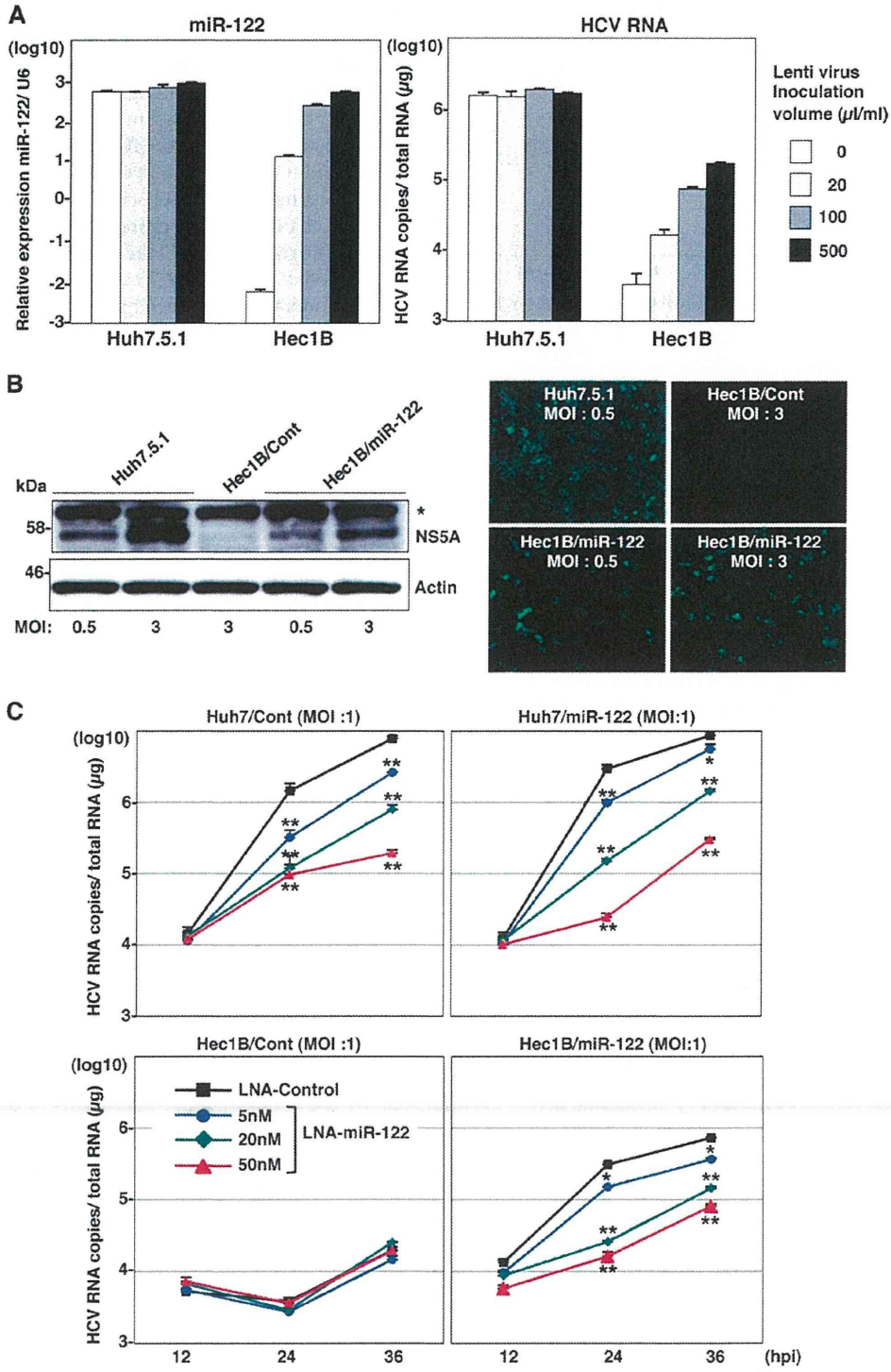

FIG 6 Expression of miR-122 is essential for the enhancement of HCV replication in the Hec1B cells. (A) Huh7.5.1 and Hec1B cells were transduced with lentiviral vectors expressing miR-122 in a dose-dependent manner and infected with HCVcc at an MOI of 1. Intracellular miR-122 and HCV RNA were determined at 24 h postinfection by qRT-PCR. (B) Huh7.5.1 and Hec1B/miR-122 cells were infected with HCVcc at an MOI of 0.5 or 3 and subjected to immunoblotting and immunofluorescence analyses using anti-NS5A antibodies at 48 h postinfection. The asterisk indicates nonspecific bands. (C) LNAs specific to miR-122 at a final concentration of 5 nM, 20 nM, or 50 nM and control (LNA alone at 50 nM) were introduced into Huh7/Cont, Huh7/miR-122, Hec1B/miR-122, and Hec1B/Cont cells by using Lipofectamine RNAiMAX transfection reagent and infected with HCVcc at an MOI of 1 at 6 h posttransfection. Intracellular HCV RNA levels were determined by qRT-PCR at 12, 24, and 36 h postinfection. Asterisks indicate significant differences (*, $P < 0.05$; **, $P < 0.01$) versus the results for control cells.

