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BRIEF REVIEW

Interaction of the hepatitis C virus (HCV) core with cellular genes in the development of HCV-induced steatosis

Mahwish Khan · Shah Jahan · Saba Khaliq · Bushra Ijaz · Waqar Ahmad · Baila Samreen · Sajida Hassan

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Abstract Hepatitis C virus (HCV) has chronically infected a large number of patients, leading to the development of steatosis, cirrhosis and, ultimately, hepatocellular carcinoma. The pathogenesis of HCV has not been fully explained, although steatosis is considered to contribute greatly to liver fibrosis progression, modulating host-cell lipid metabolism. Suspected underlying molecular mechanisms include interactions between HCV proteins and intracellular lipid metabolic pathways. Recent studies have suggested that the nucleocapsid of HCV (core) acts as a pathogenic factor involved in lipid droplet accumulation, changes in lipogenic gene expression and/or the activity of lipogenic proteins in a genotype-specific manner. In this review, we have tried to summarize the current knowledge regarding HCV-induced steatosis and the regulation of expression of host genes and receptors that aid in the viral life cycle and promote liver diseases.

Introduction

Hepatitis C virus (HCV) is a major health concern, with an estimated 3% of the world's population (~300 million individuals) chronically infected with this viral pathogen [6]. HCV causes acute and chronic hepatitis, which leads to fibrosis, steatosis, insulin resistance (IR), cirrhosis and hepatocellular carcinoma (HCC) in a significant number of patients [21, 58, 130]. Epidemiological studies reveal that about 20–30% ofchronic HCV infections are robustly associated with hepatic steatosis, type II diabetes, IR and cardiovascular disease [3, 20]. Fatty liver disease, the most frequent cause of abnormal liver function, is a pathological condition ranging from simple fat accumulation (hepatic steatosis) to hepatic steatosis with inflammation (steatohepatitis), which leads to fibrosis and HCC [118]. Two forms of steatosis, metabolic and HCV-induced, occur. Metabolic steatosis occurs in the setting of obesity, hyperlipidemia, and IR, whereas HCV-induced steatosis is known as the sole route for a direct cytopathic effect caused by HCV [42]. Hepatic steatosis is a frequent histological feature of chronic HCV infection, and there is increasing evidence that HCV infection by itself is an independent predictor of steatosis [113].

Numerous studies illustrate the involvement of HCV in steatosis. However, the fundamental cellular events involved are still poorly understood due to the unavailability of an efficient experimental model. Some insights into the pathways of steatohepatitis are defined by impaired lipid accumulation due to hepatic loss of adiponectin receptors, which play an important role in fatty acid accumulation by elevating the expression level of the enzymes AMP-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), liver gluconeogenic enzyme and phosphoenol pyruvate carboxy kinase (PEPCK) due to HCV infection. In addition, transcription factors such as sterol regulatory element binding protein (SREBP) and peroxisomal proliferator activator receptors (PPARs) are also key players in HCV-induced steatosis. SREBP activates the enzymes involved in the fatty acid/cholesterol synthesis pathway, such as ACC, FAS and 3-hydroxy-3-methylglutaryl CoA reductase (HMGR). PPARs controls fatty acid oxidation, and its deficiency results in defective fatty acid oxidation [80, 90].

M. Khan · S. Jahan · S. Khaliq · B. Ijaz · W. Ahmad · B. Samreen · S. Hassan (✉)
Applied and Functional Genomics Lab,
Centre of Excellence in Molecular Biology,
University of the Punjab, 87-West Canal Bank Road Thokar Niaz Baig, Lahore, Pakistan
e-mail: sajihassan2004@yahoo.com

Apart from abovementioned players, additional host factors that are involved in HCV-induced steatotic pathways are discussed in this review, not only for a more complete understanding of the crosstalk between HCV core and host genes that may result in the development of steatosis but also for identifying potential therapeutic targets. Recently, it has been investigated whether genetic polymorphism in the HCV core protein contributes to the development of steatosis, but the mechanism involved in the development of steatosis is not fully understood [73, 177]. The main objective of this review is to discuss the molecular mechanism by which the HCV core interacts with cellular genes in the development of steatosis and the effect of genetic polymorphisms. This review also discusses the interaction of different cellular genes that are involved in HCV-core-induced steatosis, providing a molecular basis for this disease.

HCV-induced steatosis

Hepatic steatosis is the accumulation of triglycerides in hepatocytes and a frequent histological finding in chronic hepatitis C (CHC). Hepatic steatosis can develop secondary to obesity, diabetes mellitus, alcohol abuse, protein malnutrition, acute starvation, carbohydrate overload and CHC infection. Sanyal et al. and Vidali et al. found a relationship between oxidative and hepatic steatosis in the progression of CHC [150, 187]. In CHC patients, the prevalence of steatosis ranges from 40 to 80% (mean 55%). HCV is a major cause of hepatic steatosis, as epidemiological studies reveal that the prevalence of HCV-associated steatosis is 2.5 times higher than that observed in other liver diseases, e.g., 26% in hepatitis B and 17% in autoimmune liver diseases [11, 96]. Steatosis has been detected in 30–70% of HCV patients and associated with worsening fibrosis, probability of response to interferon therapy, and the risk of developing HCC [48, 197]. Moreover, the majority of patients with steatosis, approximately 78%, have mild steatosis affecting fewer than 30% of their hepatocytes [35], but in chronic HCV infection and in prolonged cases of steatosis, it leads to fibrosis and HCC. As reported, steatosis, which is common and more severe in HCV genotype 3 patients, is possibly due to the presence of steatogenic sequences within the genome of viruses of this genotype, the burden of HCV RNA load in the liver, and the sustained virological response (SVR) to treatment with pegylated interferon-α and ribavirin [88, 107, 127, 146].

HCV, which belongs to the family *Flaviviridae*, has a positive single-stranded RNA genome of 9.6 kb [37, 142]. The HCV genome has a single open reading frame encoding a large polyprotein of 3,000 amino acids, which is processed by cellular signalase and viral protease, yielding the viral structural (core, E1, E2 and possibly P7) and non-structural (NS2, NS3, NS4A, NS4B, NS5A, NS5B) proteins [142, 149].

Mechanism of HCV-associated steatosis

The interaction of HCV proteins with hepatic cellular components contributes to interference with lipid and carbohydrate metabolism, resulting in the release of cytokines, namely, tumor necrosis factor-α (TNF-α) and interleukin (IL-6 and IL-8), insulin resistance (IR), inflammation, oxidative stress and steatosis [161]. The mechanism of triglyceride accumulation induced by infection with HCV is multifactorial [35]. As reported, lipid metabolism and signaling can be modulated by HCV at three levels: firstly by impaired lipoprotein secretion, secondly by increased lipogenesis and thirdly by impaired fatty acid degradation. Impaired secretion of lipoproteins from infected hepatocytes was the first mechanism proposed to explain HCV-induced steatosis [35].

