Analysis of Interferon Signaling by Infectious Hepatitis C Virus Clones with Substitutions of Core Amino Acids 70 and 91†§

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Substitution of amino acids 70 and 91 in the hepatitis C virus (HCV) core region is a significant predictor of poor responses to peginterferon-plus-ribavirin therapy, while their molecular mechanisms remain unclear. Here we investigated these differences in the response to alpha interferon (IFN) by using HCV cell culture with R70Q, R70H, and L91M substitutions. IFN treatment of cells transfected or infected with the wild type or the mutant HCV clones showed that the R70Q, R70H, and L91M core mutants were significantly more resistant than the wild type. Among HCV-transfected cells, intracellular HCV RNA levels were significantly higher for the core mutants than for the wild type, while HCV RNA in culture supernatant was significantly lower for these mutants than for the wild type. IFN-induced phosphorylation of STAT1 and STAT2 and expression of the interferon-inducible genes were significantly lower for the core mutants than for the wild type, suggesting cellular unresponsiveness to IFN. The expression level of an interferon signal attenuator, SOCS3, was significantly higher for the R70Q, R70H, and L91M mutants than for the wild type. Interleukin 6 (IL-6), which upregulates SOCS3, was significantly higher for the R70Q, R70H, and L91M mutants than for the wild type. Interferon-inducible genes were significantly lower for the core mutants than for the wild type, suggesting interferon resistance, possibly through IL-6-induced suppression of interferon signaling. Expression levels of endoplasmic reticulum (ER) stress proteins were significantly higher in cells transfected with a core mutant than in those transfected with the wild type. In conclusion, HCV R70 and L91 core mutants were resistant to interferon in vitro, and the resistance may be induced by IL-6-induced upregulation of SOCS3. Those mechanisms may explain clinical interferon resistance of HCV core mutants.

Hepatitis C virus (HCV) is one of the most important pathogens causing liver-related morbidity and mortality. Approximately 3% of the worldwide population is infected with HCV, which represents 170 million people, and 3 million to 4 million individuals are newly infected each year (33, 47, 62). There is no therapeutic or prophylactic vaccine available for HCV. Antiviral treatment has been shown to improve liver histology and decrease the incidence of hepatocellular carcinoma in chronic hepatitis C (CHC) (17, 64). Current therapies for CHC consist of treatment with pegylated interferon (peg-IFN), which acts both as an antiviral and as an immunoregulatory cytokine, and ribavirin (RBV), an antiviral prodrug that interferes with RNA metabolism (16, 31). However, less than 50% of patients infected with HCV genotype 1 treated in this way achieve a sustained virological response (SVR) or a cure of the infection (14, 16). Given this situation, gaining a detailed understanding of the molecular mechanisms of interferon (IFN) resistance has been a high priority in academia and industry.

The response to peg-IFN-plus-RBV treatment is affected by several viral and host factors, including age, gender (22, 23), grade of liver fibrosis (21, 42), HCV genotype, and serum viral load (14, 59). Several viral genetic factors influence treatment outcomes, including mutations in NS5A-interferon sensitivity determining region (ISDR) (13, 38) and the core region (4, 6). Akuta et al. reported that HCV-core amino acid substitutions at positions 70 and 91 are significantly correlated with poor responses to peg-IFN-plus-RBV therapy (6) and with increased hepatocarcinogenesis (2, 3). Furthermore, it was reported recently that the core amino acid 70 and amino acid 91 substitutions are associated with a poor response to peg-IFN, RBV, and telaprevir combination therapy, respectively (1). However, the underlying molecular mechanisms of such distinct biological properties of the core 70/91 mutations are poorly understood.

In this study, we have analyzed virus infection and replication kinetics and response to interferon treatment using the HCV-JFH1 cell culture system (HCVcc) (60, 65). We constructed HCVcc expressing virus with substitutions of core amino acid 70 and amino acid 91 (R70Q, R70H, and L91M). The core mutant HCV clones were compared in terms of intracellular replication, infectious virus production, and sensitivity to alpha interferon (IFN-α). Here we have shown that the differences in sensitivity to IFN are attributable to upregulated overexpression of the cellular interferon signal attenuator SOCS3 and that this upregulation is caused by overexpression of interleukin-6 (IL-6).
MATERIALS AND METHODS

