link that further complicates the disease's pathogenesis.

Introduction

Chronic hepatitis C (CHC) is characterized by a prominent feature known as liver steatosis, where lipid accumulation within the liver occurs (Chang et al., 2012). In the quest to replicate efficiently, hepatitis C virus (HCV) engages with lipoproteins and apolipoproteins, creating both macrovesicular and microvesicular fatty bubbles within which HCV replication takes place (Sato et al., 2006). Despite its significance in the pathogenesis of CHC and the regulation of HCV life cycle, the

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mechanisms underlying liver steatosis induced by HCV and its implications in the prevention and treatment of CHC remain largely enigmatic (Congiu et al., 2011). Accumulating evidence highlights the complex nature of liver steatosis, emphasizing its pivotal role in the development of CHC and its intricate interplay with HCV (Khan et al., 2010).

Pathogenesis of liver steatosis induced by HCV infection

The accumulation of lipids in the liver and the subsequent formation of liver steatosis rely on the intricate dynamics of lipid metabolism, encompassing processes such as uptake, secretion, synthesis, and degradation within the liver of patients with CHC (Depla et al., 2012). Following infection, the structural and non-structural proteins of HCV profoundly influence these processes, augmenting lipid deposition in the liver, with numerous transporters and key enzymes participating in the pathogenesis of liver steatosis (Ivanov et al., 2011).

1. Increased uptake of lipid induced by HCV

The low-density lipoprotein receptor (LDL-R) serves as the primary receptor for lipid uptake from circulation. HCV proteins upregulate the expression of LDL-R on the hepatocyte surface, enhancing lipid uptake from the bloodstream. Furthermore, peroxisome proliferator-activated receptor (PPAR) α is intricately involved in the regulation of lipid intake by the liver. Tanaka et al. conducted a study using PPARα-homozygous, -heterozygous, and -null mice with liver-specific transgenic expression of the HCV core protein gene, revealing severe steatosis exclusively in Ppara(+/+):HCVcpTg mice, attributed to heightened fatty acid uptake (Tanaka et al., 2008). These observations were closely associated with sustained PPARα activation, as long-term treatment of these mice with clofibrate, a PPARα activator, induced hepatic steatosis. These findings highlight the indispensable role of persistent PPARα activation in the pathogenesis of HCV-induced hepatic steatosis. Additionally, single nucleotide polymorphisms (SNPs) of key regulatory genes often determine the effects on liver steatosis pathogenesis. For instance, the SNP of interleukin-28B (IL-28B) impacts the formation of liver steatosis in patients with non-alcoholic fatty liver disease (NAFLD) as well as liver steatosis induced by HCV infection. However, Verdi et al. demonstrated that the PPARαL162V polymorphism does not appear to be associated with NASH or genotype 1 HCV-related liver steatosis (Verdi et al., 2005).

2. Decreased secretion of lipid induced by HCV

HCV exerts its influence on host cholesterol metabolism to facilitate key processes within its life cycle, often resulting in genotype-dependent clinical consequences of hypocholesterolemia. Notably, hypocholesterolemia tends to resolve following sustained viral response (SVR), suggesting viral interference in host lipid secretion. In a study by Clark et al., a targeted cholesterol metabolomic platform was employed to evaluate changes in in vivo HCV sterol pathway metabolites in paired sera of HCV genotype 2 (G2) and genotype 3 (G3) patients (Clark et al., 2012). Baseline levels of distal metabolites such as 7-dehyrocholesterol (7DHC) and cholesterol were lower in G3 compared to G2, while the proximal metabolite lanosterol remained unaffected. Following SVR, G3 patients exhibited increased levels of cholesterol and 7DHC, whereas lanosterol remained unchanged. Notably, HCV G3 selectively interferes with the late cholesterol synthesis pathway, where normal lanosterol levels serve as a signal for continued proteolysis of HMG CoA reductase, potentially undermining other host responses aimed at increasing cholesterol synthesis. Additionally, Yamaguchi et al. observed elevated hepatic triglyceride (TG) content and hepatic thiobarbituric acid-reactive substances. They found that HCV core protein down-regulates lipid metabolism-associated gene expression, including PPARα, MDR2, acyl-CoA oxidase (AOX), and carnitine palmitoyl transferase-1 (CPT-1) . These findings provide insights into the decreased secretion of lipid from the liver.

