conditions and efficient management of ailments, difficulties in the treatment of chronic hepatitis C in elderly individuals are increasingly coming to the fore. This is attributable, at least in part, to liver fibrosis accelerating in parallel with age [11], as well as less tolerability and more side effects of combined interferon (IFN) and ribavirin in these patients [6,11,12].

These constraints notwithstanding, there is a pressing need for treatment of aged individuals with antiviral agents in order to prevent the development of cirrhosis and HCC and to promote better survival with an increased quality of life. When planning antiviral treatment of the elderly, weighing its merits against untoward effects, it is essential to understand the natural history of HCV infection in these patients. However, there have been virtually no reports on the natural history of HCV infection in older adults, nor are there any solid guidelines for antiviral treatment in these patients [13].

In the 42 years from 1964 to 2005, we have followed-up 332 patients who were persistently infected with HCV and had not received any antiviral treatment. They included the 120 patients with aspartate and alanine aminotransferase (ASAT and ALAT) levels ≦40 IU/l (group A) and the 212 with ASAT and/or ALAT ≧41 (group B), and were followed-up for 3 years or longer without receiving any antiviral treatment. It is hoped that the evolution of chronic hepatitis in these patients, with special reference to the baseline transaminase levels, will shed light on how they should be treated for the prevention of cirrhosis and HCC in the coming era of global longevity.

Material and methods

Patients

During 42 years, from 1964 through 2005, 7358 patients with HCV-RNA in the serum visited the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo. Of these patients, 843 (11.5%) were ≧65 years of age at presentation, and 512 (60.7% of the elderly) had not received antiviral agents or other drugs that might suppress the replication of HCV. In order to rule out cirrhosis, 180 patients with platelet counts $<120\times10^3/mm^3$ were excluded. The remaining 332 patients were classified into the 120 with ASAT and ALAT levels ≦40 IU/l (Group A) and the 212 with ASAT and/or ALAT levels ≧41 IU/l (group B); they included 22 patients (10.4%) with ASAT levels ≦40 IU/l and 18 (8.5%) with ALAT levels ≦40 IU/l. Baseline transaminase levels were determined at least twice, 2–3 months apart, in the course of 6 months. The patients were followed-up for 3 years or longer without receiving any antiviral treatment, and tested monthly for liver function, HCV-RNA and α-fetprotein (AFP) or protein induced by the absence of vitamin K or antagonist-II (PIVKA-II). Screening for cirrhosis and HCC was carried out yearly using ultrasonography and/or computed tomography. Angiography was implemented when HCC was strongly suspected by imaging modalities. During follow-ups, herbal medicine (intravenous Stronger Neo-Minophagen C (SNMC) or oral Shousaikotou) and/or ursodeoxycholic acid was given to 51 (42.5%) patients in group A and 139 (65.6%) patients in group B. Three (2.5%) patients in group A and 24 (11.2%) patients in group B, in whom IFN was started after they had been followed-up for 3 years or longer, left the study cohorts at the initiation of treatment. Informed consent was obtained from each patient who participated in this study, and the protocol conformed to the ethics guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Human Research Committee of the institution.

Markers of HCV infection

Qualitative assay for HCV-RNA was performed using polymerase chain reaction (PCR) with nested primers and the results were recorded as positive or negative, with the detection limit at 100 copies/ml. Quantification of HCV-RNA was carried out with the branched-DNA assay version 2.0 (Chiron Corp., Calif., USA), and the results were expressed in megaequivalents (MEq) per milliliter over a range from <0.5 to 120 MEq/ml.

Statistical analysis

Since certain data in the analysis were regarded to comply with non-Gaussian distribution, categorical variables at baseline were compared with the Fisher exact test and numerical values were analyzed with the Mann-Whitney U-test and the Kruskal-Wallis test. Cumulative rates of cirrhosis, HCC, and death were calculated using the Kaplan-Meier technique, and differences between curves were evaluated by the log-rank test. A p-value <0.05 with the two-tailed test was considered significant. All the analyses were carried out using the computer program SPSS ver.11.0 (SPSS Inc., Ill., USA).

Results

Treatment-naive patients older than 65 years infected with HCV

During the 42 years from 1964 through 2005, the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo admitted 332 patients aged 65 years or older with HCV who had not received any antiviral treatment, and in whom cirrhosis had not developed. In Table I we compare demographic, clinical, and virological characteristics between the 120 patients with baseline transaminase levels ≦40 IU/l and the 212 patients with levels ≧41 IU/l. ASAT and ALAT levels were higher, while platelet counts were lower in the patients with elevated transaminase levels compared with in patients without elevated transaminase levels.

When patients with baseline transaminase levels ≦40 IU/l were stratified by age, the median follow-up period was shorter in those aged 75–80 years than in those aged 65–69 or 70–74 years (4.5 versus 8.6 or 7.0 years, $p=0.011$) (Table II). Although the baseline transaminase levels were within normal limits in all of them, the median ASAT level was higher in patients aged 70–74 years than in those aged 65–70 or 75–80 years (35 versus 27 or 28 IU/l, $p=0.040$). In patients with baseline levels of both or either transaminase ≧41 IU/l, the median albumin level was lower in those aged 75–80 years than in those aged 65–69 or 70–74 years (3.9 versus 4.1 or 4.1 g/dl, $p=0.005$) (Table III).

Development of cirrhosis and HCC

Cirrhosis developed more frequently in elderly patients aged 65 years or older, with elevated transaminase levels at baseline, during follow-ups for longer than 3 years (Figure 1A). At 5 and 10 years of follow-up, cirrhosis developed in, respectively, 26% and 27% of the patients with the baseline transaminase levels ≧41 IU/l in contrast to only 4% and 13% of the patients with levels ≦40 IU/l ($p<0.001$). Likewise, HCC developed more frequently in elderly patients with elevated transaminase levels at baseline (Figure 1B). At 5 and 10 years of follow-up, HCC developed in, respectively, 22% and 26% of the patients with the baseline transaminase levels ≧41 IU/l, contrasting with only 3% and 5% of the patients with levels ≦40 IU/l ($p<0.001$).

Development of cirrhosis is compared between patients with and without elevated transaminase levels at baseline who were stratified by age (Figure 2). Cirrhosis developed more frequently in the patients with elevated transaminase levels than in those without elevated transaminase levels who were aged 65–69 years ($p<0.001$). In patients aged 70–74 years, cirrhosis tended to occur more often in those with elevated transaminase levels than in those without elevated transaminase levels during 5 years (27% versus 0%), but the difference fell short of being significant owing to the small number of patients in both groups.

Likewise, development of HCC is compared between patients with and those without elevated transaminase levels at baseline who were stratified by age (Figure 3). HCC developed more frequently in the patients with elevated transaminase levels than in those without elevated transaminase levels who were aged 65–69 years ($p=0.001$). In patients aged 70–74 and 75–80 years, HCC tended to occur more often in those with elevated transaminase levels than in those without elevated transaminase levels during 5 years (20% versus 5% and 19% versus 0%, respectively), but the difference was not significant, owing to the small number of patients in both groups.

Influence of gender on the development of cirrhosis and HCC

Figure 4 shows a comparison of the development of cirrhosis and HCC between 155 male and 177

Table I. Characteristics of patients with HCV-RNA aged 65 years or older with or without elevated transaminase (ASAT and ALAT) levels.

Features	≦40 IU/ml ($n=120$)	≧41 IU/l ($n=212$)	Differences p-value
Men	51 (42.5%)	104 (49.1%)	0.513
Follow-up (years)	7.8 (3–31.5)	8.7 (3–18.9)	0.181
ASAT (IU/l)	23 (6–40)	76 (27–496)	<0.001
ALAT (IU/l)	28 (11–40)	63 (22–411)	<0.001
Albumin (g/dl)	4.1 (2.4–4.9)	4.1 (3.2–5.3)	0.189
Platelets ($\times 10^3$/mm^3)	184 (120–343)	173 (120–313)	0.001
HCV RNA (MEq/ml)	4.5 (<0.5–120)	5.6 (<0.5–49)	0.168
HCV genotypes (1b:2a:2b:ND)	85:20:3:7	176:28:12:9	0.970

Abbreviations: HCV = hepatitis C virus; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; MEq = megaequivalents; ND = not determined. Data are expressed as the number (%) or the median with the range in parentheses.

