Hepatitis C Virus Induces Proteolytic Cleavage of Sterol Regulatory Element Binding Proteins and Stimulates Their Phosphorylation via Oxidative Stress

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Hepatocellular carcinoma (9). The HCV genome is a 9.6-kb positive-sense single-stranded RNA molecule containing a 5′ untranslated region (UTR) (4, 32). The 5′ UTR contains an internal ribosome entry site, which directs cap-independent translation of a polyprotein precursor of ~3,000 amino acids that is cleaved by viral proteases and host cell signal peptides into mature structural proteins (core, E1, and E2) and nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (4). An additional viral protein found by ribosomal frameshift has been reported elsewhere (43). Study of the molecular mechanisms of HCV replication and pathogenesis has been hampered by the lack of an efficient cell culture system and a small-animal model. A robust and productive HCV (genotype 2a) infection system has been demonstrated which allows the production of a3 untranslated region (UTR), a single open reading frame, and occurring in approximately 50% of HCV-infected patients

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In the present study, we investigated the mechanism(s) of SREBP-1/2 activation in response to Ca\(^{2+}\) signaling and oxidative stress induced by HCV infection. Our results show that activation of phosphatidylinositol 3-kinase (PI3-K)–Akt and LXR are involved in the activation of SREBPs. These data collectively suggest a novel mechanism(s) of SREBP activation associated with HCV-induced steatosis.

**MATERIALS AND METHODS**

Plasmids, antibodies, and reagents. The pSynSRE-Luc plasmid containing three SREs (−325 to −225 bp of the hamster high mobility group [HMG] coenzyme A [CoA] synthase promoter fused into the luciferase pGL2), the IS-15 plasmid (identical to pSynSRE, except with a double point mutation in SRE binding sites), the fatty acid synthase (FAS) promoter construct (wild-type, FAS-700-Luc), and FAS-700-SRE mut-Luc with mutated SREBP-1 binding sites were kind gifts of T. F. Osborne (University of California, Irvine, CA) (11, 21). The plasmid pLXRE-Luc containing two LXR binding sites (between bp −249 and −149) in which the LRE complex from SREBP-1c promoter was inserted into the vector was a kind gift of H. Shimano (University of Tsukuba, Tsukuba, Japan) (44). The HCV NS5A coding sequence was generated by PCR amplification of HCV plasmid pCMV729-3010 (a gift of K. Shimotohno, Kyoto University, Kyoto, Japan). The plasmid pCMV729-3010 contains coding sequences of all of the HCV genotype 1b nonstructural proteins. The HCV genotype 1b NS4B expression plasmid under the transcriptional control of the human cytomegalovirus immediate early promoter was a kind gift of V. Konan (Stanford University, CA). The pFLAG-CMV-NS4B and pFLAG-CMV-core genes derived from genotype 3 were generated by amplification of the respective genes with the PCR primers containing HindIII and Xbal restriction sites, respectively. The pFLAG-generated fragments were cloned into the HindIII and Xbal sites of pFLAG-CMV-1 vector (Sigma) to produce pFLAG-CMV-NS4B and pFLAG-CMV-core, respectively. The mammalian expression vectors pCDNA3-Flag-SREBP-1 (amino acids 2 to 490) and pCDNA3-Flag-SREBP-2 (amino acids 2 to 485) were a gift of J. Ericsson (Ludwig Institute for Cancer Research, Uppsala, Sweden) (14).