Among HCV's structural proteins, Perlemuter et al. demonstrated that the core protein inhibits microsomal triglyceride transfer protein (MTTP) activity and very low-density lipoprotein (VLDL) secretion (Perlemuter et al., 2002). Utilizing a transgenic murine model expressing the HCV core protein, it was observed that core protein overexpression hampers hepatic assembly and secretion of triglyceride-rich VLDL. This interference resulted in reduced MTTP activity and smaller particle size of nascent hepatic VLDL, without affecting the accumulation of MTTP and protein disulfide isomerase. However, hepatic human apolipoprotein AII (apo AII) expression in double-core/apo AII transgenic mice counteracted intrahepatic core protein accumulation and nullified its effects on VLDL production. Immunohistochemical analysis confirmed the colocalization of apo AII and HCV core protein in HCV-infected liver biopsies, highlighting the significance of this interaction in productive HCV infection. These results propose a novel pathophysiological animal model elucidating the induction of viral-related steatosis, wherein the core protein of HCV targets MTTP activity and alters hepatic VLDL assembly and secretion.

MTTP is a critical player in the assembly and secretion of ApoB lipoproteins, with its activity directly influencing the efficiency of lipid secretion from the liver. HCV impairs MTTP activity, leading to a significant reduction in hepatic

lipid transfer. Hence, liver steatosis caused by HCV infection is often referred to as a storage liver disease (Mirandola et al., 2009). In CHC patients, higher degrees of hepatic steatosis were observed in individuals carrying the T allele of the MTTP -493G/T polymorphism, which correlates with increased MTTP transcription.

Mirandola et al. investigated liver MTTP gene expression and its lipid transfer activity in untreated patients infected with major HCV genotypes (Mirandola et al., 2009). The study revealed a statistically significant inverse correlation between MTTP mRNA levels and the degree of steatosis, independent of the HCV genotype. Furthermore, MTTP mRNA levels exhibited an inverse correlation with serum insulin, homeostasis model assessment-insulin resistance (HOMA-IR) in HCV-1 and HCV-2 patients, and with serum HCV-RNA in HCV-3 patients. Notably, liver MTTP-specific activity was significantly reduced in HCV-3 patients compared to those with other HCV genotypes, and this reduction correlated with decreased serum cholesterol, apo B, and low-density lipoproteins. Ryan et al. assessed gene expression involved in lipid metabolism, including MTTP, in liver biopsies from HCV genotype-1 and -3 infected patients (Ryan et al., 2011). They identified a significant genotype-independent correlation between the expression of APOB, MTTP, PRKAA1, and PPARα, highlighting the functional networks of these genes in HCV-infected liver. Moreover, Mirandola et al. genotyped for MTTP -493G/T variants in 298 CHC patients and found that patients with steatosis grades 2/3 had lower MTTP mRNA expression. The MTTP T allele emerged as an independent risk factor for steatosis grades 2/3, particularly in non-HCV genotype 3 patients. However, Petit et al. reported a lack of association between MTTP gene polymorphism and liver steatosis in HCV-infected patients (Petit et al., 2006).

3. Increased synthesis of lipid induced by HCV

3.1 SREBP family members in steatosis

The Sterol regulatory element binding protein (SREBP) family comprises several members, including SREBP-1c, SREBP-1, SREBP-2, and SREBPs act as key regulators of lipid metabolism. Kim et al. investigated the impact of HCV core protein on the expression and activity of SREBP1 (Kim et al., 2010). Their study demonstrated that HCV core protein increases the gene expression of SREBP1, while also enhancing its transcriptional activity. Another study by Jackel-Cram et al. revealed that HCV-3a core protein upregulates the promoter activity of fatty acid synthase (FASN) through SREBP-1, and the mature form of SREBP-1 is enhanced by HCV-3a core protein (Jackel-Cram et al., 2010). Inhibition of PI3K and Akt-2 resulted in reduced SREBP-1 activation by HCV-3a core protein. Similarly, Park et al. demonstrated that HCV NS4B protein increases the transcriptional activities of both SREBP and FASN, with phosphatidylinositol 3-kinase (PI3K) activity playing a crucial role (Park et al., 2009). Furthermore, NS4B protein synergistically elevates the transcriptional activity of HCV core-mediated SREBP-1. Xiang et al. found that nuclear, mature SREBP-1c levels increase in the nucleus of replicon cells expressing HCV-3a NS5A, and transcription factor Sp1 is implicated in SREBP-1c activation by HCV-3a NS5A (Xiang et al., 2010). However, McPherson et al. did not observe a significant difference in hepatic expression of SREBP-1c mRNA between subjects with HCV and normal liver, and a negative relationship was found between hepatic SREBP-1c mRNA expression and the severity of steatosis, fibrosis stage, and inflammation (McPherson et al., 2008). Furthermore, Waris et al. investigated whether genotype 2a-based HCV infection induces the expression and posttranslational activation of SREBPs and revealed that oxidative stress, PI3-K-Akt pathway activation, and inactivation (phosphorylation) of PTEN mediate HCV-induced transactivation of SREBPs (Waris et al., 2007). The activities of HCV-induced SREBP-1 and -2 were sensitive to antioxidants.