Table II. Characteristics of patients aged 65 years or older with HCV-RNA and without elevated baseline transaminase levels (ASAT and ALAT ≤40 IU/l) stratified by the age.

Features	65–69 years (n=79 (65.8%))	70–74 years (n=25 (20.8%))	75–80 years (n=16 (13.3%))	Differences p-value
Men	29 (36.7%)	11 (44.0%)	11 (68.8%)	0.062
Follow-up (years)	8.6 (3–31.5)	7.0 (3–12.6)	4.5 (3–17.6)	0.011
ASAT (IU/l)	27 (11–39)	35 (16–40)	28 (15–40)	0.004
ALAT (IU/l)	22 (6–40)	25 (9–40)	22 (9–37)	0.604
Albumin (g/dl)	4.1 (3.2–4.9)	4.1 (3.0–4.4)	4.0 (2.4–4.5)	0.247
Platelets ($\times 10^5$/mm^3)	193 (120–298)	177 (120–343)	182 (120–263)	0.408
HCV RNA (MEq/ml)	4.2 (<0.5–34.6)	6.5 (<0.5–120)	4.0 (<0.5–17.1)	0.181
HCV genotypes (1b:2a:2b:ND)	51:19:2:4	21:1:1:1	13:0:0:2	0.074

Abbreviations: HCV =hepatitis C virus; ASAT =aspartate aminotransferase; ALAT =alanine aminotransferase; MEq =megaequivalents; ND =not determined. Data are expressed as the number (%) or the median with the range in parentheses.

female patients aged 65 years or older. Cirrhosis tended to occur more frequently in male than in female patients. There were marked gender differences in the development of HCC. At 5 and 10 years of follow-up, HCC occurred more frequently in men than in women (18% and 25% versus 9% and 9%, respectively, p=0.033).

Complications and death in patients with the baseline transaminase levels ≤40 IU/l and ≥41IU/l

Of the 120 patients with baseline transaminase levels ≤40 IU/l, 33 (27.5%) developed complications during follow-up (hypertension in 9 (27%), diabetes in 7 (21%), both complications in 1 (3%), pulmonary disease in 4 (12%), heart disease in 4 (12%), and other illnesses in the remaining 8 (24%)). At 5, 10, and 15 years of follow-up, respectively, death occurred more frequently in the patients with complications than in those without complications (10%, 18%, and 45% versus 0%, 5%, and 5%, p=0.015) (Figure 5).

Among 9 of the 120 (7.5%) patients who died, liver disease was the cause of death in only one. Of the remaining 8 (89%) patients, 4 died of heart failure or infarction, and one each of pneumonia, cerebral hemorrhage, renal insufficiency, and decrepitude. Death was more frequent in the patients aged ≥70 years than in those aged <70 years at presentation (p=0.006) (Figure 6).

Complications and death in patients with the baseline transaminase levels ≥41 IU/l

Of the 212 patients with baseline tranasaminase levels ≥41 IU/l, 83 (39.2%) developed complications during follow-up (hypertension in 18 (22%), diabetes in 23 (28%), both complications in 10 (12%), extrahepatic malignancies in 12 (15%), and other diseases in the remaining 20 (24%)). There were no differences in the frequency of death between the patients with and those without complications, however (Figure 7).

Among 34 of the 212 (14.0%) patients who died, liver disease was the most frequent cause of death and occurred in 20 (59%); the frequency was higher than that (11% (1/9)) in the patients with transaminase levels ≤40 IU/l at baseline (p=0.021). There were no differences in the frequency of death among

Table III. Characteristics of patients with HCV-RNA aged 65 years or older and with elevated baseline transaminase levels (ASAT and/or ALAT ≥41 IU/l) stratified by the age.

Features	65–69 years (n=140 (66.0%))	70–74 years (n=48 (22.6%))	75–80 years (n=24 (11.3%))	Differences p-value
Men	63 (45.0%)	25 (52.1%)	16 (66.7%)	0.707
Follow-up (years)	9.0 (3–18.9)	8.4 (3–17.2)	7.7 (3–14.7)	0.061
ALAT (IU/l)	82 (28–496)	74 (27–440)	64 (30–269)	0.959
ASAT (IU/l)	67 (22–411)	67 (34–309)	71 (35–172)	0.201
Albumin (g/dl)	4.1 (3.2–5.3)	4.1 (3.4–4.6)	3.9 (3.4–4.7)	0.005
Platelets ($\times 10^5$/cm^5)	171 (120–313)	180 (120–289)	157 (120–263)	0.398
HCV RNA (MEq/ml)	5.9 (<0.5–44.8)	5.6 (<0.5–30.0)	3.0 (<0.5–49.0)	0.251
HCV genotypes (1b:2a:2b:ND)	121:19:8:6	37:7:4:1	18:2:0:2	0.294

Abbreviations: HCV =hepatitis C virus; ASAT =aspartate aminotransferase; ALAT =alanine aminotransferase; MEq =megaequivalents; ND =not determined.

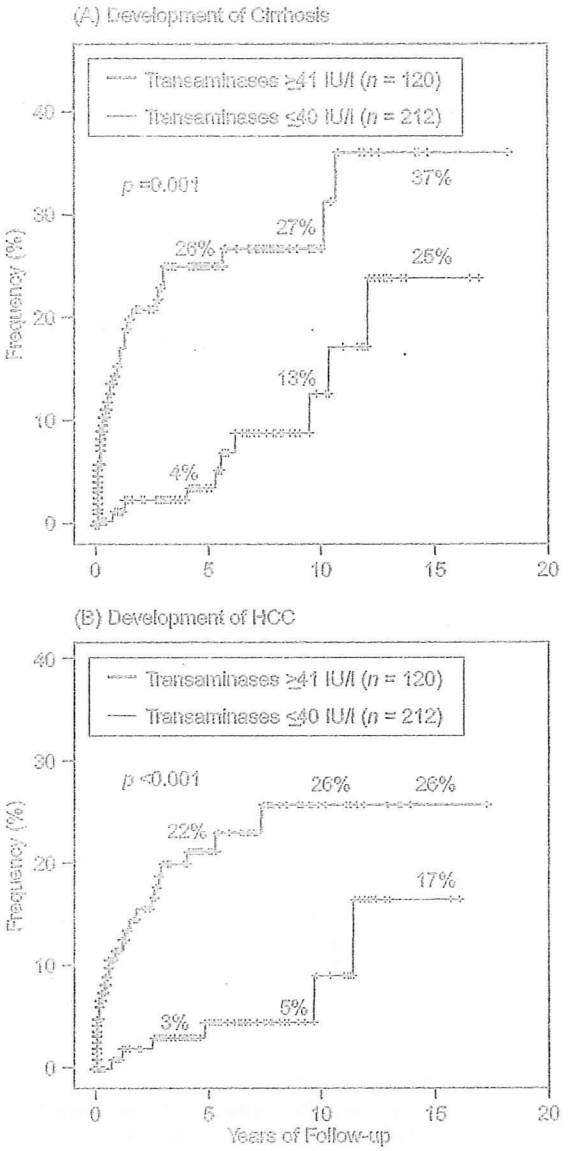

Figure 1. Development of cirrhosis (A) and HCC (hepatocellular carcinoma) (B) in patients over 65 years of age with chronic hepatitis C who were followed-up without receiving antiviral treatment. Patients with and without elevated baseline transaminase levels are compared.

the patients in distinct age groups who had elevated baseline transaminase levels at baseline (Figure 8).

