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Research Article

Cancer Epidemiology, Biomarkers & Prevention

Prediction of Hepatocellular Carcinoma Development by Plasma ADAMTS13 in Chronic Hepatitis B and C

Hitoshi Ikeda[1,2], Ryosuke Tateishi[2], Kenichiro Enooku[1,2], Haruhiko Yoshida[2], Hayato Nakagawa[1,2], Ryota Masuzaki[2], Yuji Kondo[2], Tadashi Goto[2], Shuichiro Shiina[2], Yukio Kume[1], Tomoaki Tomiya[2], Yukiko Inoue[2], Takako Nishikawa[2], Natsuko Ohtomo[2], Yasushi Tanoue[2], Tomoko Ono[3], Kazuhiko Koike[2], and Yutaka Yatomi[1]

Abstract

Background: Chronic liver injury evokes a wound healing response, promoting fibrosis and finally hepatocellular carcinoma (HCC), in which hepatic stellate cells play an important role. Although a blood marker of hepatic stellate cells is not known, those cells importantly contribute to the regulation of plasma a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 (ADAMTS13) activity, a defect of which causes thrombotic thrombocytopenic purpura.

Methods: Plasma ADAMTS13 was evaluated in chronic hepatitis B or C patients with or without HCC.

Results: Plasma ADAMTS13 activity significantly correlated with serum aspartate aminotransferase and alanine aminotransferase, liver stiffness value, and aspartate aminotransferase-to-platelet ratio index, irrespective of the presence of HCC, suggesting that it may reflect hepatocellular damage and subsequent wound healing and fibrosis as a result of hepatic stellate cell action. During the three-year follow-up period for patients without HCC, it developed in 10 among 81 patients. Plasma ADAMTS13 activity was significantly higher in patients with HCC development than in those without and was a significant risk for HCC development by univariate and multivariate analyses. Furthermore, during the one-year follow-up period for patients with HCC treated with radiofrequency ablation, HCC recurred in 55 among 107 patients. Plasma ADAMTS13 activity or antigen level was significantly higher in patients with HCC recurrence than in those without and was retained as a significant risk for HCC recurrence by multivariate analysis.

Conclusions: Higher plasma ADAMTS13 activity and antigen level was a risk of HCC development in chronic liver disease.

Impact: Plasma ADAMTS13 as a potential marker of hepatic stellate cells may be useful in the prediction of hepatocarcinogenesis. *Cancer Epidemiol Biomarkers Prev; 20(10); 2204–11. ©2011 AACR.*

Introduction

It is well known that chronic wound healing generally provides a microenvironment that gives rise to cancer (1). Indeed, chronic injury in the liver evokes a perpetuating wound healing response, promoting the development of fibrosis and finally hepatocellular carcinoma (HCC; ref. 2). Among the cells in the liver, hepatic stellate cells are known as a main effector of wound healing and fibrosis following liver injury of any etiology (3), however, a useful blood marker to reflect the activity of those cells has not been found yet in the clinical setting.

In this context, we have focused on a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 (ADAMTS13), a defect of which increases unusually large multimers of von Willebrand factor in the plasma, causes platelet thrombosis under high sheer stress, and results finally in thrombotic thrombocytopenic purpura (4–6). With regard to the site of production, *ADAMTS13* mRNA expression was shown exclusively in the liver (7–9) and then both *ADAMTS13* mRNA expression and ADAMTS13 activity were determined primarily in hepatic stellate cells among the liver cells in mice (10). ADAMTS13 expression was also detected in hepatic stellate cells in human and thereby ADAMTS13 is reportedly produced in those cells (11). To elucidate a regulatory mechanism of plasma ADAMTS13 activity, we previously determined that selective hepatic stellate cell damage caused by dimethylnitrosamine in rats leads to decreased plasma ADAMTS13 activity (12). On the other hand, plasma ADAMTS13 activity was upregulated during the process of liver fibrosis due to cholestasis caused by bile duct ligation and steatohepatitis induced by a choline-deficient L-amino acid–defined diet in rats, in which hepatic stellate cells actively proliferate (13).

Authors' Affiliations: Departments of [1]Clinical Laboratory Medicine and [2]Gastroenterology, Graduate School of Medicine, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo; and [3]Mitsubishi Chemical Medicine Corporation, Ohwadashinden, Yachiyo-shi Chiba, Japan

Corresponding Author: Hitoshi Ikeda, Department of Clinical Laboratory Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Phone: 81-3-3815-5411; Fax: 81-3-5689-0495; E-mail: ikeda-1im@h.u-tokyo.ac.jp

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These results indicate that hepatic stellate cells play an important role in the regulation of plasma ADAMTS13 activity, although other sources of ADAMTS13 were reported (14–16).

On the basis of these previous findings, we wondered whether plasma ADAMTS13 could be a blood marker of hepatic stellate cells. To examine this, plasma ADAMTS13 was evaluated in patients with chronic hepatitis B or C, in whom chronic wound healing and fibrosis are observed with a high risk of HCC development (17), in which hepatic stellate cells play an important role (3). In this study, we have found that plasma ADAMTS13 was increased in relation with serum levels of aspartate aminotransferase (AST) or alanine aminotransferase (ALT), and the markers of liver fibrosis and that higher plasma ADAMTS13 was more frequently found in patients who later developed HCC.

Patients and Methods

Patients

Eighty-one patients with chronic hepatitis B and C, who visited the Department of Gastroenterology, the University of Tokyo Hospital, Tokyo, Japan, between April and August in 2007, were first enrolled. Chronic hepatitis B was defined as hepatitis B surface antigen (HBsAg) positivity, and chronic hepatitis C was defined as serum anti-hepatitis C virus antibody (HCVAb) positivity and a detectable HCV RNA level, having persistent liver damage for more than 6 months. Patients with HCC at the time of enrollment or with past history of HCC were excluded from this analysis.

Next, between July and September in 2009, 107 consecutive patients with chronic hepatitis B and C with HCC who were scheduled to undergo radiofrequency ablation (RFA) for HCC were enrolled.

All the studies were carried out in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and were approved by the Institutional Research Ethics Committee of the Faculty of Medicine of the University of Tokyo. Informed consent from the patients was obtained for the use of the samples in this study.

Measurement of ADAMTS13 activity

ADAMTS13 enzymatic activity was measured manually using a chromogenic ELISA kit, ADAMTS13-act-ELISA (Kainos Inc./Technoclone GmbH), which captures products cleaved by ADAMTS13 using a sandwich method, and expressed as percentage of healthy control. The very high correlation of the values measured by classical VWF multimer assay and this novel chromogenic ADAMTS13-act-ELISA was reported previously (18).

Measurement of ADAMTS13 antigen level

ADAMTS13 antigen level was measured by a latex photometric immunoassay, in which suspended polystyrene latex particles coated with polyclonal antibody F(ab')2 fragment against ADAMTS13 were employed. Antisera against ADAMTS13 were obtained by immunization with pCAG-ADAMTS13 plasmid DNA (donated by Dr. Soejima from The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) using electroporation. Latex agglutination was analyzed using LPIA-A700 (Mitsubishi Chemical Medience Co.), a fully automated quantitative latex photometric immunoassay instrument. ADAMTS13 antigen level in sample of each patient was expressed as the percentage of that in pooled normal human plasma.

Measurement of liver stiffness

Liver stiffness was measured by transient elastography (FibroScan 502; EchoSens) as described previously (19–21). Briefly, the measurements were done in the right lobe of the liver through the intercostal spaces, with the patient lying in the dorsal decubitus position, and were considered valid only when at least 10 acquisitions were successful, with a success rate of at least 60% and the ratio of interquartile range to the median value was larger than 30%. Liver stiffness value was expressed in kilopascals (kPa).

Patient follow-up and diagnosis of HCC

Patients without HCC were followed up at the outpatient clinic with monthly blood tests, including tumor markers and ultrasonography every 4 to 6 months. Contrast-enhanced computed tomography (CT) was done when serum alpha-fetoprotein (AFP) levels and/or plasma des-gamma-carboxy prothrombin (DCP) levels showed an abnormal rise and/or tumors were detected as possible HCC on ultrasonography. The diagnosis of HCC was based on typical findings on CT, that is, hyperattenuation in the arterial phase and hypoattenuation in the equilibrium phase (22–24).

The end points consisted of the interval between the first measurement of plasma ADAMTS13 activity and the detection of HCC development, death without HCC development, or the last examination until 30 July 2010, whichever came first. Death without HCC development was treated as censored data.

Radiofrequency ablation, patient follow-up, and analysis of HCC recurrence

The detailed procedure of RFA was meticulously described elsewhere (25). The indication criteria for RFA consisted of total bilirubin concentration less than 3.0 mg/dL and platelet count more than $5 \times 10^4/\mu L$. Patients with portal vein tumor thrombosis, massive refractory ascites, or extrahepatic metastasis were excluded. In general, RFA was done on patients with 3 or fewer lesions, each less than 3.0 cm in diameter. However, RFA was also done on patients who did not meet these criteria when complete ablation could be anticipated in all tumors without deteriorating liver function. After RFA, dynamic CT was done to evaluate treatment efficacy. Complete ablation was defined as hypoattenuation of the whole lesion together with the surrounding liver parenchyma as a safety margin.

Patients received additional RFA until complete ablation was confirmed for each HCC nodule.