Reagents. Recombinant human IFN-α2b was from Schering-Plough (Ken- nいち), NJ). Beta-mercaptoethanol was from Wako (Osaka, Japan). Antibodies used were SOCS3 and SOCS1, which were from Cell Signaling (Beverly, MA), HCV core (Abcam, Cambridge, MA), N5SA (BioDesign, Saco, ME), GRP78, GADD153/CHOP (Santa Cruz Biotechnology, Santa Cruz, CA), disulffide isomerase (PDI) (Stressgen Biotechnologies, Victoria, British Columbia, Can- ada), and beta-actin antibody (Sigma). Secondary antibodies were peroxidase- labeled anti-mouse, anti-rabbit antibody (GE Healthcare, Connecticut), donkey anti-goat IgG-horseradish peroxidase (HRP) antibody (Santa Cruz Biotechnol- ogy, Santa Cruz, CA), and Alexa 405-labeled goat anti-mouse and Alexa 568- labeled donkey anti-rabbit IgG antibodies (Invitrogen, Carlsbad, CA).

Cells and cell culture. Huh7 cells were maintained in Dulbecco’s modified minimal essential medium (DMEM) (Sigma Chemical Co, St. Louis, MO) sup- plemented with 2 mmol/l glucose and 10% fetal bovine serum at 37°C under 5.0% CO2.

Sequence analyses. Nucleotide sequences were read from both strands using BigDye Terminator cycle sequencing ready reaction kits (Applied Biosystems, Foster City, CA) and an automated DNA sequencer (ABI Prism 310 genetic analyzer; Applied Biosystems).

Establishment of mutant HCV clones. In order to introduce various mutations into the core region of JFH1, plasmid pJFH1full was digested with EcoRI and BsiWI, and then the DNA fragment encompassing nucleotides 1 to 456 was subcloned into the pGEM-T Easy vector (Promega, Madison, WI). The follow- ing mutations were introduced into the DNA fragment in the subcloning vector by site-directed mutagenesis (Quick-Change II site-directed mutagenesis kit; Stratagene, La Jolla, CA). In order to construct JFH1 core mutants, the HCV RNA transfection, Huh7 cells were washed twice in phosphate-buffered saline (PBS), and 5 x 104 cells were suspended in Opti-MEM I (Invitrogen, Carlsbad, CA) containing 10 μg of HCV RNA, transferred into a 4-mm electroporation cuvette, and finally subjected to an electric pulse (1,050 V/ cm). After 48 h, the levels of HCV replication and viral protein expres- sion were detected by real-time PCR and Western blotting.

In vitro RNA synthesis and transfection. Full-length HCV expression plasmids were as follows: pJFH1full, which encodes the full-length HCV-JFH1 sequence (60), and pJFH1full-C1C, which encodes the full-length HCV-JFH1 sequence with the C1 and C2 deletions (48). rP70Q, pR70Q, pL91M, and pL778K, were linearized at their 3’ ends and used as templates for HCV RNA synthesis using the RibopMax large-scale RNA production system (Promega, Madison, WI). After DNase I (RNase-free DNAse; Promega) treatment, the transcribed HCV RNA was purified using Isogen (Nippon Gene, Toyko, Japan). For the RNA transfection, Huh7 cells were transfected using the RiboMax Large Scale RNA Production System (Promega) and incubated under normal culture conditions in a 10-cm-diameter cell culture dish. Forty-eight hours after transfection, the levels of HCV replication and viral protein expres- sion were detected by real-time PCR and Western blotting.

HCVcc infection analyses. Huh7 cells were plated on 12-well plates at a density of 1 x 105 cells per well. Supernatants from HCV RNA-transfected cells were inoculated onto each well at a titer of 8 x 104 copies/well (quantified by real-time reverse transcriptase PCR [RT-PCR]). Forty-eight hours after infection, various amounts of interferon were added, and the cells were harvested after 72 h of the interferon treatment (48).

RNA extraction, cDNA synthesis, and real-time RT-PCR analysis. For the detection of HCV RNA in culture supernatant, the supernatant was passed through a 0.45-μm filter (Millipore, Bedford, MA) and stored at −80°C until use. Protocols and primers for the real-time RT-PCR analysis of HCV RNA have been described previously (48). For the detection of endoge- nous mRNAs, total cellular RNA was isolated using an RNeasy Mini kit (Qiagen, Valencia, CA). Two micrograms of total cellular RNA was used to generate cDNA from each sample using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA). Expression of mRNAs was quantified using the TaqMan universal PCR master mix (Applied Biosystems, Foster City, CA) and the ABI 7900 real-time PCR system (Applied Biosystems, Foster City, CA).