Discussion

The World Health Organization defines elderly individuals as those aged ≥65 years. In general, IFN is indicated for patients under 65 years of age, in view of frequent side effects and safety precautions. HCC develops increasingly with age and in the majority after 65 years, and in Japan approximately

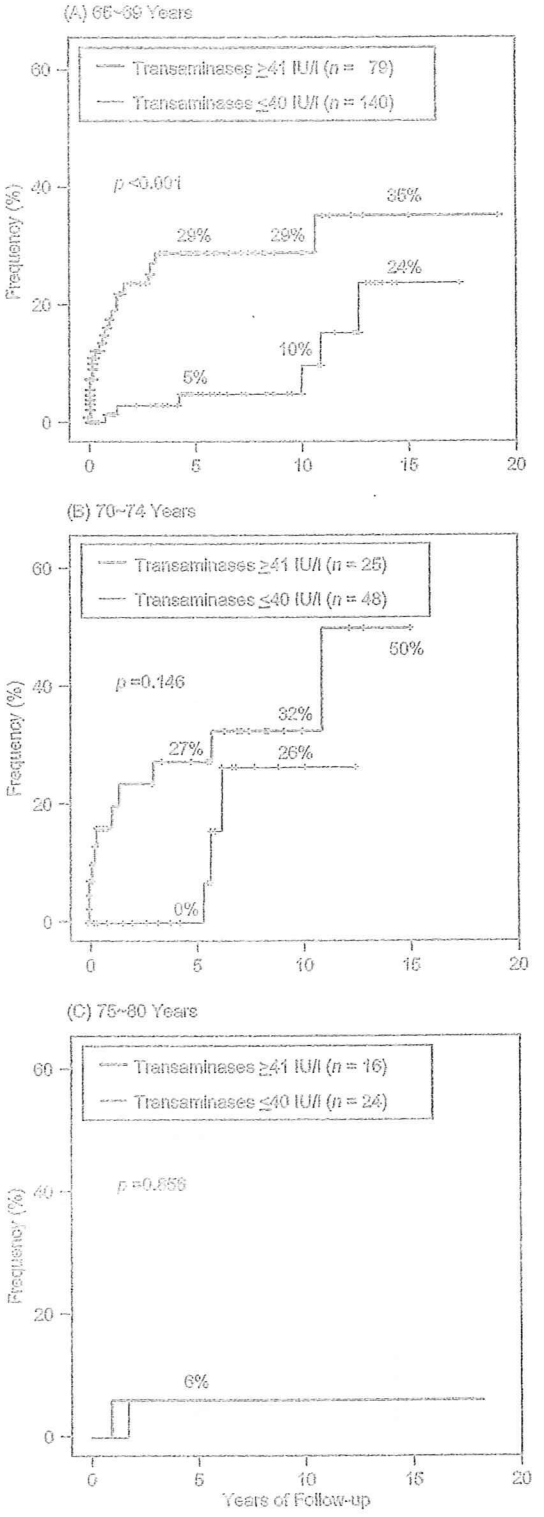

Figure 2. Development of cirrhosis in patients of more than 65 years of age with chronic hepatitis C who were followed-up without receiving antiviral treatment. Patients in different age groups are compared between those with and those without elevated transaminase levels.

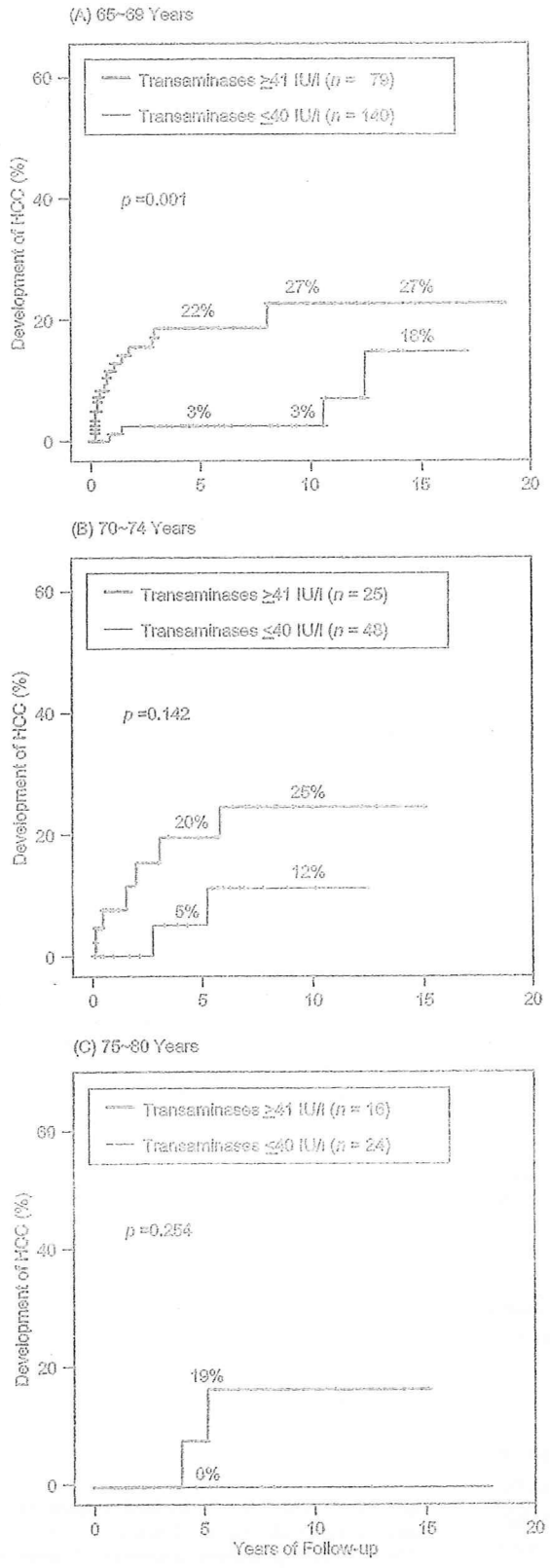

Figure 3 (*Continued*)

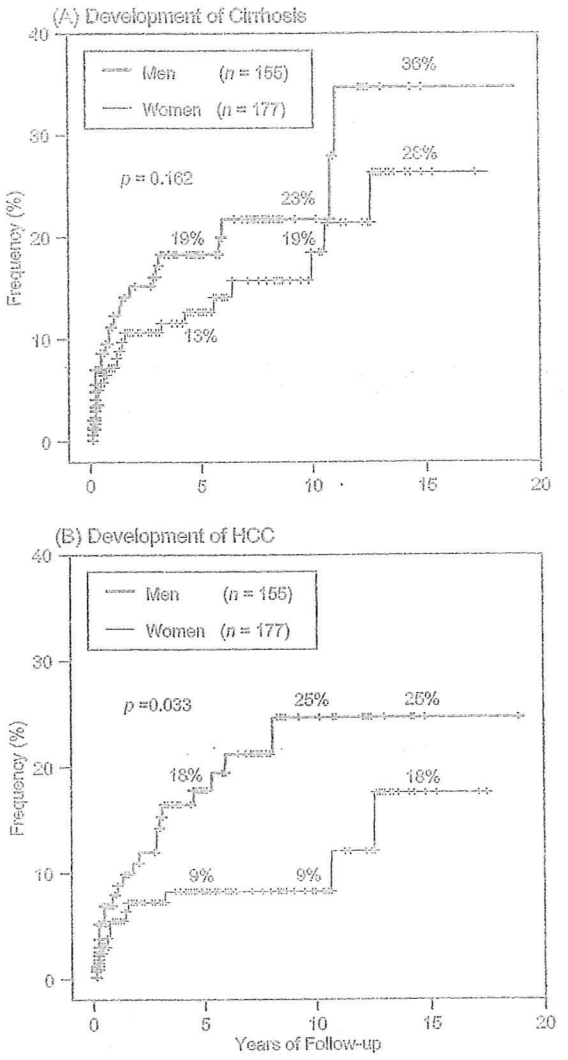

Figure 4. Development of cirrhosis (A) and HCC (hepatocellular carcinoma) (B) in patients over 65 years of age with chronic hepatitis C who were followed-up without receiving antiviral treatment. Male and female patients are compared.