The HCV core is known as an inducer of oxidative stress, steatosis and HCC [115]. In several studies, both cell culture and transgenic mouse models have been used to investigate whether the HCV core protein is sufficient to induce lipid accumulation in liver, and it has been found that the core protein causes intracellular lipid accumulation as well as malignant transformation in these models [14, 79, 141]. It has been found that genotype 3a is more efficient at causing fat accumulation in hepatocytes than the HCV genotype 1a. Expression of the genotype 3 core protein results in about three times more fat accumulation than that of genotype 1 [1]. According to clinical data based on transgenic mice as an experimental model, HCV core protein has been shown to inhibit microsomal triglyceride transfer protein (MTP) activity [132]. This enzyme plays a rate-limiting role in very-low-density lipoprotein (VLDL) assembly and ApoB secretion. Thus, its inhibition results in the accumulation of triglycerides, which causes steatosis. Moreover, data on human liver suggest that the MTP mRNA level is reduced in the liver of chronic HCV patients, particularly in genotype 3 patients with steatosis [108].

In accordance with another proposed mechanism, the HCV core as well as NS proteins may accumulate and interact with mitochondria and the endoplasmic reticulum, thus inducing liver damage by the production of reactive oxygen species (ROS) such as carbon tetrachloride [91]. The production of these ROS results in the peroxidation of membrane lipids and structural proteins that are involved in the trafficking and secretion apparatus, blocking VLDL secretion and causing mitochondrial dysfunction. DNA and cellular protein damage further aggravate oxidative stress, which leads to steatosis. Moreover, ROS production causes

Kupffer cells to burst, resulting in the release of the cytokines TNF-α, IL-6 and IL-8 [161]. TNF-α further downregulates adiponectin protein receptors, thus inducing IR and steatosis. HCV core and NS proteins also upregulate the SREBP-1c signaling pathway [174, 195]. In the nucleus, SREBP-1c transcription activates the enzymes ACC, FAS, sterol CoA dehydrogenase 4 (SCD4), which are required for lipogenesis [61], and favors the production of saturated and monosaturated fatty acid and intracellular accumulation of triglycerides in the liver. HCV core protein also binds to the active DNA-binding domain of retinoid X receptor-α (RXR-α), a transcriptional regulator that controls many cellular functions and lipid synthesis and upregulates the enzymes cellular retinol-binding protein II and acyl CoA oxidase (AOX), resulting in increased oxidative stress and decreased β-oxidation, which may cause steatosis [184]. PPAR-α is an important transcription factor for regulation of several genes that are responsible for fatty acid degradation in cellular organelles involved in lipid homeostasis and insulin sensitivity. Liver cells transfected with HCV core protein show reduced expression of PPAR-α, which might be responsible for progression of steatosis [184]. PPAR-α mRNA levels are significantly lower in the liver of HCV patients infected with genotype 3 than in those infected with HCV genotype 1 [35].

Moreover, it has been observed that RXR-α and PPAR-α are involved in downregulation of carnitine palmitoyl transferase-1 (CPT-1). This is a rate-limiting enzyme that increases fatty acid transport into the mitochondria for β-oxidation, which is the main catabolic pathway of fatty acid and AOX [30]. Reduced expression of CPT-1 in the liver of HCV patients has been observed [204]. Downregulation of CPT-1 causes mitochondrial dysfunction and further activates the substitution pathways of lipid oxidation in peroxisomes and the ER. A reduced level of CPT-1 leads to formation of 4-hydroxynonenal and malondialdehyde, which further exacerbate the oxidative stress that leads to steatosis [26]. Microarray studies also reveal a significant role of HCV in induction of transcription of several genes involved in lipid metabolism in the liver [22, 158]. Among these is soluble CD4 (SCD4), a rate-limiting enzyme in the synthesis of monosaturated fats [22]. Reduced expression of SCD4 in the liver of obese ob/ob mice has been shown to ameliorate hepatic steatosis significantly.

HCV model system

Studying the mechanism of HCV pathogenesis is quite complicated due to the lack of a suitable animal model and a competent in vitro cell culture system for sustaining the complete HCV life cycle and enabling the production of infectious virus particles. In fact, humans are the only known natural hosts for HCV. Studying HCV in a human model is not ideal because of the lack of information about the exact time period, route and source of infection, the unavailability of liver samples, and the fact that the acute stage of the infection is not obvious at an early time [15, 160]. Chimpanzees can be infected experimentally and are still considered to be the best animal model available in a clinically controlled environment, because their genetic structure is closest to that of humans, HCV RNA is detectable within a few days of infection, and liver samples are readily available before and after infection. However, due to many differences in HCV disease outcome and patterns, such as a lower HCV chronicity rate, the absence of fibrosis and cirrhosis, limited development of hepatocellular carcinoma, and inadequate therapy outcomes in chimpanzees makes them inadequate for understanding the complete behavior of HCV virus in humans [43, 99]. They are also not widely applicable due to ethical and economic concerns [27, 181]. Other primate models, like Callithrichidae and tree shrews have also been used to study HCV. These models are also too expensive and not available in most laboratories [176, 203].

As an alternative approach, the use of transgenic mice with the potential to be infected with HCV has met with moderate success [69, 82, 89, 106, 217]. Unfortunately, the existing mouse models have limited usefulness for drug screening and the study of HCV biology because they are expensive and technically challenging. Transgenic animals are tolerant to the transgenic protein, leading to an insufficient immune response and uncontrolled overexpression of viral proteins. A Cre/loxP recombination system has been developed to eliminate this problem and has allowed the expression of core, E1, E2 and NS2 protein in a controlled environment [189].

The recent introduction of a cDNA expression system and subgenomic replicons in an Huh-7 cell line have allowed researchers to study various aspects of the viral life cycle [85, 95, 190]. Moreover, Huh-7 cells are the most widely used for the study of liver-associated diseases and are fundamental to the development of the HCV infectious cell culture system [93, 214]. These models also have some drawbacks, such as low efficiency of HCV replication and artificial culture conditions that may influence final results [124]. Cellular models, such as pseudo-particles, subgenomic replicons, immortalized hepatocytes, productive replicons and hepatocyte cultures, have been used in different studies [16, 60]. Pseudo-particles are not natural virons, and they do not replicate. Subgenomic replicons have abnormal antiviral activity and no drug metabolism [19]. Immortalized cells are cell-cycle-dependent, and hepatocyte cultures using serum infections are still debatable [213]. Data collected from serum infection in primary hepatocytes do not confirm the role of HCV-LP or HCV-pp [110].

Most of the present-day knowledge about HCV pathogenesis discussed here is obtained from cell culture and mouse models, which, to some extent, display the HCV life cycle and host-virus interactions. The use of these systems has many drawbacks, as overexpression of viral proteins, either in cell culture or in transgenic mice, produces ER stress [39, 94]. Cells respond to ER stress by activating two major pathways: the unfolded protein response (UPR) and the ER overload response (EOR). An insufficient response may convert physiological mechanisms into pathogenesis of liver disorders like viral hepatitis, inflammation, steatosis and insulin resistance [74]. Viruses inhibit these responses to translate viral protein and virus production [193, 194]. The HCV structural proteins core and E2 accumulate and assemble in the ER. HCV core protein depletes calcium stored in the ER, which leads to apoptosis [18]. Christen et al. reported that the core protein activates phosphate 2A, which inhibits interferon-c signaling [31]. UPR expression has also been observed in HCV-infected cells [33, 154] and in cells expressing HCV viral proteins [186], but Deng et al. [36] did not see this phenomenon. While Tardif et al. [179] demonstrated that HCV induces ER stress but suppresses XBP (X-box-binding protein)-1 transcription factor and the downstream ERAD (ER-associated protein degradation) pathway to downregulate degradation of misfolded ER proteins and facilitate its own replication. ER stress also results in the activation of SREBP and results in non-HCV-specific SREBP-induced fatty acid synthesis. Detailed study is required to determine the role of UPR in the regulation and maintenance of homeostasis of the ER.