Pyrrolidone dithiocarbamate (PDTC) and anti-FLAG monoclonal antibody were purchased from Sigma Chemical Co. Anti-SREBP-1/2 monoclonal antibodies (immunoglobulin G Ig G1) and anti-SREBP-1 polyclonal antibodies were obtained from MBL, Japan, and Santa Cruz Biotechnology, CA. Antibodies to Akt, phospho-Akt-Ser473, PTEN (phosphatase and tensin homologue), and phospho-PTEN were obtained from Cell Signaling Technology. Anti-HCV core, anti-FAS, and antiphosphoerinosine monoclonal antibodies were purchased from AbDinnoReagent, CO; BD Transduction Labs, CA; and Alexis, CA, respectively. Anti-NS4B polyclonal antisem was a kind gift of M. Kohara (Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan). LY294002, N-acetyl-Leu-Cho, and L,2-bis[aminoalkoxy]ethane-N,N',N'-tetraacetic acid-tetra(2-ethylhexyl) ester (BAPTA-AM) were purchased from Calbiochem Novabiochem Corp. (San Diego, CA). HCV cell culture infection system. The JFH-1 genomic RNA (HCV genotype 2a) was transcribed and delivered into Huh-7 cells by electroporation or liposome-mediated transfection as described previously (44). For electroporation, cells were suspended in Cytofex buffer at 10\(^7\) cells/ml. JFH-1 RNA (8 to 10 \(\mu\)g) was mixed with 0.2 ml of the cells in a 4-mm cuvette; a Bio-Rad Gene Pulser system was used to deliver a single pulse at 0.27 kV and 960 \(\mu\)F, and the cells were plated in 100-mm dishes. Liposome-mediated transfection was performed with Lipofectamine 2000 (Invitrogen) and 8 to 10 \(\mu\)g RNA (46). Cells were then plated in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, penicillin (75 units/ml), streptomycin (50 units/ml) at 37°C. Cells (about 5 \(\times\)10\(^4\) confluent) were transfected with 0.2 ml of the cells in a 4-mm cuvette; a Bio-Rad Gene Pulser system was used to deliver a single pulse at 0.27 kV and 960 \(\mu\)F, and the cells were plated in 100-mm dishes. Liposome-mediated transfection was performed with Lipofectamine 2000 (Invitrogen) and 8 to 10 \(\mu\)g RNA (46). Cells were then plated in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, and passed every 2 to 3 days; the presence of HCV RNA in the cells and corresponding cell supernatants was determined by quantitative reverse transcription PCR (RT-qPCR). The expression of HCV core or NS4A proteins was analyzed using Western blotting. The HCV cell culture supernatant was used to infect the naive Huh-7 cells at appropriate dilutions for 5 to 6 h of incubation at 37°C and 5% CO\(_2\) (39, 46). The level of HCV infection and the proteolytic processing of SREBPs were examined at days 2, 3, 5, and 7.

**RESULTS**

In this study, we investigated the mechanism of activation of SREBPs, the master regulators of cholesterol/lipid metabolism in HCV-infected cells. To initiate this study, we incubated Huh-7 cells with HCV cell culture supernatant (HCV virions) derived from a recently described HCV genotype 2a infection system (27, 39, 46). Forty-eight hours postinfection, the levels of HCV infection were measured by analyzing HCV RNA levels in infected cells. An increased level of HCV RNA in cells infected with HCV was observed by quantitative RT-PCR (data not shown) that is consistent with previous studies (39, 46).

To examine whether HCV infection induces SREBP-1/2 proteolytic processing leading to the liberation of the N-terminus domain (68 kDa), cellular lysates from naive Huh-7 and HCV-infected cells were fractionated by SDS-PAGE and immunoblotted with anti-SREBP-1/2 antibodies. Western blot analysis of HCV-infected cellular lysates revealed an accumulation of mature forms of SREBP-1 (Fig. 1A, lane 2) compared to
to uninfected Huh-7 cells (Fig. 1A, lane 1). The expression of HCV core protein in HCV-infected cells represents the level of HCV infection (Fig. 1A, lane 2, bottom panel). Similarly, we observed the accumulation of the mature forms of SREBP-2 in HCV-infected cells (Fig. 1B, lane 2). Huh-7 cells incubated with delipidated medium for 24 h show a similar pattern of