The follow-up consisted of monthly blood tests and monitoring of tumor markers at the outpatient clinic, with ultrasonography and dynamic CT scan done every 4 months. HCC recurrence was diagnosed on the basis of the criteria as described earlier.

The end points consisted of the interval between the first ablation and the detection of HCC recurrence, death without recurrence, or the last examination until 30 September 2010, whichever came first. Death without recurrence was treated as censored data.

Statistical analysis

Comparisons between groups were made using Student's t test or χ^2 test. The correlation between 2 groups, in which the data points were distribution free, was analyzed using Spearman's rank correlation coefficient (ρs). The cumulative incidence of HCC was estimated using the Kaplan–Meier method. In the analysis of risk factors for hepatocarcinogenesis, we tested the following variables obtained at the time of entry in univariate and multivariate Cox proportional hazard regression analyses: age, sex, positivity for HBsAg and HCVAb, albumin, total bilirubin, AST, ALT, prothrombin time, platelet counts, liver stiffness value, APRI, AFP, DCP, and either plasma ADAMTS13 activity or antigen level. Multichotomous categorical variables were represented by corresponding binary dummy variables. Factors that had a $P < 0.2$ in univariate analysis were subsequently included in a multivariate Cox proportional hazard regression model, with stepwise selection of variables based on the Akaike information criterion (AIC). Data processing and analysis were done by using the S-plus Ver. 7 (TIBCO Software Inc.).

Results

Characteristics of the patients without HCC and correlation between plasma ADAMTS13 activity and clinical variables

The characteristics of the patients, who were first enrolled for the measurement of plasma ADAMTS13 activity, are summarized in Table 1. There were 21 patients with chronic hepatitis B and 60 patients with chronic hepatitis C. All the patients were outpatients without HCC at the time of enrollment and past history of HCC.

Plasma ADAMTS13 activity in these patients was 114.0 ± 45.4% (mean ± SD) of control, ranged from 28.0% to 221.5%, as shown in Table 1. Relationships between plasma ADAMTS13 activity and clinical variables are shown in Table 2. The significant correlations were determined between plasma ADAMTS13 activity and serum AST and ALT levels ($P < 0.001$). On the other hand, the significant correlations were also determined between plasma ADAMTS13 activity and the variables predicting the stage of liver fibrosis, liver stiffness value ($P < 0.001$), and aspartate aminotransferase-to-platelet ratio index (APRI; $P = 0.027$). Of note is the finding that plasma ADAMTS13 activity significantly correlated with serum AFP level ($P < 0.001$).

HCC development and risk analysis

Next, a potential link between plasma ADAMTS13 activity and HCC was examined. During the mean follow-up period of 35.4 months, one patient had been lost to follow-up evaluation and one patient died before HCC was identified. By the end of the follow-up, HCC developed in 10 patients, among whom 2 patients died of HCC. The cumulative incidence rates of HCC at

Table 1. Characteristics of patients without HCC or with HCC

Variables	Patients without HCC	Patients with HCC
Age (y)	63 ± 12 (23–85)	68.9 ± 8.5 (43–86)
Man/Woman	49/32	68/39
HBV/HCV	21/60	15/92
Albumin (g/dL)	4.1 ± 0.4 (3.1–4.9)	3.7 ± 0.6 (2.0–5.1)
AST (U/L)	48 ± 35 (3–270)	61.4 ± 39.2 (16–289)
ALT (U/L)	53 ± 66 (11–542)	54.1 ± 38.6 (11–276)
Platelet count (× 10^4/μL)	15.2 ± 6.3 (3.4–30.8)	10.8 ± 4.7 (3.4–25.2)
Prothrombin time (%)	87.7 ± 11.6 (49.2–100.0)	98.3 ± 5.2 (73.0–100.0)
Plasma ADAMTS13 activity (%)	114.0 ± 45.4 (28.0–221.5)	125.0 ± 32.4 (62.0–223.0)
Plasma ADAMTS13 antigen level (%)	Not measured	128.6 ± 39.6 (48.9–258.3)
Liver stiffness (kPa)	11.4 ± 9.2 (3.1–48.0)	28.5 ± 17.9 (6.1–75.0)
APRI	1.07 ± 1.00 (0.08–5.92)	1.89 ± 1.44 (0.22–7.81)
AFP (ng/mL)	12.6 ± 38.2 (1–319)	99.4 ± 361.2 (1–3,399)
DCP (mAu/mL)	18.1 ± 17.6 (10–165)	70.7 ± 194.3 (8–1,462)
Maximum size of HCC (mm)	Not available	17.8 ± 6.0 (6.0–33.0)

NOTE: Values are expressed as the mean ± SD (range).

Table 2. Relation between plasma ADAMTS13 activity and clinical variables in patients without HCC or with HCC

Variables	Patients without HCC		Patients with HCC	
	ρs[a]	*P*	ρs[a]	*P*
Age	−0.067	0.554	−0.030	0.760
AST (U/L)	0.360	<0.001	0.531	<0.001
ALT (U/L)	0.426	<0.001	0.519	<0.001
Albumin (g/dL)	−0.114	0.309	−0.146	0.133
Platelet count (× 10^4/μL)	−0.091	0.418	−0.129	0.185
Prothrombin time (%)	−0.343	<0.005	−0.029	0.764
Liver stiffness (kPa)	0.379	<0.001	0.216	0.026
APRI	0.245	0.027	0.403	<0.001
AFP (ng/mL)	0.465	<0.001	0.554	<0.001
DCP (mAu/mL)	0.135	0.230	−0.281	0.003
Size of HCC (mm)[b]	Not available		−0.075	0.571

[a]Spearman's rank correlation coefficient.
[b]Analyzed in patients with single nodule of HCC.

1, 2, and 3 years estimated by the Kaplan–Meier method were 4.9%, 9.1%, and 11.1%, respectively, as shown in Figure 1A. In these patients who developed HCC, plasma ADAMTS13 activity was significantly higher than that in patients who did not develop HCC ($P < 0.001$), as depicted in Table 3; plasma ADAMTS13 activity was 161.9 ± 33.8% in patients who developed HCC and 108.8 ± 42.2% in patients who did not develop HCC. Liver stiffness value was also significantly higher in patients with HCC development, and serum albumin level and prothrombin time (%) were significantly lower in those patients. Then, univariate analyses showed that the higher plasma ADAMTS13 activity was a risk for HCC development ($P < 0.001$; Table 4). Other significant risk factors for HCC included lower albumin level, higher ALT level, lower prothrombin time (%), and higher liver stiffness value. Next, stepwise variable selection with AIC was used to find the best model in multivariate analysis (Table 4), which revealed that the higher plasma ADAMTS13 activity ($P = 0.03$) and the higher liver stiffness value ($P = 0.03$) were the significant risk factors for HCC. These results suggest that plasma ADAMTS13 activity may predict HCC development in patients with chronic hepatitis B or C.

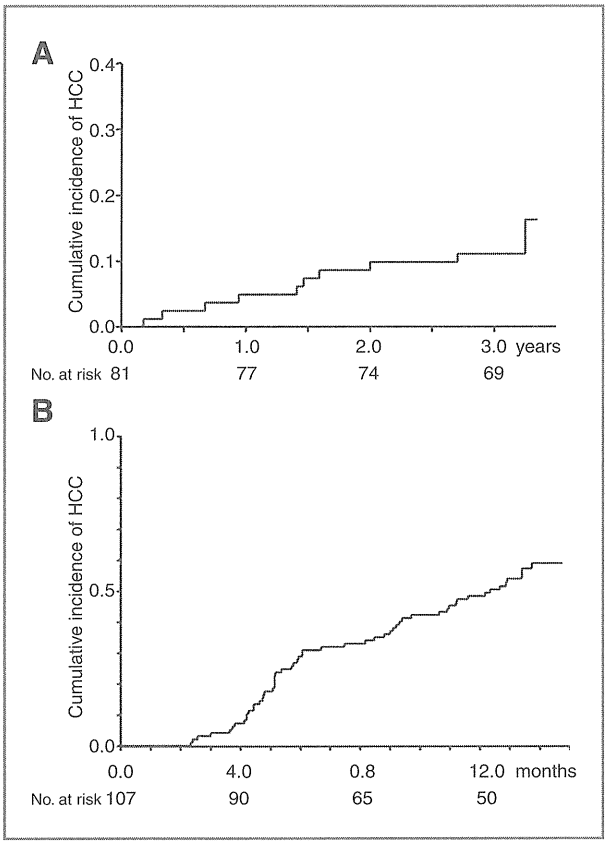

Figure 1. Cumulative incidence of HCC development (A) and recurrence (B).

