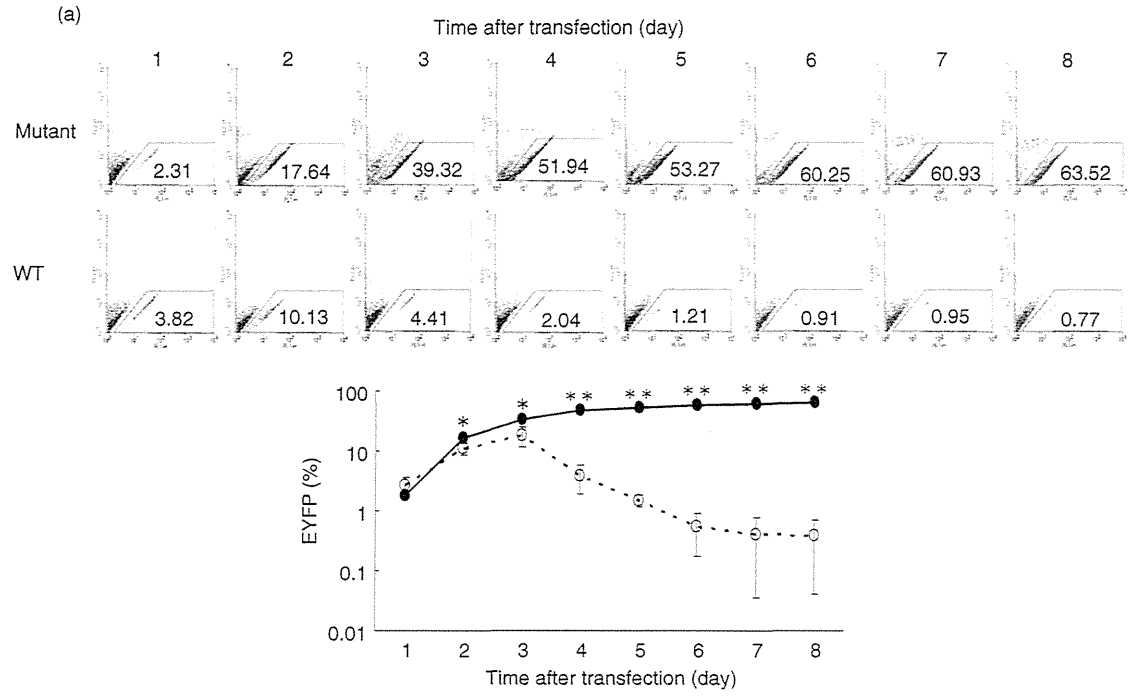
directly visualized by fluorescence microscopy on days 1–7. As shown in Figure 2(a), the number of cells positive for both JFH1-EYFP and JFH1-AsRed mutants, but not for JFH1-EYFP and JFH1-AsRed-infected cells, increased in a time-dependent manner. In JFH1-EFYP mutant-transfected cells, the proportion of EYFP-positive cells on days 3, 5 and 7 post-infection was 4.4%, 29% and 41%, respectively. In contrast, only 4.9% of JFH1-EYFP-transfected cells became EYFP-positive at 3 days post-infection, and the percentage of these fluorescence-positive cells decreased rapidly thereafter (Fig. 2b). Similarly, the percentage of cells infected with JFH1-AsRed mutant but not JFH1-AsRed increased exponentially. These results indicated that the two fluorescence virus clones with mutations are able to secrete infectious virus particles. We next compared levels of HCV core antigen in culture medium of cells infected with JFH1, JFH1-EYFP, JFH1-EYFP mutant, JFH1-AsRed, and JFH1-AsRed mutant viruses. The mutant viruses, but not the wild-type, produced amounts of core protein comparable to that of the parental JFH1 (Fig. 2C). In HCV-JFH1, JFH1-EYFP mutant, and JFH1-AsRed mutant-transfected cells, the core protein reached a peak of 1.25, 1.35 and 1.34 fmol/L, respectively, at 14 days post-transfection, while that of JFH1-EYFP JFH1-AsRed-transfected cells became undetectable at 10 days post-transfection. These results indicated that the mutant type is capable of producing an amount of viral particles comparable to that of the parental JFH1.

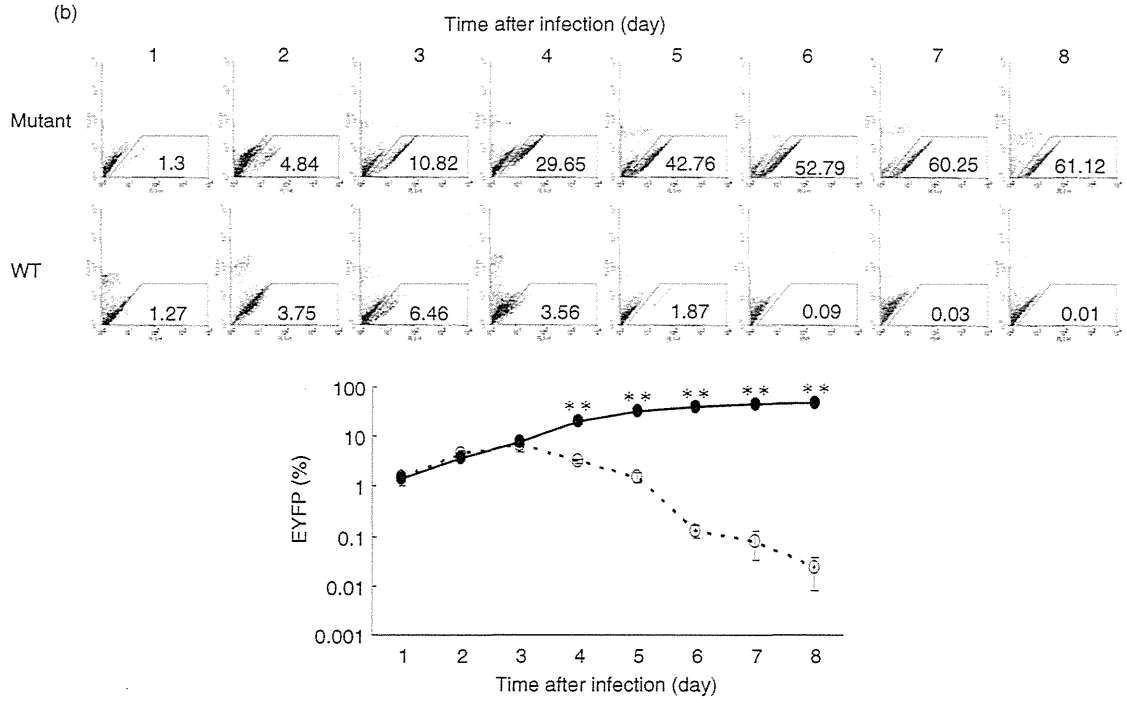
Using the two fluorescence-tagged viruses, we conducted co-infection of two virus strains, JFH1-AsRed mutant, which secreted infectious virus particles, and JFH1-EYFP, in which virus particle secretion was impaired. We collected culture media from cells transfected with JFH1-AsRed-mutant or JFH1-EYFP on day 2 post-transfection and infected both media onto uninfected Huh7.5.1 cells at a multiplicity of infection (moi, focus forming unit per cell) of 0.01. The number of JFH1-AsRed mutant-infected cells increased exponentially until day 3 but reached a plateau on days 5 and 7 post-infection. Interestingly, the number of cells positive for viral secretion-impaired JFH1-EYFP also increased in a manner similar to that of the JFH1-AsRed mutant (Fig. 3a). The percentage of AsRed mutant-positive cells was 4.6%, 6.7% and 14.8% at days 3, 5 and 7, respectively, while the percentage of EYFP-positive cells at the corresponding days was 3.1%, 4.8% and 5.1%, respectively (Fig. 3b). These results suggest that, in the co-culture of two HCV clones with and without virus particle secretion, a secretion-impaired virus clone is able to replicate and produce infectious particles possibly through the complementation of the intact virus.