Pathogenic effects of steatosis in HCC progression

Until recently, it has been unclear whether the two forms of steatosis, i.e., metabolic and virus-related steatosis, induce fibrosis progression and which of them is more efficient. A few studies have shown that steatosis is more closely associated with fibrosis progression in HCV patients infected with genotype 3, i.e., patients with virus-induced steatosis [147, 197]. However, other investigators have reported that metabolic steatosis is significantly associated with more severe fibrosis [56, 127]. Unfortunately, none of these studies could clearly distinguish which form of steatosis induces fibrosis, so it is possible that these two forms of steatosis act synergistically.

HCV proteins interact with mitochondria and the endoplasmic reticulum of liver cells, generating ROS, which disrupts the host cellular machinery, causing mitochondrial dysfunction, exacerbating oxidative stress, and leading to the release of TNF-α [161]. TNF-α induces IR by inhibiting tyrosine phosphorylation of insulin receptors IRS-1 and IRS-2, which downregulates adiponectin [10, 125, 185]. A high level of TNF-α downregulates adiponectin, which induces IR in CHC patients [53, 83, 185]. IR is known to be a major factor in HCV-induced steatosis, specifically in genotype 1 patients [161]. Clinical studies have demonstrated that chronic HCV infection can result in steatosis, IR and impaired IRS-1/PI3 kinase (phosphotidyl inositol-3) responses [3, 10, 66]. HCV core protein is mainly implicated in the pathogenesis of steatosis and IR [79, 91, 113]. Recent studies suggest that NS5A can co-localize with the core protein on lipid droplets and interacts with apolipoproteins, signifying a role in lipid metabolism contributing to the stimulation of hepatic IR [143, 163]. Several studies have revealed that IR is also associated with non-alcoholic steatotic hepatits (NASH) and with a high risk of development of HCC in CHC patients [41, 123], but the mechanism through which hepatic steatosis leads to HCC is still controversial.

HCC is one of the most common causes of malignancy-related death in Africa and Asia [79]. Oxidative stress and steatosis are collectively believed to play a pivotal role in the development of liver injury or HCC in chronic HCV infection [115]. HCV genotype 3a is mostly involved in oxidative stress, lipid peroxidation and HCV-induced steatosis, which contribute to the development of HCC in CHC patients [114, 146]. The additive effects of oxidative stress caused by the inflammatory process and induced by HCV proteins may further exert synergistic effects with alterations in intracellular signaling systems, such as mitogen-activated protein kinase, that are also induced by HCV proteins. These synergistic effects may be responsible for rare characteristics, that is, the high incidence and multicentric nature of hepatocarcinogenesis in HCV infection.

Viral and host factors affecting steatosis in HCV infection

Numerous studies have revealed that both host and viral factors may contribute to the development of steatosis, varying with HCV genotype [112]. Host factors that are responsible for the development of steatosis are alcohol consumption, being overweight, hyperlipidaemia, diabetes and IR, while the viral factor for steatosis is HCV genotype 3 and its structural as well as non-structural genes (core, NS4B and NS5A). Hui et al. and Sheikh et al. found that increased body mass index (BMI) is associated with the severity of steatosis in HCV non-3-genotype patients, while the overall steatosis does not significantly associate with BMI [66, 161]. Some specific viral genetic polymorphism may also play a vital role in the pathogenesis of steatosis. Moreover, hyperhomocysteinemia induces ER stress, which further downregulates the endogenous sterol response

pathway through SREBP, increasing hepatic biosynthesis and uptake of cholesterol and triglycerides, which causes steatosis [3]. A decreased apolipoprotein B (ApoB) concentration is considered to be an independent risk factor for severity of steatosis in HCV genotype 3 patients [157], and disappearance of steatosis correlates with the normalization of ApoB and cholesterol levels [138]. These findings suggest that steatosis is viral or cytopathic in genotype 3 and metabolic in non-genotype 3 patients.

Genetic polymorphism in HCV core protein induces steatosis

It is well known that the HCV core protein interacts with several pathways, including lipid metabolism, in the development of steatosis. In recent studies, it has been demonstrated that the core gene of genotype 3 more efficiently induces steatosis than that of genotype 1, but the mechanism underlying this process of inducing steatosis is still not clear. One of the factors in the production of steatosis in hepatocytes may be variation in the HCV core region. Jhaveri et al. [73] identified amino acid substitutions at position 182 and 186 of the HCV genotype 3a core protein that affect lipid metabolism and contribute to the development of steatosis. Moreover, it was observed that mutations introduced at positions 182 and 186 resulted in a decrease in the amount of lipid accumulation, which suggests that a domain of the HCV core protein plays a vital role in regulating lipid metabolism or trafficking. A significant association has been found between the prevalence of steatosis and amino acid substitutions in the viruses of patients with steatosis. Amino acid substitution at the sequence YATG (1b) and FATG (3a) of the HCV core gene has been found to be important for FAS activation in a genome-specific manner [72]. Previously, Hourioux et al. [64] showed a greater involvement of these HCV 3a amino acid sequences in lipid accumulation and steatosis in a cell culture system. Tachi et al. [177] reported that 46.7% of HCV patients with steatosis had the amino acid glutamine at position 70, and only 9.1% with this mutation were without steatosis, while arginine was found to be a major amino acid 70 of genotype 3a, which is also defined as a non-Q group. Hence, HCV genotype 1b with the amino acid 70/Q, which is not common in genotype 3a, enhanced the lipid accumulation that causes steatosis with substitution in amino acid 70 of the HCV core region. Therefore, polymorphism of the HCV core protein could be one of the main reasons for development of hepatic steatosis in CHC.

Association of the HCV core with lipid droplets

Core and E1 glycoprotein have signal peptide translational sequences that direct the viral polyprotein to the ER membrane. Release of the core protein from the polyprotein involves proteolysis at this signal peptide by two cellular enzymes, signal peptidase and signal peptide peptidase [67, 105, 149]. The release of the protein from the ER is targeted to lipid droplets, which is also a critical step for viral protein cleavage [105]. In mutation studies, it has been demonstrated that the truncated form of the core protein (domain 2 absent) is unable to attach to lipid droplets and that key residues binding to lipid droplets reside within D2 [59]. The earliest report describing a core-lipid droplet association under infectious conditions came from examination of liver biopsies isolated from HCV-infected chimpanzees [163]. In the cell, the core protein is co-localized with lipid droplets in Japanese fulminant hepatitis-1 (JFH1)-infected or -producing cells and also at punctuate sites juxtaposed to the organelles at later stages of infection, suggesting that it is targeted first to loading site on droplets, from which it progressively covers the whole organelle [23, 145, 159].