Then, the relation between plasma ADAMTS13 activity and HCC development was analyzed separately in patients with chronic hepatitis B and with chronic hepatitis C. In patients with chronic hepatitis B ($n = 20$), plasma ADAMTS13 activity was significantly higher in patients who developed HCC than that in patients who did not develop HCC ($P < 0.005$); plasma ADAMTS13 activity was 158.9 ± 36.7% in patients who developed HCC and 95.3 ± 35.0% in patients who did not develop HCC. Then, univariate analyses showed that the higher plasma ADAMTS13 activity was a risk for HCC development ($P < 0.001$), and further multivariate analysis revealed that the higher plasma ADAMTS13 activity was a significant risk factor for HCC ($P = 0.03$) in these patients. In patients

Table 3. Characteristics of patients according to HCC development and recurrence

Variables	Development (−)	Development (+)	*P*	Recurrence (−)	Recurrence (+)	*P*
Age (y)	63.0 ± 12.4	61.4 ± 12.4	0.695	70.2 ± 7.5	68.3 ± 9.2	0.267
Man/Woman	40/29	6/4	0.82	23/19	36/19	0.39
HBV/HCV	16/53	4/6	0.45	7/35	7/48	0.80
Albumin (g/dL)	4.1 ± 0.3	3.8 ± 0.6	0.024	3.8 ± 0.5	3.6 ± 0.6	0.296
AST (IU/L)	46.9 ± 36.6	55.1 ± 23.0	0.494	61.0 ± 46.5	60.5 ± 33.3	0.951
ALT (IU/L)	52.0 ± 68.8	63.2 ± 46.9	0.620	56.9 ± 46.7	49.7 ± 29.5	0.359
Platelet count ($\times 10^4/\mu L$)	15.7 ± 6.5	12.2 ± 3.9	0.097	10.9 ± 5.5	10.5 ± 4.3	0.667
Prothrombin time (%)	89.5 ± 10.5	74.7 ± 11.6	<0.001	98.9 ± 3.5	97.5 ± 6.5	0.188
Plasma ADAMTS13 activity (%)	108.8 ± 42.2	161.9 ± 33.8	<0.001	116.8 ± 28.5	130.0 ± 30.8	0.039
Plasma ADAMTS13 antigen (%)	Not measured	Not measured		118.9 ± 35.4	134.3 ± 36.1	0.037
Liver stiffness (kPa)	9.2 ± 5.7	22.6 ± 13.7	<0.001	23.4 ± 15.0	30.6 ± 18.4	0.053
APRI	1.03 ± 1.03	1.35 ± 0.80	0.35	1.57 ± 0.81	1.47 ± 0.77	0.517
AFP (ng/mL)	10.7 ± 38.1	27.4 ± 41.4	0.203	131.5 ± 546.0	81.2 ± 158.1	0.521
DCP (mAu/mL)	17.9 ± 18.8	19.7 ± 8.2	0.766	114.0 ± 297.6	41.7 ± 64.3	0.082

NOTE: Values are expressed as the mean ± SD (range).

with chronic hepatitis C ($n = 59$), plasma ADAMTS13 activity was significantly higher in patients who develop HCC than that in patients who did not develop HCC ($P < 0.01$); plasma ADAMTS13 activity was 163.9 ± 35.2% in patients who developed HCC and 112.9 ± 43.6% in patients who did not develop HCC. Then, univariate analyses showed that the higher plasma ADAMTS13 activity was a risk for HCC development ($P < 0.001$), and multivariate analysis revealed that the higher plasma ADAMTS13 activity was a significant risk factor for HCC ($P = 0.02$) in these patients.

Characteristics of the patients with HCC and correlation between plasma ADAMTS13 activity or antigen level and clinical variables

To further examine a potential link between plasma ADAMTS13 and HCC, plasma ADAMTS13 activity and antigen level were measured in 107 patients with HCC. Their characteristics are summarized in Table 1. There were 15 patients with chronic hepatitis B and 92 patients with chronic hepatitis C.

Plasma ADAMTS13 activity in these patients was 124.9% ± 32.3% (mean ± SD) of control, ranged from 62.0% to 223.0%, and plasma ADAMTS13 antigen level, 128.3% ± 39.3% (mean ± SD) of control, ranged from 48.9% to 258.3%, respectively (Table 1). Of note, the strong correlation between plasma ADAMTS13 activity and plasma ADAMTS13 antigen level was observed (Spearman's rank; $\rho s = 0.803$, $P < 0.00001$, $n = 107$). Relationships between plasma ADAMTS13 activity and clinical variables are shown in Table 2. Same as in patients without HCC, the significant correlations were determined between plasma ADAMTS13 activity and serum AST and ALT levels ($P < 0.001$), liver stiffness value ($P = 0.026$), APRI ($P < 0.001$), and serum AFP level ($P < 0.001$). Of note, there was no significant correlation between plasma ADAMTS13 activity and maximum tumor size in patients with single nodule, suggesting that plasma ADAMTS13 activity is not a tumor marker of HCC.

Table 4. Risk factors for HCC development–univariate and multivariate analyses

Variable	HR (95% CI)	*P*
Univariate analysis		
ADAMTS13 (per 10% increase)	1.29 (1.11–1.50)	<0.001
Age (per 1 year increase)	0.990 (0.943–1.04)	0.68
Sex (male vs. female)	1.07 (0.563–2.02)	0.84
Hepatitis virus (HCV vs. HBV)	0.718 (0.380–1.36)	0.31
Albumin (per 1 g/dL increase)	0.208 (0.0477–0.905)	0.04
AST >40 U/L	1.73 (0.881–3.41)	0.11
ALT >40 U/L	2.06 (1.05–4.07)	0.04
PLT <15 $\times 10^4/\mu L$	1.97 (0.908–4.29)	0.09
Prothrombin time (%; per 10% increase)	0.490 (0.324–0.743)	<0.001
Liver stiffness (per 10% increase)	1.16 (1.07–1.26)	<0.001
APRI (per 10% increase)	1.05 (0.983–1.13)	0.14
AFP >20 ng/mL	1.71 (0.785–3.71)	0.18
DCP >40 mAU/mL[a]	NA	
Multivariate analysis		
ADAMTS13 (per 10% increase)	1.20 (1.02–1.40)	0.03
Liver stiffness (per 10% increase)	1.12 (1.01–1.23)	0.03

[a]Not accessed as only DCP was more than 40 mAU/mL in only 1 patient.

HCC recurrence and risk analysis

During the follow-up period of 12 months, 1 patient died without HCC. Two patients who developed extrahepatic recurrence and 3 patients who developed recurrence at a site adjacent to the treated site were excluded from the analysis. Four patients who were treated with IFN were not analyzed because IFN is known to reduce the risk of HCC development in chronic hepatitis B and C (26, 27). By the end of the follow-up, HCC recurrence was determined in 55 patients. The cumulative recurrence rates of HCC by the Kaplan–Meier method are shown in Figure 1B. The characteristics of patients with or without HCC recurrence are shown in Table 3. Among the various parameters, plasma ADAMTS13 activity ($P = 0.039$) and antigen level ($P = 0.037$) were significantly higher in patients with HCC recurrence than those in patients without HCC recurrence (Table 3). No significant differences were determined in other parameters between patients with and without HCC recurrence. Although there was no significant risk factor for HCC recurrence in univariate analyses (Table 5), plasma ADAMTS13 activity was retained as a significant risk factor of HCC recurrence ($P = 0.028$) in the multivariate Cox proportional hazard model, as shown in Table 5. When plasma ADAMTS13 antigen level was analyzed instead of plasma ADAMTS13 activity level, plasma ADAMTS13 antigen level was also a significant risk factor of HCC recurrence ($P = 0.007$) in multivariate analysis. These results suggest that plasma ADAMTS13 activity may predict HCC recurrence in patients with chronic hepatitis B or C.

Table 5. Risk factors for HCC recurrence–univariate and multivariate analyses

Variable	HR (95%CI)	*P*
Univariate analysis		
ADAMTS13 activity (per 10% increase)	1.106 (0.997–1.228)	0.052
Age (per 1 year increase)	0.982 (0.953–1.013)	0.25
Sex (male vs. female)	1.22 (0.70–2.13)	0.49
Hepatitis virus (HCV vs. HBV)	1.10 (0.50–2.44)	0.81
Albumin (per 1g/dL increase)	0.723 (0.432–1.210)	0.22
AST > 40 IU/L	1.35 (0.75–2.42)	0.31
ALT > 40 IU/L	1.00 (0.58–1.71)	0.99
PLT < 15 × 10^4/μL	1.25 (0.65–2.43)	0.51
Prothrombin Activity (per 10% increase)	0.88 (0.42–1.07)	0.20
Liver stiffness (per 10% increase)	1.12 (0.98–1.28)	0.11
APRI (per 10% increase)	1.00 (0.973–1.04)	0.81
AFP > 20 ng/mL	1.24 (0.73–2.11)	0.42
DCP > 40 mAU/mL	1.17 (0.64–2.14)	0.62
Multivariate analysis		
ADAMTS13 activity (per 10% increase)	1.14 (1.01–1.29)	0.028

The relation between plasma ADAMTS13 activity and HCC recurrence was also analyzed separately in patients with chronic hepatitis B and with chronic hepatitis C. In patients with chronic hepatitis B ($n = 14$), plasma ADAMTS13 activity or antigen level was not different between patients with (105.0 ± 34.0% or 97.3 ± 24.7%) and without HCC recurrence (104.0 ± 16.3% or 98.9 ± 14.4%), possibly because the number of patients analyzed was small. On the other hand, in patients with chronic hepatitis C ($n = 83$), plasma ADAMTS13 activity or antigen level was significantly higher in patients with HCC recurrence (133.2 ± 28.9% or 139.7 ± 34.4%) than that in patients without HCC recurrence (119.4 ± 29.8% or 122.9 ± 37.1; $P = 0.037$ or $P = 0.036$). Multivariate analysis revealed that the higher plasma ADAMTS13 activity or antigen level was a significant risk factor for HCC ($P = 0.024$ or $P = 0.005$) in these patients.