3.2 FASN in steatosis

Fatty acid synthase (FASN) is a crucial enzyme involved in fatty acid synthesis. In the livers of patients with CHC, FASN activity is upregulated, leading to enhanced fatty acid synthesis. Jackel-Cram et al. demonstrated that both HCV-3a and -1b core proteins upregulate the FASN promoter, and this upregulation is dependent on SREBP-1 (Jackel-Cram et al., 2007). Mutational analysis revealed that the processing of HCV core proteins of different genotypes differentially contributes to FASN promoter upregulation. While lipid droplet localization of HCV core protein does not play a significant role in FASN upregulation, a specific amino acid residue (Phe(164)) within the FATG lipid droplet localization sequence of HCV-3a core protein significantly influences the stronger FASN activation by HCV-3a core. Furthermore, Oem et al. demonstrated that HCV NS2 protein activates SREBP-1c transcription, and the sterol regulatory element (SRE) and liver X receptor element (LXRE) in the SREBP-1c promoter are involved in SREBP-1c activation by HCV NS2. Additionally, HCV NS2 expression upregulates FASN transcription in an SREBP-1-dependent manner, suggesting that HCV NS2 contributes to HCV-associated steatosis through the upregulation of SREBP-1c and FASN.

3.3 HMG CoA in steatosis

HMG-CoA reductase (HMGCR) is the rate-limiting enzyme in the cholesterol biosynthetic pathway and serves as a target for statins in clinical practice. Honda et al. reviewed the roles of cholesterol in HCV infection, highlighting the alterations in host lipid metabolism caused by HCV, leading to steatosis

and hypocholesterolemia (Honda et al., 2011). While hepatic fatty acid and triglyceride syntheses are upregulated in chronic hepatitis C patients, direct evidence of increased hepatic de novo cholesterol biosynthesis remains elusive. Impaired VLDL secretion from hepatocytes is suggested to increase intracellular cholesterol concentrations, potentially leading to hypocholesterolemia. Notably, lower serum cholesterol levels are associated with lower rates of sustained virological responses (SVR) to pegylated-interferon plus ribavirin therapy, though the precise reasons for this association are still unclear. The antiviral actions of statins are thought to be driven by the inhibition of geranylgeranyl pyrophosphate synthesis rather than their cholesterol-lowering effects. Additionally, other compounds that target various steps of cholesterol metabolic pathways have been investigated as potential strategies for complete eradication of the virus.

3.4 PPARγ in liver steatosis

Kim et al. found that the NS5A protein of HCV increases hepatic lipid accumulation by inducing the activation and expression of peroxisome proliferator-activated receptor gamma (PPARγ) (Kim et al., 2009). NS5A augmented the transcriptional activity and gene expression of PPARγ, while also enhancing its ability to recruit the transcriptional coactivator PGC-1s to the peroxisome proliferator response element (PPRE) with PPARγ. Moreover, NS5A showed increased interaction with PPARγ2 and PGC-1alpha. These findings suggest that NS5A employs multiple strategies to enhance PPARγ-induced lipid accumulation.