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Sense primer</th>
<th>Antisense primer</th>
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<tr>
<td>SREBP-1c</td>
<td>5'-GCCATGGATTGACCTTT-3'</td>
<td>5'-CAAGAGAGGCCCTCACATG-3'</td>
</tr>
<tr>
<td>SREBP-2</td>
<td>5'-CTTTGATATACCAGAATGCAG-3'</td>
<td>5'-TAGTTGAGGGCCTCACACCA-3'</td>
</tr>
<tr>
<td>LXR</td>
<td>5'-ATCCCCATGACCGACTGATGT-3'</td>
<td>5'-TGCAGCTACATGCAATGGGCA-3'</td>
</tr>
<tr>
<td>HMG CoAR</td>
<td>5'-GGTCCAGAGGAGCTCAATG-3'</td>
<td>5'-AGCAGTGCTCTAGGCTACACT-3'</td>
</tr>
<tr>
<td>Squalene synthase</td>
<td>5'-CGAGGACACTTGGATGCATC-3'</td>
<td>5'-AGCAGTGCTCTAGGCTACACT-3'</td>
</tr>
<tr>
<td>ACL</td>
<td>5'-CTTTGATATACCAGAATGCAG-3'</td>
<td>5'-TAGTTGAGGGCCTCACACCA-3'</td>
</tr>
<tr>
<td>ACC1</td>
<td>5'-TTGGTGGAGATGTGCTCACG-3'</td>
<td>5'-AGCAGTGCTCTAGGCTACACT-3'</td>
</tr>
<tr>
<td>FAS</td>
<td>5'-CGAGGAGCCCTTTGGATGACATC-3'</td>
<td>5'-AGCAGTGCTCTAGGCTACACT-3'</td>
</tr>
<tr>
<td>HPRT</td>
<td>5'-TTGGTGGAGATGTGCTCACG-3'</td>
<td>5'-AGCAGTGCTCTAGGCTACACT-3'</td>
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Abbreviations: CoAR, CoA reductase; ACL, ATP citrate lyase; SCD, stearoyl-CoA desaturase; ACC1, acetyl-CoA carboxylase; HPRT, hypoxanthine phosphoribosyltransferase.
proteolytic cleavage (data not shown). To determine whether HCV infection also induces the transcriptional stimulation of SREBP-1c mRNA, total cellular RNA was extracted from Huh-7 and HCV-infected cells, and the level of SREBP-1c mRNA was quantified by real-time RT-PCR. The results showed increased SREBP-1c mRNA expression in HCV-infected cells (Fig. 1C, compare bar 1 with bar 2), indicating that HCV infection also regulates the biosynthesis of SREBP-1c mRNA. Similarly, the expression of SREBP-2 mRNA was also stimulated (data not shown). These results collectively suggest that HCV genotype 2a infection can induce both the transcriptional stimulation and the proteolytic processing of SREBPs. These activities lead to the production of mature forms of the proteins.

Previously, the expression of HCV proteins (core and NS5A) derived from genotype 1b has been shown to regulate lipid metabolism (3, 35). To further demonstrate that the expression of HCV proteins from genotype 1b can induce the activation of SREBP-2, Huh-7 cells were transiently transfected with plasmid vectors expressing all the HCV nonstructural proteins (pCMV/729-3010), NS5A, and core proteins, all derived from genotype 1b. The results show that cells expressing all nonstructural proteins induce accumulation of SREBP-2 (Fig. 1D, lane 2), whereas HCV NS5A and core proteins fail to do so (Fig. 1D, lanes 6 and 7), suggesting that HCV nonstructural proteins other than NS5A might be involved in the activation of SREBP-2. To explore this possibility, we chose NS4B because the expression of NS4B has been previously shown to induce structural changes in the ER and induce ER stress (12, 45). ER stress has been demonstrated to trigger the proteolytic cleavage of precursor SREBPs into mature forms (16). Huh-7 cells were transiently transfected with vector expressing NS4B (genotype 1b). Western blot analysis of the cellular lysates shows the accumulation of mature SREBP-2 (Fig. 1D, lane 3). Since HCV genotype 3a is more commonly associated with steatosis (2), and since the HCV 3a core protein has been shown to be sufficient to induce triglyceride accumulation in vitro (1, 19), we decided to examine the effect of core and NS4B proteins derived from HCV genotype 3a on the maturation of SREBP-2. Huh-7 cells were transiently transfected with plasmid vectors expressing genotype 3a FLAG-HCV NS4B and core proteins. The results of this analysis show the accumulation of the mature form of SREBP-2 (Fig. 1D, lanes 5 and 9), suggesting that HCV genotype 3a core and NS4B proteins promote proteolytic processing of SREBP-2.