Discussion

In the current study, plasma ADAMTS13 activity or antigen level significantly correlated with serum AST and ALT levels and also the variables predicting the stage of liver fibrosis, liver stiffness value, and APRI in patients with chronic hepatitis B or C, irrespective of the presence of HCC. Serum levels of AST and ALT reflect hepatocellular damage, and higher hepatocellular damage generally induces a higher wound healing response. Thus, our current findings may be in line with our speculation that plasma ADAMTS13 activity or antigen level reflects the activity of hepatic stellate cells as a main effector of wound healing and fibrosis in the liver.

Major finding of this study is that the higher plasma ADAMTS13 activity or antigen level was a significant risk factor for HCC development. With regard to HCC development among patients with chronic hepatitis B or C without the past history of HCC, plasma ADAMTS13 activity was higher in the patients who developed HCC than in those who did not develop HCC. Among the various clinical parameters, univariate analysis revealed that the higher plasma ADAMTS13 activity was a significant risk factor for HCC development. Then, multivariate analysis showed that the higher plasma ADAMTS13 activity was a significantly predicting factor for hepatocarcinogenesis, independent of other significant risk factors for HCC development, including the variables predicting the stage of liver fibrosis. This potential link between plasma ADAMTS13 activity and HCC development was further observed in the analysis of HCC recurrence: the patients who had HCC recurrence during the 1-year follow-up period had also significantly higher plasma ADAMTS13 activity or antigen level than those who did not have HCC recurrence. Then, only plasma ADAMTS13 activity or antigen level was retained in the multivariate Cox proportional hazard model as a significant risk factor of recurrence.

Then, we wondered how HCC development might be predictable by the activity or antigen level of plasma ADAMTS13, whose source is mainly hepatic stellate cells, as a key player of liver fibrosis. To explain this, the notion that advanced liver fibrosis is the strong risk factor for HCC development (17) may be important. Furthermore, the recent evidence suggests a potential direct link between hepatic stellate cells and HCC (3), as follows.

It is well known that HCC usually develops in the liver already suffering from chronic liver disease (2). In particular, HCV-related cirrhosis is associated with an extremely high risk of HCC development, with a reported annual incidence ranging between 3% and 8% (28–30). Thus, advanced liver fibrosis is one of the strongest risk factors for HCC development. In fact, the higher liver stiffness value is reportedly a strong risk for HCC development (21). In this study, a significant correlation was observed between plasma ADAMTS13 activity or antigen level and the variables predicting the stage of liver fibrosis such as liver stiffness value. Thus, we have first speculated that plasma ADAMTS13 activity is retained as a risk factor for HCC development by univariate analysis because plasma ADAMTS13 activity may reflect liver fibrosis. However, the higher plasma ADAMTS13 activity was a significant risk factor for HCC development, independent of liver stiffness value by multivariate analysis. Furthermore, in the analysis of HCC recurrence, plasma ADAMTS13 activity or antigen level was retained as a significant risk for HCC development, but not liver stiffness value, by multivariate analysis. The current finding that plasma ADAMTS13 activity or antigen level significantly correlated with serum AST and ALT levels may explain this. Of note, it was previously shown that the higher serum ALT is associated with the higher rate of incidence of HCC development (31) and HCC recurrence after the surgical treatment (32) in HCV-related cirrhosis, suggesting that more hepatocellular damage increases a risk for HCC development in the liver of the same stage of fibrosis. Because plasma ADAMTS13 activity or antigen level reflect hepatocellular damage and subsequent wound healing as well as liver fibrosis stage, plasma ADAMTS13 activity, or antigen level may act distinctly from liver stiffness value in the risk analysis of HCC development.

Alternatively, the prediction of HCC development by plasma ADAMTS13 activity or antigen level may be explained by a potential direct link between hepatic stellate cells and HCC, which has been recently reported (3). This concept is suggested based on the findings that hepatic stellate cells express the stem cell marker of CD133 (33) and both hedgehog (34, 35) and Wnt signaling (36) are found in hepatic stellate cells, two pathways implicated in stem cell differentiation and cancer (37). Furthermore, the direct promotion of tumorigenicity of HCC by hepatic stellate cells has been reported (38).

In human studies, the alteration of plasma ADAMTS13 activity in chronic liver disease has already been reported (39–44). In patients with liver cirrhosis, plasma ADAMTS13 activity was shown to be decreased (39) in relation to the severity of cirrhosis (44), although the wide range of values were detected compared with normal controls (43). In contrast, Lisman and colleagues showed that plasma ADAMTS13 activity in patients with liver cirrhosis was highly variable and not significantly different from that in normal controls (42). In line with the latter report, plasma ADAMTS13 activity in chronic hepatitis B and C was variable in the current study. We speculate that these distinct results of plasma ADAMTS13 activity in chronic liver disease may be caused by the characteristics of the patients enrolled in the analysis. The patients with reduced plasma ADAMTS13 activity in the previous reports (39, 43, 44) might have minimal hepatitis activity, that is, minimal wound healing response. Highly variable activity of plasma ADAMTS13 in liver cirrhosis (42) might also be explained by the variable hepatitis activity in those patients. This issue should be further clarified.

In conclusion, the higher plasma ADAMTS13 activity or antigen level was a significantly independent risk factor for HCC development in chronic hepatitis B or C, suggesting that plasma ADAMTS13 activity and antigen level may be useful in the prediction of hepatocarcinogenesis in chronic liver disease. It should be further evaluated whether plasma ADAMTS13 activity and antigen level could be useful as a predictor of HCC development with a larger sample size and also with other etiology of underlying chronic liver disease such as NASH.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Original Article

Cancer preventive effect of pegylated interferon α-2b plus ribavirin in a real-life clinical setting in Japan: PERFECT interim analysis

Sumio Watanabe,[1] Nobuyuki Enomoto,[2] Kazuhiko Koike,[3] Namiki Izumi,[4] Hajime Takikawa,[5] Etsuko Hashimoto,[6] Fuminori Moriyasu,[7] Hiromitsu Kumada,[8] Michio Imawari[9] and PERFECT Study Group

[1]*Department of Gastroenterology, Juntendo University School of Medicine, Tokyo,* [2]*First Department of Internal Medicine, Faculty of Medicine, University of Yamanashi, Yamanash,* [3]*Department of Gastroenterology, Graduate School of Medicine, the University of Tokyo, Tokyo,* [4]*Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo,* [5]*Department of Medicine, Teikyo University School of Medicine, Tokyo,* [6]*Department of Medicine and Gastroenterology, Tokyo Women's Medical University, Tokyo,* [7]*Department of Gastroenterology and Hepatology, Tokyo Medical University, Tokyo,* [8]*Department of Hepatology, Toranomon Hospital, Tokyo,* [9]*Department of Gastroenterology, Showa University School of Medicine, Tokyo, Japan*

Aim: This study was conducted to clarify the incidence of hepatocellular carcinoma (HCC) and the factors contributing to its occurrence by following chronic hepatitis C patients who received pegylated interferon (PEG-IFN) α-2b plus ribavirin (RBV) combination therapy.

Methods: Patients who received PEG-IFN α-2b and RBV combination therapy with no history of HCC or HCC within 3 months after the start of treatment were observed for the onset of HCC at 67 centers.

Results: Sustained virological response (SVR) was observed in 999 (53.5%) of 1865 patients eligible for analysis. During the observation period (median duration: 4 years and 3 months), HCC developed in 59 patients (3.1%). A significant difference was observed in the 5-year cumulative incidence of HCC between SVR and non-SVR patients (1.1% vs. 7.1%). Factors contributing to HCC selected in multivariate analysis were therapeutic efficacy, sex, age, alanine aminotransferase (ALT) level at 24 weeks after the end of treatment, and platelet count. Non-SVR patients with ALT improvement after the end of treatment had a significantly lower 5-year cumulative incidence of HCC than those without (3.4% vs. 11.0%). HCC developed in 10 patients who achieved SVR, and multivariate analysis indicated that ALT level at 24 weeks after the end of treatment was the only significant factor contributing to HCC.

Conclusion: Several known risk factors for HCC contributed to HCC in patients who received PEG-IFN α-2b and RBV combination therapy, and ALT abnormality after the end of treatment contributes to the onset of HCC in both non-SVR and SVR patients.

Key words: alanine aminotransferase, chronic hepatitis C virus, hepatocellullar carcinoma, pegylated inteferon, ribavirin

Abbreviations: AFP, alpha fetoprotein; ALT, alanine aminotransferase; BR, biochemical response; CHC, chronic hepatitis C; HCC, hepatocellular carcinoma; IFN, interferon; LVR, late virological response; NR, no response; NVR, non-virological response; PEG-IFN, pegylated interferon; ; RBV, ribavirin; SVR, sustained virological response; TR, transient response.

Correspondence: Dr Sumio Watanabe, Department of Gastroenterology, Juntendo University School of Medicine, 2-1-1Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. Email: sumio@juntendo.ac.jp

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INTRODUCTION

THE INCREASE IN the incidence of hepatocellular carcinoma (HCC) in Japan peaked in 2004 and is now in a declining trend.[1] The HCC mortality rate, however, is still particularly high among developed countries,[2] and even now nearly 35 000 people die

annually from HCC. In Japan, about 70% of patients diagnosed with HCC are positive for hepatitis C virus antibody.[3] The hepatitis C virus infection rate[2] and incidence of HCC both increase with the age of the patient,[4] and curing chronic hepatitis C (CHC) to reduce HCC and deaths due to HCC is a pressing issue.