3.5 LXR in steatosis

Lima-Cabello et al. reported that liver X receptor (LXR), in addition to inducing hepatic fatty acid biosynthesis, regulates a set of inflammatory genes (Lima-Cabello et al., 2011). They evaluated the hepatic expression of LXRα and its lipogenic and inflammatory targets in CHC patients, revealing that the LXRα gene and its lipogenic targets, including PPAR-γ, SREBP-1c, SREBP-2, and FASN, were overexpressed in the livers of HCV patients with steatosis. The abnormally increased hepatic expression of LXRα and its related lipogenic and inflammatory genes suggests a potential role of LXRα in the pathogenesis of hepatic steatosis. Additionally, Moriishi et al. demonstrated that knockout of the PA28γ gene leads to the accumulation of HCV.

3.6 RXRα in steatosis

Retinoid X receptor alpha (RXRα), a transcriptional regulator with essential roles in cell proliferation, differentiation, and lipid metabolism, has been implicated in HCV-induced steatosis. Tsutsumi et al. discovered a direct interaction between HCV core protein and RXRα, whereby the core protein binds to the DNA-binding domain of RXRα, leading to increased DNA binding of RXRα to its responsive element (Tsutsumi et al., 2002). They also demonstrated that the core protein activates RXRα in cells expressing the protein, as well as in the livers of core-transgenic mice, which develop hepatic steatosis and hepatocellular carcinoma (HCC) later in life. Moreover, their study showed that the core protein enhances transcriptional activity regulated by both RXRα homodimers and heterodimers with peroxisome proliferator-activated receptor alpha (PPARα), using the promoter genes of cellular retinol binding protein II (CRBPII) and acyl-CoA oxidase as reporters. Additionally, the expression of CRBPII was up-regulated in the livers of HCV core-transgenic mice.

3.7 PA28γ in steatosis

The nuclear proteasome activator PA28γ/REGγ, which specifically binds to the HCV core protein, plays a crucial role in the pathogenesis of HCV-related steatosis. Understanding the mechanisms by which the core protein participates in these conditions may provide valuable insights for the development of novel therapeutic strategies for chronic hepatitis C (Mori et al., 2008).

3.8 ACC1 in steatosis

Fukasawa et al. discovered that the HCV core protein enhances fatty acid biosynthesis in core-expressing cells by up-regulating the expression of fatty acid biosynthetic enzymes, particularly acetyl-CoA carboxylase 1 (ACC1) (Fukasawa et al., 2006). The up-regulation of de novo fatty acid biosynthesis mediated by the HCV core protein impacts cellular lipid metabolism, resulting in the accumulation of neutral lipids in HCV-infected cells.

3.9 CB(1) in steatosis

Activation of hepatic CB(1) receptors (CB(1)) has been associated with steatosis and fibrosis in experimental liver diseases. van der Poorten et al. measured CB(1) receptor mRNA levels in liver tissue extracted from patients with chronic hepatitis C (CHC) and in the Huh7/JFH1 HCV cell culture model (van der Poorten et al., 2010). They found that CB(1) was expressed in all patients with CHC and increased with fibrosis stage. Notably, even in mild CHC cases without steatosis, CB(1) levels remained significantly elevated. Huh7 cells infected with JFH-1 HCV showed a significant upregulation of CB(1), indicating that CB(1) is an HCV-inducible gene. Furthermore, CB(1) expression significantly increased with steatosis grade, primarily driven by patients with genotype 3 CHC. In genotype 3 patients, CB(1) correlated with the activation of sterol regulatory element-binding protein-1c (SREBP-1c) and its downstream target fatty acid synthase (FASN). These findings highlight the up-regulation of CB(1) in CHC and its association with increased steatosis in genotype 3 HCV.

3.10 δ-9 desaturase in steatosis

Lipid metabolism disturbance is a prominent feature of chronic hepatitis C, influencing the progression of liver

fibrosis. Miyoshi et al. investigated the activities of fatty acid metabolizing enzymes and fatty acid compositions in HepG2 cells with or without HCV core protein expression (Miyoshi et al., 2011). Their study revealed a significant accumulation of triglycerides in core-expressing HepG2 cells. Notably, the core-expressing cells exhibited a marked accumulation of downstream product 5,8,11-eicosatrienoic acid (20:3(n-9)). Activation of delta-9 desaturase, a fatty acid enzyme, was attributed to the HCV core protein, and the administration of polyunsaturated fatty acids effectively countered its impact on lipid metabolism. These findings provide new insights into the mechanisms underlying lipid metabolism disorders associated with HCV infection and offer potential directions for the development of novel therapeutic approaches.