Phosphorylation of SREBPs via mitogen-activated protein (MAP) kinase and PI3-K–Akt has been shown to be necessary for transcriptional activation (24, 40). Our previous studies have shown that HCV-induced reactive oxygen synthase (ROS) and Ca²⁺ signaling activate cellular kinases (15, 41, 42). To demonstrate the role of HCV-induced activation of cellular kinases in inducing SREBP phosphorylation, HCV-infected cells were transiently transfected with the N-terminal domain of FLAG-tagged SREBP-2 and HCV core protein during Western blot analysis. (C) In vivo phosphorylation of SREBP-1 in HCV-infected cells. HCV-infected cells were metabolically labeled with [³²P]orthophosphate (100 μCi for 6 h); a calcium chelator, BAPTA-AM (50 μM for 2 h); and a PI3-K inhibitor, LY294002 (50 μM for 12 h), respectively. The bottom panels represent the expression of N-terminal FLAG-tagged SREBP-1/2 and HCV core protein during Western blot analysis.

FIG. 2. HCV induces phosphorylation of SREBP-1/2. (A and B) Huh-7 cells and HCV-infected cells were transiently transfected with N-terminus FLAG-tagged SREBP-1/2. Whole-cell lysates were immunoprecipitated with anti-FLAG monoclonal antibody, fractionated by SDS-PAGE, and immunoblotted with antiphosphoserine monoclonal antibody. Lanes 1 and 2, lysates from Huh-7 and HCV-infected cells expressing FLAG-SREBP-1 (A) and FLAG-SREBP-2 (B). Lanes 3, 4, and 5, HCV-infected cells were treated with an antioxidant, PDTC (100 μM for 6 h); a calcium chelator, BAPTA-AM (50 μM for 2 h); and a PI3-K inhibitor, LY294002 (50 μM for 12 h), respectively. The bottom panels represent the expression of N-terminal FLAG-tagged SREBP-1/2 and HCV core protein during Western blot analysis. (C) In vivo phosphorylation of SREBP-1 in HCV-infected cells. HCV-infected cells were metabolically labeled with [³²P]orthophosphate (100 μCi) for 4 h. Cellular lysates were immunoprecipitated with anti-SREBP-1 antibody and subjected to SDS-PAGE followed by autoradiography.
toradiography. The SREBP-1 was $^{32}$P labeled in vivo in HCV-infected cells (Fig. 2C, lane 2). These results together demonstrate that HCV stimulates the phosphorylation of SREBPs via oxidative stress and calcium signaling.

To provide biological evidence for SREBP-2 activation, cell-based luciferase reporter assays were performed. The luciferase reporter gene (pSynSRE-Luc) under the control of the SRE derived from the HMG-CoA synthase gene and a mutant plasmid, pJS-15, that contains point mutations in the SREs, were used (11). These reporter plasmids were transfected into HCV-infected cells as well as cotransfected with the plasmid vectors encoding individual HCV proteins (NS4B, NS5A, and core). Thirty-six hours posttransfection cellular lysates were assayed for luciferase activity. The results displayed increased activity of FAS-mut-Luc promoter reporter in the presence of all the nonstructural proteins (Fig. 3A, bar 4), NS4B (1b) (bar 2), NS4B (3a) (bar 5), and core (3a) (bar 6). The expression of NS5A and core derived from genotype 1b did not stimulate the luciferase activity (Fig. 3A, bars 3 and 7). Similar induction of FAS activity was mediated via Ca$^{2+}$ signaling. These results unambiguously establish that HCV induces activation of SREBP target genes.