The precise purpose of attachment of core protein to lipid droplets as part of the HCV life cycle is not fully clear. Non-structural proteins and viral RNA are found in association with lipid droplets coated with the core protein, whereas removing core protein decreases association with lipid droplets and the number of RNA synthesis sites [8, 109, 145, 180]. Moreover, mutation in the D2 domain or core signal peptidase eliminates detection of HCV RNA near lipid droplets [109, 180]. The association between core protein and lipid droplets shows the necessary components required to initiate viral assembly at lipid droplets. Mutations in non-structural gene NS5A domain 3 also eliminate the interaction of viral RNA with lipid droplets, which may be required for the viral replication complex [103]. Since lipid droplets in hepatocytes provide the bulk of the lipid incorporated into nascent VLDL, which may lead to steatosis [98, 199], it has been proposed that this pathway may be utilized by the virus as a mechanism for transport of virions out of the cell [7]. Moreover, by inhibiting expression of apolipoprotein B (ApoB), apolipoprotein E (ApoE) and microsomal triglyceride transfer protein (MTP), the assembly and release of infectious virus was blocked [29, 47, 65, 117]. Both ApoB and ApoE are structural components of very-low-density lipoprotein (VLDL) [49, 87, 162], while MTP is required for transfer of triglycerides to the ER lumen for VLDL assembly [139].

Role of HCV non-structural proteins in steatosis

The role of the HCV core in the development of steatosis is well established. Nevertheless, the nonstructural proteins NS4B and NS5A have also been shown to regulate lipogenesis. Park et al. [126] has shown that NS4B synergistically enhanced the transcriptional activities of

HCV-core-induced SERBP-1 and FAS via Akt signaling pathway, but the other non-structural proteins play no role in its regulation in Huh-7 cells. They observed a genotype-specific response, as the expression was higher in the 1b than in the 2a (JFH-1) expression system. Presently, Akt [46] and liver X receptor [57] are thought to regulate SERBP expression; however, the mechanism is not fully known.

NS5A also induces a range of pathological conditions in host cells. Wang et al. [191] has observed lipid accumulation and production of ROS in NS5A transgenic mice. In addition to lipid accumulation, high levels of NFkB and STAT3 were also observed in hepatocytes of NS5A transgenic mice. This may play a role in steatosis and hepatocellular carcinoma. Kim et al. [81] have reported recently that NS5A uses multiple strategies that produce PPAR-γ-related lipogenesis. These studies show that non-structural proteins, in addition to the HCV core, also take part in the development of steatosis. However, future studies are required to fully understand the exact nature of their role in steatosis.

Role of host cell receptors in HCV-associated steatosis

An interaction between the HCV proteins and cellular receptors acting as transcriptional regulators with many cellular functions has been proposed, depending on their importance in steatosis progression with lipid metabolism.

Adiponectin and its receptors

Adiponectin is a 30-kDa, insulin-sensitizing, soluble matrix protein that is abundantly expressed in white adipose tissue [97]. Adiponectin improves hepatic insulin sensitivity, decreases lipid accumulation in macrophages and has anti-inflammatory properties, such as induction of IL-10 [201, 210]. In mice, administration of adiponectin reduces hepatomegaly, steatosis and attenuates inflammation [202]. Adiponectin is reported to exert its action via its two receptors, adiponectin receptor1 (Adipo R1) and Adipo R2. In mice, Adipo R1 is expressed abundantly in skeletal muscles, while Adipo R2 is considered the primary transcript in liver [206]. The expression of both receptors has been reported to be regulated by insulin in animal models and cell culture systems [183], but the role of these receptors in human liver disease is still unclear. Adipo R1 is reported to be associated with AMPK activation, whereas Adipo R2 is associated with PPAR-α. Moreover, adenovirus-mediated overexpression of both receptors in liver inverted the phenomena of IR associated with steatosis [207]. Adiponectin in human serum depends on metabolic activity, and is present as LMW (low-molecular-weight) and HMW (high-molecular-weight) multimers composed of hexamers. HMW has been found to be responsible for hepatic and whole-body insulin sensitivity and the anti-inflammatory action of adiponectin. Wang et al. [192] hypothesized that, in chronic HCV patients, the anti-inflammatory property of adiponectin might leesen liver disease severity. They found that insulin sensitivity is correlated with HMW adiponectin only in HCV patients with genotype 3, whereas in HCV genotype 1 infection, the serum adiponectin level correlated inversely with steatosis.

Recently, Wedemeyer et al. [196] reported that both the serum adiponectin level and the free fatty acid (FFA) level increased in chronic HCV infection, while it decreased in obesity. Moreover, HCV core protein may provoke steatosis and production of adiponectin. In contrast, several studies have demonstrated that the serum adiponectin level decreases in NASH, type II diabetes mellitus and coronary artery diseases [50, 63, 66, 198]. The adiponectin level correlated inversely with BMI, percentage of body fat, fasting insulin concentration and plasma triglyceride level [17].

A low serum adiponectin concentration and an increased collagen IV and IR-HOMA (Insulin Resistance Homeostatic Model Assessment) index are the indirect indexes to differentiate simple steatosis from early-stage NASH [164]. Adiponectin is believed to protect hepatocytes from triglyceride accumulation by increasing β-oxidation of free fatty acid and/or decreasing de novo free fatty acid (FFA) production in hepatocytes [211]. Steatosis may activate hepatocytes to upregulate FAS/CD95 and thus increase vulnerability to apoptosis, inflammation and fibrosis. Adiponectin protects hepatocytes from FFA-triggered CD95 expression and induction of apoptosis [196]. Death-receptor-mediated apoptosis occur through the CD95/CD95 ligand system, which is significant for non-alcoholic fatty acid liver disease (NAFLD) and HCV-related liver injury [13]. It has been demonstrated that both CD95 expression and secretion of CD95 ligand by T cells and Kupffer cells are greatly enhanced in chronic HCV patients [13, 136]. Interestingly, it has been observed that the adiponectin level is significantly reduced in patients with NAFLD, while in CHC it correlates inversely with the development of steatosis. Moreover, impairment of serum adiponectin secretion also depends upon the HCV genotype [40, 135].

The biological effect of adiponectin and its receptors and their hepato-protective role in fatty liver diseases suggest that controlling the level of adiponectin receptors, specifically adipo-R2, might be an important therapeutic target for the treatment of fatty liver diseases. Since very little information is available on the subject of interaction between steatosis and adiponectin receptors expression in human hepatic cells, further studies are necessary in order to understand the molecular mechanism that regulates Adipo R2 protein turnover in HCV-induced steatosis.