Luciferase assays. Luciferase activities were measured using a luminometer (Lumat LB9501; Promega) using the Dual-Luciferase reporter assay system (Promega). Assays were performed in triplicate.

Western blot analysis. Western blotting was carried out as described previ- ously (24, 53, 63). Briefly, 10 μg of total cell lysate was separated using NaPAGE 4%–12% Bis-Tris gels (Invitrogen) and blotted onto a polyvinylidene fluoride (PVDF) Western blotting membrane (Roche). The membrane was incubated with the primary antibodies followed by a peroxidase-labeled anti-IgG antibody and visualized by chemiluminescence using the ECL Western blotting analysis system (Amersham Biosciences, Buckinghamshire, United Kingdom).

RESULTS

HCV core 70/91 mutants show resistance to IFN treatment. First, we investigated sensitivity to IFN treatment of the HCV core mutant R70Q, R70H, and L91M virus clones and compared them to the wild type. The wild type and core mutants were transfected into Huh7 cells, which were cultured in the presence of various concentrations of IFN-α for 48 h. RNA was extracted from the cells and culture supernatant, and the level of HCV RNA was quantified by real-time RT-PCR. Al- though the levels of supernatant HCV RNA did not differ between the wild type and core mutants (Fig. 1A), the levels of cellular HCV RNA showed that all three core mutants were significantly resistant to IFN compared to the wild type, with EC50 of 5.0 IU/ml, 48 IU/ml, 32 IU/ml, and 47 IU/ml for the R70Q, R70H, L91M, and mutants and the wild type, respec- tively (Fig. 1B). To exclude the possible effects on interferon signaling by the input HCV RNA, we performed interferon sensitivity analyses by HCVcc infection. As shown in Fig. 1C, the interferon sensitivities of HCV core mutants and the wild type were consistent with the results of HCV RNA transfection. Similarly, according to Western blotting, the core mutants were more resistant to IFN treatment than the wild type (Fig. 1D).

Core mutants show decreased secretion of viral particles. To determine the mechanisms underlying the resistance to interferon, we compared baseline virus expression levels in cells and culture supernatants. The three core mutants, carry- ing R70Q, R70H, and L91M, expressed significantly higher levels of intracellular HCV RNA than the wild type, as well as the 7780K clone. (Fig. 2A). 7780K was a negative-control clone that lacked virus particle secretion (37). On the contrary, these core mutants released significantly smaller amounts of HCV RNA than the culture supernatant than the wild type, as well as the negative-control 7780K clone. (Fig. 2B). Consistent with the HCV RNA data, Western blotting showed that cellular HCV core protein levels were higher for the core amino acid 70/91 mutants than the wild type (Fig. 2C). These results sug- gested that the core 70/91 mutant clones were partially defec- tive in the secretion of infectious virus particles.

Subcellular localization of wild-type and mutant core pro- teins and lipid droplets. It has been reported that HCV core protein localizes on the cellular LD membrane and may mediate encapsidation of viral genomic RNA and subsequent virus assembly (35, 36). Therefore, we visualized the subcellular localization of wild-type and mutant core proteins in rela-
FIG. 1. Comparison of interferon sensitivity between HCV wild type and core mutant clones. The wild type and core mutants were transfected into Huh7 cells and cultured in the presence of IFN-α2b at concentrations ranging from 0 to 100 U/ml. (A) The culture supernatant of HCV-transfected Huh7 cells was collected 72 h after transfection, and the levels of HCV core antigen in the culture supernatant were measured. The values are displayed as percentages of those for the IFN-untreated control. The experiments were repeated three times, and representative results are shown. (B) Expression of intracellular HCV RNA. Cellular RNA was harvested at 72 h posttransfection. HCV RNA was quantified by real-time RT-PCR. The values are displayed as percentages of those for the IFN-untreated control. (C) Expression of intracellular HCV RNA. Cellular RNA was harvested at 72 h postinfection. HCV RNA was quantified by real-time RT-PCR. The values are displayed as percentages of those for the IFN-untreated control. In panels A through C, asterisks indicate P values of less than 0.05, compared to results for the interferon-negative control. (D) Western blotting was performed to assess intracellular suppression of HCV core protein. Ten micrograms of harvested cell lysates were subjected to Western blotting using anti-HCV core antibodies. Densitometry of core protein was performed, and results are shown as percentages of the results for an IFN-negative sample.
tion to that of LDs and the ER by indirect immunofluorescence and confocal microscopy. Consistent with previous reports, core proteins were colocalized with LDs but not with an ER-located protein, PDI, in the HCV-transfected cells (see the figure in the supplemental material). There were no obvious differences in colocalization of core and LDs or core and ER between the wild type and mutant core proteins.