With the discovery of interferon (IFN), CHC became a curable disease, and with the addition of ribavirin (RBV), therapeutic outcomes have improved dramatically. Currently, about 50%[5–8] of patients with HCV genotype 1b and high virus load and more than 80%[9] of genotype 2 patients achieve sustained virologic response (SVR), and the SVR rate is reported to improve further with long-term treatment[10,11] and with combination therapy plus a statin.[12]

The efficacy achieved with these IFN therapies is also reported to lead to the inhibition of the onset of HCC and deaths due to HCC[13-19], but only a few reports are available of long-term observation of patients receiving PEG-IFN α plus RBV combination therapy.

We therefore examined the HCC preventive effect of combination therapy in 1865 patients who received PEG-IFN α-2b and RBV.

METHODS

Patients and treatment

PERFECT (THE PEG-IFN and Ribavirin, Find Evidence of Chronic Hepatitis C Therapy in Tokyo) Study Group, consisting of 67 centers in Tokyo and Yamanashi Prefecture, conducted a retrospective study to investigate the efficacy and safety of PEG-IFN α-2b plus RBV in CHC patients in a real-life clinical setting. The participating centers, targeted patients, and the treatment method have already been reported[10] and are summarized below.

Patients seen from December 2004 who completed PEG-IFN α-2b plus RBV combination therapy by September 2007 were registered regardless of genotype, history of IFN treatment, or alanine aminotransferase (ALT) levels. Excluded from this study were pregnant or possibly pregnant and lactating women, and patients with severe heart disease, chronic kidney failure or creatinine clearance of ≤50 mL/min, current or history of severe psychiatric disorder, and autoimmune hepatitis. Doses of PEG-IFN α-2b and RBV and dose adjustment followed the Japanese package insert. The duration of treatment was 48 weeks, the standard of care for patients with genotype 1 and high virus load. In patients with late viral response (LVR) who did not achieve viral negativity by week 12, treatment could be extended up to 72 weeks. Patients other than those with genotype 1 and high virus load were treated for 24 weeks.

Included in this analysis were the patients registered in the PERFECT Study who had no history of HCC and for whom SVR/non-SVR status could be confirmed. The patients who developed HCC within 3 months of the start of treatment were excluded from analysis to rule out the possibility of inclusion of patients with HCC already present at the start of treatment.

The start of the follow-up period was defined as the first day of PEG-IFN α-2b and RBV treatment. The patients were monitored for the onset of HCC by routine follow-up methods practiced by each center. The diagnosis of HCC was based on the presence of typical hypervascular characteristics on angiography in addition to the findings on computed tomography and ultrasonography. Microscopic examination of fine-needle biopsy specimens was performed in patients whose angiograms did not demonstrate a typical image of HCC.

This multicenter study was approved by the institutional review board of each participating center. The study protocol was carried out according to the ethical guidelines of the 1975 Declaration of Helsinki, and informed consent was obtained from each patient.

Statistical analysis

All statistical analyses were performed using SAS, version 9.13 (SAS Institute, Cary, NC, USA). Intergroup comparison of background variables was performed by Fisher's exact test and Mann–Whitney *U*-test.

The cumulative incidence of HCC was calculated by the Kaplan–Meier method, and intergroup comparison was conducted using the log-rank test. The determination of the factors contributing to HCC was conducted by Cox proportional hazards regression model using a stepwise procedure, incorporating the factors exhibiting $P < 0.2$ by the log-rank test and excluding factors with more than 30% of values missing. The determination of factors associated with biochemical response (BR) was conducted by a stepwise procedure using the results of logistic univariate analysis ($P < 0.2$) in logistic multivariate analysis.

All tests were two-sided, with a significance level set at $P < 0.05$.

RESULTS

Study population

A TOTAL OF 1865 subjects, consisting of 999 SVR patients (SVR rate 53.5%) and 866 non-SVR patients, were eligible for analysis. Of the non-SVR patients, 441 had transient response (TR) defined as viral negativity achieved during treatment (relapse: 408, virus breakthrough: 33), 400 patients had non-virological response (NVR) defined as viral negativity not being achieved, and the change in viral load during treatment was not known for 25 patients.

The duration of observation ranged from 3 months to 5 years and 8 months, with a median of 4 years and 3 months.

During the observation period, HCC developed in 59 patients (3.1%). Between patients who developed HCC and those who did not, significant differences in background factors were detected in age ($P < 0.0001$), hepatic fibrosis ($P = 0.0002$), virological efficacy ($P < 0.0001$), ALT levels ($P = 0.0089$), ALT level at 24 weeks after the end of treatment (≤40 vs. >40 IU/L) ($P < 0.0001$), platelet count ($P = 0.0001$), serum albumin ($P = 0.0062$), and alpha fetoprotein (AFP) ($P < 0.0001$) (Table 1).

Virological efficacy and incidence of HCC

The 5-year cumulative incidence of HCC by the Kaplan–Meier method was 1.1% in SVR patients and 7.1% in non-SVR patients, a difference that was significant ($P < 0.001$) (Fig. 1). No significant difference was observed in the incidence of HCC between TR and NVR patients among non-SVR patients, but the difference between TR and SVR patients was significant ($P < 0.0001$) (Fig. 2). This trend was also observed regardless of gender, with no significant difference in the incidence of HCC observed between TR and NVR in either male or female patients and a significant difference observed between TR and SVR in both male patients ($P = 0.0007$) and female ($P = 0.0065$) patients.

Factors contributing to HCC

The factors contributing to HCC selected in the multivariate analysis were therapeutic efficacy (SVR vs. NVR), sex, age (<60 vs. ≥60 years), ALT level at 24 weeks after the end of treatment (≤40 vs. >40 IU/L), and platelet count (<10 vs. ≥10 × 10 000/mm^3) (Table 2).

Biochemical response and incidence of HCC in non-SVR patients

Since ALT levels at 24 weeks after the end of treatment was selected as one factor contributing to HCC, the changes in ALT levels and onset of HCC were examined in 514 non-SVR patients with a pretreatment ALT level of more than 40 IU/L whose ALT level at 24 weeks after the end of treatment was obtained. Of these 514

Table 1 Patient background by onset of hepatocellular carcinoma (HCC) (1865 patients)

Factor	With onset of HCC ($n = 59$)	Without onset of HCC ($n = 1806$)	*P*-value
Gender (male/female)	40/19	1014/792	0.0832
Age	62 (44–74)	56 (17–77)	<0.0001
Diabetes (yes/no/unknown)	6/33/20	100/1040/666	0.1539
Hypertension (yes/no/unknown)	4/6/49	116/569/1121	0.0763
Alcohol abuse (yes/no/unknown)	11/16/32	195/493/1118	0.1930
Fibrosis (0/1/2/3/4/unknown)	0/12/13/15/4/15	57/573/355/205/56/560	0.0002
Genotype (1/2/3/unknown)	52/5/0/2	1421/365/2/18	0.0876
Effect of IFN (SVR/non-SVR)	10/49	989/817	<0.0001
Body mass index (kg/m^2)	22.6 (14.2–34.0)	22.9 (14.9–41.2)	0.8546
ALT (IU/L)	79 (24–343)	60 (8–984)	0.0089
ALT at 24 weeks after end of treatment (IU/L) (≤40/>40/unknown)	16/30/13	1105/352/349	<0.0001
Platelet count (×10 000/mm^3)	13.3 (4.3–22.2)	16.3 (3.6–213.3)	0.0001
Serum albumin (g/dL)	3.9 (2.9–4.7)	4.1 (2.8–5.9)	0.0062
AFP (ng/mL)	13 (2.2–327.9)	5 (0–875)	<0.0001

Median (minimum – maximum).
AFP, alpha fetoprotein; ALT, alanine aminotransferase; IFN, interferon; SVR, sustained virological response.

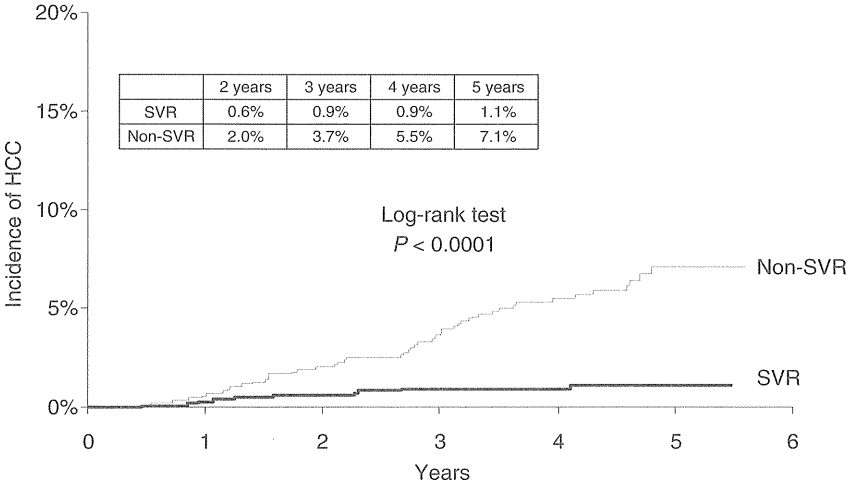


Figure 1 Onset of hepatocellular carcinoma (HCC) by therapeutic efficacy (1865 patients) (sustained virological response [SVR] vs. non-virological response [NVR]). The cumulative incidence of HCC was calculated by the Kaplan–Meier method. The difference between SVR and non-SVR was examined using the log-rank test.

patients, ALT level at 24 weeks after the end of treatment was reduced to less or equal to 40 IU/L (biochemical response: BR) in 234 patients, and the remaining 280 patients had values of more than 40 IU/L (non-BR). There were significant differences between BR and non-BR patients in the background factors of pretreatment ALT level, age, hepatic fibrosis, platelet count, AFP, and treatment duration. Selected as the factors contributing to BR in non-SVR patients in the multivariate analysis were TR, long treatment duration, and high platelet count before the start of treatment (Table 3).