PPAR-α in pathogenesis

PPAR-α is a member of the nuclear hormone receptor superfamily that is required for the differentiation of normal adipocytes [182]. The major function of nuclear transcription factor PPAR-α is to control fatty acid oxidation and activation. For the transcriptional activation of the adiponectin gene, PPAR-α plays an important role, enhancing mRNA expression and the serum adiponectin concentration [38, 71]. PPAR-α deficiency results in defective fatty acid oxidation in the liver [80, 90]. Transfection of liver cells with the gene for the HCV core protein leads to reduced expression of PPAR-α, which further downregulates several genes involved in the fatty acid pathway [30], e.g., AOX and CPT-1, which are rate limiting enzymes of mitochondrial β-oxidation [184].

PPAR-α mRNA expression was found to be significantly reduced in the liver of chronic HCV patients infected with genotype 3 compared to HCV genotype 1 [35, 37]. To understand the role of PPAR-α in pathogenesis of steatosis and HCC, Tanaka et al. [178] conducted a study in which they mated a core gene transgenic mouse with a PPAR-α knockout (KO) mouse and observed that one-third of transgenic mice with PPAR-α and an intact core gene developed HCC at the age of 9 or 24 months. A thorough understanding of the physiological role of PPAR-α and HCV protein interaction in modulating lipid metabolism and HCC may be helpful in developing therapies against HCV-induced steatosis.

Retinoid X receptor-alpha (RXR-α)

Retinoid X receptor (RXR), a member of the nuclear hormone receptor superfamily of ligand-controlling transcription factors, controls gene expression by binding cooperatively as a dimer to hormone responsive elements [100]. RXR forms homodimers and is involved in 9-*cis* retinoic acid-mediated gene activation. It also interacts with either trans-retinoic acid receptor and PPAR [100, 101]. There are three isoforms of RXR, namely, RXR α, β and γ, with RXR α most abundantly expressed in the liver, regulating cell proliferation and differentiation.

Zhu et al. have reported that HCV-induced steatosis aggravates oxidative stress. For instance, HCV core protein may interfere with the oxidant/antioxidant state in the liver and may interacts with RXR-α, a transcription regulator that controls cell proliferation and differentiation and lipid metabolism, thus inducing HCC [216]. HCV core protein could possibly compete with p50 and p65 for direct interaction with RXR α, influencing signaling of NF-κB, a transcriptional regulator that controls many cellular functions and lipid synthesis, and up regulate the enzymes cellular retinol binding protein II and acyl CoA oxidase (AOX), resulting in increased oxidative stress and decreased β-oxidation, which may causes steatosis [184].

Role of LDL-R in pathogenesis

Liver is a key element in the control of plasma cholesterol, regulated by hepatic LDL-Rs [24]. The mechanism by which HCV binds to and enters cells appears to be complex [4]. Several cellular receptors have been proposed to mediated the entry of HCV into cells, including the CD81 receptor [137], the scavenger receptor class B type I receptor [152], and the LDL-R [111]. The LDL-R is an endocytic receptor that transports lipoproteins, mainly the cholesterol-rich lipoprotein LDL, into cells through receptor-mediated endocytosis [32, 120]. This process involves the cell-surface receptor recognizing an LDL particle, followed by its internalization through clathrin-coated pits [173, 202]. It has been suggested that HCV might enter cells via the LDL-R [4, 111].

Some studies have reported a higher prevalence of hypocholesterolemia and hypobetalipoproteinemia in HCV- infected patients than in control groups [134, 157]. Sidorkiewicz et al. [171] reported that a high level of LDL-C in serum might be responsible for inhibition of HCV binding to LDL-R on PBMCs, which in turn leads to restriction on HCV propagation in PBMCs in vivo. Perlemuter et al. [132] demonstrated that hepatic overexpression of HCV core protein interferes with the hepatic assembly and secretion of VLDL and inhibits MTP activity, which results in lipid accumulation and leads to steatosis.

Role of host genes in HCV-induced steatosis

AMPK

AMPK is a serine/threonine protein kinase, a heterotrimeric protein that serves as a sensor of the cellular energy level [54] and mediates cellular adaptation to environmental or nutritional stress factors and changes in energy metabolism [55]. AMPK is activated by increased cellular AMP levels, a marker of decreased cellular energy stores. Activated AMPK inhibits energy-consuming biosynthetic pathways, for instance, fatty acid and sterol synthesis, whereas it activates ATP-producing catabolic pathways, such as fatty acid β oxidation and inhibits ATP-consuming processes such as lipogenesis, directly, by phosphorylating regulatory proteins, and indirectly, by affecting expression level of genes in these pathways [54]. AMPK phosphorylates and inactivates ACC directly, thus decreasing malonyl CoA formation, increasing fatty acids transport into the mitochondria and β-oxidation and restoring the energy balance in the cell [200]. Moreover, AMPK activation inhibits ACC activity indirectly by suppression of the SREBP-1c gene [215].

Hepatic AMPK can be activated by nutrient deprivation and starvation [9], hypoxia and ischemia [131], oxidative/hyperosmotic stress [55] and chronic alcohol consumption [211]. It can also be activated by adiponectin [205] and the antidiabetic drugs metformin [215] and thiazolidinediones (TZDs) [148]. Treatment of ob/ob mice with metformin significantly reduced hepatic steatosis, and its administration in humans with NASH enhanced LFT levels and decreased liver size [102, 149]. Kohjima et al. [84] found that AMPK gene expression was unchanged, or increased in NAFLD so that SREBP-1c and ACC expression also increased in parallel, which revealed negative feedback regulation through AMPK. In other words, negative feedback regulation of SREBP-1c through AMPK failed in NAFLD.

ACC

A biotin-containing multifunctional enzyme system, ACC catalyzes the synthesis of malonyl-CoA, which is both an intermediate in fatty acid synthesis and an allosteric inhibitor of CPT1 [116]. CPT1 controls the transfer of long-chain acyl-CoAs from the cytosol into the mitochondria, where they are oxidized. Malonyl-CoA is therefore a key physiological regulator of both fatty acid synthesis [188] and oxidation [104]. Two isoforms of ACC, ACC1 and ACC2, are present in rodents and humans. ACC1 is highly expressed in liver and adipose tissue, whereas ACC2 is predominantly expressed in heart and skeletal muscle and, to a lesser extent, in the liver [2]. The primary structural difference between the two is an extra N-terminal hydrophobic domain in ACC2 that appears to facilitate ACC2 localization to the mitochondrial membrane [2], where it is believed to regulate local malonyl-CoA levels, CPT1 activity, and fat oxidation.

Enzymatic activity of ACC1 is significantly enhanced in HCV-core-expressing cells. Thus, the higher expression of ACC1 contributes to the increased fatty acid biosynthesis in HCV-core-expressing cells [45]. Suppression of ACC1 inhibits lipogenesis, while ACC2 reduction has no effect on lipogenesis. Savage et al. [151] observed increased expression of ACC in the liver of NAFLD patients, indicating enhanced fatty acid synthesis in hepatocytes, which further stimulates fatty acid accumulation leading to steatosis. Moreover, the SREBP-1c gene in the liver of NAFLD positively upregulated ACC gene expression [84].