It is known that Akt can increase the expression of the SREBP-1 gene (30). Previously, we have shown that HCV activates the PI3-K–Akt pathway (15). To demonstrate if HCV-induced Akt activation (via phosphorylation) can stimulate the expression of FAS protein in HCV-infected cells, cellular lysates from cells infected with HCV were subjected to SDS-PAGE followed by Western blot analysis. The results further indicate a functional role of PI3-K in HCV-mediated activation of SREBPs, most likely via direct phosphorylation. The pJS-15 reporter plasmid harboring mutated SREBP-2 binding sites did not show stimulation of luciferase activity (Fig. 3A, bar 8).

Similarly, to assess the transactivity of SREBP-1 in HCV-infected cells, the luciferase reporter gene under the control of SREBP-1 derived from the FAS gene (FAS-700-Luc) and a plasmid (FAS-mut-Luc) that contains mutated SREBP-1 binding sites were used (21). Huh-7 and HCV-infected cells were transiently transfected with these luciferase reporter plasmids and treated with antioxidant (PDTC), Ca$^{2+}$-chelator (BAPTA-AM), and PI3-K inhibitor (LY294002). Thirty-six hours posttransfection cellular lysates were assayed for luciferase activity. The results displayed increased activity of FAS-700-Luc promoter reporter in HCV-infected cells (Fig. 4A, bar 2) and were dramatically reduced in the presence of antioxidant (PDTC), Ca$^{2+}$-chelator (BAPTA-AM), and PI3-K inhibitor (LY294002) (Fig. 4A, bars 3 to 5), suggesting that SREBP-1-mediated FAS-Luc activity was mediated via Ca$^{2+}$ signaling, ROS, and PI3-K-Akt pathway activity. The expression of FAS-mut-Luc did not show stimulation of luciferase activity (Fig. 4A, bar 6). These results unambiguously establish that HCV induces activation of SREBP-1 and -2, via phosphorylation leading to stimulation of SREBP target genes.
show enhanced expression of FAS in HCV-infected cells (Fig. 4B, lane 2). An activated form of Akt (serine phosphorylated) is also produced in HCV-infected cellular lysates (Fig. 4B, lane 2). Since the tumor suppressor protein PTEN regulates the activity of PI3-K–Akt, we examined the status of PTEN in HCV-infected cells. The Western blot analysis of HCV-infected Huh-7 cells revealed the presence of a phosphorylated form of PTEN (Fig. 4B, lane 2), suggesting that phospho-PTEN (inactive PTEN) might favor the activity of PI3-K–Akt in HCV-infected cells (13). PTEN was not phosphorylated in uninfected Huh-7 cells.

Recently, LXR has been shown to induce SREBP-1c at the transcriptional level (17). To demonstrate if the activation of SREBP-1c in HCV-infected cells was upregulated through LXR activation, we first examined the mRNA levels of LXR in HCV-infected cells. The quantitative real-time PCR results demonstrate transcriptional stimulation of LXR mRNA in HCV-infected cells (Fig. 4C, lane 2). To examine if induced LXR in HCV-infected cells can stimulate SREBP-1c, a luciferase reporter gene derived from SREBP-1c promoter/enhancer, which contains the binding site for LXR (pLXRE-Luc), was transfected into Huh-7 and HCV-infected cells. Luciferase activity was assayed at 36 h posttransfection. LXRE-Luc activity was stimulated in HCV-infected cells (Fig. 4D, bar 2). These results suggest that HCV gene expression stimulates the activation of SREBP-1c at the transcriptional level through LXR receptor.