The 5-year cumulative incidence of HCC was 3.4% in BR patients and 11.0% in non-BR patients, and the difference in incidence was significant ($P = 0.0012$) (Fig. 3). The 5-year cumulative incidence of HCC in male patients was 3.6% in BR patients and 13.9% in non-BR patients, and the difference was significant ($P = 0.0012$). In female patients, however, it was 3.5% in BR patients and 7.6% in non-BR patients, and although the incidence of HCC was lower in BR patients, the difference was not significant ($P = 0.0706$).

Incidence of HCC in patients with normal pretreatment ALT levels

When the incidence of HCC was compared between SVR (288) and non-SVR (214) patients among 502 patients with pretreatment ALT levels less or equal to 40 IU/L, the 5-year cumulative incidence of HCC was 0% in SVR patients and 4.8% in non-SVR patients, indicating a significant difference ($P = 0.0005$) between the groups

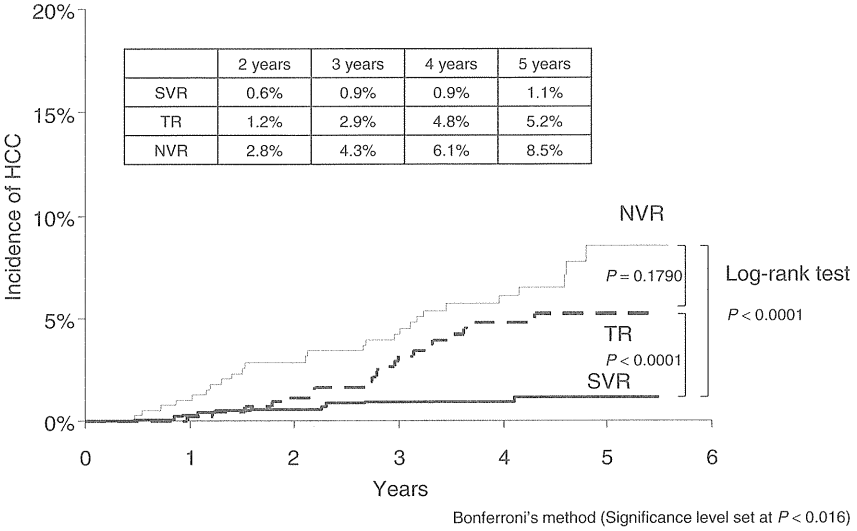


Figure 2 Onset of hepatocellular carcinoma (HCC) by therapeutic efficacy (sustained virological response [SVR] vs. transient response [TR] vs. non-virological response [NVR]). The cumulative incidence of HCC was calculated by the Kaplan–Meier method. The difference between each group was examined using the log-rank test (Bonferroni's Method, significance level set at $P < 0.016$).

Table 2 Factors contributing to hepatocellular carcinoma (all patients) Cox regression analysis (multivariate)

		Hazard ratio	95% confidence interval	*P*-value
Therapeutic efficacy	SVR	1		
	TR	2.055	0.709–5.955	0.1845
	NVR	2.985	1.036–8.601	0.0428
Sex	Male	1		
	Female	0.486	0.243–0.969	0.0405
Age	<60	1		
	≥60	2.005	1.035–3.883	0.0391
ALT at 24 weeks after end of treatment (IU/L)	≤40	1		
	>40	3.940	1.754–8.850	0.0009
Platelet count (×10 000/mm^3)	<10	1		
	≥10	0363	0.169–0.779	0.0093
Serum albumin (g/dL)	<4	1		
	≥4	0.594	0.310–1.140	0.1175

Factors examined: Of the 15 factors exhibiting $P < 0.2$ by log-rank test (therapeutic efficacy [1: SVR, 2: TR, 3: NVR], genotype [1: 1, 2: 2 or 3], sex [1: male, 2: female], age [1: <60, 2: ≥60], pre ALT [1: ≤40, 2: >40], +24 w ALT [1: ≤40, 2: >40], pre PLT [1: <10, 2: ≥10], pre ALB [1 <4, 2: ≥4], pre AFP [1: <20, 2: ≥20], grade [1: A0–1, 2: A2–3], stage [1: F0–1, 2: F2–4], hypertension [1: absent, 2: present], diabetes [1: absent, 2: present], heavy drinking [1: absent, 2: present], and treatment duration [1: ≤48 W, 2: >48 W]), nine factors were examined. Excluded were factors for which approximately 30% of values were missing (AFP, grade, stage, diabetes, hypertension, and heavy drinking).
AFP, alpha fetoprotein; ALB, albumin; ALT, alanine aminotransferase; NVR, non-virological response; PLT, platelet count; SVR, sustained virological response; TR, transient response.

(Fig. 4). This tendency is also observed with the 280 patients having pretreatment ALT levels of less or equal to 30 IU/L.

Onset of HCC in SVR patients

Hepatocellular carcinoma developed in 10 patients who achieved SVR. Multivariate analysis indicated that in SVR patients, the ALT level at 24 weeks after the end of treatment was the only significant factor contributing to HCC ($P = 0.0007$) (Table 4). In SVR patients with an ALT level of more than 40 IU/L at 24 weeks after the end of treatment, the 5-year cumulative incidence of HCC was 5.6% while the incidence in patients with an ALT level of less or equal to 40 IU/L was 0.7%, indicating a significant difference ($P = 0.0004$) between the groups (Fig. 5).

DISCUSSION

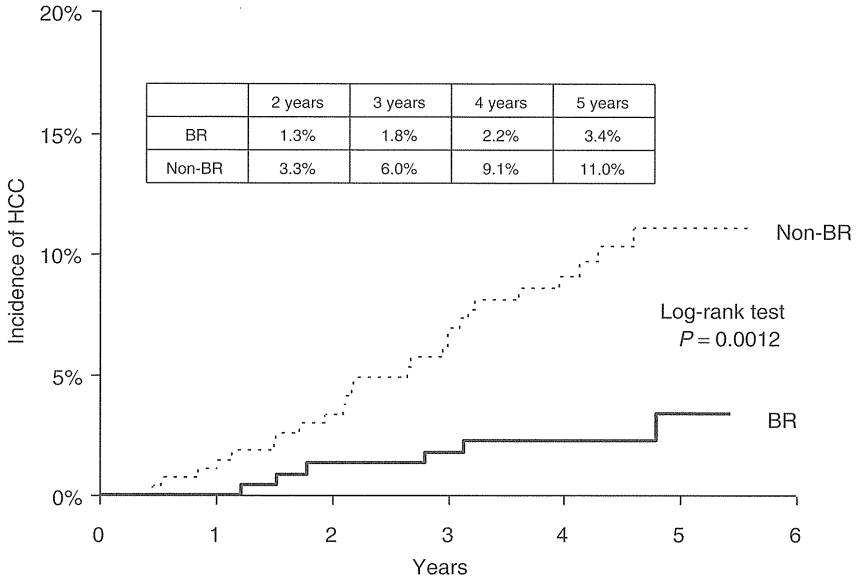
THIS STUDY INDICATED that the risk factors for HCC after PEG-IFN α-2b plus RBV combination therapy are NVR, male sex, older age, low platelet count, and an ALT level of more than 40 IU/L at 24 weeks after the end of treatment.

Kurokawa *et al.*[16] tracked 403 patients receiving PEG-IFN α-2b plus RBV combination therapy for a median

Table 3 Factors contributing to biochemical response in non-sustained virological response patients Logistic regression analysis (multivariate)

		Odds ratio	95% confidence interval	*P*-value
Virological response	NVR	1	1.480–3.203	0.0001
	TR	2.177		
Treatment duration	per week	1	1.000–1.022	0.0424
		1.011		
Platelet count	per 10 000/mm^3	1	1.018–1.099	0.0043
		1.058		

Factors examined were those exhibiting $P < 0.2$ by log-rank test: Genotype, virological response (TR/NVR), treatment duration, pre platelet count, diabetes, stage, and alanine aminotransferase (ALT).