Expression and subcellular localization of NS5A-fluorescence proteins

We next used fluorescence microscopy to study the subcellular localization of fluorescence and viral proteins. In cells transfected with JFH1-EYFP, JFH1-AsRed, and the respective mutants, EYFP and AsRed, were clearly visualized as dot-like structures in the perinuclear area (Fig. 4a). To determine if the NS5A-AsRed fusion protein indicates the subcellular localization of NS5A, we performed immunofluorescence staining of JFH-AsRed- and JFH1-AsRed mutant-infected cells using NS5A and HCV-core antibodies. Fluorescence of AsRed was co-localized precisely with NS5A and partially with core proteins. The fluorescence intensities of the JFH1-EYFP and -AsRed mutants within the cells were equal to that of the wild-type constructs. EYFP-NS5A of wild type and mutant JFH1 were localized in the ER (Fig. 4b). Western blotting was performed by using anti-GFP and anti-HCV-NS5A antibodies. As shown in Figure 4(c), three bands of the expected molecular weights of 27, 58 and 85 kDa, which corresponded to EGFP, NS5A and NS5A-EYFP fusion protein, were detected in EGFP, JFH1, JFH1-EYFP, JFH1-AsRed, JFH1-EYFP mutant, and JFH1-AsRed mutant-transfected cells. The expression levels of core protein in JFH1-EYFP mutant- and JFH1-AsRed mutant-transfected cells were almost the same as those transfected with parental JFH1. These results indicate

Figure 5 Kinetics of hepatitis C virus (HCV)-infected cells. (a) Huh7.5.1 cells were infected with JFH1-EYFP or JFH1-EYFP mutant HCV RNA. At the days indicated, cells were harvested and subjected to flow cytometry. EYFP-positive cells were sorted based on EYFP activating (*x*-axis) and staining with a marker of dead cells (*y*-axis). The results are depicted as density plots. The ratios of EYFP-positive cells vs time are shown below. Assays were carried out in triplicate and the results are expressed as mean ± standard deviation. $^{*}P < 0.05$. $^{**}P < 0.01$. —•—, JFH1-EYFP mutant; ··◇··, JFH1-EYFP. (b) Culture media from JFH1-EYFP or mutant-transfected cells were added onto uninfected Huh7.5.1 cells at a moi of 0.01. At the days indicated, infected cells were analyzed using flow cytometry. The results are depicted as density plots. The ratio of EYFP-positive cells vs time are shown below. Assays were carried out in triplicate and the results are expressed as mean ± standard deviation. $^{*}P < 0.05$. $^{**}P < 0.01$. WT, wild type. —•—, JFH1-EYFP mutant; ··◇··, JFH1-EYFP.





that the fusion proteins of NS5A and the fluorescent proteins remain intact within cells and serve as accurate markers of infection and as indicators of the subcellular localization of HCV-NS5A proteins.

Kinetics of HCV infection

Using those fluorescence-tagged HCV constructs, we analyzed more precisely the ratio and kinesics of HCV RNA-transfected cells and virus-infected cells by flow cytometry. After HCV RNA transfection, the percentages of JFH1-EYFP and JFH1-EYFP mutant-transfected cells were almost equivalent up to 2 days. Thereafter, JFH1-EYFP-positive cells began to decrease in number, while the mutant-transfected cells increased exponentially until 5 days post-transfection and then reached a plateau, when 52.2% of the cells were EYFP-positive (Fig. 5a). We collected the media from JFH1-EYFP and mutant-transfected cells at 2 days post-transfection, and added it onto uninfected Huh7.5.1 cells at a moi of 0.01. Similar to the results of the transfection assay, the population of JFH1-EYFP mutant-infected cells increased exponentially and reached a stable state at 6 days post-infection, when 39.2% of the cells were EYFP-positive (Fig. 5b). Calculating from the above data, the rate of expansion of HCV-infected cells was $2^{1.5}$/day. The cell-to-cell expansion of the JFH1-EYFP mutant infection was blocked by prior treatment of cells with anti-CD81 antibody (data not shown). This finding indicated that the expansion of the EGFP-positive cells was due to cell–cell spread of EYFP-tagged HCV and not the division of the virus-positive cells.

To further refine the calculation of the rate of cell-to-cell spread of infection, we carried out JFH1-EYFP mutant infection of uninfected Huh7.5.1 cells seeded at various densities from 2×10^3 to 2×10^5 cells/mL (Fig. 6). Flow cytometry showed that the rates of expansion of HCV-infected cells were $2^{1.5}$, $2^{2.3}$ and $2^{2.5}$/day at 2×10^3, 2×10^4 and 2×10^5 cells/cm^2, respectively. The ability of JFH1-EYFP to spread is greater in cells seeded at higher density. The maximum rate of expansion of HCV-infected cells was calculated as $2^{2.5}$/day.

Effects of antiviral drugs on HCV-infected cells

We next investigated the effects of antiviral agents on the infection kinetics of tagged-HCV. Eighteen hours after transfection of EYFP-tagged HCV RNA, the cells were treated with 10, 30 or 50 U/mL of IFN-α-2b or with 10 μM of protease inhibitor, BILN2061. JFH1-EYFP mutant-transfected cells were analyzed using flow

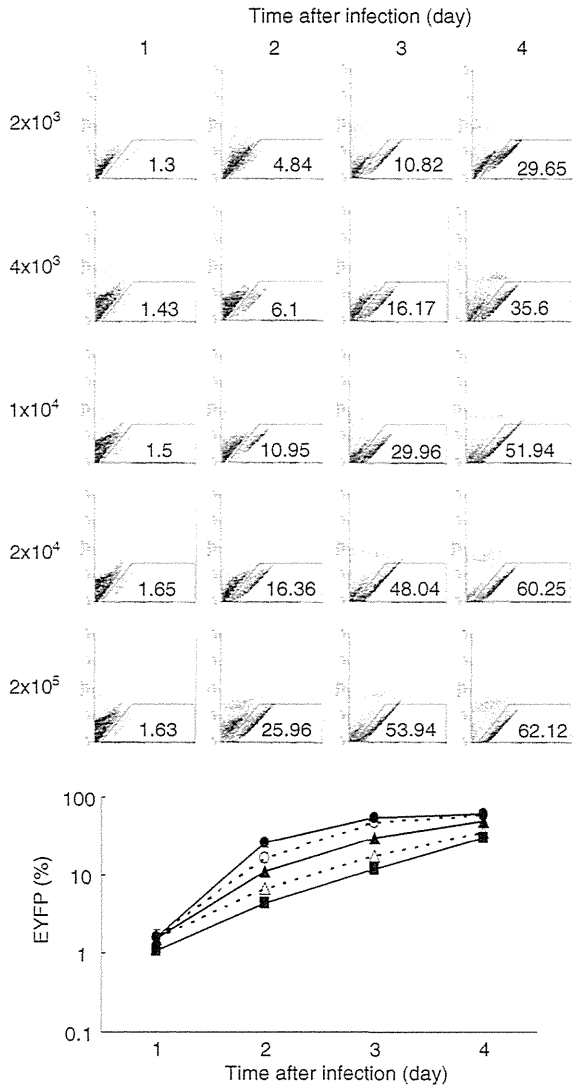


Figure 6 Rate of expansion of hepatitis C virus (HCV)-infected cells. The medium from JFH1-EYFP mutant was inoculated onto Huh7.5.1 cells seeded at different densities (2×10^3, 4×10^3, 1×10^4, 2×10^4 and 2×10^5 cells/cm^2) with core antigen adjusted doses. The results of flow cytometric analysis are depicted as density plots. The ratios of EYFP-positive cells vs time are shown beneath. Assays were carried out in triplicate and the results are expressed as mean ± standard deviation. —■—, 2×10^3; ···◇···, 4×10^3; —▲—, 1×10^4; ···◇···, 2×10^4; —●—, 2×10^5.

cytometry. As shown in Figure 7, treatment of cells with the two compounds suppressed the time-dependent increase of HCV propagation. In addition, IFN-α-2b suppressed the dose-dependent increase of HCV