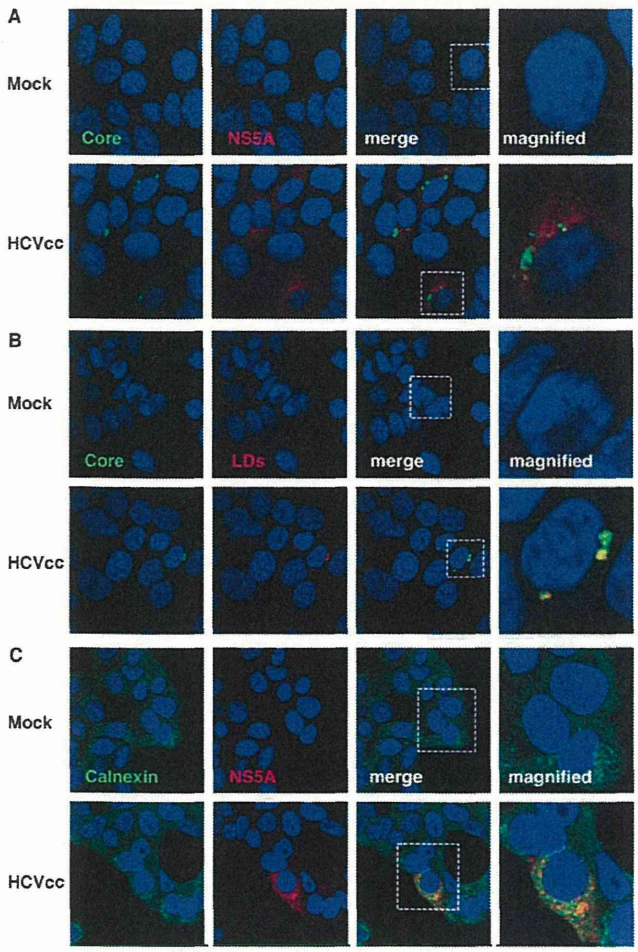

FIG 7 Subcellular localization of core and NS5A proteins in Hec1B/miR-122 cells infected with HCVcc. Hec1B/miR-122 cells infected with or without HCVcc at an MOI of 1 were fixed with 4% PFA at 48 h postinfection and stained with appropriate antibodies to core and NS5A proteins (A), core protein and lipid droplets (B), and NS5A and calnexin (C). The boxes in the merged images were magnified, and the images are displayed on the right.

MTTP, in nonhepatic cells were significantly lower than those in hepatic cells (Fig. 10). Collectively, these results suggest that intracellular functional lipid metabolism, including the biosynthesis of lipid droplets and the production of VLDL, participates in the assembly of HCV.

Establishment of HCV replicon in Hec1B/miR-122 cells. It was previously shown by using RNA replicon cells based on the JFH1 strain that expression of miR-122 enhanced the translation of HCV RNA in HEK293 cells and MEFs (8, 35). We tried to establish HCV replicon cells based on genotype 1b Con1 and genotype 2a JFH1 strains in Hec1B/miR-122 and HEK293 cells stably expressing miR-122 (HEK293/miR-122). To examine the colony formation efficiency of the HCV RNAs of the Con1 and JFH1 strains, SGR RNA was electroporated into the cell lines and selected by G418 for 3 weeks. Expression of miR-122 in Hec1B cells significantly enhanced the colony formation of SGR of the Con1 strain (Fig. 11A), suggesting that the expression of miR-122 in Hec1B cells supports the efficient replication of SGR. HCV replication in 20 replicon clones established by the transfection with SGR RNA of the Con1 strain in Hec1B/miR-122 cells was examined by qRT-PCR and immunoblotting. All clones contained high levels of HCV RNA (3×10^6 to 5×10^7 copies per μg of total RNA) (Fig. 11B), and expression of NS5A was well correlated with the levels of HCV RNA in the clones (Fig. 11C). Two replicon clones (clones 2 and 10) in Hec1B/miR-122 cells exhibiting high levels of RNA replication and NS5A expression further confirmed the high level of expression of NS5A by immunofluorescent microscopy (Fig. 11D). These results suggest that expression of miR-122 facilitates the efficient replication of SGR of at least two HCV genotypes in Hec1B cells.

Our previous reports showed that HCV NS proteins were colocalized with dsRNA and cochaperone molecules, FK506-binding protein 8 (FKBP8), in dot-like structures on the ER membrane of Huh7 replicon cells (59). Colocalization of NS5A with dsRNA or FKBP8 was observed in the dot-like structures in not only Huh7 SGR cells but also Hec1B/miR-122 SGR cells (Fig. 12A), suggesting that the dot-like structure required for efficient viral replication is also generated in Hec1B/miR-122 replicon cells. It has been shown that HCV replication induces the formation of convoluted membranous structures, called membranous webs, in Huh7 cells (13, 45). FM-EM techniques revealed the localization of NS5A on the convoluted structures in Hec1B/miR-122 replicon cells (Fig. 12B). These results suggest that the replication complex required for viral replication was also generated in the Hec1B/miR-122 replicon cells, as was seen in the Huh7 replicon cells.

miR-122 is a crucial determinant of HCVcc propagation. It has been shown that the infectivity of HCVcc in cured cells, established when IFN treatment induces the elimination of the viral genome from the Huh7 replicon cells harboring an HCV RNA, is significantly higher than that in parental Huh7 cells (2, 66). Therefore, we tried to establish Hec1B-based cured cells from the Con1 SGR clones harboring a high copy number of HCV RNA. Treatment with cyclosporine and the protease inhibitor of HCV suppressed NS5A expression in Hec1B/miR-122 SGR clone 2 in a dose-dependent manner (Fig. 13A), whereas no reduction was observed by the IFN treatment due to a lack of an IFN receptor, as shown in Fig. 4C. It was reported that monotherapy by the HCV protease inhibitor induces the emergence of resistant breakthrough viruses (34, 55). Therefore, we treated five Hec1B/miR-122 SGR clones (clones 2, 5, 10, 14, and 16) with 1 μg/ml cyclosporine and 100 nM protease inhibitor for HCV. Viral RNA was determined by qRT-PCR every 5 days posttreatment. Elimination of viral RNA was achieved in four clones (clones 2, 5, 10, and 14) within 20 days posttreatment (Fig. 13B). Replication of HCV RNA in the cured cells infected with HCVcc at an MOI of 0.5 was 2- to 30-fold higher than that in parental cells at 24 h postinfection (Fig. 13C). In addition, replication of HCV RNA in cured clone 2 infected with HCVcc at an MOI of 0.1 was comparable to that in Huh7.5.1 cells until 24 h postinfection (Fig. 13D). Expression of NS5A was significantly increased in cured clone 2 compared to that in the parental Hec1B/miR-122 cells (Fig. 13E and F). It was previously shown that the increased permissiveness of Huh7-derived cured cells, Huh7.5 cells, is attributable to a mutation in the RIG-I gene (58). To examine the innate immune response in the parental and cured Hec1B/miR-122 cells, induction of IFN-stimulated gene 15 (ISG15) was determined upon stimulation with IFN-α or VSV. Although induction of ISG15 was not observed in either parental or cured cells upon stimulation with IFN-α due to a lack of an IFN receptor (11) (Fig. 14A), it was