**Induction of interferon-stimulated genes following treatment of HCV-transfected cells with interferon.** To investigate the mechanism of the relative IFN resistance of the core 70/91 mutants, as demonstrated in Fig. 1, we analyzed the cellular IFN signaling pathway. First, we assessed the expression and IFN-mediated induction of the mRNA transcripts of the IFN-stimulated genes (ISGs), encoding P56, double-stranded RNA-dependent protein kinase R (PKR), and 2-5'-oligo-adenylate synthetase (25AS), which mediate direct antiviral effects on HCV expression (24, 25). Cellular expression of PKR, P56, and 25AS was substantially increased in HCV-transfected cells, as well as naive cells, following IFN treatment. However, the levels of induction were significantly lower in the three HCV core mutant-transfected cells than in wild-type-transfected cells (Fig. 3A, B, and C). We next detected IFN-induced phosphorylation of STAT1 and STAT2 in the mutant and wild-type HCV-expressing cells. Our previous experiments showed that the levels of phosphorylated STAT1 and STAT2 (pSTAT1 and pSTAT2, respectively) increased within minutes of the addition of IFN and decreased subsequently at 8 h (25). Therefore, we detected pSTAT1 and pSTAT2 levels before and at 15 min after the addition of IFN. As shown in Fig. 3D and E, levels of pSTAT1 and pSTAT2 were lower in core mutant-transfected and -infected cells after IFN treatment than in wild-type-transfected cells and naive cells. These findings indicate that the differences in sensitivity to interferon of core mutant clones and the wild type were associated with attenuation of the cellular IFN signaling pathway.

**SOCS3 is upregulated in core mutant clones-transfected, IFN-resistant cells.** We examined next the effects of HCV replication on the expression of SOCS1 and SOCS3, proteins that suppress IFN receptor-mediated signaling (50, 58). There was no significant difference in expression levels of SOCS1
FIG. 3. Interferon-induced expressional induction of the ISGs, P56, PKR, and 25AS in Huh7 cells transfected or infected with wild-type and core mutant JFH1 clones. Two days posttransfection, cells were treated with 50 IU/ml of IFN-α. After 8 h, total cellular RNA was extracted and mRNAs of P56 (A), PKR (B), or 25AS (C) were quantified by real-time RT-PCR analyses. The values are displayed as ratios of IFN-untreated control values. Experiments were repeated three times, and representative results are shown. Asterisks indicate \( P \) values of less than 0.05 compared to results for the wild type. (D) Western blotting. Expression of total and phosphorylated STAT1 and STAT2 proteins in cells transfected with the wild type and core mutant HCV clones. (E) Western blotting. Expression of total and phosphorylated STAT1 and STAT2 proteins in cells infected with the wild type and core mutant HCV clones. Densitometries for pSTAT1 and pSTAT2 were performed, and results are shown as percentage of results for HCV-negative samples.
mRNA between cells transfected with the wild type and the core mutant clones. In contrast, the SOCS3 mRNA expression level was significantly higher in core mutant-transfected cells than in wild-type-transfected cells (Fig. 4A and B). It is known that SOCS3 is induced principally by phosphorylated STAT3 (pSTAT3) (18) and that interleukin-6 (IL-6) is a strong inducer of pSTAT3 via receptor-mediated Janus kinase activation in the liver (41, 51). On that basis, we investigated whether overexpression of SOCS3 is associated with increased pSTAT3 and with overproduction of IL-6. The pSTAT3 level was significantly higher in core mutant-transfected cells than in JFH1-transfected cells and naive Huh7 cells (Fig. 5A). Moreover, cellular IL-6 mRNA expression was significantly higher in core mutant-transfected cells than in wild-type-transfected cells (Fig. 5B). These findings suggested that upregulation of cellular SOCS3 is associated with the resistance to IFN of the core 70/91 mutant HCV clones and that this effect is mediated partly by overproduction of IL-6.