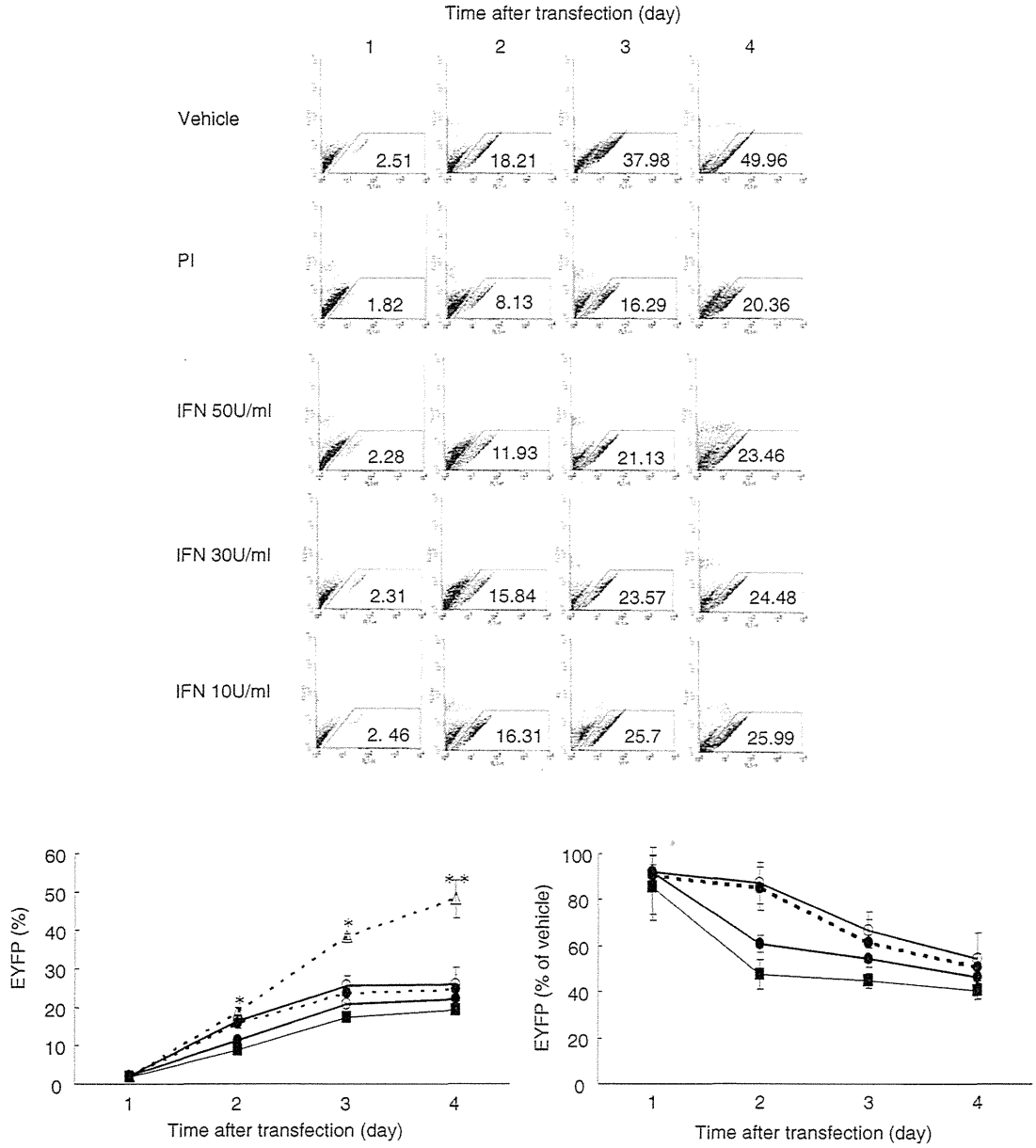


Figure 7 Effect of antiviral drugs on hepatitis C virus (HCV)-infected cells. Huh7.5.1 cells were transfected with JFH1-EYFP mutant RNA. Eighteen hours after transfection, cells were cultured with 10, 30 or 50 U/mL of interferon (IFN)-α-2b or 10 μM of the protease inhibitor BILN-2061. The cells were harvested at the days indicated and flow cytometry was performed. Ratios of EYFP-positive cells over time are shown at lower left. Plot values of 100% in each curve represent the EYFP expression levels in untreated cells (lower right). Assays were carried out in triplicate and the results are expressed as mean ± standard deviation. $^*P < 0.05$. $^{**}P < 0.01$. PI, protease inhibitor. ·-·-·, vehicle; —•—, 50 U/mL; ·-•-·, 30 U/mL; —○—, 10 U/mL; —■—, PI.

propagation. At all time points, the number of infected cells was significantly lower in the culture treated with the two compounds than in the untreated culture. The protease inhibitor suppressed infection faster than did IFN-α-2b. These data indicate that IFN and the protease inhibitor were not only able to suppress intracellular HCV replication levels but also to inhibit virus particle secretion and expansion of HCV-infected cell populations.

DISCUSSION

IN THIS STUDY, we used fluorescence-tagged HCV, in which virus assembly, particle secretion and re-infection functions are fully preserved (Fig. 1).[17] Utilizing the fluorescence-tagged HCV, we analyzed the rate of expansion of HCV infection using live-cell flow cytometric analyses (Figs 5–7). In the early periods of infection, the expansion of the virus-positive cell population increased exponentially and the maximum rate of expansion was calculated as $2^{2.5}$/day. It is not clear why HCV propagation reaches a plateau, but this observation, where HCV replication is limited in confluent cells, has been made previously.[26] Possible explanations include cell death due to over-confluence, depletion of the nucleoside triphosphate pools in resting cells, and/or cell cycle-dependent effects on virus RNA replication and translation.

Co-infection of the two virus clones, EYFP-JFH1 and AsRed mutant JFH1, showed that viruses with impaired particle secretion were able to replicate and expand virus-infected cells (Fig. 3). Although we have found no clear mechanism to explain those effects, we speculate that the secretion-defective virus (JFH1-EYFP) may assemble into infectious virus through *trans*-complementation of virus proteins via co-infection in a single cell or recombination of mutant and wild-type virus genomic RNA. The co-infection experiment showed that the increase of JFH1-AsRed mutant-positive cells was slower than in the single clone infection experiment (Fig. 2a). These findings suggest that viruses with impaired particle secretion (JFH1-EYFP) partially suppressed expansion of viruses with intact particle secretion (JFH1-AsRed mutant) through *trans*-suppression of cellular virus replication or competitive binding to cellular virus entry receptors.

After the development of HCV-JFH1 cell culture,[11] many variations of HCV cell culture systems have been developed. Lindenbach *et al.* developed a genotype 2a intragenotypic chimera, J6/JFH, in which the JFH1 structural region was replaced with that of J6, isolated from a patient with chronic hepatitis.[27] The J6/JFH chimera is able to produce virus particles more efficiently than JFH1 but does not produce virus-induced cytopathic effects (CPE). Several marker protein-tagged viruses have been reported, in which viral infection could be visualized readily in living cells. A subgenomic replicon that expressed an NS5A-GFP fusion protein was reported first.[12] However, the clone lacked the structural regions that are required for virus propagation. Subsequently, full-length HCV reporter viruses were developed in which the EGFP gene was inserted into the NS5A-C-terminus of JFH1[13] or JC-1.[14] Jones *et al.* inserted the *renilla* luciferase gene into P7 of J6/JFH.[15] Unfortunately, the efficiency of virus production by the recombinant reporter viruses was greatly reduced compared to wild-type viruses. Very recently, it has been reported that a JFH1-based adaptive strain of a HCV reporter virus can produce infectious HCV particles as robustly as the JFH1 wild-type strain.[17] This virus system has overcome the serious limitations associated with the use and application of other reporter viruses.

Compared with the other HCV reporter viruses, the JFH1-EYFP/AsRed mutant is capable of producing amounts of HCV virus equivalent to that of the parental JFH1, which enables continuous passage of infection in cell culture and analyses using various research modalities, including flow cytometry and live-cell microcopy. Considering the current situation regarding the lack of singly effective, proven antiviral agents against HCV, other than IFN formulations, the search for potential antiviral agents will continue to be a dominant goal of research to improve clinical anti-HCV chemotherapeutics. This tagged HCV culture system may provide a very convenient tool for studies of the complete virus life cycle in live cells and of virus–host interactions, and it may be useful for high-throughput screening of drugs.

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Sequences in the Interferon Sensitivity-Determining Region and Core Region of Hepatitis C Virus Impact Pretreatment Prediction of Response to PEG-Interferon Plus Ribavirin: Data Mining Analysis

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The aim of the present study was to clarify the significance of viral factors for pretreatment prediction of sustained virological response to pegylated-interferon (PEG-IFN) plus ribavirin (RBV) therapy for chronic hepatitis C using data mining analysis. Substitutions in the IFN sensitivity-determining region (ISDR) and at position 70 of the HCV core region (Core70) were determined in 505 patients with genotype 1b chronic hepatitis C treated with PEG-IFN plus RBV. Data mining analysis was used to build a predictive model of sustained virological response in patients selected randomly (n = 304). The reproducibility of the model was validated in the remaining 201 patients. Substitutions in ISDR (odds ratio = 9.92, $P < 0.0001$) and Core70 (odds ratio = 1.92, $P = 0.01$) predicted sustained virological response independent of other covariates. The decision-tree model revealed that the rate of sustained virological response was highest (83%) in patients with two or more substitutions in ISDR. The overall rate of sustained virological response was 44% in patients with a low number of substitutions in ISDR (0–1) but was 83% in selected subgroups of younger patients (<60 years), wild-type sequence at Core70, and higher level of low-density lipoprotein cholesterol (LDL-C) (≥120 mg/dl). Reproducibility of the model was validated ($r^2 = 0.94$, $P < 0.001$). In conclusion, substitutions in ISDR and Core70 of HCV are significant predictors of response to PEG-IFN plus RBV therapy. A decision-tree model that includes these viral factors as predictors could identify patients with a high probability of sustained virological response. ***J. Med. Virol. 83:445–452, 2011.***

KEY WORDS: data mining; decision-tree model; ISDR; core region; PEG-interferon

INTRODUCTION

The combination of pegylated-interferon (PEG-IFN) plus ribavirin (RBV) is currently the most effective therapy for chronic hepatitis C, but the rate of sustained virological response after 48 weeks of therapy is about 50% in patients with HCV genotype 1b and a high HCV

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RNA titer [Manns et al., 2001; Fried et al., 2002]. The most reliable means to predict sustained virological response is to monitor the viral response during the early weeks of treatment. The early virological response, defined as undetectable HCV RNA at week 12, is associated with a high rate of sustained virological response [Davis et al., 2003; Lee and Ferenci, 2008]. The rapid virological response, defined as undetectable HCV RNA at week 4 of therapy, is even more predictive of sustained virological response than the early virological response [Jensen et al., 2006; Yu et al., 2008; Izumi et al., 2010]. However, there is no established means that predicts the virological response before commencing treatment. Recent reports have revealed that single nucleotide polymorphisms located near the *IL28B* gene show a strong association with the response to PEG-IFN plus RBV therapy [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Kurosaki et al., 2010c]. These findings indicate that the host factor is an important determinant of the treatment response. On the other hand, the present study's authors have reported that a stretch of 40 amino acids in the NS5A region of HCV, designated as the interferon sensitivity-determining region (ISDR), has a close association with the virological response to interferon mono-therapy [Enomoto et al., 1995, 1996; Kurosaki et al., 1997]. More recently, amino acid substitutions at positions 70 and 91 of the core region have been reported to be associated with response to PEG-IFN plus RBV combination therapy [Akuta et al., 2005, 2007a]. The impact of these HCV substitutions on treatment response is yet to be validated.