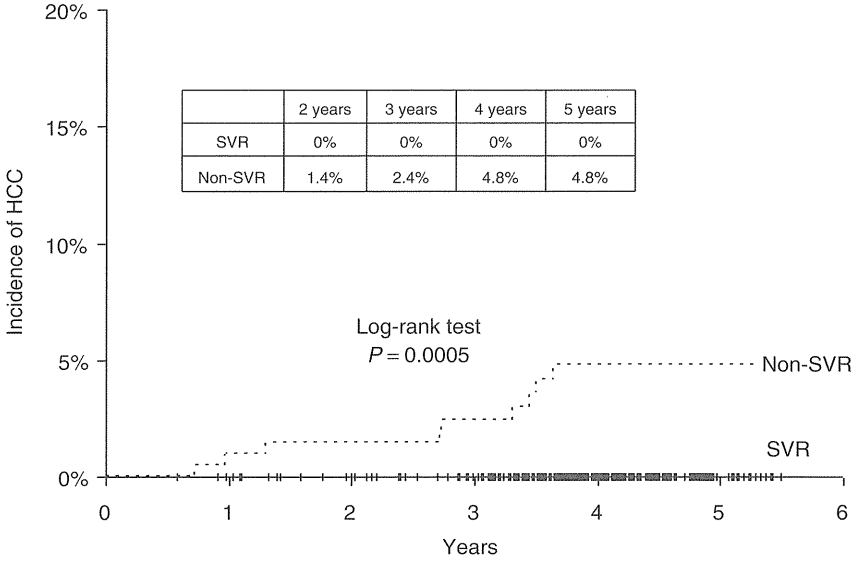


	2 years	3 years	4 years	5 years
BR	1.3%	1.8%	2.2%	3.4%
Non-BR	3.3%	6.0%	9.1%	11.0%

Figure 3 Alanine aminotransferase (ALT) normalization and hepatocellular carcinoma (HCC) in non-virological response [NVR] patients. The cumulative incidence of HCC was calculated by the Kaplan–Meier method. Log-rank test was used to study the difference between biochemical response (BR) and non-BR.

duration of 36.5 months and reported that in multivariate analysis, virological efficacy (SVR vs. non-SVR), age, and hepatic fibrosis were selected as the factors contributing to HCC. Arase *et al.*[15] tracked 500 patients 60 years of age and older receiving IFN alone or in combination with RBV for an average duration of 7.4 years and also reported that the factors contributing to HCC are virological efficacy (SVR vs. non-SVR), age, and hepatic fibrosis. In our study, hepatic fibrosis was not tested with multivariate analysis because more than 30% of values were missing, but it was selected as a significant factor in the univariate analysis. Platelet count was selected in multivariate analysis, and the results in our study are therefore considered to be generally consistent with these reports.

The results of the present study indicated no significant difference between TR and NVR in non-SVR in stratified cumulative incidence of HCC, and although there was a significant difference between SVR and both TR and NVR, TR was not significant against SVR in multivariate analysis, and NVR was the only significant factor. Kurokawa *et al.*[16] reported the same results by



	2 years	3 years	4 years	5 years
SVR	0%	0%	0%	0%
Non-SVR	1.4%	2.4%	4.8%	4.8%

Figure 4 Therapeutic efficacy and hepatocellular carcinoma (HCC) in patients with pretreatment alanine aminotransferase (ALT) of ≤40. The cumulative incidence of HCC was calculated by the Kaplan–Meier method. Log-rank test was used to study the difference between sustained virological response (SVR) and non-virological response (NVR).

Table 4 Factors contributing to hepatocellular carcinoma (sustained virological response [SVR] patients) Cox regression analysis (multivariate)

		Hazard ratio	95% confidence interval	*P*-value
ALT at 24 weeks after end of treatment (IU/L)	≤40	1		
	>40	16.054	3.235–79.681	$P = 0.0007$
Serum albumin (g/dL)	<4	1		
	≥4	0.196	0.036–1.073	$P = 0.0603$

Factors examined: Of the 10 factors exhibiting $P < 0.2$ by log-rank test (Genotype [1: 1, 2: 2 or 3], age [1: <60, 2: ≥60], pre ALT [1: ≤40, 2: >40], +24 w ALT [1: ≤40, 2: >40], pre PLT [1: <10, 2: ≥10], pre ALB [1: <4, 2: ≥4], pre AFP [1: <20, 2: ≥20], grade [1: A0–1, 2: A2–3], stage [1: F0–1, 2: F2–4], and diabetes [1: absent, 2: present]), 5 factors were examined. Excluded were pre ALT, with which HCC did not occur in the ≤40 group, and AFP, grade, stage, and diabetes, the factors for which approximately 30% of values were missing.
ALB, albumin; ALT, alanine aminotransferase; PLT, platelet count;

comparing cumulative incidences of HCC among SVR, TR and NVR (the results of multivariate analysis are not known). On the other hand, Morgan *et al.*,[19] in their follow-up study of the HALT-C Trial, reported that there was no difference between TR and NVR in the incidence of HCC or death related to hepatic disease/liver transplantation, but when all hepatic-related outcomes were examined, a significantly superior inhibition was observed with TR compared to NVR. Our results also demonstrate that although the difference is not significant, the cumulative incidence of HCC is lower in TR patients than in NVR patients, especially in male patients (5-year cumulative incidence of HCC: 6.0% vs. 10.7%). It is therefore necessary to continue to observe this for an extended number of years.

Our results study indicated that in non-SVR patients, whether or not ALT level is normalized after treatment is a greater contributing factor for the onset of HCC than virological response. Normalization of ALT has already been reported to contribute to the inhibition of the onset of HCC even under HCV-positive conditions,[13,20] and this was found to apply also to non-SVR patients receiving PEG-IFN α plus RBV combination therapy.

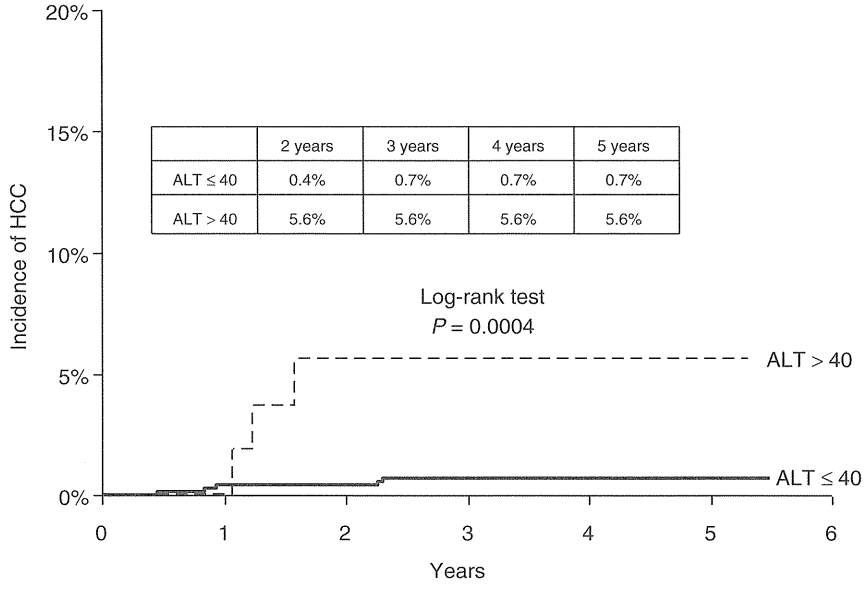


Figure 5 Alanine aminotransferase (ALT) levels at 24 weeks after end of treatment and hepatocellular carcinoma (HCC) in patients with sustained virological response (SVR). The cumulative incidence of HCC was calculated by the Kaplan–Meier method. Log-rank test was used to study the difference between SVR patients with an ALT level of more than 40 IU/L at 24 weeks after the end of treatment and those with an ALT level of less or equal to 40 IU/L.

Our investigation also indicated that abnormal ALT levels also contribute to the onset of HCC in SVR patients. In multivariate analysis, the only contributing factor to the development of HCC in SVR patients was ALT levels at 24 weeks after the end of treatment. However, the onset of HCC is also observed in patients who achieve ALT normalization after treatment, and it is therefore difficult to conclude that ALT is the only risk factor for the onset of HCC in SVR patients. The potential involvement of hepatic fibrosis as well as hepatic steatosis, which persists after viral clearance[21] and small amounts of virus remaining in the liver[22] have also been suggested as risk factors for the onset of HCC in SVR patients. Further detailed investigation is therefore necessary. Nevertheless, regardless of whether or not SVR is achieved, it is clear that abnormal ALT is a factor affecting the onset of HCC. Careful monitoring of changes in ALT and instituting measures to normalize ALT are therefore important regardless of whether or not SVR is achieved.

With the administration of PEG-IFN α plus RBV combination therapy tailored for individual patients and the addition of direct-acting antivirals to current combination therapy, the therapeutic outcomes for CHC will continue to further improve, and the number of patients who develop hepatic cirrhosis and HCC from hepatitis C can be expected to decrease in the future. HCC can occur even in patients achieving SVR, and even if SVR is not achieved, as long as the possibility to inhibit the onset of HCC remains, there will be a need for various treatment innovations to achieve the prevention of HCC, the ultimate goal of treatment of CHC.