**UPRs are enhanced in core mutant-transfected cells.** We have reported that HCV causes direct cytopathic effects on host cells and that these effects are mediated by HCV-induced unfolded protein responses (UPRs) (48). Therefore, we detected the expression of UPR-related proteins, GRP78 and CHOP, in cells expressing wild-type HCV and the core 70/91 mutants. As shown in Fig. 6, HCV-transfected cells showed higher expression levels of GRP78 and CHOP than untreated cells. Furthermore, cells transfected with HCV core 70/91 mutant clones expressed larger amounts of GRP78 and CHOP than the wild-type-transfected cells. Because IL-6 is principally expressed following UPR induction (Fig. 5B), these data indicate that HCV-induced UPR may be involved in the IFN resistance of core mutant clones.

**DISCUSSION**

In this study, we used a virus cell culture system to investigate the characteristics of R70Q, R70H, and L91M HCV core mutant viruses, which were clinically resistant to peg-IFN-plus-RBV treatment, and found that these core mutant clones showed resistance to IFN *in vitro*, consistent with the clinical findings (Fig. 1). These differences in the IFN sensitivity of the core mutant clones led us to conduct a series of experiments to investigate the molecular mechanisms of IFN-related response pathways. We found that IFN-α receptor-mediated signaling was attenuated in wild-type HCV-infected and core mutant-infected cells compared to that in uninfected cells and that the suppression of IFN signaling was more potent for core mutant clones than for the wild type. The differences in the interferon-mediated antiviral effects were demonstrated further by the difference in the induction rates of IFN-inducible P56, PKR, and 25AS mRNAs (Fig. 3A, B, and C) and IFN-induced phosphorylation of STAT1 and STAT2 (Fig. 3D and E). Furthermore, the expression levels of an interferon signal attenuator, SOCS3, were significantly higher in core mutant-transfected cells than in wild-type-transfected cells. Moreover, cellular expression of IL-6, which induces SOCS3 expression through phosphorylation of STAT3 (18, 41), was significantly higher in the core mutant-transfected cells than in wild-type-transfected cells (Fig. 5A). Taking all these things together, it is suggested strongly that the IFN resistance of core mutant clones is due to
SOCS3-mediated attenuation of IFN responses and that, more importantly, upregulation of cellular IL-6 is attributable to emergence of IFN resistance (Fig. 7).

Miyanari et al. demonstrated that core protein, which is localized in LD-associated membrane, recruits HCV nonstructural (NS) proteins and replication complexes to LD and that this recruitment is critical for producing infectious viruses (35). Furthermore, Masaki et al. reported that the NS5A protein interacts with core at its C-terminal serine cluster and this NS5A-core interaction is crucial for the production of virus particle (32). In this study, there was no difference between the core mutants and the wild-type virus in terms of the pattern of colocalization of core protein with LDs and also the ER membrane (see the figure in the supplemental material). These results suggest that the core amino acid substitutions at positions 70 and 91 do not alter the characteristics of the core protein in terms of subcellular localization. Murray et al. conducted a comprehensive alanine substitution scan of the core protein to search for domains that are essential for virion production. They showed that substitutions of amino acids 70 and 91 spared but slightly decreased the capacity for virus particle production (37), which is consistent with our present results. Those mutations may cause accumulation of virus and core protein in the LDs and ER membrane and may elicit UPRs and IFN resistance.

Type I IFNs and their responsive ISGs are the principal mediators of host defense against virus infections, including HCV (10, 26, 44). Upon binding of IFNs to their receptors, IFNAR1 and IFNAR2, Janus kinases (Jak)1 and 2 phosphorylate STAT1 and STAT2 to form ISGF-3, which translocates to the nucleus and activates transcription of ISGs (46, 54, 55). Members of the SOCS family are potent inhibitors of type I and type III IFN-induced activation of the Jak-STAT pathway and subsequent expression of ISGs (58). HCV, on the other hand, counteracts such IFN-mediated antiviral pathways through its interaction with various steps of IFN signaling. The HCV NS5A and E2 proteins interfere with the action of IFN by inhibiting the activity of PKR (20, 56). NS5A also induces expression of IL-8 and attenuates expression of ISGs (40). HCV core protein has been reported to interfere with the antiviral actions of IFN. Core protein binds the STAT1-SH domain (29) and destabilizes STAT1 (28) to block IFN signaling. Blindenbacher et al. (8) showed that STAT signaling was strongly inhibited in the hepatocytes of HCV core transgenic mice. Bode et al. showed that HCV core protein induced SOCS3 expression and inhibited tyrosine phosphorylation of STAT1 in HepG2 cells (9). In this study, we used full-length HCV cell culture and found that SOCS3 expression is upregulated at different rates, depending on the genetic sequences of HCV strains, and that these differences in SOCS3 expression are associated with sensitivity to IFN. These results indicate that the IFN resistance of HCV-infected cells is mediated by overexpression of SOCS3, which may be upregulated by HCV proteins, as previously reported (9, 27). Only one amino acid difference, R70Q, R70H, or L91M, might have affected cellular responses to interferon.