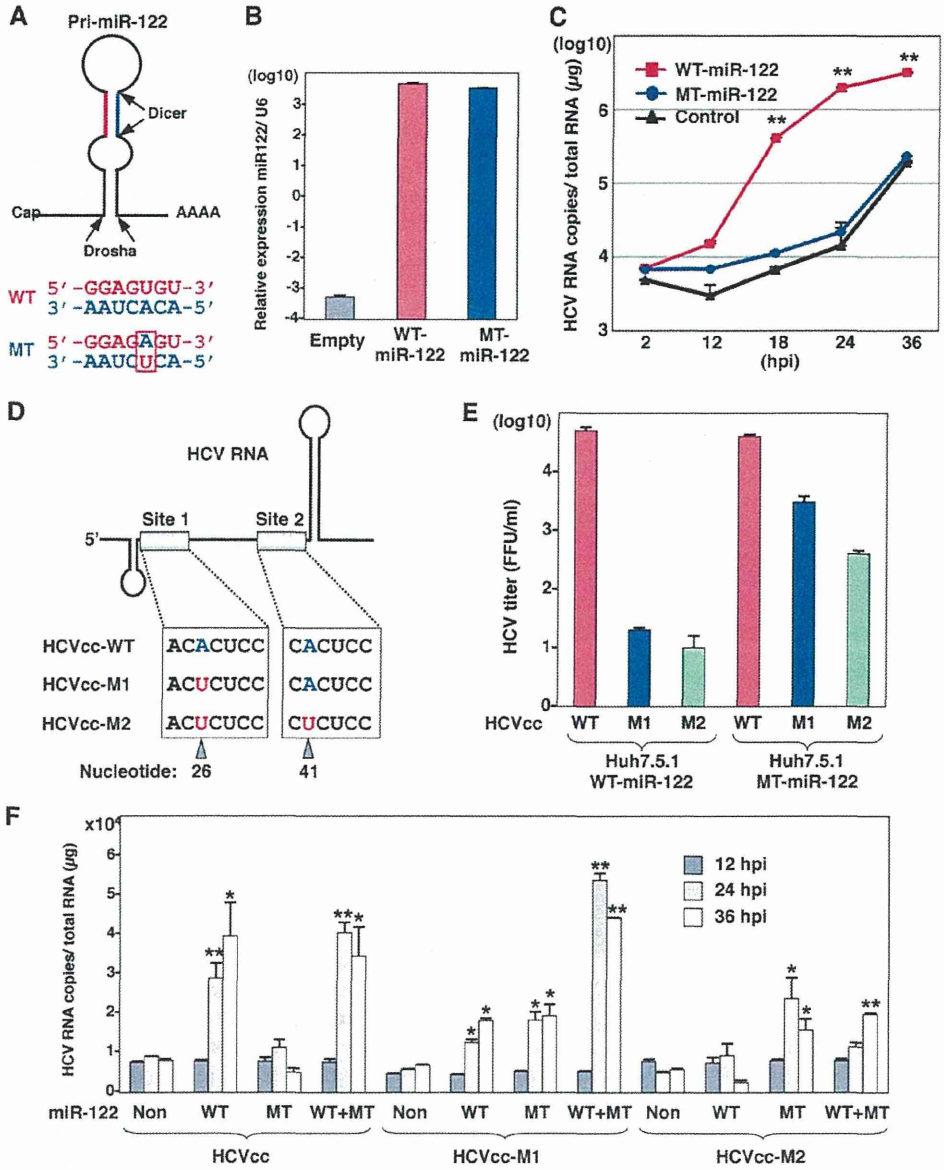

FIG 8 Specific interaction between miR-122 and the 5′ UTR of HCV is required for HCV replication. (A) Structures of pri-miR-122 and the nucleotide sequences of WT and MT miR-122, which has a substitution of uridine to adenosine in the seed domain and an additional complementary substitution of adenosine to uridine for stable expression. (B) WT or MT miR-122 was introduced into Hec1B cells by a lentiviral vector, and miR-122 expression levels were determined by qRT-PCR. (C) HCVcc was inoculated into Hec1B cells expressing either WT or MT miR-122 and control cells at an MOI of 1, and the intracellular HCV RNA levels were determined by qRT-PCR. (D) Diagram of mutant viruses HCVcc-M1 and HCVcc-M2 carrying complementary substitutions in the miR-122-binding site 1 alone and both sites 1 and 2 in the 5′ UTR of HCV, respectively. (E) Viral RNA of HCVcc, HCVcc-M1, or HCVcc-M2 was electroporated into Huh7.5.1 cells expressing either WT or MT miR-122, and infectious titers of the viruses recovered in the culture supernatants at 72 h postinfection of the second passage were determined by a focus-forming assay in cells expressing either WT or MT miR-122. Red, blue, and green bars, infectious titers of HCVcc, HCVcc-M1, and HCV-M2, respectively. (F) HCVcc, HCVcc-M1, or HCV-M2 was inoculated into Hec1B cells expressing either or both WT and MT miR-122 at an MOI of 0.5, and intracellular HCV RNA levels were determined at 12, 24, and 36 h postinfection by qRT-PCR. Asterisks indicate significant differences (*, $P < 0.05$; **, $P < 0.01$) versus the results for control cells.

detected in both cells infected with VSV (Fig. 14B). Therefore, other mechanisms should be involved in the enhancement of permissiveness of Hec1B-derived cured cells. Ehrhardt et al. showed that the expression levels of miR-122 in Huh7-derived cured cells, including Huh7.5, Huh7.5.1, and Huh7-Lunet cells, are significantly higher than those in parental Huh7 cells (14). In addition, our recent study indicated that levels of expression of miR-122 in the cured Huh7 and Hep3B/miR-122 cells were higher than those in parental cells (29). Levels of expression of miR-122 in the Hec1B-based cured cell clones are also higher than those in parental Hec1B/miR-122 cells (Fig. 13G). These results suggest that a high level of miR-122 expression is a crucial determinant of high susceptibility to HCVcc propagation in the cured cells.