30,000 patients infected with HCV die yearly [14]. Furthermore, HCC is steadily increasing in the United States, and the incidence is expected to double or triple in the next two decades [15]. Hence, HCV carriers aged 65 years or older should be given IFN treatment, which is proven to be efficacious in preventing the development of HCC [16,17]. Previously, we have evaluated the efficacy and safety of IFN monotherapy in patients aged 65 years or older [18]. Of the 84 patients studied, the sustained virological response was reached in 30 (36%), while

Figure 3. Development of hepaptocelluar carcinoma (HCC) in patients over 65 years of age with chronic hepatitis C who were followed-up without receiving antiviral treatment. Patients in different age groups are compared between those with and those without elevated transaminase levels.

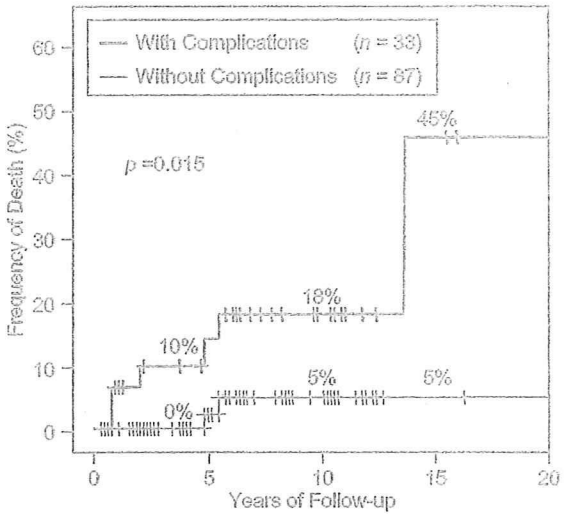

Figure 5. Deceased patients without elevated baseline transaminase levels (ASAT and ALAT <40 IU/l). Patients with and without complications other than liver disease are compared.

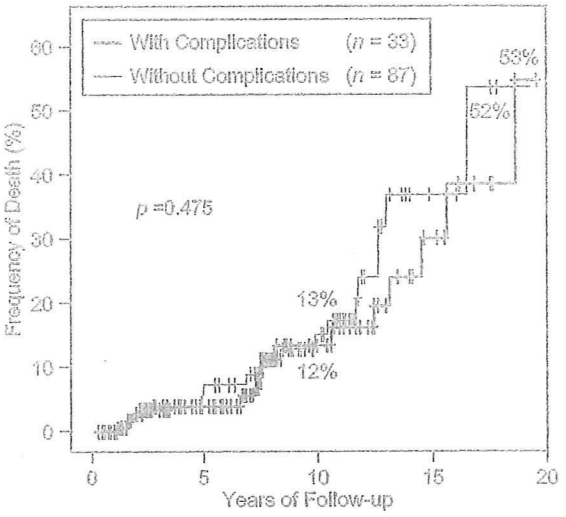

Figure 7. Deceased patients without elevated baseline transaminase levels (ASAT and ALAT <40 IU/l). Patients with and without complications other than liver disease are compared.

IFN was discontinued owing to adverse events in 11 (13%). Remarkably, the sustained virological response to combined IFN and ribavirin was comparable between the 66 patients aged ≥60 years and the 154 aged <60 years (31.8% versus 38.3%), although ribavirin had to be discontinued more frequently in the older patients (33.3% versus 20.8%, $p<0.05$) [19].

HCV spread widely in Japan around the end of World War II, at least 20 years earlier than in the other countries [4,14]. As a consequence, patients given combined IFN and ribavirin are 10–15 years older than those in Western countries [20–22]. Throughout the world, there are increasing numbers of individuals who are infected with HCV and entering the elder years. By the year 2010, the number of the elderly infected with HCV is estimated to account for 0.48 (54%) of the entire 0.89 million infected in Japan, and that in the United States for 0.78 (22%) of the 3.61 million [2–4]. These numbers will continue to increase for some time thereafter. As sequellae to this, cirrhosis and HCC will continue to increase, demanding higher medical costs. In the USA already, HCV-related

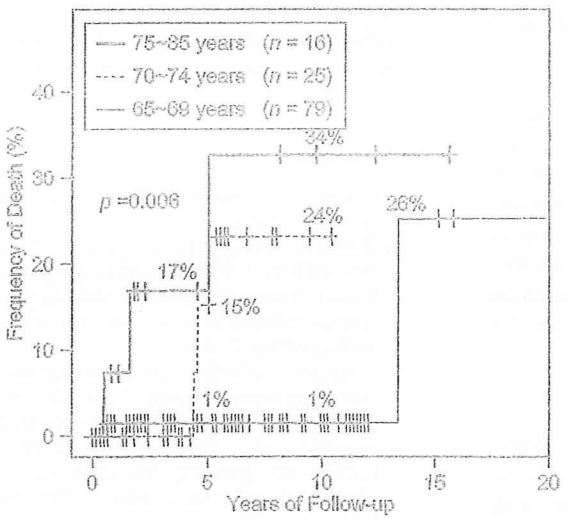

Figure 6. Deceased patients with elevated baseline transaminase levels (ASAT and/or ALAT >41 IU/l). Patients in the different age groups are compared.

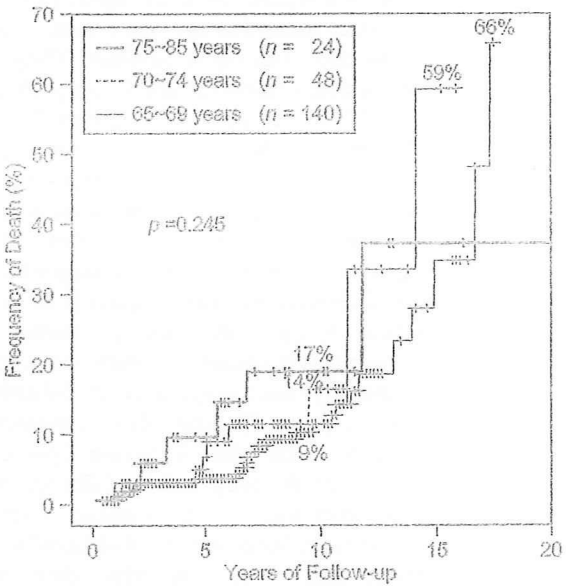

Figure 8. Deceased patients with elevated baseline transaminase levels (ASAT and/or ALAT >41 IU/l). Patients in the different age groups are compared.

end-stage liver disease is the leading cause of orthotopic liver transplantation [23]. This background demands that immediate measures should be taken to prevent fibrosis developing in the elderly with chronic hepatitis C by initiating the appropriate treatment; pegylated IFN combined with ribavirin can eliminate HCV efficiently [24,25].

Management of antiviral treatment in the elderly, however, is not without difficulties. Discontinuation of therapy or dose reduction was required frequently in the Japanese patients older than 60 years with chronic hepatitis C [21]. It is obvious that antiviral treatment needs to be administered with caution in aged patients with chronic hepatitis C, with the indication restricted to those who are likely to derive benefit from it. Early virological response at 12 weeks of treatment is predictive of sustained virological response [26]. The influence of HCV genotypes on the response to combined therapy, which increases with age [27], would have to be taken into consideration, also. In the Japanese patients infected with HCV genotype 1b, substitutions of amino acids at positions 70 and 91 are associated with a better response to combined treatment [28]. In view of the more frequent and serious side effects in elderly patients, these predictors would need to be taken into account when deciding whether to continue or discontinue combined treatment with IFN and ribavirin in elderly patients with chronic hepatitis C.