Decision-tree analysis is a core component of data mining analysis that can be used to build predictive models [Breiman et al., 1980]. This method has been used to define prognostic factors in various diseases such as prostate cancer [Garzotto et al., 2005], diabetes [Miyaki et al., 2002], melanoma [Averbook et al., 2002; Leiter et al., 2004], colorectal carcinoma [Zlobec et al., 2005; Valera et al., 2007], and liver failure [Baquerizo et al., 2003]. The major advantage of decision-tree analysis over logistic regression analysis is that the results of analysis are easy to understand. The simple allocation of patients into subgroups by following the flowchart form could define the predicted possibility of outcome [LeBlanc and Crowley, 1995].

Decision-tree analysis was used for the prediction of early virological response (undetectable HCV RNA within 12 weeks of therapy) to PEG-IFN and RBV combination therapy in chronic hepatitis C [Kurosaki et al., 2010a], and more recently for the pretreatment prediction of sustained virological response [Kurosaki et al., 2010b]. In the latter model, simple and noninvasive standard tests were used as parameters; specialized tests such as viral mutations and host genetics, or invasive tests such as liver histology, were not included because the aim of that model was for use in general medical practice, especially in some countries or areas where resources are limited. Thus, the impact of viral mutations or liver histology was not considered in that model.

The present study examined whether including viral substitutions in ISDR and the core region of HCV in the decision-tree model could improve its predictive accuracy over the previous model to identify chronic hepatitis C patients who are likely to respond to PEG-IFN plus RBV therapy.

MATERIALS AND METHODS

Patients

This multicenter retrospective cohort study included 505 chronic hepatitis C patients who were treated with PEG-IFN alpha-2b and RBV at Musashino Red Cross Hospital, Toranomon Hospital, Tokyo Medical and Dental University, Osaka University, Nagoya City University Graduate School of Medical Sciences, Yamanashi University, Osaka City University, and their related hospitals. The inclusion criteria were: (1) genotype 1b, (2) HCV RNA titer higher than 100 kIU/ml by quantitative PCR (Cobas Amplicor HCV Monitor v 2.0, Roche Diagnostic Systems, Pleasanton, CA), (3) no co-infection with hepatitis B virus or human immunodeficiency virus, (4) no other causes of liver disease, (5) patients having undergone liver biopsy prior to IFN treatment, (6) number of substitutions in ISDR having been determined, (7) substitutions in the amino acid positions 70 and 91 of the core region having been determined, and (8) completion of at least 12 weeks of therapy. Patients were treated with PEG-IFN alpha-2b (1.5 μg/kg) weekly plus RBV. The daily dose of RBV was adjusted by weight: 600 mg for <60 kg, 800 mg for 60–80 kg, and 1,000 mg for >80 kg. For the analysis, patients were assigned randomly to either the model building (304 patients) or validation (201 patients) groups. There were no significant differences in the clinical backgrounds between these two groups (Table I). Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committees of all concerned hospitals.

Laboratory Tests

Hematological tests, blood chemistry, and HCV RNA titer were analyzed before therapy and at least once every month during therapy. Sequences of ISDR and the core region of HCV were determined by direct sequencing after amplification by reverse transcription and polymerase chain reaction as reported previously. At position 70 of the core region (Core70), arginine was defined as the wild type, and glutamine or histidine was defined as the mutant type. At position 91 of the core region, leucine was defined as the wild type and methionine was defined as the mutant type, as described previously [Akuta et al., 2005]. Fibrosis and activity were scored according to the METAVIR scoring system [Bedossa and Poynard, 1996]. Fibrosis was staged on a scale of 0–4: F0 (no fibrosis), F1 (mild fibrosis), F2 (moderate fibrosis), F3 (severe fibrosis), and F4 (cirrhosis). Activity of necroinflammation was graded on a scale of

TABLE I. Comparison of Pretreatment Factors Between Model Building and Validation Patients

	Model (n = 304)	Validation (n = 201)	*P*-value
Age (years)	55.6 (9.4)	56.0 (12.2)	0.80
Male (%)	53 (%)	55 (%)	0.13
Body mass index (kg/m^2)	23.1 (3.1)	23.1 (4.0)	0.99
Albumin (g/dl)	4.0 (0.3)	4.0 (0.3)	0.47
Creatinine (mg/dl)	0.72 (0.15)	0.72 (0.14)	0.62
AST (IU/L)	63.3 (45.6)	58.9 (46.4)	0.91
ALT (IU/L)	78.7 (58.6)	74.5 (67.5)	0.68
GGT (IU/L)	53.2 (49.1)	57.4 (63.5)	0.43
Total cholesterol (mg/dl)	170.9 (32.6)	169.4 (34.1)	0.33
Triglyceride (mg/dl)	107.0 (44.7)	105.7 (48.0)	0.90
LDL-C (mg/dl)	95.5 (28.0)	96.4 (28.8)	0.34
White blood cell count (/μl)	4,902 (1,489)	4,906 (1,319)	0.86
Hemoglobin (g/dl)	14.1 (1.3)	14.3 (1.4)	0.09
Platelets (10^9/L)	164 (56)	172 (55)	0.68
HCVRNA (10^3 IU/ml)	1,859 (1,468)	2,021 (1,393)	0.09
ISDR mutations: ≥2 (%)	15 (%)	20 (%)	0.11
Core70: mutant (%)	36 (%)	29 (%)	0.22
Core91: mutant (%)	40 (%)	36 (%)	0.20
Fibrosis: F2–4 (%)	49 (%)	48 (%)	0.36
Activity: A2–3 (%)	42 (%)	34 (%)	0.10

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; LDL-C, low-density-lipoprotein-cholesterol; ISDR, interferon sensitivity-determining region.
Data expressed as mean (SD).

0–3: A0 (no activity), A1 (mild activity), A2 (moderate activity), and A3 (severe activity). Sustained virological response was defined as undetectable HCV RNA by qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor, Roche Diagnostic Systems) at week 24 after the completion of therapy.

Statistical Analysis

A database of pretreatment variables included hematological tests (hemoglobin level, white blood cell count, and platelet count), blood chemistry tests (serum levels of creatinine, albumin, aspartate aminotransferase, alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C)), viral factors (HCV RNA titer, number of substitutions in ISDR, substitutions in the amino acid positions 70 and 91 of the core region), histological findings (stage of fibrosis and grade of activity) and patient characteristics (age, sex, and body mass index). Based on this database, decision-tree analysis was used to define a predictive model for sustained virological response.

Student's *t*-test was used for the univariable comparison of quantitative variables and Fisher's exact test was used for the comparison of qualitative variables. For the multivariable analysis for factors associated with sustained virological response, logistic regression models with backward selection were used to identify independent predictors of sustained virological response. Variables that showed significant association with sustained virological response by univariable analysis were included in the multivariable analysis. IBM-SPSS software v.15.0 (SPSS, Inc., Chicago, IL) was used for these analyses. For the decision-tree analysis [Segal and Bloch, 1989], the data mining software IBM SPSS Modeler 13 (IBM SPSS, Inc.) was used, as reported previously [Kurosaki et al., 2010a,b]. In brief, the software searched for the optimal split variables to build a decision-tree structure. The entire study population was first evaluated to determine the variables and cut-off points for the most significant division into two subgroups having different probabilities of sustained virological response. Thereafter, analysis was repeated on all subgroups in the same way until either no additional significant variable was detected or the sample size was below 20.