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APPENDIX I

IN ADDITION TO the study authors, the investigators in the PEG-IFN and Ribavirin, Find Evidence of Chronic Hepatitis C Therapy in Tokyo (PERFECT) Study Group included: Hiroyasu Adachi, Department of Internal Medicine, Tobu Chiki Hospital; Yoshio Aizawa Department of Internal Medicine, The Jikei University School of Medicine, Aoto Hospital; Masatoshi Akamatsu, Department of Gastroenterology, JR Tokyo General Hospital; Masahiro Arai, Department of Gastroenterology, Toshiba General Hospital; Yasuhiro Asahina, Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital; Yoshimichi Chuuganji, Department of Gastroenterology, Tokyo Metropolitan Bokutoh Hospital; Yoshiyuki Fujita, Department of Gastroenterology, St. Luke's International Hospital; Yukiya Hakozaki, Department of Internal Medicine, Self-Defence Forces Central Hospital; Naoaki Hashimoto, Department of Gastroenterology, Tokyo Teishin Hospital; Katsuya Hattori, Department of Gastroenterology, Kohsei Chuo General Hospital; Seishu Hayashi, Division of Hepatology, Tokyo Metropolitan Komagome Hospital; Masanori Hirano, Department of Gastroenterology Tokyo Metropolitan Police Hospital; Keiichi Hirata, National Hospital Organization Disaster Medical Center; Department of Gastroenterology; Toshiya Horibe, International University of Health & Welfare Mita Hospital, Gastroenterology Center; Kazuhiko Hosoda, Department of Gastroenterology and Hepatology Yamanashi Hospital of Social Insurance; Hiroaki Igarashi, Department of Gastroenterology, Kawakita General Hospital; Yoshida Ikuma, Department of Internal Medicine, Kasai Cardiology & Neurosurgery Hospital; Tetsuya Irie, Department of Internal Medicine, Nakano General Hospital; Koji Ishii, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Toho University School of Medicine; Takayoshi Ito, Department of Gastroenterology, Department of Medicine, Showa University School of Medicine; Naohiro Kawamura, The Third Department of Internal Medicine, Kyorin University School of Medicine; Tateo Kawase, Department of Gastroenterology, Kanto Central Hospital of the Mutual Aid Association of Public School Teachers; Hirokazu Komeichi, Department of Internal Medicine, Division of Cardiology, Hepatology, Geriatrics and Integrated Medicine, Nippon Medical School; Sadanori Kubo, Department of Internal Medicine, Showa University Toyosu Hospital; Naohiko Masaki, Division of Gastroenterology, International Medical Center of Japan, Toyama Hospital; Akihisa Miyazaki, Department of Gastroenterology, Juntendo University Nerima Hospital; Mitsuhiko Moriyama, Division of Gastroenterology and Hepatology, Department of Medicine, Nihon University of School of Medicine; Naoya Murashima, Department of Gastroenterology, Mishuku Hospital; Hikaru Nagahara, Department of Gastroenterology, Aoyama Hospital Tokyo Women's Medical University; Hisato Nakajima, Department of Gastroenterology and Hepatology, Jikei University School of Medicine Daisan Hospital; Ikuo Nakamura, Department of Gastroenterology, Tokyo Medical University; Ryo Nakata, Department of Gastroenterology, Japanese Red Cross Medical Center; Katsuhisa Nakatsuka, Division of Gastroenterology, Department of Internal Medicine Nippon Medical School; Yasuhiro Nishizaki, Department of Gastroenterology, Tokai University Tokyo Hospital; Osamu Noguchi, Division of Gastroenterology and Hepatology, Ome Municipal General Hospital; Toshihiko Nouchi, Department of Gastroenterology, Showa General Hospital; Yuki Ogura, Department of Medicine, Tokyo Metropolitan Fuchu Hospital; Masanaru Ozawa, Yoshikawa Hospital; Shigehiko Sainokami, Fussa Hospital; Naoya Sakamoto, Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University; Minoru Sakamoto, Department of Internal Medicine, Faculty of Medicine, University of Yamanashi; Mina Sasaki, Department of Gastroenterology, Tokyo Metropolitan Geriatric Hospital; Yoshiyuki Sato, Department of Internal Medicine, Tokyo Kosei Nenkin Hospital; Koichi Shiraishi, Division of Gastroenterology and Hepatology, Tokai University Hachioji Hospital; Satoko Suzuki, Department of Gastroenterology, Juntendo University School of Medicine; Tomohiko Suzuki, Department of Internal Medicine, Tokyo Metropolitan Health and Medical Treatment Corporation Ohkubo Hospital;

Fumitaka Suzuki, Department of Hepatology, Toranomon Hospital; Kazumi Tagawa, Department of Gastroenterology, Mitsui Memorial Hospital; Ichiro Takagi, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Jikei University School of Medicine; Seiichirou Takahashi, Department of Internal Medicine, Fujiyoshida Municipal Medical Center; Atsushi Tanaka, Department of Medicine, Teikyo University School of Medicine; Takuma Teratani, Department of Gastroenterology, Kanto Medical Center NTT EC; Katsutoshi Tokushige, Department of Medicine and Gastroenterology, Tokyo Women's Medical University; Masahiko Tomimatsu, Department of Medicine, Tokyo Women's Medical University Medical Center East; Shigeki Tsukada, Department of Gastroenterology, Juntendo Tokyo Koto Geriatric Medical Center; Hiroyuki Watanabe; Department of Gastroenterology, Yamanashi Red Cross Hospital; Michiyasu Yagura, Department of Gastroenterology, National Hospital Organization, Tokyo National Hospital; Haruki Yamada, Department of Internal Medicine, Social Insurance Central General Hospital; Toshio Yamada, Department of Gastroenterology, Tokyo Rinkai Hospital; Taro Yamanaka, Department of Gastroenterology, Itabashi Chuo Medical Center; Kiyomi Yasuda, Department of Hepatology, Kiyokawa Hospital; Yuji Yoshikawa, Department of Gastroenterology, Sanraku Hospital; Yoko Yoshioka, Department of Gastroenterology, Shiseikai-Daini Hospital; Hiroshi Yotsuyanagi, Department of Infectious Diseases, Internal Medicine, Graduate School of Medicine, University of Tokyo; Mikio Zeniya, Department of Gastroenterology, Jikei University Graduate School of Medicine.

Receptor for Activated Protein Kinase C: Requirement for Efficient MicroRNA Function and Reduced Expression in Hepatocellular Carcinoma

Motoyuki Otsuka[1]*, Akemi Takata[1], Takeshi Yoshikawa[1], Kentaro Kojima[1], Takahiro Kishikawa[1], Chikako Shibata[1], Mutsuhiro Takekawa[2], Haruhiko Yoshida[1], Masao Omata[1¤], Kazuhiko Koike[1]

1 Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan, **2** Department of Cell Signaling and Molecular Medicine, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

Abstract

MicroRNAs (miRNAs) are important regulators of gene expression that control physiological and pathological processes. A global reduction in miRNA abundance and function is a general trait of human cancers, playing a causal role in the transformed phenotype. Here, we sought to newly identify genes involved in the regulation of miRNA function by performing a genetic screen using reporter constructs that measure miRNA function and retrovirus-based random gene disruption. Of the six genes identified, RACK1, which encodes "receptor for activated protein kinase C" (RACK1), was confirmed to be necessary for full miRNA function. RACK1 binds to KH-type splicing regulatory protein (KSRP), a member of the Dicer complex, and is required for the recruitment of mature miRNAs to the RNA-induced silencing complex (RISC). In addition, RACK1 expression was frequently found to be reduced in hepatocellular carcinoma. These findings suggest the involvement of RACK1 in miRNA function and indicate that reduced miRNA function, due to decreased expression of RACK1, may have pathologically relevant roles in liver cancers.

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* E-mail: otsukamo-tky@umin.ac.jp

¤ Current address: Yamanashi Prefectural Hospital Organization, Yamanashi, Japan

Introduction

MicroRNAs (miRNAs) are short (20-23-nt), endogenous, single-stranded RNA molecules, that regulate gene expression and control physiological and pathological processes, such as development and cancer. Mature miRNAs and Argonaute (Ago) proteins form the RNA-induced silencing complex (RISC), a ribonucleoprotein complex that mediates post-transcriptional gene silencing [1] and then, complementary base-pairing of miRNAs guides the RISC to target messenger mRNAs, which are subsequently destabilized and sequestered from the translational machinery by Ago proteins [2–5]. Although insights into the regulatory function of miRNAs are beginning to emerge, their mechanisms of action and the genes involved in miRNA pathway have not yet been fully determined [6,7].

A large body of evidences suggests that the multigene regulatory capacity of miRNAs is dysregulated and exploited in cancer. Although several miRNAs are upregulated in specific tumors [8], a global reduction of miRNA abundance appears a general trait of human cancers, playing a causal role in the transformed phenotype [9,10]. In fact, the enzymes and cofactors involved in miRNA processing pathways may be targets of genetic disruption, further enhancing cellular transformation [9]. Moreover, the disruption of Dicer in mice promotes hepatocarcinogenesis [11] and the truncating mutations in TARBP2, which causes a defect in the processing of miRNAs, were identified in sporadic and hereditary colon carcinomas [12].

Retroviral insertion-mediated random gene disruption can be used to generate null alleles, resulting in diminished endogenous gene expression [13]. The use of such retroviral integration methods, combined with appropriate reporter constructs, has provided an efficient, comprehensive gene screening method [14,15]. In this study, we sought to identify new genes involved in miRNA function and determine its role in live cancers. To this end, we established reporter cell lines in which cellular miRNA function could be assessed by expression of a drug resistance gene. Using these cell lines and a random gene disruption method, we identified genes that have not previously been implicated in the regulation of miRNA function. We subsequently determined the role of one of these genes, RACK1, which encodes "receptor for activated protein kinase C" (RACK1), in miRNA function, as well as its expression in liver cancers. Collectively, our data suggest the potential involvement of RACK1 in pathological processes.