SREBP

SREBP plays an important role in regulation of lipid synthesis and cholesterol metabolism [25]. The human genome encodes three isoforms of SREBP: SREBP-1a, SREBP-1c and SREBP-2 [25]. SREBP-1c regulates genes that are involved in the lipid synthesis pathway [168], whereas SREBP-2 specifically regulate genes involved in cholesterol synthesis [62], and these two forms of SREBPs are expressed in the liver. SREBP-1a is an activator of both the fatty acid synthesis and cholesterol pathways and is expressed at reduced levels in the liver of adult mice, rats and humans [61, 167].

It has been observed that the fatty acid synthesis rate increases in the insulin-resistant liver. The ability of insulin to transcriptionally activate the pathway of lipogenesis is mediated by a membrane-bound transcription factor called SREBP-1c [44, 168]. SREBP-1c belongs to the basic helix-loop-helix-leucine zipper family of transcription factors [25] and is a major regulator of fatty acid synthesis. Its expression is markedly higher in NAFLD, up to fivefold higher than control [84]. Horton et al. [61] demonstrated that SREBP transcription in nucleus activates all genes involved in lipogenesis, and overexpression of these proteins in transgenic mouse liver caused the development of fatty liver by increasing lipogenesis [166]. Insulin stimulates the transcription and proteolytic maturation of hepatic SREBP, which results in an increased rate of de novo fatty acids biosynthesis [168, 195]. A positive correlation was found between IRS-1 and SREBP, whereas IRS-2 expression was found to be decreased by 50%. IRS-2 exerts its effect on lipid metabolism. In contrast, IRS-1 is probably involved in glucose metabolism. Specifically, knockdown of IRS-2 results in up-regulation of SREBP and FAS [84].

Current studies have revealed that HCV infection enhances the proteolytic cleavage of SREBP precursors in hepatic cells [195], and HCV NS2 and NS4B proteins can upregulate SREBP-1c at the transcriptional level [126]. Interestingly, NS4B-induced SREBP activation requires the activation of the Akt signaling pathway [126]. Consequently, the promoter activity of FAS, one of the target genes of SREBP-1c, is up-regulated upon expression of NS2 [121] and NS4B [126] as well as the HCV core protein [72]. Lerat et al. have observed that several HCV proteins in transgenic mice play a role in the synthesis of triglycerides through the induction of SREBP-1c, independent of ER stress. Transgenic mice have low plasma triglyceride levels, with development of hepatic steatosis, as observed in infected patients [92].

A role of SREBP-1c in accumulation of triglycerides in insulin-resistant liver of ob/ob mice has been observed [53]. It has also been found that, in the liver of ob/ob mice, inactivation of this gene resulted in an approximately 50 percent reduction in triglyceride accumulation [155]. This shows that SREBP plays an important role in the development of hepatic steatosis in insulin-resistant animals. SREBP upregulates the enzymes ACC and FAS, which are involved in lipogenesis. ACC catalyzes the formation of malonyl CoA [2], and increased production of malonyl

CoA results in decreased fatty acid oxidation due to the inhibition of CPT-1, which transports the fatty acids into the mitochondria [104], and decreased fatty acid β-oxidation causes accumulation of hepatic triglycerides. SREBP is downregulated by AMPK [84].

Furthermore, mouse and chimpanzee models have been used to investigate the effect of HCV core and NS proteins on SREBP gene regulation, and it has been observed that HCV proteins interfere with SREBP processing, which leads to steatosis [122, 195]. A better understanding of the genes that are involved in lipid biosynthesis and their complex molecular interaction with HCV proteins may help in developing novel therapeutic drugs that reduce IR and steatosis.

FAS

FAS, a multifactorial protein that is directly linked to intracellular lipid synthesis, plays a central role in triglyceride accumulation in liver cells by catalyzing the conversion of acetyl CoA and malonyl CoA to saturated fatty acid, i.e., palmitic acid (C16:0), which is further converted to triglycerides after etherification [156]. FAS expression is also positively regulated by SREBP-1c [84, 167]. It has been reported that alterations in the SREBP-1-FAS pathway can result in steatosis and diabetes [61]. Jackel-Cram and colleagues [72] have reported that HCV 3a is a stronger steatogenic factor than 1b, as expression of the HCV-3a core protein enhanced FAS promoter activity more than the core protein of genotype 1b. They further reported that a single amino acid, phenylalanine at position 164 of the HCV 3a core protein, is critical for upregulation of FAS promoter activity. A short amino acid stretch, YATG in1b and FATG in 3a, is responsible for FAS upregulation. Indeed, replacing the phenylalanine with tyrosine in the HCV 3a core protein significantly reduced the level of FAS promoter activity. Although these findings reveal the involvement of phenylalanine of HCV-3a in FAS activation, the molecular mechanism underlying this process is not known. Therefore, further research is required to clarify this issue. Recent confirmation of the upregulation of FAS by HCV has also been provided using the in vitro infectious system [208]. An increased intrahepatic activity of enzymes involved in lipogenesis such as ATP citrate lyase, which is also regulated by SREBP-1c, was observed in chimpanzees experimentally infected with HCV [174].

PEPCK

PEPCK is a member of the phosphoenolpyruvate carboxykinase (GTP) family and a key enzyme of gluconeogenesis in the liver. PEPCK is a mitochondrial enzyme that catalyzes the conversion of oxaloacetate to phosphoenolpyruvate in the presence of GTP. The hepatic PEPCK enzyme has no known allosteric or covalent modifiers. Its mRNA has a short life of 30 min, and thus the level of this enzyme and its activity are mostly determined by changes in the rate of transcription of the gene [76, 170]. Hepatic PEPCK gene expression is normally suppressed by insulin, but in the case of hepatic insulin resistance, suppression is released and PEPCK gene expression increases. Adiponectin receptors (Adipo R1 and Adipo R2) displayed a significant association with hepatic PEPCK gene expression. Adipo R1 is strongly and positively associated with PEPCK, while Adipo R2 is negatively associated with PEPCK [75]. Despite these findings, the role of HCV proteins in the regulation of PEPCK gene expression and its effects on lipiogenesis are unclear.

HMGR

Cholesterol synthesis in the liver is controlled by the microsomal enzyme HMGR. This enzyme catalyzes the reduction of HMGR CoA to mevalonate, a rate-limiting step in the synthesis of cholesterol and nonsterol isoprenoids [51]. HMGR is the primary means for controlling the level of cholesterol biosynthesis. Moreover, the enzyme is controlled by four distinct mechanisms: feedback inhibition, control of gene expression, rate of enzyme degradation and phosphorylation/dephosphorylation. Sidorkiewicz et al. [171] showed that modulation of the mevalonate pathway is associated with the presence of HCV RNA in PBMCs, which results in up-regulation of HMGR expression. The cytosolic HMGR-CoA concentration is sensitive to changes in the supply/demand of acetyl-CoA, and HMGR increases in liver cells in response to a shortage of cholesterol precursors caused by ablation or inhibition of enzyme HMG-CoA synthase [140, 153]. Morbid obesity is also associated with an increased level of HMGR mRNA [172]. Horton et al. [62] reported that SREBP-2 activates genes involved in cholesterol synthesis, such as HMG-CoA synthase and reductase. Overexpression of SREBPs in transgenic mice increases the level of HMGR mRNA [165]. However, the transcriptional control of HMGR by SREBP is not fully understood.