RESULTS

Generation of the Decision-Tree Model

The decision-tree analysis selected five predictive variables to produce six subgroups of patients (Fig. 1). The number of substitutions in ISDR was selected as the best predictor of sustained virological response. The possibility of achieving sustained virological response was 83% for patients with two or more substitutions in ISDR compared with 44% for patients with a single or no substitution. Among patients with a single or no substitution in ISDR, age, with an optimal cut-off of 60 years, was selected as the variable of second split. Patients younger than 60 had the higher probability of sustained virological response (55%) compared with those older than 60 years (31%). Among younger patients, amino acid substitution at Core70 was selected as the third variable of split—wild-type sequence being the predictor of favorable response compared with the mutant type (65% vs. 36%). Among patients with wild-type Core70, the level of serum LDL-C was selected as the fourth variable of split, with an optimal cutoff of

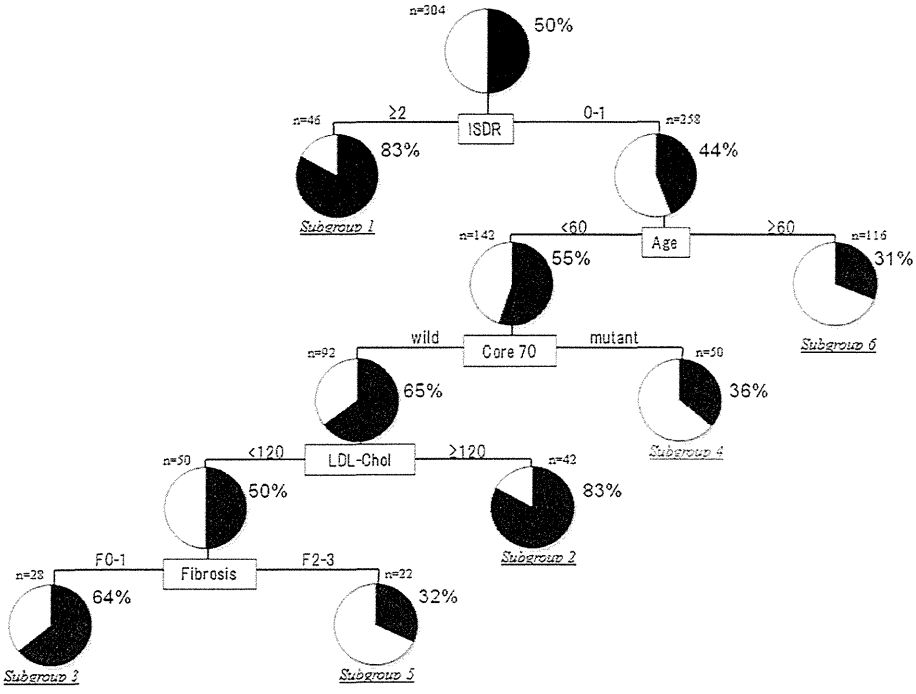

Fig. 1. Decision-tree model. Boxes indicate the factors used for splitting and the cutoff value for the split. Pie charts indicate the rate of sustained virological response for each group of patients after splitting. Terminal subgroups of patients discriminated by the analysis are numbered from 1 to 7. The rate of sustained virological response was >80% in subgroups 1 and 2, 64% in subgroup 3, and 31–36% in subgroups 4, 5, and 6. LDL-C represents low-density lipoprotein cholesterol and Core70 represents amino acid substitution at position 70 of the core region.

120 mg/dl. Patients with higher LDL-C level had the higher probability of sustained virological response (83% vs. 50%). The stage of fibrosis was selected as the final variable of split, with significant fibrosis (F2–4) being the predictor of lower sustained virological response probability (64% vs. 32%).

Among the six subgroups derived by this decision tree, the subgroup of patients with two or more substitutions in ISDR (subgroup 1) or with a single or no substitution in ISDR but younger than 60 years of age, having the wild-type Core70 and high serum level of LDL-C (≥120 mg/dl) (subgroup 2) showed the highest probability of sustained virological response (83%).

Validation of the Decision-Tree Model

The decision-tree model was validated using a validation dataset of 201 cases that were not included the model-building dataset. Each patient in the validation set was allocated to subgroups 1–6 using the flowchart form of the decision tree. The rates of sustained virological response were 75% for subgroup 1, 73% for subgroup 2, 65% for subgroup 3, 41% for subgroup 4, 46% for subgroup 5, and 33% for subgroup 6. The rates of sustained virological response for each subgroup of patients were correlated closely between the model building dataset and the validation dataset ($r^2 = 0.94$) (Fig. 2).

The six subgroups were reconstructed into three groups according to their rate of sustained virological response: the high-probability group consisted of subgroups 1 and 2, the intermediate-probability group consisted of subgroup 3, and the low-probability group consisted of subgroups 4, 5, and 6. The rate of sustained virological response in the high-probability group was high on a consistent basis: 83% for model-building patients and 74% for validation patients. The rate of sustained virological response in the intermediate-probability group was 64% for model building patients and 65% for internal validation patients. The rate of sustained virological response in the low-probability group was low on a consistent basis: 32% for model-building patients and 36% for internal validation patients (Fig. 3). Thirty percent of the patients were classified into the high-probability group and 10% of the patients were classified into intermediate-probability group, which means that about 40% of patients with higher than average probability of achieving sustained virological response were identified.

Effect of Dose Reductions of PEG-IFN and RBV

The possible effect of drug reductions was analyzed in the three groups of patients divided by decision tree (low-, intermediate-, and high-probability groups)

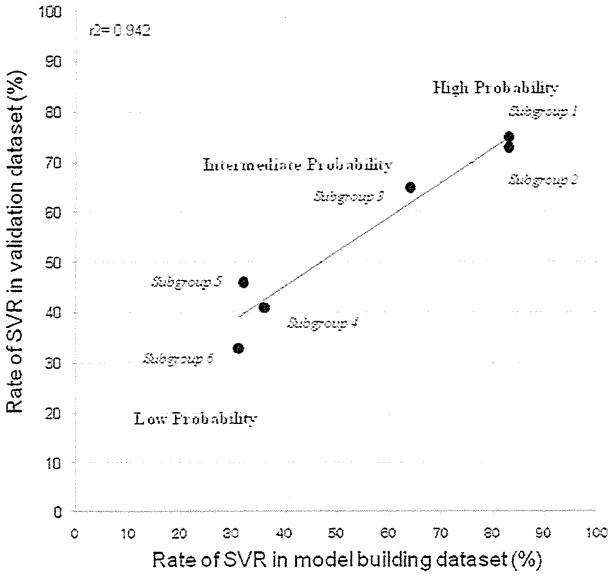

Fig. 2. Validation of the decision-tree analysis: Subgroup-stratified comparison of the rate of sustained virological response. Each patient in the validation set was allocated to subgroups 1–6 by following the flowchart form of the decision tree, and the rates of sustained virological response were then calculated and plotted for each subgroup. The *x*-axis represents the rate of sustained virological response in the model-building datasets and the *y*-axis represents the rate of sustained virological response in the validation datasets. The rates of achieving sustained virological response in each subgroup of patients correlated closely between the model-building dataset and the validation dataset (correlation coefficient: $r^2 = 0.94$).

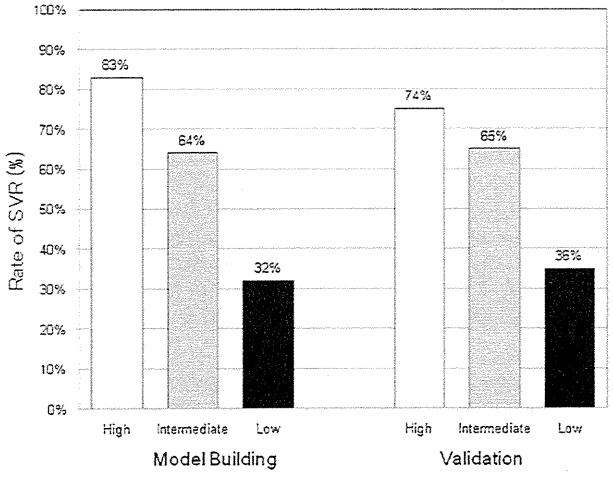

Fig. 3. Comparison of sustained virological response rates between groups divided by the decision tree. The rate of sustained virological response was compared between three groups of patients as divided by the decision-tree analysis. Black, gray, and white boxes indicate the low-probability group (subgroup 4, 5, and 6), intermediate-probability group (subgroup 3), and high-probability group (subgroup 1 and 2), respectively. The rate of sustained virological response showed significant difference between the three groups.

(Fig. 4). Patients were stratified according to the cumulative drug exposure with PEG-IFN and RBV: the good adherence group consisted of patients who took ≥80% planned doses of both PEG-IFN and RBV; the poor adherence group consisted of patients who took <80% of planned doses of both PEG-IFN and RBV. Even after adjustment for drug adherence, the three groups of patients divided by decision-tree analysis still had low, intermediate, and high probability of achieving sustained virological response, respectively, indicating that this model predicts sustained virological response independent of drug exposure.

Multivariable Logistic Regression Analysis

Age, sex, serum levels of creatinine, ALT, GGT, LDL-C, hemoglobin, platelet count, HCV RNA titer, ISDR substitution, substitution at Core70, substitution at Core91, histological stage of fibrosis, and grade of activity were found to be associated with sustained virological response by standard univariable analysis. Multivariable analysis including these factors showed that age, sex, LDL-C levels, GGT levels, platelet count, ISDR substitution, and substitution at Core70 showed independent associations with sustained virological response (Table II). Substitution in ISDR had the highest odds ratio, at 9.92. Fibrosis, which was selected as a significant predictor of response in the decision-tree analysis, was not found to be an independent predictor of response in standard multivariable analysis, indicating that the decision-tree analysis could identify significant predictors that would apply specifically to selected patients.

DISCUSSION

The present study revealed that viral factors such as substitutions in ISDR and Core70 are significant and independent predictors of sustained virological response to PEG-IFN plus RBV in chronic hepatitis C. In a decision-tree model for the pretreatment prediction of sustained virological response, the number of substitutions in ISDR was the best predictor of sustained virological response, followed by younger age, wild-type sequence at Core70, higher level of LDL-C, and absent fibrosis. This decision-tree model could identify patients with high probability of sustained virological response (83%) among difficult-to-treat genotype 1b chronic hepatitis C patients. Using this model, rapid estimates of the response before treatment can be made by allocating patients to specific subgroups with a defined rate of response simply by following the flowchart form. Because more potent therapy, such as a combination of protease inhibitor, PEG-IFN, and RBV, is under clinical trial and may become available in the near future [Hezode et al., 2009; McHutchison et al., 2009], pretreatment prediction of the likelihood of sustained virological response may be useful for both patients and physicians to support clinical decisions whether to start current standard therapy or to wait for emerging new therapies.