To determine if HCV-infected cells can induce lipogenic
target genes and cholesterol/lipid biosynthetic pathways, we analyzed the transcripts of lipogenic genes by quantitative RT-PCR analysis. The mRNAs analyzed include HMG-CoA reductase, squalene synthase, ATP citrate lyase, acetyl-CoA carboxylase, FAS, and stearoyl-CoA desaturase. HCV infection generally enhanced levels of these lipogenic transcripts (Fig. 5). These data collectively indicate that HCV infection transcriptionally stimulates hepatic lipid biosynthesis.

**DISCUSSION**

Several studies have suggested that HCV alters the expression of genes associated with lipid metabolism (6, 26, 37). Microarray analysis of liver biopsy samples from HCV-infected chimpanzees provided the evidence for the induction of genes involved in lipid metabolism and cholesterol/fatty acid biosynthesis (6, 37). HCV induces the formation of cytosolic lipid droplets onto which HCV structural and nonstructural proteins have been shown to colocalize (35). In chronic hepatitis C, genotype 3a is more commonly associated with steatosis than other genotypes (2), suggesting the presence of steatogenic sequences in the viral genome. The transgenic mice expressing whole HCV genome or core gene derived from genotype 1 developed steatosis (26, 29). HCV genotype 3-infected patients who respond to antiviral therapy reverse steatosis, suggesting a direct causal relationship between HCV infection and hepatic accumulation of lipid (31, 33).

We previously reported that HCV subgenomic replicon (genotype 1b) affects apolipoprotein B-100 secretion and MTP activity (10). In support of this observation, MTP gene expression and enzymatic activity in liver biopsy specimens from patients with chronic hepatitis C were inversely correlated with the histological grade of steatosis (28). The molecular mechanism(s) underlying the altered cholesterol/lipid homeostasis in response to HCV gene expression has not been characterized.

In the present study, we investigated the mechanism of activation of SREBPs in the course of HCV infection in the cell culture system. The results described here show that the expression of HCV proteins derived from genotype 1b can induce proteolytic processing of SREBP-2 (Fig. 1D). Here, we show that NS4B derived either from genotype 1 or from genotype 3 is capable of proteolytic cleavage of SREBP-2, probably through ER stress and structural changes that are known to be caused in the ER membrane by this protein. ER stress triggers the proteolytic cleavage of precursor SREBPs into mature forms (16, 23). We have previously described the ability of the HCV nonstructural proteins to induce ER stress (38). NS4B is an integral membrane protein that has been shown to induce the formation of an ER-derived membranous web-like structure and induce ER stress (12, 45). It is also possible that the induction of the membranous web as a novel platform for HCV replication activities demands higher levels of cholesterol. Stimulation of transcriptionally active SREBPs meets that challenge. We observed that HCV core and NS4B proteins derived from genotype 3a were more efficient in the proteolytic processing of SREBP-2 (Fig. 1D). This is consistent with the previous studies in which HCV core protein derived from genotype 3a was shown to induce significant accumulation of triglycerides (1, 20). The molecular mechanisms underlying the significant involvement of HCV genotype 3 compared to other genotypes in steatosis remain to be characterized. The severity of steatosis in patients with genotype 3 has been shown to correlate with higher viral load (33).

Recently, evidence has been accumulating that SREBPs are
not only involved in cholesterol-regulated events but are also regulatory targets of MAP kinase and the PI3-K–Akt pathway. We have previously shown that HCV gene expression induces activation of cellular kinases such as JAK, Src, MAP kinase, and PI3-K–Akt via oxidative stress and calcium signaling (41, 42). In this study, we observed an increase in serine phosphorylation of SREBPs in HCV-infected cells (Fig. 2), which is mediated by HCV-induced calcium signaling, subsequent elevation of ROS levels, and activation of PI3 kinase. Phosphorylation of SREBPs may aid in the generation of homo- and heterodimers, which in turn influence the transactivation potential of SREBPs (24, 30). Previously, MAP kinase and PI3-K–Akt cascades have been shown to be necessary for transcripational activation of SREBPs (24, 30, 40). Recently, the activity of mature SREBP-1 was shown to be regulated by hyperphosphorylation during the cell cycle, suggesting that SREBP-1 may provide a link between lipid synthesis, proliferation, and cell growth (5).