IL-6 is the principal activator of STAT3 in hepatocytes (18, 41). It has been reported that plasma IL-6 levels are elevated in CHC patients (30). Basu et al. have conducted DNA microarray analyses in HCV core-expressing cells and demonstrated that genes including those encoding IL-6 and STAT3 were upregulated by core protein (7). Consistent with these findings, we found that cellular IL-6 expression levels were elevated in HCV-transfected cells in the order (from lowest to highest levels) uninfected, wild type, and then core mutants, which correlated well with SOCS3 expression (Fig. 4B) and with cellular responses to IFN (Fig. 1B and C). The inducers of IL-6 remain to be clarified. IL-6 is secreted in response to cellular steatosis and insulin resistance (45). Hepatic steatosis is found in 70% of CHC patients (57) and those with obesity; steatosis or insulin resistance is refractory to IFN treatment (43). Such patients show higher levels of hepatic SOCS3 ex-
expression than those without obesity or insulin resistance (34, 61). We reported previously that a series of genes involved in fatty acid and cholesterol synthesis are upregulated in HCV replicon-expressing and HCV-JFH1-infected cells and increased cellular LĐs (39). Such lipogenic cellular processes may be the cause of the upregulated expression of IL-6. Alternatively, UPRs may produce IL-6. Chen et al. have reported that UPRs are coupled with TNF-α and IL-6 production in human macrophages (11). In this study, transfection of HuH7 cells by HCV induced the expression of UPR genes, and their expression levels were significantly higher in mutant core protein-transfected cells than in wild type-transfected cells (Fig. 6).

The differences in ISG expression levels between the HCV wild type and core mutants were significant but small (Fig. 3A, B, and C). As shown in Fig. 3D, and E and 4B, the interclone differences in pSTAT and SOCS3 were significant but relatively small, which may explain the small differences in ISG levels. Similarly, the clinical difference in interferon treatment outcomes between core 70/91 mutants and wild types are significant but are around the sustained viral clearance rates of 32.4% versus 53.5% in core 70 or 91 mutants and wild types, respectively (19), which might be consistent with our present results.

In clinical settings, IFN resistance of the core amino acid 70/91 mutants has been reported for genotype 1b strains (5). At present, there is no report that these mutations are associated with IFN treatment responses to other genotypes, including genotype 2a, which we used in this study. Because HCV strains other than genotypes 1 and 4 are generally sensitive to IFN, the core 70/91 mutations might not affect final treatment outcomes. We have conducted preliminary experiments using genotype 1b infectious clones with low levels of replication and found that these mutations did not significantly affect sensitivity to IFN in culture. It may be necessary to investigate IFN sensitivity when efficient cell culture systems have been developed for HCV genotype 1.

In addition to the poor virological responses of HCV core amino acid 70/91 mutants to peg-IFN-plus-RBV treatment (4, 6, 12), patients infected with the core mutants showed increased incidence of hepatocellular malignancies (2, 15, 49). It has been reported that the HCV core R70 but not L91 mutant frequently causes steatosis and increased hepatic oxidative stress (52). It is possible that core 70/91 mutations not only induce IFN resistance but also may cause other pathophysiological conditions, such as carcinogenesis and disorders of lipid metabolism.

In conclusion, our study demonstrates that the IFN resistance of HCV core mutants may be, for the most part, determined by cellular expression levels of SOCS3 and IL-6. Therapeutic targeting of IL-6 potentially may be a key to targeting IFN resistance and improving antiviral chemotherapeutics against HCV.

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