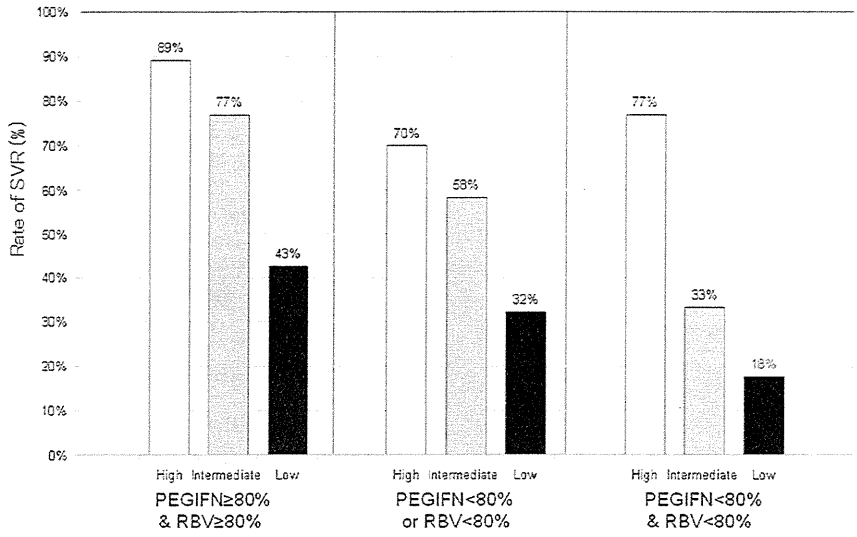

Fig. 4. Comparison of the rate of sustained virological response between the decision-tree groups stratified by drug adherence. The three groups of patients divided by the decision tree (black, gray, and white boxes indicating the low-, intermediate-, and high-probability groups, respectively) were further stratified according to cumulative drug exposure to PEG-IFN and RBV.

Two or more substitutions in ISDR had a strong impact on sustained virological response, because this factor was selected as a top variable in decision-tree analysis and had the highest odds ratio in multivariable analysis. Moreover, even among patients with unfavorable ISDR (0 or 1 mutation), younger patients (<60 years) with the wild-type sequence at Core70 and high level of LDL-C (≥120 mg/dl) had a high rate of sustained virological response. The sustained virological response rate of these two subgroups of patients was 83% in the model-building patients and 75% in the validation patients. Thus, patients with high possibility of sustained virological response could be extracted by the combined analysis of ISDR and Core70. These patients may be the best-suited candidates for treatment with the current combination therapy. Conversely, the following patients with 0–1 mutation in ISDR had a low probability of sustained virological response (32–35%): (1) older (>60 years); or (2) younger (<60 years) patients but having mutant-type sequence at Core70; or (3) younger (<60 years) patients having a wild-type sequence at Core70, but having a low level of LDL-C (<120 mg/dl) and advanced fibrosis. These patients may be advised to wait for a more effective therapy. Decision may be made on a case-by-case basis, taking into account the potential risk of disease progression while waiting.

In a previous decision-tree model using simple and noninvasive standard tests that are available readily worldwide [Kurosaki et al., 2010b], the rate of sustained virological response was at most 65–76% among those in the high-probability group. That model focused on use by general physicians in routine general practice, especially where specialized resources, such as liver biopsy or determination of viral sequences, are not available. In that model, younger age, male sex, higher platelet counts, lower alpha-fetoprotein (AFP) levels, and lower GGT levels were identified as favorable predictive parameters. Higher AFP levels and lower platelet counts that are hallmarks of advanced fibrosis [Shiratori and Omata, 2000; Akuta et al., 2007b] were associated with low probability of sustained virological response in that model. On the other hand, the present analysis aimed to clarify the significance of viral factors for pretreatment prediction of sustained virological response, and to build an advanced model that may be used by specialist physicians engaged in the

TABLE II. Multivariable Logistic Regression Analysis for Factors Associated With SVR

Parameter		Odds	95% CI	*P*-value
Age (years)	<60 vs. ≥60	2.28	1.31–3.94	0.003
Sex	Male vs. female	3.36	1.87–5.99	<0.0001
GGT (IU/L)	<40 vs. ≥40	2.65	1.45–4.85	0.002
LDL-C (mg/dl)	≥120 vs. <120	1.79	0.91–3.53	0.094
Platelets (109/L)	≥120 vs. <120	2.69	1.22–5.90	0.014
ISDR mutations	≥2 vs. 0–1	9.92	3.71–26.54	<0.0001
Core70	Wild vs. mutant	1.92	1.07–3.47	0.030

GGT, gamma-glutamyltransferase; LDL-C, low-density-lipoprotein-cholesterol; ISDR, interferon sensitivity-determining region.

treatment of hepatitis. In the present model, stage of fibrosis was selected as a predictive factor, but at lower level of significance than HCV mutations. The predicted rate of sustained virological response in the high-probability group of the present model is higher than that in the previous model (75–83% vs. 65–76%). These results indicate that substitutions in ISDR and Core70 were important pretreatment predictors of sustained virological response. Determination of these viral factors is not available readily in clinical practice, but is of value for improving the accuracy of pretreatment prediction of sustained virological response.

Substitutions in ISDR and Core70 have been reported previously to be associated with efficacy of IFN therapy. The association between the number of substitutions in ISDR and response to therapy was demonstrated originally in patients treated with IFN mono-therapy [Enomoto et al., 1995, 1996; Kurosaki et al., 1997], but recent studies have reported a positive correlation with PEG-IFN and RBV combination therapy as well [Munoz de Rueda et al., 2008; Shirakawa et al., 2008; Ikeda et al., 2009]. Another important viral factor relevant to treatment response is amino acid substitution in Core70. The sequence of this amino acid was reported originally to be associated with nonresponse to therapy [Akuta et al., 2005], but subsequent studies confirmed the positive correlation of a wild-type Core70 with sustained virological response [Akuta et al., 2009]. The multiple logistic regression analysis showed that ISDR and Core70 were independent factors associated with sustained virological response along with host factors. How these important viral factors and other host factors can be combined to predict response to PEG-IFN plus RBV is an important clinical question. Decision-tree modeling can make the response probability apparent by combining all these factors. Some factors that may be associated with treatment outcome, such as levels of ferritin or homocysteine, were not included. This may be a potential limitation of the present study.

It is of interest that a recent study by Li et al. [2010] has shown that a high serum level of LDL-C is linked to the *IL28B* major allele (CC in rs12979860). In that study, a high serum level of LDL-C was associated with sustained virological response, but it was no longer significant when analyzed together with the *IL28B* genotype in multivariate analysis. Thus, the association between treatment response and LDL cholesterol levels in the present study may reflect the underlining link of LDL cholesterol levels to the *IL28B* genotype. Recent reports indicate that the *IL28B* genotype and HCV substitutions are correlated closely [Akuta et al., 2010; Kurosaki et al., 2010c]. Still, Core70 [Akuta et al., 2010] or ISDR [Kurosaki et al., 2010c] were predictors of response to therapy independent of *IL28B* genotype. Future study is needed to elucidate the possible mechanisms underlying the association between HCV sequences and host genetic factors, and also the role of host and viral factors for the prediction of treatment response.

In conclusion, a data mining analysis emphasized the impact of substitutions in ISDR and Core70 on pretreatment prediction of sustained virological response to PEG-IFN plus RBV therapy. A decision-tree model that includes substitutions in ISDR and Core70 of HCV could identify patients with high probability of sustained virological response, and could thereby improve the predictive accuracy over predictions that are based on standard tests.