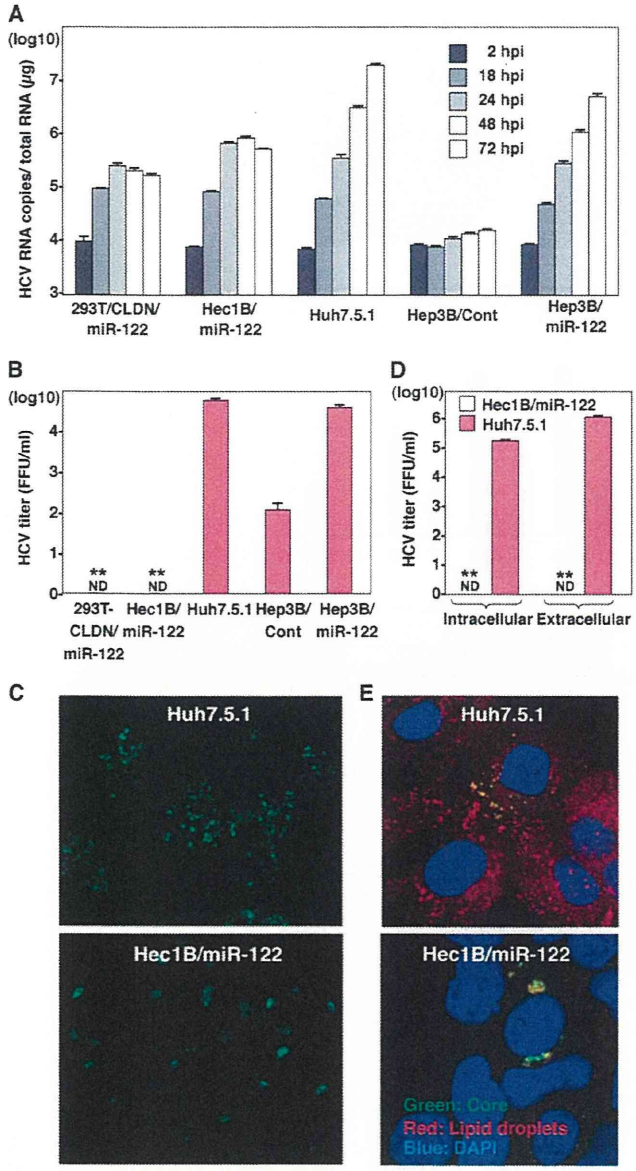

FIG 9 Viral particle formation in hepatic and nonhepatic cells. (A) HCVcc was inoculated into 293T-CLDN/miR-122, Hec1B/miR-122, Hep3B/Cont, and Hep3B/miR-122 cells at an MOI of 1 and into Huh7.5.1 cells at an MOI of 0.1. HCV RNA levels (copies/μg) in cells at 2, 18, 24, 48, and 72 h postinfection were determined by qRT-PCR. (B) HCVcc was inoculated into 293T-CLDN/miR-122, Hec1B/miR-122, Hep3B/Cont, and Hep3B/miR-122 cells at an MOI of 1 or into Huh7.5.1 cells at an MOI of 0.1, and infectious titers in the culture supernatants were determined at 72 h postinfection by a focus-forming assay in Huh7.5.1 cells. ND, not determined. (C) Huh7.5.1 and Hec1B/miR-122 cells were infected with HCVcc at MOIs of 0.1 and 1, respectively, incubated with 1% methylcellulose in DMEM containing 10% FCS for 72 h, fixed with 4% PFA, and subjected to immunofluorescence analysis using anti-NS5A antibody. (D) Hec1B/miR-122 and Huh7.5.1 cells were infected with HCVcc at MOIs of 1 and 0.1, respectively, and infectious titers in cells and supernatants were determined by a focus-forming assay at 72 h postinfection. (E) Huh7.5.1 and Hec1B/miR-122 cells were infected with HCVcc at MOIs of 0.1 and 1, respectively, fixed with 4% PFA, and subjected to immunofluorescence assay using anti-core protein antibody (green). Lipid droplets and cell nuclei were stained with BODIPY (red) and DAPI (blue), respectively. Asterisks indicate significant differences (**, $P < 0.01$) versus the results for Huh7.5.1 cells.

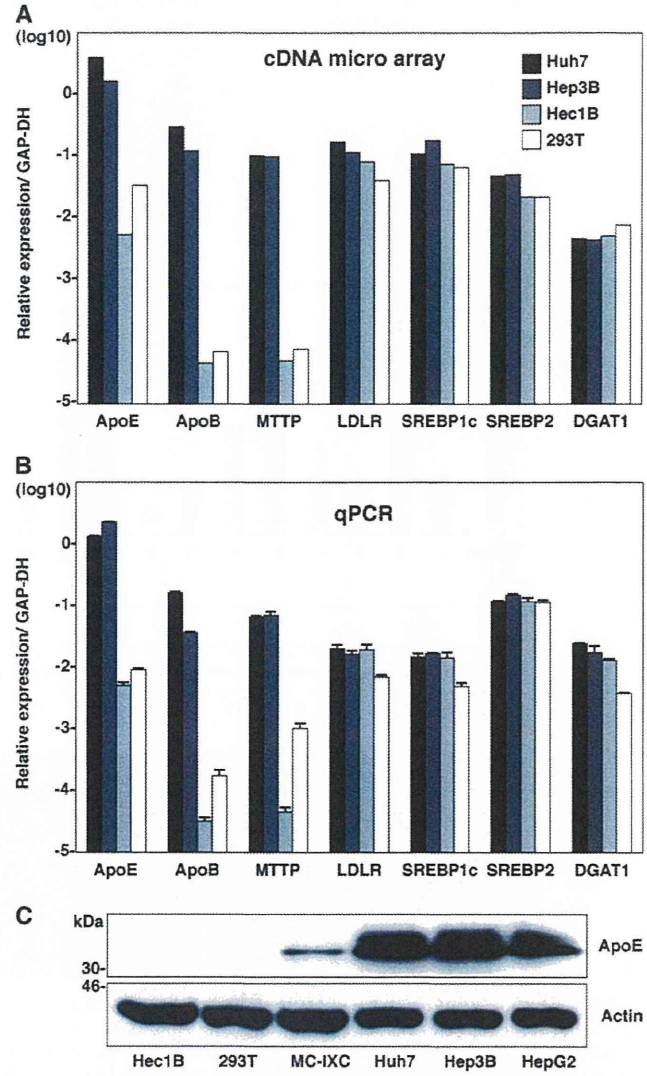

FIG 10 Expression of lipid metabolism-associated proteins in hepatic and nonhepatic cells. (A) Expression levels of ApoE, ApoB, MTTP, LDLR, SREBP1c, SREBP2, and DGAT1 were compared among hepatic (Huh7 and Hep3B) and nonhepatic (Hec1B and 293T) cells using cDNA microarray analyses. (B) Total RNA was extracted from the cells, and expression levels of ApoE, ApoB, MTTP, LDLR, SREBP1c, SREBP2, and DGAT1 gene were determined by qPCR. (C) Nonhepatic (Hec1B, 293T, and MC-IXC) and hepatic (Huh7, Hep3B, and HepG2) cells were subjected to immunoblotting using anti-ApoE antibody.