Under certain physiological conditions, upregulation of HMGR may be a natural response to prevent an early decline in cholesterogenic flux, which affects the activity of pathways that produce and utilize acetyl-CoA. Conversely, downregulation of HMGR may produce changes in non-cholesterogenic pathways, which tend to increase flux into acetyl-CoA. This association indicates that a doubling of the acetyl-CoA concentration would cause an eight-fold increase in the HMG-CoA concentration [153]. It is known that the SREBP gene controls both the lipid and

cholesterol pathways; however, the coordination between these two pathways is still controversial. Thus, more investigations are needed to understand the contribution of genes that are involved in the mevalonate pathway in the progression of HCV-induced steatosis.

Relationship between HCV-induced steatosis and insulin resistance

HCV infection is thought to be responsible for insulin resistance in patients with chronic liver diseases with fibrosis. Insulin resistance is a common metabolic disorder in the pre-diabetic state. Diabetes is more often seen with HCV infection (20–25%) and hepatitis B (10%) than with any other liver diseases [212]. HCV seems to cause insulin resistance by targeting intracellular insulin signaling, mainly the serine phosphorylation of the insulin receptor-1 (IRS-1) pathway [12]. Transgenic mice expressing HCV core protein develop insulin resistance, which does not occur in wild-type animals [169]. During HCV replication, HCV core protein induces mitochondrial permeability transition, calcium accumulation, stimulation of electron transport and ROS production, as well as promoting glutathione depletion and release of cytochrome C [86, 175]. Moreover, HCV core protein inhibits PPAR-α and γ, the main players of steatosis, which are expressed in hepatocytes and adipocytes, promoting IRS-1 degradation and insulin resistance [35]. The HCV core protein induces the overproduction of TNF-α, which is responsible for phosphorylation of serine residues of IRS-1, IRS-2 and downregulation of glucose transporter gene expression. TNF promotes hyperinsulinemia and hyperglycaemia and has been linked to an increased risk of development of diabetes and HCC [70]. A high level of TNF-α downregulates adiponectin, which induces IR in CHC patients [53, 83, 185]. HCV core protein interferes with in vitro insulin signaling by genotype-specific mechanisms. Pazienza et al. [128] studied the effect of the transient expression of the core protein of genotypes 3a and 1b on insulin signaling and found that the IRS-1 protein level was significantly reduced in Huh-7 cells expressing the core protein of both genotypes 3a and 1b. The core protein of genotype 3a promoted IRS-1 degradation through the downregulation of PPAR-γ and by upregulating the suppressor of cytokine signal 7 (SOCS-7), whereas the core protein of genotype 1b targeted rapamycin (mTOR), demonstrating a genotype-specific interaction between viral core protein and IRS-1 degradation and steatosis [128]. Suppressor of cytokine signaling protein (SOCS) upset intracellular insulin signaling by inhibiting the phosporylation of Akt, phosphatidyl inositol 3 kinase and Glut-4. High-level expression of SOCS-3 also has a role in insulin and interferon resistance [133]. Insulin resistance was not seen in transgenic mice that were unable to express SOCS-3 and expressed the HCV core protein [79]. HCV itself induces insulin resistance through several factors that are also implicated in interferon resistance, allowing the virus to resist antiviral treatment and to promote fibrosis progression [144]. Contrary to these observations, Pazienza and coworkers have shown that SOCS-1 and SOCS-3 mRNA levels did not change after transfection with both core proteins from genotypes 1b and 3a. However, cells transfected with core protein of HCV genotype 3a had higher levels of expression of SOCS-7 than those expressing core protein 1b. IRS-1 downregulation by SOCS-7 has been confirmed using siRNA in core 3a-transfected cells. The mechanism of IRS-1 degradation by genotype 3a seems to be quite different from that of genotype 1b [128]. Recently, Pazieza et al. [129] explored whether the expression of SOCS-1, SOCS-3 and SOCS-7 is activated by STAT3 in the in vitro model of Huh-7 cells expressing the HCV core protein of genotype 3a but concluded that, in contrast of the other members of the SOCS family (1 and 3), which are regulated by STAT3 activation, SOCS-7 expression appears to be STAT3-independent and is instead regulated by PPAR-gamma. All of these studies indicate that HCV-core-induced insulin resistance and steatosis are related to the genes involved in pathogenesis caused by HCV.

HCV life cycle and therapeutic approaches for steatosis

HCV infection leads to imbalances in lipid metabolism, resulting in increased lipogenesis, reduced secretion and β-oxidation and steatosis. The lipid content of the infected hepatocytes is critical for viral replication. Viral core and NS5A protein interactions with lipid storage organelles and association with ER membranes are important elements for viral replication [8, 23].

Several lipids are required for the virus life cycle, and inhibitors of lipid and fatty acid biosynthesis pathways can be used as therapy. HCV co-localizes with VLDL and controls its secretion for its own secretion. Treatment of Huh-7 cells producing infectious particles with an inhibitor of MTP or siRNA reduces the amount of HCV released as it lowers the levels of the proteins apoB, apoE and MTP, which are required for VLDL assembly [29, 47, 65]. CD81, LDL and SR-B1 receptors are involved in HCV entry. HCV particles isolated from HCV-infected patients are found to be correlated with LDL receptor activity [110]. However, HCV pseudo-particles containing E1 and E2 proteins do not require LDL receptor for their entry. Affected patients have an elevated level of LDL, and inhibition of LDL receptor and HCV binding can protect from viral infection. The efficacy of HCV treatment can be monitored by measuring the serum lipoprotein concentration. It has been reported

that patients with genotype 1 and 2 infections and with high LDL and cholesterol levels respond better to anti-HCV therapy [52]. HCV particles are found as a heterogeneous population of varying densities in blood [119]. These particles are rich in apolipoproteins and cholesterol and resemble VLDL particles. They are precipitated on treatment with antibodies against ApoB and ApoE. HCV-induced steatosis and insulin resistance are ways for the virus to escape interferon therapy and produce fibrosis. Ye et al. observed that inhibition of HMG CoA reductase using a lovastation drug inhibited RNA replication, and similar results have been obtained by others [68, 77]. The direct role of cholestrol in HCV replication is still debatable, as different results are obtained in different studies. Ye et al. [209] observed that addition of cholesterol to levostatin-treated cells did not help to rescue HCV replication. However, Aizaki et al. and Kapadia et al. reported that depletion of cholesterol by treating with beta cyclodextrin either did not effect replication or reduced it to 50% [5, 78]. This variation is because of the different HCV genotypes used in these studies. HCV replication occurs in association with the membrane-bounded structure. These vesicles containing HCV replicons are rich in apoB, apoE and, which are required for VLDL assembly. Targeting ApoB using siRNA or inhibiting MTP, reduced secretion of both HCV and VLDL in HCV infected Huh-7 cells [58].