In order to plan the treatment of elderly patients, the natural history of HCV infection in these patients needs to be elucidated, which has not been done as yet. In the present study, we have followed-up treatment-naive patients aged $\geq$65 years without antiviral treatment for more than 3 years. None of them had cirrhosis at baseline. They were stratified by baseline transaminase levels $\leq$40 IU/l (group A (n=120)) and $\geq$41 IU/l (group B (n=212)) and classified further into the three age groups, 65–69, 70–74, and 75–85 years. Cirrhosis and HCC developed more frequently in the patients in group B than those in group A ($p<0.001$ for both). Of the patients aged 65–69 years at entry, in particular, cirrhosis and HCC developed more frequently in group B than in group A ($p<0.001$ and $p=0.001$, respectively). Liver-related causes of death were more common in group B than in group A (20/34 (59%) versus 1/9 (11%), $p<0.05$), and HCC developed more frequently in men than in women ($p=0.021$).

Despite the progression of fibrosis that is accelerated with age [6], liver-related deaths were infrequent in patients with normal baseline transaminase levels and much less often than in those with elevated baseline transaminase levels (1/120 (0.8%) versus 20/212 (9.4%), $p=0.002$). Development of cirrhosis or HCC was no different between patients in groups A and B who were aged 70 years or older at entry. Taken altogether, elderly patients with elevated transaminase levels who are younger than 70 years would be the best candidates for antiviral treatment. They would need to be treated, even when side effects appear, by modifying the doses of IFN and ribavirin. In contrast, antiviral treatment may not be necessary for elderly patients with normal ALAT levels, or can be discontinued in these patients when side effects emerge.

There has been some controversy over antiviral treatment for elderly patients with chronic hepatitis C, and no specific guidelines have been drawn up so far [29]. The sustained virological response to antiviral treatment in aged patients is reported to be either poorer than [30–32] or comparable with that in younger patients [19,33]. The difference is most likely ascribed to careful selection of the aged patients who would benefit from treatment [13]. Based on the natural history of elderly patients with chronic hepatitis C described herein, those with elevated transaminase levels would need treatment to prevent progression to cirrhosis and HCC, while others with normal levels may not require treatment. It is to be hoped that the results in this study might be of help in planning a reasonable treatment strategy towards the longevity, without development of cirrhosis or HCC, in elderly patients with chronic hepatitis C, whose numbers are expected to increase progressively in the foreseeable future.

Acknowledgements

This work was supported in part by grants from the Ministry of Health, Labor, and Welfare of Japan.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Cohen J. The scientific challenge of hepatitis C. Science 1999;285:26–30.

[2] Tanaka J, Kumagai J, Katayama K, Komiya Y, Mizui M, Yamanaka R, et al. Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,648 first-time blood donors during 1995–2000. Intervirology 2004;47:32–40.

[3] Wong JB, McQuillan GM, McHutchison JG, Poynard T. Estimating future hepatitis C morbidity, mortality, and costs in the United States. Am J Public Health 2000;90:1562–9.

[4] Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. Ann Intern Med 2006;144:705–14.

[5] Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. N Engl J Med 1999;340:1228–33.

[6] Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 1997;349:825–32.
[7] Vogt M, Lang T, Frosner G, Klingler C, Sendi AF, Zeller A, et al. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med 1999;341:866–70.
[8] Feray C, Gigou M, Samuel D, Paradis V, Mishiro S, Maertens G, et al. Influence of the genotypes of hepatitis C virus on the severity of recurrent liver disease after liver transplantation. Gastroenterology 1995;108:1088–96.
[9] Garcia-Samaniego J, Soriano V, Castilla J, Bravo R, Moreno A, Carbo J, et al. Influence of hepatitis C virus genotypes and HIV infection on histological severity of chronic hepatitis C. Am J Gastroenterol 1997;92:1130–4.
[10] Pageaux GP, Ducos J, Mondain AM, Costes V, Picot MC, Perrigault PF, et al. Hepatitis C virus genotypes and quantitation of serum hepatitis C virus RNA in liver transplant recipients: relationship with severity of histological recurrence and implications in the pathogenesis of HCV infection. Liver Transpl Surg 1997;3:501–5.
[11] Wali M, Harrison RF, Gow PJ, Mutimer D. Advancing donor liver age and rapid fibrosis progression following transplantation for hepatitis C. Gut 2002;51:248–52.
[12] Kumada T, Toyoda H, Honda T, Kuzuya T, Katano Y, Nakano I, et al. Treatment of chronic hepatitis C with interferon alone or combined with ribavirin in Japan. Intervirology 2006;49:112–8.
[13] Marcus EL, Tur-Kaspa R. Chronic hepatitis C virus infection in older adults. Clin Infect Dis 2005;41:1606–12.
[14] Yoshizawa H, Tanaka J, Miyakawa Y. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. Intervirology 2006;49:7–17.
[15] El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. Ann Intern Med 2003;139:817–23.
[16] Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, et al. Interferon-induced prolonged biochemical response reduces hepatocarcinogenesis in hepatitis C virus infection. J Med Virol 2007;79:1485–90.
[17] Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 1999;29:1124–30.
[18] Koyama R, Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, et al. Efficacy of interferon therapy in elderly patients with chronic hepatitis C. Intervirology 2006;49:121–6.
[19] Honda T, Katano Y, Urano F, Murayama M, Hayashi K, Ishigami M, et al. Efficacy of ribavirin plus interferon-alpha in patients aged (60 years with chronic hepatitis C. J Gastroenterol Hepatol 2007;22:989–95.
[20] Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. N Engl J Med 1998;339:1493–9.
[21] Iwasaki Y, Ikeda H, Araki Y, Osawa T, Kita K, Ando M et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology 2006;43:54–63.
[22] Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 1998;352:1426–32.
[23] Verna EC, Brown RS Jr. Hepatitis C and liver transplantation: enhancing outcomes and should patients be retransplanted? Clin Liver Dis 2008;12:637–59.
[24] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347: 975–82.
[25] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–65.
[26] Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38:645–52.
[27] Antonucci G, Longo MA, Angeletti C, Vairo F, Oliva A, Comandini UV, et al. The effect of age on response to therapy with peginterferon alpha plus ribavirin in a cohort of patients with chronic HCV hepatitis including subjects older than 65 years. Am J Gastroenterol 2007;102:1383–91.
[28] Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol 2007;79:1686–95.
[29] Wright TL. Treatment of patients with hepatitis C and cirrhosis. Hepatology 2002;36:S185–94.
[30] Floreani A, Minola E, Carderi I, Ferrara F, Rizzotto ER, Baldo V. Are elderly patients poor candidates for pegylated interferon plus ribavirin in the treatment of chronic hepatitis C? J Am Geriatr Soc 2006;54:549–50.
[31] Hayashi J, Kishihara Y, Ueno F, Yamaji K, Kawakami Y, Furusyo N, et al. Age-related response to interferon alfa treatment in women vs men with chronic hepatitis C virus infection. Arch Intern Med 1998;158:177–81.
[32] Hiramatsu N, Oze T, Tsuda N, Kurashige N, Koga K, Toyama T, et al. Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy? Hepatol Res 2006;35:185–9.
[33] Tsui JI, Currie S, Shen H, Bini EJ, Brau N, Wright TL. Treatment eligibility and outcomes in elderly patients with chronic hepatitis C: results from the VA HCV-001 Study. Dig Dis Sci 2008;53:809–14.