DISCUSSION

Although multiple epidemiological studies have revealed that HCV infection induces several EHMs, they have not well elucidated the molecular mechanisms of the EHMs induced by HCV infection (19). Indeed, HCVcc does not infect PBMCs (38). It has been shown that two neuroepithelioma cell lines permit HCVcc infection at low levels (17) and lymphotropic strains or quasispecies of HCV exist in infected individuals (12, 52). Furthermore, many molecules involved in the entry, replication, and assembly of HCVcc have been identified, although these molecules are not sufficient to explain the liver tropism of HCV. Recently, a liver-specific microRNA, miR-122, was shown to facilitate the efficient

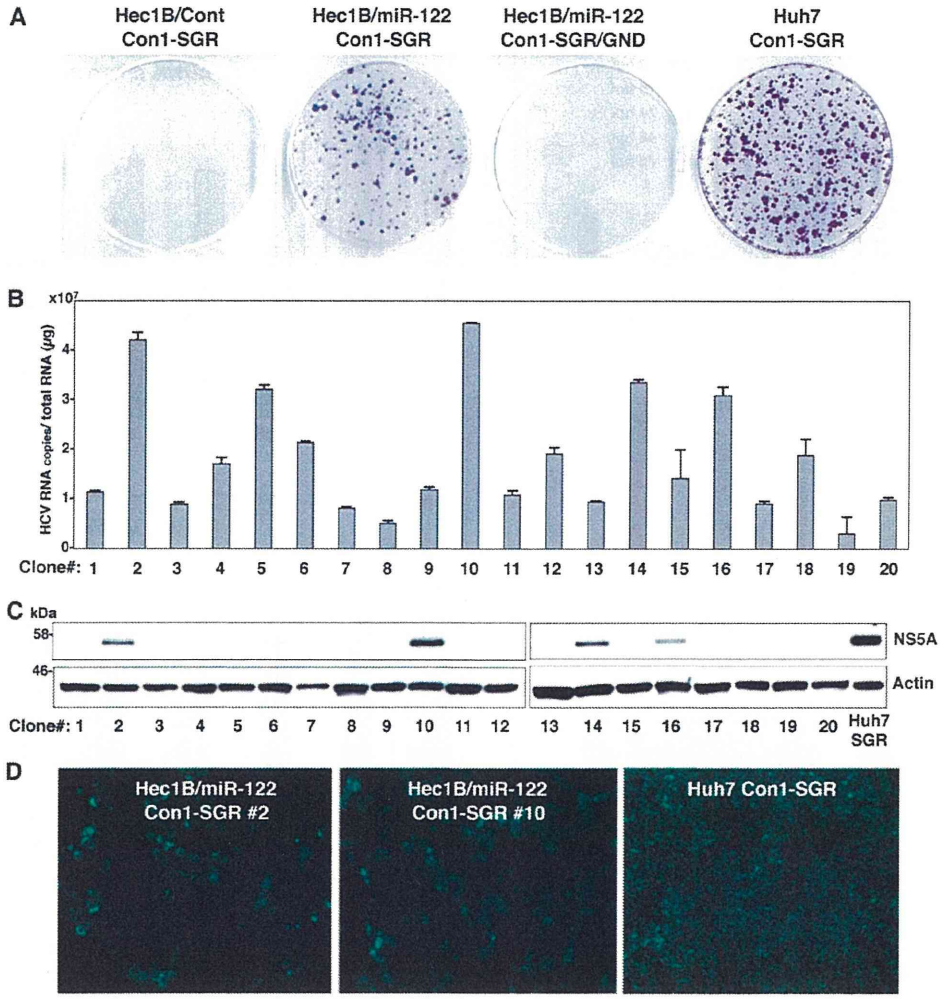

FIG 11 Establishment of Con1-based HCV replicon cells by using Hec1B cells. (A) WT or replication-defective SGR RNA of the HCV Con1 strain was electroporated into Hec1B/Cont, Hec1B/miR-122, and Huh7 cells, and the medium was replaced with DMEM containing 10% FCS and 1 mg/ml G418 at 24 h postelectroporation. Colonies were stained with crystal violet after 3 weeks of selection with G418. (B) Total RNAs of 20 selected clones were extracted and subjected to qRT-PCR. (C) The 20 SGR clones were subjected to immunoblotting using anti-NS5A antibody. Huh7-derived Con1-based SGR cells were used as a positive control. (D) NS5A proteins in SGR clones 2 and 10 were stained with appropriate antibodies and examined by fluorescence microscopy. Huh7-derived Con1-based SGR and parental Hec1B cells were used for positive and negative controls, respectively.

replication of HCV through a specific interaction with the complementary sequences in the 5′ UTR of HCV RNA (21, 25, 27, 36). In addition, exogenous expression of miR-122 facilitates the replication of SGR of the JFH1 strain in HEK293 cells (8) and the propagation of HCVcc in HepG2 and Hep3B nonpermissive hepatoma cells (29, 43), suggesting that the expression of miR-122 is required for the efficient replication of HCV. However, HCV replicon cells have also been established in HeLa and LI90 cells derived from stellate cells in which no exogenous miR-122 is expressed (30, 63). In this study, naïve Hec1B cells also exhibited a low level of replication upon infection with HCVcc (Fig. 4B), and this replication was resistant to treatment with an inhibitor of miR-122, LNA-miR-122 (Fig. 6C), suggesting that miR-122 expression is not a necessary condition but is required for the enhancement of HCV replication and that HCV is capable of replicating in nonhepatic cells in an miR-122-independent manner. Although the application of miR-122-specific LNAs to chronic hepatitis C patients is now in progress (32), further studies are needed to clarify the mechanisms underlying the miR-122-independent replication of HCV in more detail.