The biological activity of SREBP-2 was demonstrated by using cell-based luciferase reporter assays. We observed an increased activity of SRE-controlled luciferase activity in the presence of the HCV proteins NS4B (genotypes 1b and 3a) and core (genotype 3a), suggesting that individual HCV proteins, regardless of HCV genotype, are able to induce the transactivation activities of SREBPs.

In the present study, we set out to investigate the possible mechanism by which SREBPs trans-activate gene expression in the nucleus. Our results demonstrate that pSynSRE-Luc activities were reduced in the presence of antioxidants and Ca2+-chelator, suggesting that SREBP transactivation is mediated via Ca2+ signaling and oxidative stress induced by HCV. It has been postulated that constitutive activation of the PI3-K–Akt pathway may be involved in fatty acid and cholesterol accumulation in several pathologies and cancers (30). It is known that the PI3-K–Akt kinases increase the expression of the SREBP-1 gene (30). Previously, we have shown that HCV activates the PI3-K–Akt pathway (41). In this study, we observed that pSynSRE-Luc activity was reduced in the presence of PI3-K inhibitor, suggesting that the PI3-K–Akt pathway stimulates the phosphorylation of SREBPs. Previously, the role of PI3-K–Akt in SREBP-1-mediated FAS promoter activity has been demonstrated (20, 30). Furthermore, our results revealed the presence of phosphorylated PTEN during the course of HCV infection. Phospho-PTEN is an inactive form of PTEN phosphatase, which favors the activation of PI3-K–Akt in HCV-infected cells (13).

The regulation of SREBP-1a and SREBP-2 proteolytic cleavage by cellular sterol content is well defined, but less is known about the regulation of SREBP-1c, the predominant isoform in the liver (36). Recently, LXR has been shown to induce SREBP-1c at the transcriptional level (17). In this analysis, we observed transcripational stimulation of LXR mRNA as well as increased pLXRE-Luc activity in HCV-infected cells. These results suggest that HCV gene expression stimulates the activation of SREBP-1c at the transcriptional level at least in part through LXR transcriptional activation. How HCV induces LXRE activation and subsequently SREBP-1c is not clearly understood.

It is known that HCV is dependent on the cholesterol/fatty acid synthetic pathway for efficient RNA replication (37). Genome-wide transcriptional analyses of HCV infection suggest that a high-level of HCV replication is associated with the modulation of genes involved in lipid biosynthesis (37). In this study, HCV infection generally enhanced the levels of lipogenic transcripts (Fig. 5). These results are consistent with the previous studies conducted with chimpanzees, transgenic mice, and Huh-7 cells expressing the genotype 1b HCV replicons (6, 22, 26). These results are also consistent with the previous studies in which Huh-7 cells expressing the genotype 1b HCV replicon induced lipogenic genes (22). These data collectively indicate that HCV infection transcriptionally stimulates hepatic lipid biosynthesis. Increased cholesterol and fatty acid synthesis may play a key role(s) in efficient HCV replication.

This study demonstrates that HCV infection based on genotype 2a leads to stimulation of lipogenic genes through activation of all three isoforms of SREBPs. Our results demonstrate that mature forms of SREBP-1 and -2 are further activated via oxidative stress induced by HCV. This stimulation occurs by the action of activated PI3-K. Moreover, NS4B and core proteins derived from genotype 3a also contribute to the activation of SREBPs through proteolytic cleavage. The transcriptional stimulation of FAS and SREBP-1c suggests a possible model of HCV-mediated lipogenesis through LXR activation. Activated SREBP-1c may represent a new potential therapeutic target in the pathogenesis of HCV infection associated with steatosis.

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