免疫抑制・化学療法により発症するB型肝炎対策
—厚生労働省「難治性の肝・胆道疾患に関する調査研究」班劇症肝炎分科会および「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」班合同報告—

坪内　博仁　熊田　博光　清澤　研道　持田　智　坂井田　功
田中　榮司　市田　隆文　溝上　雅史　鈴木　一幸　輿芝　眞彰
森脇　久隆　日比　紀文　林　紀夫　國土　典宏　藤澤　知雄
石橋　大海　菅原　寧彦　八橋　弘　井戸　章雄　滝川　康裕
井上　和明　桶谷　真　宇都　浩文　中山　伸朗　内木　隆文
多田慎一郎　木曽　真一　矢野　公士　遠藤　龍人　田中　靖人
梅村　武司　熊谷公太郎

「肝臓」第50巻　第1号（2009）別刷

肝臓　50巻1号　38-42（2009）

＜特別寄稿＞

免疫抑制・化学療法により発症するB型肝炎対策 —厚生労働省「難治性の肝・胆道疾患に関する調査研究」班 劇症肝炎分科会および「肝硬変を含めたウイルス性肝疾患の 治療の標準化に関する研究」班合同報告—

坪内　博仁[1)*]　熊田　博光[2)]　清澤　研道[3)]　持田　智[4)]　坂井田　功[5)]
田中　榮司[6)]　市田　隆文[7)]　溝上　雅史[8)]　鈴木　一幸[9)]　與芝　眞彰[10)]
森脇　久隆[11)]　日比　紀文[12)]　林　紀夫[13)]　國土　典宏[14)]　藤澤　知雄[15)]
石橋　大海[16)]　菅原　寧彦[14)]　八橋　弘[16)]　井戸　章雄[1)]　滝川　康裕[9)]
井上　和明[10)]　桶谷　真[1)]　宇都　浩文[1)]　中山　伸朗[4)]　内木　隆文[11)]
多田慎一郎[12)]　木曽　真一[13)]　矢野　公士[16)]　遠藤　龍人[9)]　田中　靖人[8)]
梅村　武司[6)]　熊谷公太郎[1)]

索引用語：劇症肝炎　HBV再活性化　*de novo* B型肝炎　核酸アナログ製剤　リツキシマブ

近年，化学療法，免疫療法，移植療法の進歩に伴い，多様な抗癌剤や免疫抑制剤を使用する機会が増加している．以前よりB型肝炎ウイルス（HBV）キャリアに合併した悪性腫瘍患者に対し，ステロイドを併用した化学療法を施行した場合，HBVの急激な増殖すなわちHBVの再活性化（reactivation）により致死的な重症肝炎が発症することが知られていた[1)2)]．HBV遺伝子にはglucocorticoid enhancement elementが存在するため[3)]，ステロイドにより直接的にウイルス複製が助長されるだけでなく，化学療法による免疫抑制や治療終了後に生じる免疫学的な均衡の破綻により，HBVの増殖とともに広範な感染肝細胞の破壊を伴う重症肝炎が惹起される．このようなHBVキャリアに対する化学療法時にはラミブジンなどの核酸アナログを予防投与してHBV再活性化を避けることが必要である[4)]．

一方，HBs抗原陰性でHBc抗体ないしHBs抗体陽性例は従来HBV既往感染とされ，臨床的には治癒の状態と考えられてきた．しかしこのような既往感染例でも肝臓や末梢血単核球中では低レベルながらHBV-DNAの複製が長期間持続することが明らかになっている[5)–7)]．最近，移植後やB細胞表面抗原CD20に対する抗体であるリツキシマブなど強力な免疫抑制剤の使用により，このような既往感染例からもHBV再活性化により重症肝炎が発症することが報告され，de novo B型肝炎と呼ばれている[8)–10)]．厚生労働省「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」班の全国調査によりこのようなde novo B型肝炎は通常のB型肝炎

1）鹿児島大学大学院消化器疾患・生活習慣病学
2）国家公務員共済組合連合会虎の門病院
3）長野赤十字病院内科
4）埼玉医科大学消化器内科・肝臓内科
5）山口大学大学院消化器病態内科学
6）信州大学医学部消化器内科
7）順天堂大学医学部附属静岡病院消化器内科
8）名古屋市立大学大学院臨床分子情報医学
9）岩手医科大学第一内科
10）昭和大学藤が丘病院消化器内科
11）岐阜大学大学院消化器病態学
12）慶應義塾大学医学部消化器内科
13）大阪大学大学院消化器内科学
14）東京大学大学院肝胆膵外科学・人工臓器移植外科学
15）済生会横浜市東部病院こどもセンター
16）国立病院機構長崎医療センター臨床研究センター
*Corresponding author:
htsubo@m2.kufm.kagoshima-u.ac.jp
＜受付日2008年10月23日＞＜採択日2008年12月8日＞

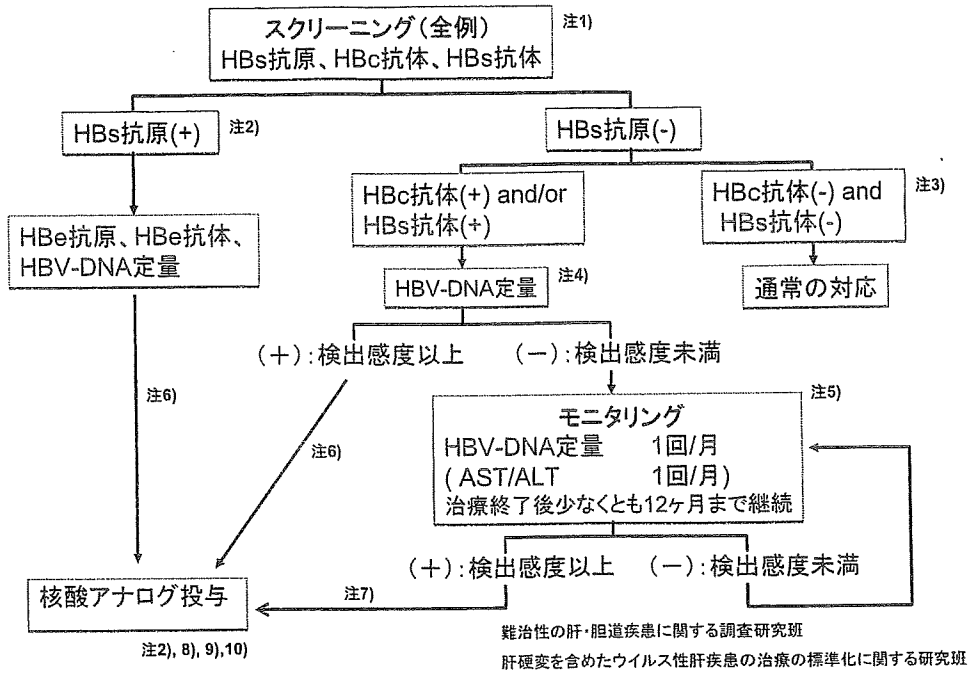

Fig. 1　免疫抑制・化学療法により発症するB型肝炎対策ガイドライン*

補足

*血液悪性疾患に対する強力な免疫・抑制化学療法中あるいは終了後にHBs抗原陽性あるいはHBs抗原陰性例の一部にHBV再活性化によりB型肝炎が発症し，その中には劇症化する症例があり，注意が必要である．その他の疾患においても治療によるHBV再活性化のリスクを考慮して対応する必要がある．また，ここで推奨する核酸アナログ予防投与のエビデンスはなく，劇症化予防効果を完全に保証するものではない．

注1)　CLIA法で測定することが望ましい．

注2)　HBs抗原陽性例は肝臓専門医にコンサルトすること．全ての症例で核酸アナログ投与にあたっては肝臓専門医にコンサルトするのが望ましい．

注3)　初回治療時にHBc抗体，HBs抗体未測定の再治療例では抗体価が低下している場合があり，HBV-DNA定量検査などによる精査が望ましい．

注4)　PCR法およびリアルタイムPCR法により実施する．より検出感度の高いリアルタイムPCR法が望ましい．

注5)　リツキシマブ・ステロイド使用例，造血細胞移植例はHBV再活性化の高リスクであり，注意が必要である．フルダラビンは強力な免疫抑制作用を有するが，HBV再活性化のリスクは不明であり，今後注意が必要である．

注6)　免疫抑制・化学療法を開始する前，できるだけ早期に投与を開始するのが望ましい．

注7)　免疫抑制・化学療法中はHBV-DNA定量検査が検出感度以上になった時点で直ちに投与を開始する．

注8)　核酸アナログはエンテカビルの使用を推奨する．

注9)　下記の条件を満たす場合には核酸アナログ投与の終了を検討して良い．
スクリーニング時にHBs抗原（+）例ではB型慢性肝炎における核酸アナログ投与終了基準を満たす場合．スクリーニング時にHBc抗体（+）and/or HBs抗体（+）例では，(1) 免疫抑制・化学療法終了後，少なくとも12カ月間は投与を継続すること．(2) この継続期間中にALT（GPT）が正常化していること．(但しHBV以外にALT異常の原因がある場合は除く) (3) この継続期間中にHBV-DNAが持続陰性化していること．