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特集・C型肝炎―新時代の治療戦略

C型肝炎治療における治療効果予測と副作用対策

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Summary

C型慢性肝炎の治療にあたっては，発癌リスクの高い高度線維化症例では，たとえ高齢者であっても積極的な治療介入が必要である。しかし，一方で副作用による中止減量が治療効果の低下につながることが報告されており，高齢患者に対する抗ウイルス治療導入の適応基準が問題となっている。IL28BやITPAを含めた詳細な宿主因子およびウイルス因子解析により治療効果や副作用を事前予測し，抗ウイルス療法導入症例を慎重に見極めることが重要であり，導入症例では治療完遂を目指し，適宜投薬調整を行い，薬物治療を含めた早期の副作用対策を行うことが重要である。

Key Words　C型慢性肝炎／GWAS／IL28B／ITPA

はじめに

高齢化社会が進むにつれ本邦におけるC型慢性肝炎（CHC）・肝硬変患者の平均年齢は上昇し，高齢患者からの肝発癌が増加しているなかで，治療効果と安全性を的確に評価し治療方針を決定する必要がある。C型肝炎の自然経過をみると，平均8～10年で線維化ステージは1段階上昇し，線維化が進むにつれ発癌率が上昇すること，症例により進展度の早い群と遅い群が存在することが報告されているが[1,2]，発癌リスクの高い高度線維化症例では，たとえ高齢者であっても積極的な治療介入が必要であると考えられる。しかし，一方でCHCに対する根治療法であるインターフェロン（IFN）併用治療は副作用による中止減量で十分な投薬量を確保できないことが治療効果の低下につながることが報告されており，高齢患者に対する抗ウイルス治療導入の適応基準が問題となっている。今後，詳細なウイルスおよび宿主因子解析による効果および副作用予測，それに基づく治療法の選択が，さらに重要性を増してくることが予想される。

C型肝炎の自然経過・治療効果とゲノムワイド解析

2009年ゲノムワイド関連分析法（genome-

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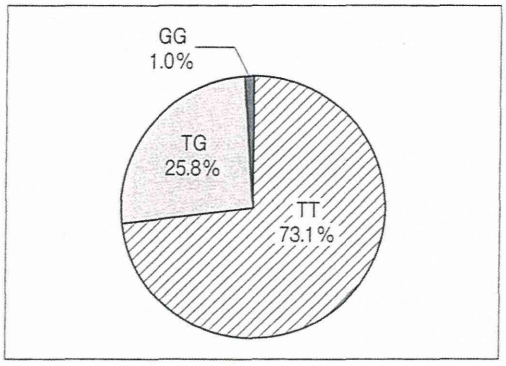

図1　C型慢性肝炎患者におけるIL28B SNP（rs8099917）の頻度
IL28Bゲノタイプの頻度はTT：73.1%，TG：25.8%，GG：1.0%であり，マイナーアリル（G）頻度は0.139であった（自験例）。

wide association study：GWAS）により，19番染色体上のIL28B遺伝子周辺に治療無効に強く関連する一連の有意な遺伝子多型（SNP）が存在することが報告された[3]。治療効果予測とIL28Bの関連を示す同様の報告は，わが国を含めて3つの独立した研究グループからほぼ同時期に報告され，人種を越えた遺伝要因であることが証明され[4,5]，さらにThomasらによりIL-28B SNPがC型急性肝炎の自然治癒率にも関連することが報告された[6]。

IL28B遺伝子座にはIFN治療効果に強く関連する複数のSNPが存在するが，これらはすべてが強い連鎖不平衡を伴っておりハプロタイプを形成している。したがって，最も関連の強い特定の一つのSNP（タグSNP）を調べれば残りのSNPを推測することができる。このタグSNPとしては，現在のところrs12979860[5]，またはrs8099917[3,4]の2ヵ所がde facto standardとして使用され，それぞれ固有の核酸がメジャーアリルおよびマイナーアリルを代表している。SNPの検出法にはいくつかの方法があるが，少数の特定のSNPを検出するにはシーケンス法，Taqmanアッセイなどが一般的に用いられる。ゲノタイピングを含む遺伝子検査では生涯変化しない個人の重要な遺伝学的情報が扱われるため，検査実施時に，個人の遺伝学的情報の保護，検査に用いた生体試料の取り扱い，検査前後の遺伝カウンセリングなどを被検者に十分に説明し，インフォームド・コンセントを取った上で行わなければならない。また厚生労働省の「ヒトゲノム・遺伝子解析研究に関する倫理指針」に則して，しかるべき施設で倫理委員会の承認を得た上で施行しなければいけない。

IL28B多型とPEG-IFN・RBV治療効果

2004年12月より本学および関連施設で行ったペグインターフェロン（PEG-IFN）/リバビリン（RBV）療法の最終効果判定可能であったGenotype 1：323例，Genotype 2：108例を対象にIL28B SNP rs8099917の解析を行い，ウイルス，宿主因子と併せて治療効果との関連を検討した。IL28Bゲノタイプの頻度はTT：73.1%，TG：25.8%，GG：1.0%

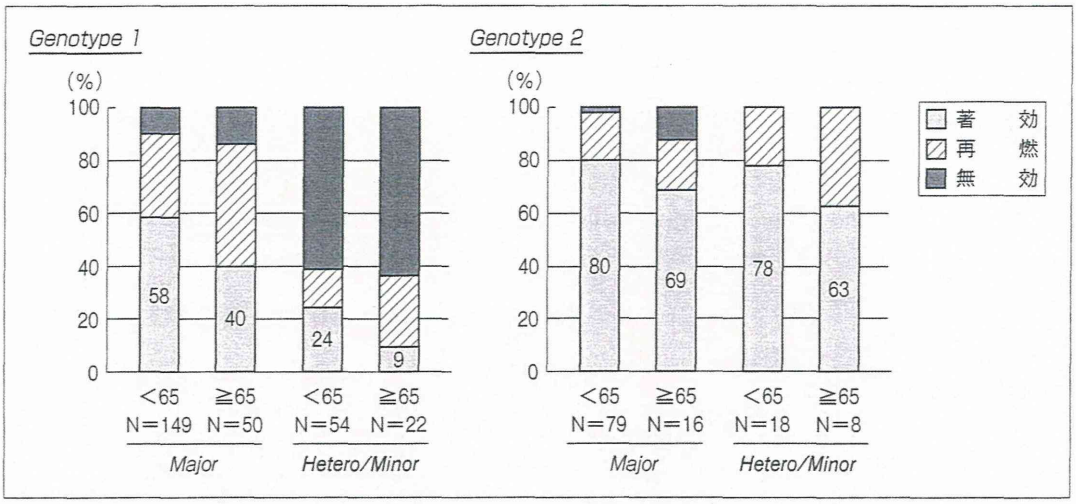

図2　C型慢性肝炎患者におけるIL28B SNP（rs8099917）とPEG-IFN/RBV治療効果（自験例）
IL28B SNPは治療効果と強く関連しており，マイナーアリルを持つ症例では60%がnull responseであった。一方，IFN高感受性のウイルスであるGenotype 2症例ではIL28B多型にかかわらず良好な治療効果が得られた。

であり，マイナーアリル（G）頻度は0.139であった（図1）。IL28B SNPは治療効果と強く関連しており，Genotype 1患者のウイルス学的著効（SVR）は非高齢者TT群58%，高齢者TT群40%に比し，非高齢者TG+GG群24%，高齢者TG+GG群9%と有意に低下し，マイナーアリルを持つ症例では60%がnull responseであった。一方，IFN高感受性ウイルスであるGenotype 2症例はIL28B多型にかかわらず良好な治療効果が得られており，高齢者TG+GG群でも比較的良好なSVRが得られた（図2）。

これまでのPEG-IFN/RBV療法における治療効果の検討では，Genotype 1症例でもISDR変異2以上の症例はGenotype 2同様に治療効果が良好であることは複数施設より報告があり，自験例でもGenotype 1かつISDR変異2以上の症例はIFN高感受性であり，Genotype 2とほぼ同等のSVRが得られることを報告したが[7]，IL28B多型別に治療中の経時的HCV陰性化率を比較するとGenotype 1かつISDR変異2以上の症例やGenotype 2など治療高感受性のウイルスでは，IL28Bが治療効果に与える影響は弱いことが確認された[8]。一方，ISDR変異1以下の症例ではIL28Bマイナーアリル症例で有意に治療効果は低下し，高率にnull responseとなる。STAT-C治療薬の第一号として認可されたテラプレビル（TPV）を加えた3剤併用療法の治験ではIL28Bマイナーアリルの症例で前治療null responseの場合，SVR 30%程度と十分な治療効果は得られておらず，ウイルス側抵抗因子であるコア変異[9,10]と宿主側抵抗因子であるIL28B変異とを組み合わせることで，TPV併用3剤併用療法の効果をより詳細に予測できることも報告されている[11]。これらの知見は臨床上も非常に有意義なデータであるとともに，治療抵抗症例に対する有効な治療法開発に向けて，IL28Bやコア変異がいかに治療抵抗機序へ関与しているか解明することが，今後の最重要事項の一つと考えられる。