The current therapies against HCV can be less effective, depending on the genotype and because of toxic effects. Various therapies targeting HCV proteins are being developed, but these are limited due to the high mutation rate of the virus. New therapies are urgently needed to overcome this deadly virus. Inhibitors of MTP are used in clinical trials, such as BMS-201038 [28] and CP-346086 [34], lower the plasma LDL, cholesterol and triglycerides in treated humans and rodents when given for short time, duration but long-term treatment results in a high amino transferase level and fat accumulation. Therefore, these drugs have not been approved for long-term treatment. The significant crosstalk between HCV proteins and the lipid metabolic pathway can be a potential antiviral strategy. Several studies are being conducted to clarify the relationship between HCV and the lipid/cholesterol metabolic pathways, and also to find potential therapeutic targets. However, various aspects of disease still need to be investigated.

Conclusion and future direction

Irrespective of the steatosis grade, HCV-induced steatosis is associated with several diseases, leading to HCC and IR in CHC patients. HCV proteins, like those of many viruses, have developed the ability to modulate intracellular metabolism and signaling of apoptosis and steatosis, ultimately leading to hepatic fibrosis and hepatocellular carcinoma. Despite the well-defined morphological features, evaluation of the cellular mechanism involved in the development of steatosis may help in the production of therapeutic drugs against virus-induced steatosis. Experimental studies have shown that the core protein is capable of inducing lipid accumulation in hepatocytes. This review discusses the possible mechanism by which the HCV core interacts with host genes involved in fatty acid synthesis, the oxidation pathway and alterations in the cellular lipid metabolism, inducing steatosis. It is the first review giving detailed information about all of theindividual genes that are involved in lipid metabolism and the interaction of these genes with other genes involved in this pathway. Some insights into the pathways of steatohepatitis are provided by impaired lipid accumulation due to HCV core protein downregulating the adiponectin receptors, which further reduce PPAR-α levels and upregulate the expression of the enzymes AMPK, ACC, FAS, liver gluconeogenic enzyme, and PEPCK, which results in activation of cell growth signaling and induces HCC. On the other hand, downregulation of the adiponectin receptor also activates hepatic stellate cells (HSC), increases net collagen synthesis and lead to fibrosis and HCC. Adipo R2 catalyzes a reaction in which AMPK is converted into acetyl CoA. Acetyl CoA is converted into malonyl Co-A by ACC, and increases in malonyl CoA and inhibition of CPT-1 result in a decrease in β-oxidation, which causes steatosis. HMGR catalyzes the reduction of HMGR CoA to mevalonate, which is then converted to cholesterol. Regulation of cholesterol and LDL is controlled by the LDL receptor. In addition, transcription factors such as SREBP and PPARs are also key players in HCV-induced steatosis. SREBP activates the enzymes involved in fatty acid/cholesterol synthesis pathway such as ACC, FAS and HMGR (Fig. 1). Although a large amount of information has been obtained, more studies are required to fully understand and correlate the expression level of host genes and the severity of steatosis. All of this information reveals that the interactions of different cellular genes involved in HCV-induced steatosis provide the molecular basis of this disease. HCV genotype 3 is more often associated with viral steatosis, and virus-related steatosis may be a risk factor for developing other diseases such as fibrosis, IR and HCC in a substantial number of HCV patients. Large clinical studies and comprehensive studies are required to understand the effect of genetic polymorphisms in the HCV core region at different positions on intracellular lipid accumulation and clinical progression towards steatosis. Attractive pharmacological approaches include new molecules that could block the receptor ligand binding of PPARs, adiponectin, RXRα, and LDL-Rs and control gene expression of

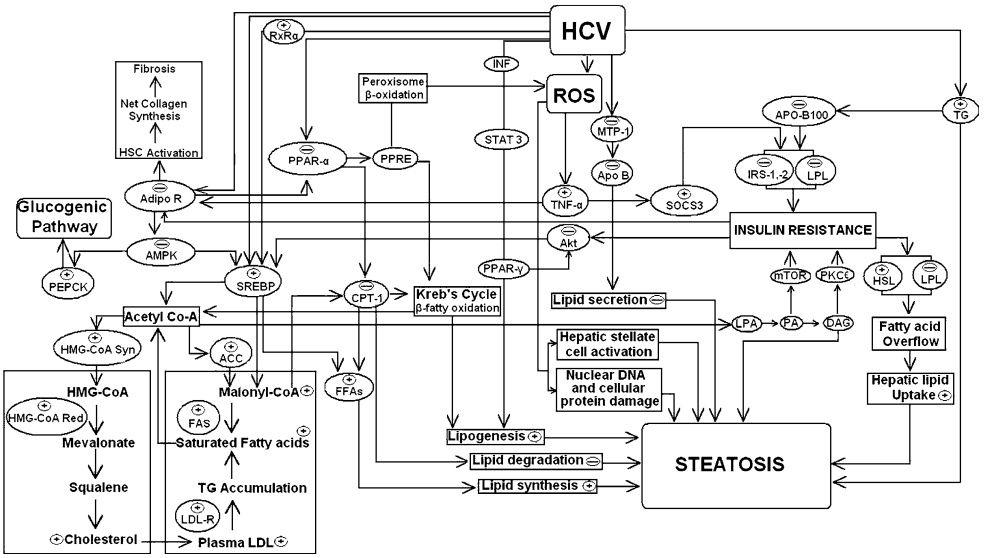

Fig. 1 Pathway of HCV-induced steatosis. HCV contributes to hepatocarcinogenesis and steatosis by modulating intracellular metabolism and signaling. The HCV core protein downregulates the adiponectin receptors, which further reduces PPAR-α and upregulates JNK, AP-1 and cyclin D1, resulting in activation of cell growth signaling and inducing HCC. On the other hand, downregulation of adiponectin receptor also activates hepatic cells HSC (hepatic stellate cells), due to which net collagen synthesis increases, and this leads to fibrosis and HCC. Adipo R2 catalyzes a reaction in which AMPK is converted to acetyl CoA. Acetyl CoA is converted to malonyl Co-A by ACC, and increases in malonyl CoA and inhibition of CPT-1 result in a decrease in β-oxidation, which causes steatosis. Moreover, adiponectin receptors display significant association with PEPCK. HMGR catalyzes the reduction of HMGR CoA to mevalonate, which is then converted to cholesterol. Regulation of cholesterol and LDL is controlled by the LDL receptor. Insulin resistance has been associated with steatosis in CHC patients. Upregulation of TNF-α activates SOC, which downregulates IRS1-2, resulting in insulin resistance. *PPAR-α* peroxisome proliferator activated receptor-α, *RxRα* retinoic acid receptor-α, *FAS* fatty acid synthase, *TNF-α* tumor necrosis factor-α, *ROS* reactive oxygen species, *CPT-1* carnitine palmitoyl transferase-1, *SREBP* sterol regulatory element binding protein, *JNK* Jun N-terminal kinase, *INF* interferon, *ACC* acetyl CoA carboxylase, *Apo* apolipoprotein

AMPK, ACC, SREBP, PEPCK, FAS, which regulate lipid metabolic pathways along with viral gene silencing. Therefore, based on the evidence reported so far, any strategy enabling fatty livers to increase their resistance to stress will protect them from inflammation and will necessarily improve the basal function of these organs.

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