Although the importance of receptor-mediated entry in the cell tropism of HCV has been evaluated (16, 65), cDNA microarray databases, including the NextBio search engine, revealed that HCV receptor candidates, including hCD81, SR-BI, CLDN1, and OCLN, are highly expressed in many nonhepatic tissues. In addition, our current data and previous reports demonstrated that many nonhepatic cells permitted the entry of the pseudotype viruses bearing HCV envelope proteins, suggesting that other host factors must be involved in the cell tropism of HCV to human hepatocytes (4, 17, 54, 61). The data in this study suggest that miR-122 expression and functional lipid metabolism play crucial roles in the determination of an efficient propagation of HCV *in vitro*. On the other hand, previous studies showed the compartmentalization of genetic variation in HCV between hepatic and

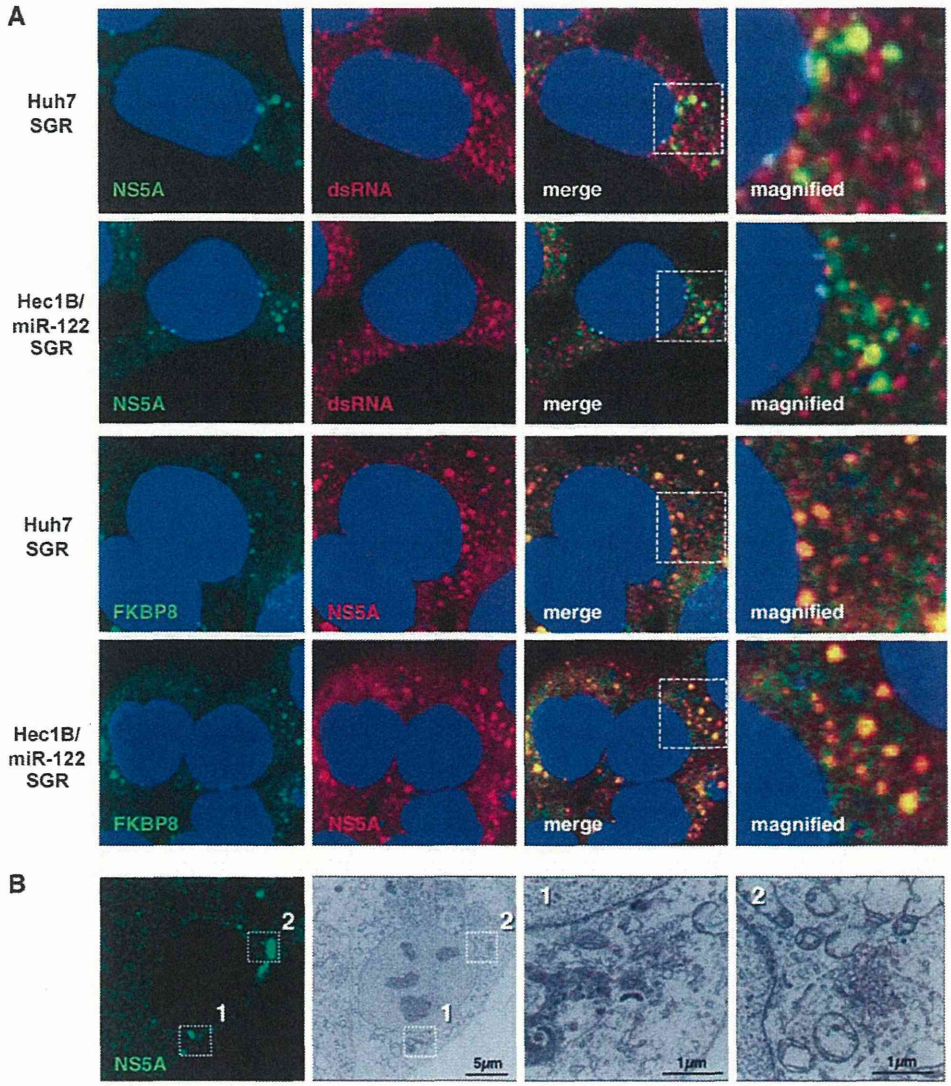

FIG 12 Replication complex in Hec1B/miR-122 replicon cells. (A) Huh7 and Hec1B/miR-122 cells harboring the Con1 SGR RNA were fixed, permeabilized, and stained with antibodies to NS5A and dsRNA or FKBP8. The boxed areas in the merged images were magnified, and the images are displayed on the right. (B) Hec1B-derived Con1 SGR cells were stained with anti-NS5A antibody. Identical fields were observed under EM by using the correlative FM-EM technique. The boxed areas are magnified, and the images displayed on the right.

nonhepatic tissues, suggesting that HCV is capable of replicating in nonhepatic tissues expressing either miR-122, ApoE, ApoB, or MTTP (52). Collectively, these results suggest that entry receptors, miR-122, and functional lipid metabolism are mainly involved in the regulation of internalization, RNA replication, and assembly of HCV, respectively, and are important factors in determining the cell tropism of HCV to hepatocytes. On the other hand, it might be feasible to speculate that EHMs observed in chronic hepatitis C patients are caused by an incomplete miR-122-independent propagation of HCV in nonhepatic cells.

In spite of the efficient replication of HCV in Hec1B and 293T-CLDN cells expressing miR-122, no infectious particle was detected, in contrast to the case with hepatic cells (Fig. 9), suggesting the involvement of liver-specific host factors and/or machineries in the assembly of infectious particles. In general, the liver plays a major role in lipid metabolism, such as in fatty acid and lipoprotein syntheses (9), and many reports have indicated the involvement of lipid metabolism, especially triglyceride metabolism, in the assembly and budding of HCV particles. Lipid droplets, MTTP, ApoB, and ApoE have been shown to participate in the assembly and secretion of infectious particles of HCVcc in Huh7 cells (20, 23, 26, 40). In the current analyses, there were fewer lipid droplets and the expression levels of ApoE, ApoB, and MTTP were lower in nonhepatic cells than in hepatic cells. Although minus-strand HCV RNA and viral proteins were detected in nonhepatic cells (33, 60), it was shown that the recurrence of HCV after liver transplantation for patients with HCV-induced liver diseases was mainly caused by HCV variants generated in the liver but not in nonhepatic tissues (50). These results support the notion that replication of HCV RNA in nonhepatic cells is unlikely to be a reservoir for persistent infection, due to the lack of infectious particle formation.