4. Decreased degradation of lipid induced by HCV

Impaired production of apolipoprotein B-100-containing lipoproteins and subsequent dysregulation of hepatic fat export contribute to the development of liver steatosis in HCV-infected individuals. Mancone et al. employed quantitative proteomics and computational biology to identify ferritin heavy chain (Fth) as a critical cellular determinant responsible for inhibiting apolipoprotein B-100 (apoB-100) production (Mancone et al., 2012). They demonstrated that HCV non-structural proteins and NS5A are sufficient to induce up-regulation of Fth, which in turn inhibits apoB-100 secretion, leading to increased intracellular degradation mediated by the proteasome. Intriguingly, silencing of intracellular Fth by siRNA restores apoB-100 secretion. The inverse correlation between ferritin and plasma apoB-100 concentrations was also observed in JFH-1 HCV cell culture systems and HCV-infected patients. Additionally, Fth expression was found to be necessary for robust HCV infection. These findings provide a novel molecular explanation for the development of liver steatosis and offer potential avenues for the development of therapeutic and antiviral strategies.

Sequence heterogeneity of HCV and liver steatosis

The polymorphism of HCV core gene in different genotypes plays a crucial role in its ability to induce liver steatosis. Tachi et al. analyzed HCV genotype 1b core region sequences from 67 patients with chronic hepatitis C (CHC) and revealed that amino acid substitutions, particularly at position 70/Q (glutamine), were significantly associated with the presence of steatosis (Tachi et al., 2010). Multivariate analysis further identified amino acid 70 substitution and triglyceride levels as independent factors related to liver steatosis. Similarly, Akuta et al. investigated the impact of amino acid substitutions in the HCV-1b core region on insulin resistance (IR) and treatment response in Japanese patients infected with HCV-1b (Akuta et al., 2009). They found that substitutions in the HCV-1b core region were important predictors of severe IR, although HOMA-IR values were not useful predictors of therapy response. In HCV genotype 3-infected patients, the etiology of steatosis appears to be closely correlated with viral factors that increase intracellular lipid levels. Depla et al. analyzed the association between specific residues in the HCV genotype 3 core protein and steatosis severity and found that host factors play a more significant role in the pathogenesis of liver steatosis (Depla et al., 2010). Additionally, polymorphisms at positions 182 and 186 of the core protein have been correlated with the presence or absence of intrahepatic steatosis (Jhaveri et al., 2008). Furthermore, the Y164F mutant HCV core protein was associated with increased lipid droplet formation compared to the wild-type protein (Hourioux et al., 2007). These findings highlight the complex interplay between viral genetic heterogeneity and host factors in the development of liver steatosis in HCV infection.

Polymorphism of IL-28B gene and liver steatosis

Genome-wide association studies (GWAS) have revealed that polymorphisms in the IL-28B gene of the host play a significant role in determining the treatment outcomes of patients with chronic hepatitis C (CHC) who receive pegylated interferon plus ribavirin therapy. These IL-28B polymorphisms have been associated with both treatment-induced and spontaneous clearance of HCV. However, the biological implications of IL-28B gene polymorphisms and their relevance to sustained virological response (SVR) in CHC patients undergoing standard-of-care (SOC) therapy remain unclear. Interestingly, recent research has identified an association between IL-28B genotype and gamma-glutamyl transferase (γ-GTP) levels. Cai et al. conducted a study to evaluate the important single nucleotide polymorphisms (SNPs) in molecules involved in the pathogenesis of liver steatosis in CHC patients, such as patatin-like phospholipase family 3 protein (PNPLA3), IL-28B, and others (Cai et al., 2011). Through the analysis of 626 Caucasian HCV-infected patients, they extracted SNPs from a genome-wide association dataset and assessed their associations with the presence and severity of steatosis using univariate and multivariate logistic regression, considering relevant covariates. The study revealed that carrying the I148M variant in PNPLA3 increased the risk of steatosis, particularly in patients infected with HCV genotypes other than 3. Weaker associations were also found for SNPs in peroxisome proliferator-activated

receptor gamma (PPARγ) and IL-28B. Furthermore, the presence of a SNP in MTTP increased the risk of steatosis, but only in patients infected with HCV genotype 3. These findings suggest that the rs738409 SNP in PNPLA3 is associated with a higher risk of steatosis in patients infected with HCV genotypes other than 3. Host genes, including PNPLA3, PPARγ, and IL-28B, influence steatosis in a genotype-dependent manner, suggesting their interaction with viral factors.