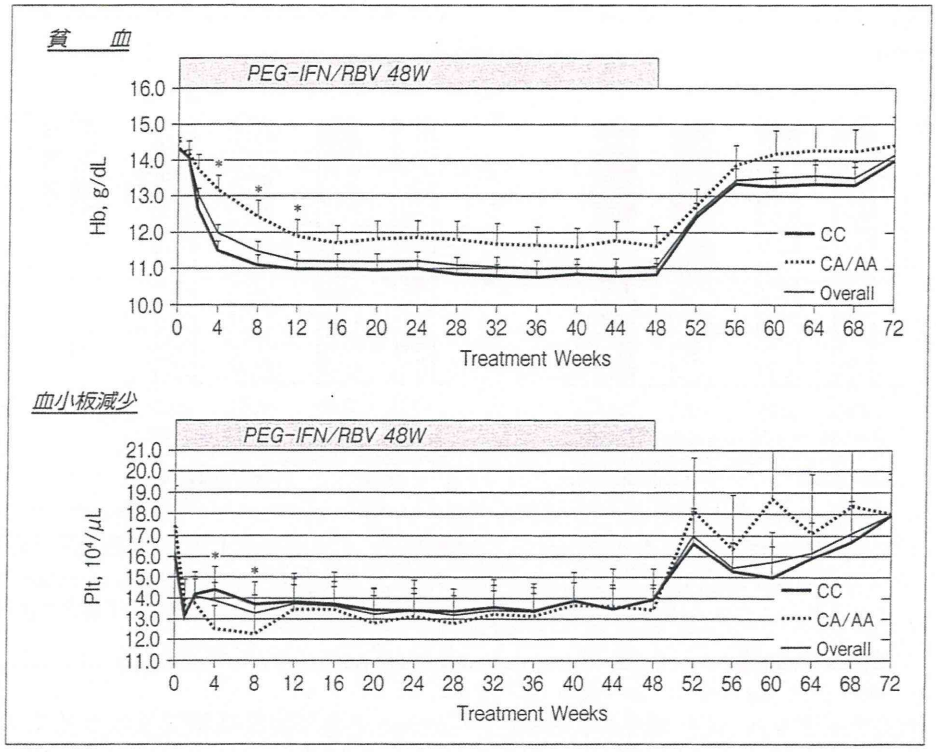

図3　ITPA多型と血球減少

Genotype 1型に対するPEG-IFN/RBV48週治療における経時的な血球減少の推移をITPA別に示してみると，貧血に関しては，ITPAマイナーアリルを持つ症例では治療早期，経過中を通じて貧血は有意に抑制されたが，血小板に関しては逆の傾向を示し，ITPAマイナーアリルを持つ症例では治療早期で血小板低下が顕著であった（＊p＜0.05）。

（文献13より）

ITPAと血球減少

2010年GWASにより，CHC治療における貧血と関連するSNPとして，Inosine triphosphatase（ITPA）遺伝子座の2つのSNP（rs11277354，rs7270101）が同定された[12]。ITPAはITPをIMPに脱リン酸化する酵素であり，マイナーアリルを持つ患者（CA/AA）はITPA活性が低下または欠損している。そもそもRBVによる溶血性貧血は，赤血球に蓄積したRBVによってIMPDH活性が抑制されることで赤血球GTPが減少し，それによりもたらされるATPレベルの低下が抗酸化ストレス作用を減弱させ，溶血をきたすと考えられている。ITPAのマイナーアリルを持つ患者はITPA活性が低いため赤血球内にITPが蓄積しGTP減少を代償するため，ATPレベルの低下が抑えられ溶血も起こりにくくなる。ITPAマイナーアリルを持つCHC患者はPEG-IFN/RBV治療早期の貧血が抑制され，自験例でも治療開始2週，4週のHb低下はITPAマイナーアリル症例で有意に小さいことが確認された（2週：$p=6.6\times10^{-13}$，4週：$p=3.0\times10^{-29}$）[13]。この結果としてRBV減量が必要になる患者の割合やRBV減量までの期間，RBV予定投与量達成率との関連も報告されている。

ITPA SNPはPEG-IFN/RBV治療中の血小板減少とも関連することが知られ，貧血と

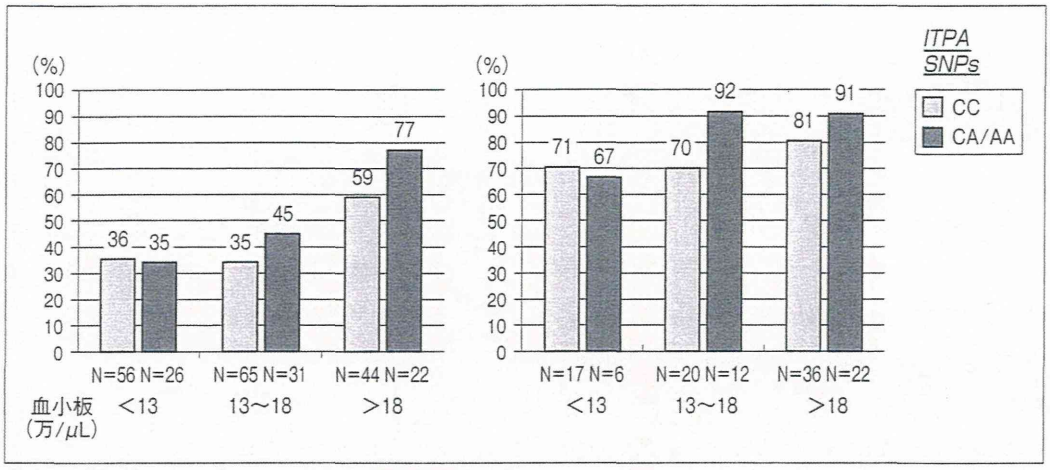

図4　ITPA多型-治療前血小板値と治療効果の関係（自験例，左：Genotype 1，右：Genotype 2）
治療前血小板値が13万/μL以下では，ITPAマイナーアリルを持つ症例はITPAメジャーアリルを持つ症例に比しSVR率は低下した。肝線維化進展のみられる血小板低値例ではITPAマイナーアリルであることが，血小板低下による薬剤減量により治療効果低下につながることが示唆された。

血小板減少は逆の相関であり，マイナーアリルを持つ患者において血小板減少が強くなる[14]。これは貧血の軽度なマイナーアリルでは，溶血性貧血に対する代償機能としての反応性造血亢進が弱くなるためと考えられているが，血小板減少とITPA SNPの関連は貧血と独立であるとの報告もあり，IFN誘導性血小板減少とITPA SNPの間の直接の相互作用も示唆されるなど，今後の解析が待たれる。自験例においてもGenotype 1型に対するPEG-IFN/RBV 48週治療における経時的な血球減少の推移をITPA別に示してみると，貧血に関しては，ITPAマイナーアリルを持つ症例では治療早期，経過中を通じて貧血は有意に抑制されたが，血小板に関しては逆の傾向を示し，ITPAマイナーアリルを持つ症例では治療早期で血小板低下が顕著であった（**図3**）[15]。白血球減少に関する同様の解析では白血球減少とITPA SNPとの関連は認められなかった。

貧血と血小板減少という，投薬量の調節に重要な血球減少が同じ宿主遺伝子で逆の動きをしていることから，実際どちらがより強く投薬調節に影響しているのか，自験例でさらに解析を進めたところ，全体としてはITPAマイナーアリルを持つ症例は貧血になりにくいことからRBV減量例が有意に低く（p＝0.012），ITPA SNPとIFN減量との間には有意な関連は認められなかったが[13]，治療前血小板値が13万/μL以下のGenotype 1症例では，ITPAマイナーアリルを持つ症例はSVRの低下を認め，肝線維化進展のみられ

表1　副作用中止（自験例656例での解析）

	65歳未満	65歳以上
食欲不振・倦怠感	14人	5人
血球減少	8人	0人
皮疹（乾癬含む）	8人	2人
精神症状	8人	4人
IFN網膜症，眼底出血	4人	4人
間質性肺炎（疑い）	4人	2人
悪性腫瘍（HCC含む）治療	6人	2人
甲状腺機能低下症	3人	0人
感染症（骨盤腹膜炎）	1人	1人
合併症悪化（DM，胆石症）	0人	1人
心疾患（胸痛，不整脈）	0人	2人
鼠径ヘルニア	0人	1人
難聴，脱毛	3人	0人

る血小板低値例ではITPAマイナーアリルであることが，血小板低下による薬剤減量により治療効果低下につながることが示唆された（図4）。

その他の副作用

従来のPEG-IFN/RBV併用療法では，上述の血球減少のほかに，さまざまな副作用が添付文書にも記載されているが，自験例で副作用中止の内訳を検討したところ，**表1**に示すように重篤な副作用はなく，いずれも治療中止により速やかに回復した。65歳以上の高齢者は非高齢者に比較して副作用中止は多い傾向にあったが有意差はなく（65歳未満 vs. 65歳以上＝11.8% vs. 15.4%，p＝0.24），適宜投薬調整を行い，薬物治療を含めた早期の副作用対策を行うことが，治療完遂につながるものと思われた。

2011年本邦でもプロテアーゼ阻害薬であるTPVが承認となり，今後HCV治療はdirect-acting antiviral（DAA）製剤を中心とした多剤併用療法の時代を迎えると思われる。TPV併用療法では，従来の2剤治療の副作用に加えて，皮疹，貧血，腎機能障害などが強く出ることが報告されているが，現在治験中の第2世代プロテアーゼ阻害薬は副作用の軽減が期待されており，1日も早い臨床現場への登場が期待される。

おわりに

CHC患者の高齢化が進むわが国においては，IL28BやITPAを含めた詳細な宿主因子およびウイルス因子解析により治療効果や副作用を事前予測し，抗ウイルス療法導入症例を慎重に見極めることが治療の質の向上に重要である。今後，新規薬剤の登場とともに高齢者でも負担の少ない治療法の開発が期待される。PEG-IFN併用療法では根治の見込める患者を高い的中率で選別し，治療高感受性で高い治癒率が期待される場合は治療導入を強く勧め，また治療抵抗性と判定された患者はSTAT-C併用療法，あるいはPEG-IFN-λ療法に振り替えるなど，治療のオーダーメイド化の展開が期待される。

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