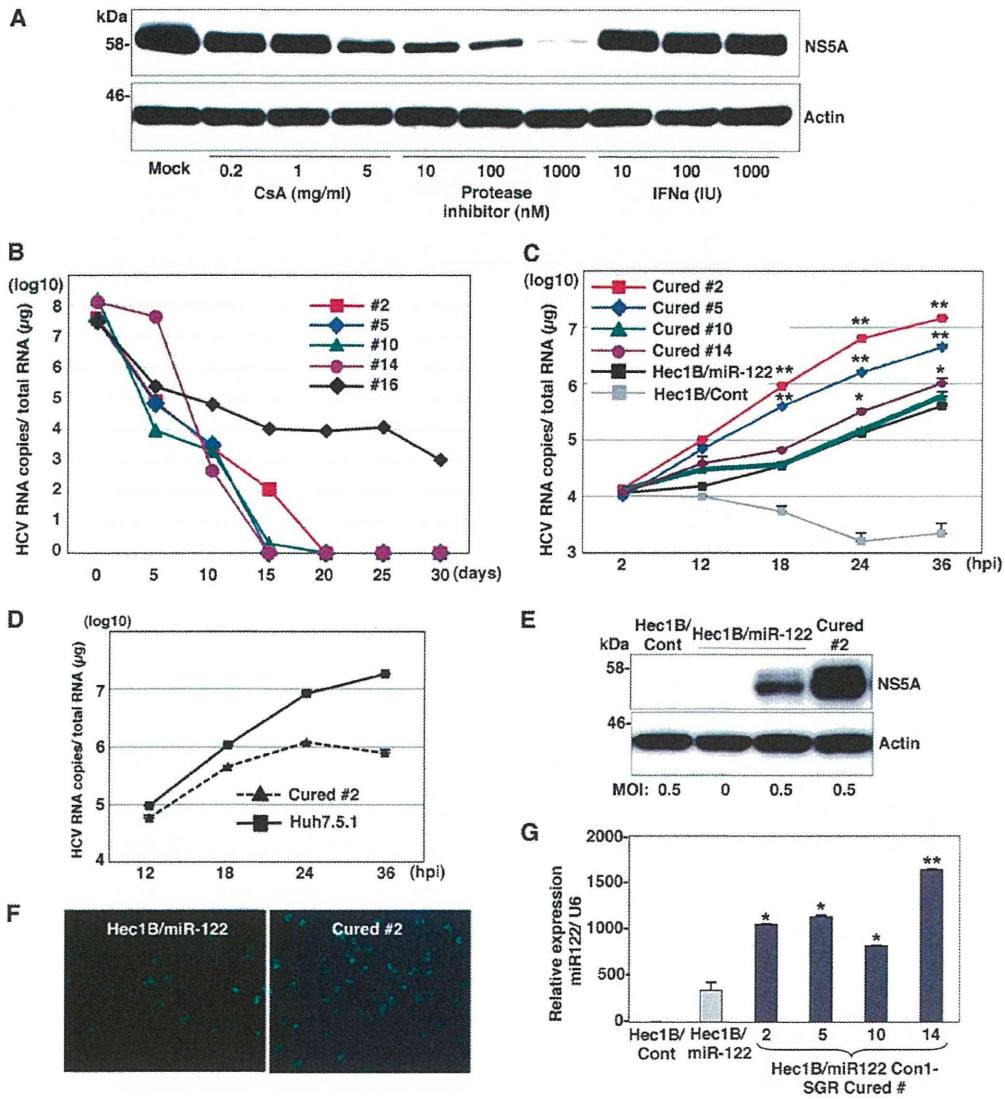

FIG 13 miR-122 is a crucial determinant for the efficient replication of HCVcc and replicon RNA. (A) Hec1B Con1 replicon clone 2 was treated with stepwise concentrations of cyclosporine (CsA; 0.2, 1, and 5 μg/ml), NS3/4A protease inhibitor (10, 100, and 1,000 nM), or IFN-α (10, 100, and 1,000 IU) and subjected to immunoblotting using anti-NS5A antibody at 48 h posttreatment. (B) Five Con1-based SGR clones were treated with the combination of 1 μg/ml cyclosporine and 100 nM HCV protease inhibitor to eliminate the HCV genome. Intracellular HCV RNA levels at 5, 10, 15, 20, 25, and 30 days posttreatment were determined by qRT-PCR analysis. (C) HCVcc was inoculated with Hec1B/Cont, parental Hec1B/miR-122, and Hec1B-based cured cells (clones 2, 5, 10, and 14) at an MOI of 1. Intracellular HCV RNA levels at 2, 12, 18, 24, and 36 h postinfection were determined by qRT-PCR analysis. (D) HCVcc was inoculated into Huh7.5.1 and Hec1B/miR-122 cured clone 2 cells at an MOI of 1. Intracellular HCV RNA levels were determined by qRT-PCR at 12, 24, 36, and 48 h postinfection. (E and F) Hec1B/Cont, parental Hec1B/miR-122, and cured cells of clone 2 were infected with HCVcc at an MOI of 0.5. After 48 h, the cells were subjected to immunoblotting and immunofluorescence analyses using appropriate antibodies. (G) Total miRNAs were extracted from Hec1B/Cont (white), parental Hec1B-miR-122 cells (gray), and four cured cell clones (black). miR-122 expression levels in these cells were determined by qRT-PCR analysis. Asterisks indicate significant differences (*, $P < 0.05$; **, $P < 0.01$) versus the results for parental Hec1B/miR-122 cells.

The endogenous expression of miR-122 is hardly detected in Hec1B cells, in contrast to the abundant expression of miR-122 in Huh7 cells. Therefore, more accurate analyses of the biological significance of the interaction between miR-122 and the 5′ UTR on the replication of HCVcc in Hec1B could be possible by introducing mutations not only into viruses but also into miR-122. Replication of HCVcc and a mutant virus bearing two mutations in the 5′ UTR (HCVcc-M2) was observed in Hec1B cells expressing WT and MT miR-122, respectively, although the level of replication was lower in cells infected with HCVcc-M2 than in those infected with HCVcc, probably due to the mutations in the 5′ UTR (Fig. 8F). In contrast, a mutant virus (HCVcc-M1) bearing a mutation in site 1 alone exhibited efficient replication in Hec1B cells expressing both WT and MT miR-122 comparable to the replication level of the wild-type virus in cells expressing WT miR-122. Furthermore, the replication level of HCVcc-M1 was low in Hec1B cells expressing either WT or MT miR-122, suggesting that interaction between miR-122 and either of the seed sequence-binding sites in the 5′ UTR has an equal ability to enhance the replication of the HCV genome. However, it was shown that the