注10)　核酸アナログ投与終了後12カ月間は厳重に経過観察する．経過観察方法は各核酸アナログの使用上の注意に基づく．経過観察中にHBV-DNA定量検査が検出感度以上になった時点で直ちに投与を再開する．

に比して劇症化する頻度が高率で，死亡率も高いことが明らかになった[11)~13)]．また，厚生労働省「難治性の肝・胆道疾患に関する調査研究」班で実施している劇症肝炎・遅発性肝不全(LOHF)の全国調査でもここ数年，特に悪性リンパ腫に対しリツキシマブとステロイドを併用したR-CHOP治療例からの劇症化やde novo B型肝炎が増加傾向にあり，予後不良であった[14)15)]．以上のような経緯から，早急なHBV再活性化対策が必要となり，両研究班が合同でワーキンググループを立ち上げ，Fig. 1に示すガイドラインを作成した．

ガイドラインの要旨は以下のとおりである．まずHBV再活性化リスク群の同定を目的にスクリーニング検査として，全ての症例にHBs抗原およびHBc抗体，HBs抗体を測定する．HBs抗原が陽性の場合はさらにHBe抗原，HBe抗体，HBV-DNA定量検査を実施する．HBs抗原陽性例では，無症候性キャリアだけではなく，慢

性肝炎，肝硬変例が含まれる可能性があるので肝臓専門医にコンサルトする必要がある．HBs抗原陽性例での再活性化のリスクは大きいので，基本的に核酸アナログの予防投与を実施する．但し，HBV再活性化のリスクが少ない悪性疾患以外の若年HBe抗原陽性無症候性キャリアに対するステロイド治療例などでは，核酸アナログ予防投与の有効性に関するエビデンスはなく経過観察など他の選択肢があり，適応は慎重に判断する必要がある．HBs抗原陰性でHBc抗体，HBs抗体いずれも陰性の場合は通常の対応とする．HBs抗原陰性でHBc抗体ないしHBs抗体が陽性，すなわち感染既往例と判断される場合は更にHBV-DNA定量検査を実施し，HBV-DNAが陽性の場合は核酸アナログの予防投与を行う．一方，HBV-DNAが陰性の場合はHBV-DNAを毎月モニタリングしながら，陽性化した時点で直ちに核酸アナログを投与する．特にリツキシマブ・ステロイド使用例，造血細胞移植例は再活性化のリスクが高いので慎重な対応が必要である．核酸アナログ予防投与例の投与中止時期に関する明確なエビデンスはないが，HBs抗原陰性，HBc抗体ないしHBs抗体陽性例では免疫抑制・化学療法終了後も12カ月間は投与を継続し，この継続期間中に一定の基準を満たせば投与終了も可能とした．以下にガイドライン作成にあたり論点になった事項を補足する．①スクリーニングにあたってはHBs抗原だけではなくHBc抗体，HBs抗体をできるだけ感度の高い検査法で実施する必要がある．HBs抗原陰性でHBc抗体，HBs抗体いずれも陰性の場合でも，患者が既に免疫抑制状態にある場合には抗体が検出されないことがあり，HBV-DNA定量検査まで測定することが望ましい．②B型キャリア例の急性増悪では発症後早期の核酸アナログ治療が有効であるが，HBV再活性化による劇症化例は発症後の核酸アナログ治療では予後不良であり，発症前の予防投与が必要である．しかし既往感染例でのHBV再活性化率は明らかでなく，また本邦におけるHBc抗体ないしHBs抗体陽性の既往感染例の頻度は高率であることより，全ての症例に核酸アナログの予防投与を実施するのは医療経済的にも困難である．Huiらの報告[16]ではHBs抗原陰性例のHBV再活性化では，HBV-DNAが陽性化し，肝炎が発症するまでに12～28週（平均18.5週）を要しており，したがってHBV-DNAをPCR法またはリアルタイムPCR法で毎月モニタリングし，検出感度以上になった時点で直ちに核酸アナログを投与しても肝炎の重症化は予防可能と推測される．③核酸アナログ製剤はB型慢性肝炎の治療ガイドライン[17]に準拠して，エンテカビル投与を推奨している．しかし，投与期間が長期に及ばない場合など，より安価なラミブジンへの代用も検討の余地がある．④核酸アナログ投与終了に関する明確な基準はない．HBs抗原陽性例では使用する各核酸アナログの投与終了基準に準ずる．HBs抗原陰性，HBc抗体ないしHBs抗体陽性例では免疫抑制・化学療法終了後も12カ月間は投与を継続し，この継続期間中にALTの正常化とHBV-DNAの持続陰性化が見られる場合は投与終了の検討も可能である．但し，HBV以外にALT異常の原因がある場合はALTの正常化は必須ではない．また，核酸アナログ予防投与終了後のHBV再活性化例の報告もあり，投与終了後も更に12カ月間は厳重な経過観察が必要である[18]．

本ガイドライン作成にあたってはワーキンググループ委員の他，名古屋市立大学腫瘍・免疫内科学および鹿児島大学大学院消化器疾患・生活習慣病学血液内科グループの協力および助言を得た．今後は本ガイドラインを血液内科をはじめとする関係領域に周知させていくとともに，各分野と協力して本ガイドラインの有効性を検証していくことが重要である．

謝辞：本研究は厚生労働省科学研究費難治性疾患克服研究事業および肝炎等克服緊急対策研究事業からの助成金によって支援された．

文　献

1) Lok AS, Liang RH, Chiu EK, et al. Reactivation of hepatitis B virus replication in patients receiving cytotoxic chemotherapy. Report of a prospective study. Gastroenterology 1991; 100: 182—188
2) Yeo W, Johnson PJ. Diagnosis, prevention and management of hepatitis B virus reactivation during anticancer therapy. Hepatology 2006; 43: 209—220
3) Chou CK, Wang LH, Lin HM, et al. Glucocorticoid stimulates hepatitis B viral gene expression in cultured human hepatoma cells. Hepatology 1992; 16: 13—18
4) Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007; 45: 507—539
5) Kuhns M, McNamara A, Mason A, et al. Serum and liver hepatitis B virus DNA in chronic hepatitis B after sustained loss of surface antigen. Gastroenterology 1992; 103: 1649—1656
6) Fong TL, Di Bisceglie AM, Gerber MA, et al. Per-