Ohnishi et al. also discovered an association between IL-28B polymorphisms and fatty changes in the livers of patients with chronic hepatitis C (Ohnishi et al., 2012). They observed that vesicular changes in liver tissue were significantly associated with body mass index (BMI), HCV RNA titer, serum aspartate aminotransferase, gamma-glutamyl transferase (γ-GTP), IL-28B genotype, and liver fibrosis level. Clear cell changes, on the other hand, showed significant associations with serum aspartate aminotransferase, γ-GTP, and IL-28B genotype. IL-28B genotype, liver fibrosis, and BMI were identified as significant independent factors for vesicular changes, while IL-28B genotype played a role in clear cell changes.

Tillmann et al. were the first to observe that LDL cholesterol levels were higher in CHC patients with the CC genotype at the rs12979860 polymorphism, which is located proximal to the IL-28B gene (Thompson et al., 2010). Subsequently, they investigated the association between IL-28B genotype and steatosis in treatment-naïve CHC patients. The study included two cohorts comprising a total of 325 CHC patients, and it assessed the presence and severity of steatosis in relation to the rs12979860 polymorphism at the IL-28B locus. The CC genotype was found to be associated with a lower prevalence of steatosis. In the first cohort, steatosis was observed in 47.6% of IL-28B non-CC patients compared to only 22.5% in CC patients. Similarly, in the second cohort, steatosis was present in 67.4% of non-CC patients compared to only 39.6% of CC patients. These results indicate that the IL-28B CC genotype is associated with milder disturbances in lipid metabolism, as evidenced by serum lipoprotein levels and hepatic steatosis, in patients with HCV infection.

Liver steatosis and HCV life cycle regulation

HCV infection disrupts both lipid and glucose metabolism, contributing to the development of steatosis, insulin resistance, and type 2 diabetes. These metabolic perturbations not only influence disease pathogenesis but also impact viral replication. HCV manipulates liver steatosis to enhance its own replication (Clément et al., 2011; Wedemeyer et al., 2009; Piodi et al., 2008; Bernsmeier et al., 2008; Kim et al., 2007). AMP-activated protein kinase (AMPK), a key regulator of lipid and glucose metabolism, plays a pivotal role in this intricate interplay. Mankouri et al. demonstrated that in cells infected with HCV or harboring HCV subgenomic replicons, the phosphorylation and activity of AMPK at threonine 172 were dramatically reduced (Mankouri et al., 2010). They found that this effect was mediated by the activation of protein kinase B, which phosphorylates serine 485 and inhibits AMPK. The physiological significance of this inhibition was highlighted by the observation that pharmacological restoration of AMPK activity not only abolished lipid accumulation in virus-infected and replicon-harboring cells but also efficiently inhibited viral replication. These findings underscore the requirement for AMPK inhibition in HCV replication.

Yang et al. identified 175 proteins, among which fatty acid synthase (FASN) was highly enriched, from a cell culture supernatant fraction containing HCV genotype 2a (JFH1) virus (Yang et al., 2008). Subsequent studies demonstrated increased FASN expression in Huh7 cell lines. Pharmacological inhibition of FASN activity with C75, a potent FASN inhibitor, resulted in decreased HCV production. Moreover, silencing FASN through RNA interference suppressed viral replication in both replicon and infection systems. Notably, FASN appeared to be selectively required for the expression of claudin-1, a tight junction protein identified as an entry co-receptor for HCV, but not for CD81, another HCV co-receptor. Inhibition of FASN led to reduced claudin-1 expression and a decrease in transepithelial electric resistance of Huh7 cells, indicating a loss of tight junction integrity. Consequently, the entry of human immunodeficiency virus-HCV pseudotypes was significantly inhibited in C75-treated Huh7 cells. This represents the first evidence demonstrating that HCV infection directly induces FASN expression, potentially elucidating the mechanism by which HCV alters cellular lipid profiles and contributes to diseases such as steatosis (Durante-Mangoni et al., 2006; Shi et al., 2002).

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