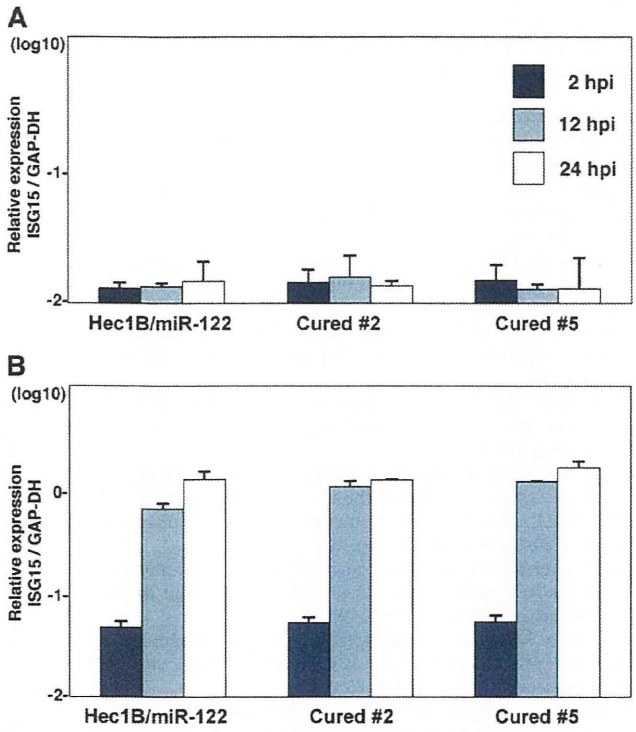

FIG 14 Innate immune responses in parental and cured Hec1B/miR-122 cells. Parental and cured Hec1B/miR-122 cells were stimulated with 100 U of IFN-α (A) or VSV (B). The expression levels of IFN-stimulated gene 15 (ISG15) were determined by qRT-PCR at 2, 12, and 24 h posttreatment.

ability of miR-122 to promote the growth of a laboratory strain of HCV (HJ3-5) is dependent upon its direct interaction with both seed sequence-binding sites in the 5′ UTR and that the binding to site 1 is more important for efficient replication than the binding to site 2 (25). Recently, it was shown that the binding of miR-122 to the 5′ UTR of the HCV genome masks the 5′-terminal sequences of the viral genome through the 3′ overhanging nucleotides of miR-122 (36). It is necessary to evaluate the importance of this enhancement mechanism on mutant HCVcc infection in Hec1B cells.

In summary, we demonstrated that HCV is capable of replicating at a low level in nonhepatic cells and that exogenous expression of miR-122 facilitates efficient viral replication but not the production of infectious particles, probably due to the lack of hepatocytic lipid metabolism in nonhepatic cell lines. These results suggest that miR-122 plays a crucial role in determination of the cell tropism of HCV and the possible involvement of incomplete propagation of HCV in the development of EHM in hepatitis C patients.

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CD44 Participates in IP-10 Induction in Cells in Which Hepatitis C Virus RNA Is Replicating, through an Interaction with Toll-Like Receptor 2 and Hyaluronan

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The mechanisms of induction of liver injury during chronic infection with hepatitis C virus (HCV) are not well understood. Gamma interferon (IFN-γ)-inducible protein 10 (IP-10), a member of the CXC chemokine family, is expressed in the liver of chronic hepatitis C (CHC) patients and selectively recruits activated T cells to the sites of inflammation. Recently, it was shown that a low plasma concentration of IP-10 in CHC patients was closely associated with the outcome of antiviral therapy. In this study, we examined the role of the Toll-like receptor (TLR) pathway on IP-10 production in cells replicating HCV. Among the CXC chemokines, the expression of IP-10 was specifically increased in cells replicating HCV upon stimulation with conventional TLR2 ligands. The enhancement of IP-10 production upon stimulation with TLR2 ligands in cells replicating HCV induced CD44 expression. CD44 is a broadly distributed type I transmembrane glycoprotein and a receptor for the glycosaminoglycan hyaluronan (HA). In CHC patients, the expression of HA in serum has been shown to increase in accord with the progression of liver fibrosis, and HA also works as a ligand for TLR2. In the present study, IP-10 production upon HA stimulation was dependent on the expression of TLR2 and CD44, and a direct association between TLR2 and CD44 was observed. These results suggest that endogenous expression of HA in hepatocytes in CHC patients participates in IP-10 production through an engagement of TLR2 and CD44.

Hepatitis C virus (HCV) infects 170 million people worldwide and frequently leads to the development of cirrhosis and hepatocellular carcinoma (32). The current combination therapy of pegylated interferon (IFN) and ribavirin is effective in fewer than 50% of patients infected with HCV of genotype 1. Histological analyses of the liver biopsy specimens of chronic hepatitis C (CHC) patients have revealed the infiltration of mononuclear cells, including T and B lymphocytes, natural killer (NK) and NKT cells, and virus-specific cytotoxic T lymphocytes (2, 26, 42, 47). Long-term infection by HCV is associated with progressive infiltration of the liver parenchyma by the mononuclear cells, fibrosis, cirrhosis, and, finally, the development of hepatocellular carcinoma. Although the factors that regulate the recruitment of mononuclear cells and the other components of the inflammatory response to the HCV-infected liver cells are not well characterized, it has been hypothesized that chemokines and other inflammatory cytokines play fundamental roles in the immune cell recruitment.

Chemokines, small chemotactic cytokines (approximately 8 to 10 kDa) that act to guide leukocytes to sites of inflammation, are important determinants of the development of intrahepatic inflammation in chronic HCV infection (16). Although chemokines play crucial roles in viral elimination, persistent expression of chemokines may induce tissue damage and inflammation in chronic infection. CXCR3 is a receptor for the CXC chemokines, including IP-10 (also known as CXCL10), MIG (also known as CXCL9), and I-TAC (also known as CXCL11). Recent studies have shown that the CXCR3 ligands are elevated in the livers and sera of CHC patients (12–14, 17, 33, 36, 40, 49), and IP-10 was shown to correlate with treatment response. In addition, several studies suggested a significant association between the expression of the CXC chemokines and the development of progressive liver injury in CHC patients (23, 49). In CHC patients, these chemokines are expressed in hepatocytes, hepatic stellate cells, and sinusoidal endothelial cells (12, 14, 33, 42, 49), and the majority of intrahepatic mononuclear cells express CXCR3, suggesting that the CXC chemokine network plays a pivotal role in the migration of mononuclear cells to the liver and in the subsequent intrahepatic inflammation.

Among chemokines, IP-10 plays a central role in liver inflammation, and it is expressed in the liver of hepatitis C patients (12, 33, 42). Several independent studies indicate that elevated plasma levels of IP-10 predict the failure of combination therapy (3, 5, 40). In addition, a recent study suggests that IP-10 in the plasma of many hepatitis C patients is cleaved by DPP4 (also known as CD26) and that the truncated IP-10 works as an IP-10 receptor antagonist (4). In contrast to these clinical observations, little is known about the expression of the CXC chemokines in cells replicating HCV.

Production of the inflammatory chemokines upon viral infec-

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