sistence of hepatitis B virus DNA in the liver after loss of HBsAg in chronic hepatitis B. Hepatology 1993; 18: 1313—1318
7) Michalak TI, Pasquinelli C, Guilhot S, et al. Hepatitis B virus persistence after recovery from acute viral hepatitis. J Clin Invest 1994; 93: 230—239
8) Hui CK, Sun J, Au WY, et al. Occult hepatitis B virus infection in hematopoietic stem cell donors in a hepatitis B virus endemic area. J Hepatol 2005; 42: 813—819
9) Kawatani T, Suou T, Tajima F, et al. Incidence of hepatitis virus infection and severe liver dysfunction in patients receiving chemotherapy for hematologic malignancies. Eur J Haematol 2001; 67: 45—50
10) Dhédin N, Douvin C, Kuentz M, et al. Reverse seroconversion of hepatitis B after allogeneic bone marrow transplantation: a retrospective study of 37 patients with pretransplant anti-HBs and anti-HBc. Transplantation 1998; 66: 616—619
11) 清澤研道，梅村武司，熊田博光，他．免疫抑制・化学療法中に発生するde novo B型急性肝炎の発症機序の検討．「厚生労働省肝炎等克服緊急対策事業「B型及びC型肝炎ウイルスの感染者に対する治療の標準化に関する臨床的研究」班　平成18年度研究報告書」2007，p30—32
12) 田中榮司，梅村武司，清澤研道，他．de novo B型急性肝炎の全国調査成績．「厚生労働省肝炎等克服緊急対策事業「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」班　平成19年度研究報告書」2008，p34—35
13) Umemura T, Tanaka E, Kiyosawa K, et al. Mortality secondary to fulminant hepatic failure in patients with prior resolution of hepatitis B virus infection in Japan. Clin Infect Dis 2008; 47: e52—56
14) 坪内博仁，桶谷　真，井戸章雄，他．劇症肝炎及び遅発性肝不全の全国集計（2005年）．「厚生労働省難治性疾患克服研究事業「難治性の肝・胆道疾患に関する調査研究」班　平成18年度研究報告書」2007，p90—100
15) 坪内博仁，桶谷　真，井戸章雄，他．劇症肝炎及び遅発性肝不全の全国集計（2006年）．「厚生労働省難治性疾患克服研究事業「難治性の肝・胆道疾患に関する調査研究」班　平成19年度研究報告書」2008，p83—94
16) Hui CK, Cheung WW, Zhang HY, et al. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. Gastroenterology 2006; 131: 59—68
17) 熊田博光．肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究．「厚生労働省肝炎等克服緊急対策事業「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」班　平成19年度研究報告書」2008，p1—11
18) Dai MS, Chao TY, Kao WY, et al. Delayed hepatitis B virus reactivation after cessation of preemptive lamivudine in lymphoma patients treated with rituximab plus CHOP. Ann Hematol 2004; 83: 769—774

Prevention of immunosuppressive therapy or chemotherapy-induced reactivation of hepatitis B virus infection —Joint report of the Intractable Liver Diseases Study Group of Japan and the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis—

Hirohito Tsubouchi[1]*, Hiromitsu Kumada[2], Kendo Kiyosawa[3], Satoshi Mochida[4], Isao Sakaida[5], Eiji Tanaka[6], Takafumi Ichida[7], Masashi Mizokami[8], Kazuyuki Suzuki[9], Shinsyo Yoshiba[10], Hisataka Moriwaki[11], Toshifumi Hibi[12], Norio Hayashi[13], Norihiro Kokudo[14], Tomoo Fujisawa[15], Hiromi Ishibashi[16], Yasuhiko Sugawara[14], Hiroshi Yatsuhashi[16], Akio Ido[1], Yasuhiro Takikawa[9], Kazuaki Inoue[10], Makoto Oketani[1], Hirofumi Uto[1], Nobuaki Nakayama[4], Takafumi Naiki[11], Shinichiro Tada[12], Shinichi Kiso[13], Koji Yano[16], Ryujin Endo[9], Yasuhito Tanaka[8], Takeji Umemura[6], Kotaro Kumagai[1]

Key words: fulminant hepatitis　HBV reactivation　*de novo* hepatitis B　nucleoside analog　rituximab

Kanzo 2009; 50: 38—42

1) Digestive Disease and Life-style related Disease, Kagoshima University Graduate School of Medical and Dental Sciences
2) Department of Hepatology, Toranomon Hospital
3) Department of Internal Medicine, Nagano Red Cross Hospital
4) Division of Gastroenterology and Hepatology, Department of Internal Medicine, Faculty of Medicine, Saitama Medical University
5) Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine
6) Department of Internal Medicine, Hepatology, and Gastroenterology, Shinshu University School of Medicine
7) Department of Gastroenterology, Juntendo University Shizuoka Hospital
8) Department of Clinical Molecular Informative Medicine, Nagoya City University Graduate School of Medical Sciences
9) First Department of Internal Medicine, Iwate Medical University
10) Division of Gastroenterology, Showa University Fujigaoka Hospital
11) Department of Gastroenterology, Gifu University Graduate School of Medicine
12) Department of Internal Medicine, School of Medicine, Keio University
13) Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine
14) Hepato-Biliary-Pancreatic Surgery Division, Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, University of Tokyo
15) Children's Center for Health and Development; Yokohama City Tobu Hospital
16) Clinical Research Center and Clinical Laboratory, NHO Nagasaki Medical Center
*Corresponding author: htsubo@m2.kufm.kagoshima-u.ac.jp

肝臓　50巻10号　588-589（2009）

＜速　報＞

核酸アナログ療法中のB型関連肝癌に対する肝癌再発予測マーカーとしてのHBコア関連抗原の有用性

保坂　哲也[1)*]　鈴木　文孝[1)]　小林　正宏[1)]　平川　美晴[1)]　川村　祐介[1)]
八辻　寛美[1)]　瀬崎ひとみ[1)]　芥田　憲夫[1)]　鈴木　義之[1)]　斎藤　聡[1)]
荒瀬　康司[1)]　池田　健次[1)]　小林万利子[2)]　熊田　博光[1)]

緒言：B型肝疾患に対する核酸アナログ療法の有効性は広く知られており，ラミブジンにおいては投与により発癌率を抑制することが既に報告されている[1)2)]．しかしながら経過観察期間が長くなるにつれ肝発癌例も増加しつつある．また血中HBV-DNA量が抑制されているにもかかわらず，肝癌根治後の再発例も散見される．そこで今回我々は核酸アナログ投与中の肝癌について，肝癌根治療法後の再発予測マーカーとしてのHBコア関連抗原（HBcrAg）の有用性を検討した．

対象と方法：2001年～2008年までに当院で初発の肝細胞癌と診断されたB型肝癌症例で核酸アナログ投与中に肝発癌した54例を対象とした．肝癌発症時の核酸アナログ投与内容の内訳はラミブジン29例，ラミブジン＋アデフォビル併用17例，エンテカビル8例であった．肝癌治療法の内訳は外科切除36例，経皮的局所治療18例であった．HBcrAg測定は既報のごとくCLEIA法を[3)]，HBV-DNA量はアンプリコア法を用いた．肝癌根治後の再発に寄与する因子についてCox比例ハザードモデルを用いて，単変量及び多変量解析を行い検討した．

結果：発癌時のAST/ALT値は31/29 IU/l(中央値)，genotype Cが92.6%（50/54）で，HBe抗原陽性例は42.6%（23/54），血清HBV-DNA量は＜2.6 log copies/ml（中央値）であった．血清HBcrAg量は5.0 logU/ml（中央値）であった．血清HBV-DNA量＜2.6 log copies/mlであった症例35例中，HBcrAg量≧3.0 logU/mlであった症例が29例（82.9%），≧4.8 logU/mlであった症例は13例（37.1%）であった．核酸アナログ投与開始から発癌までの投与期間は2.2年（中央値）であった．

肝癌再発は38.9%（21/54）で認め，根治後から再発までの期間は14カ月（中央値）であった．再発に寄与する因子について単変量解析を行ったところ，HBV-DNA量≧3.0 log copies/ml，HBcrAg≧4.8 logU/ml，腫瘍数多発，門脈浸潤ありの4因子が抽出され，さらに多変量解析を行ったところ，独立因子としてHBcrAg≧4.8 logU/ml，門脈浸潤の2因子が抽出された(Table)．

考察：今回の検討では核酸アナログ投与中の発癌例は血清HBV-DNA量が低値に抑制されているにもかかわらず，HBcrAg量は十分抑制されていない例が認められた[4)]．核酸アナログが投与されていないB型肝癌において，血清HBV-DNA量が肝癌再発に関係するという報告はされている[5)]．しかしながら今回の対象症例のように核酸アナログ投与中の場合はHBV-DNA量よりHBcrAg量の方が肝癌根治後の再発予測マーカーとして有用であると考えられる．

索引用語：HBコア関連抗原，肝癌再発予測，核酸アナログ

文献：1) Liaw YF, Sung JJ, Chow WC, et al. N Engl J Med 2004; 351: 1521—1531　2) Matsumoto A, Tanaka E, Rokuhara A, et al. Hepatol Res 2005; 32: 173—184　3) Kimura T, Rokuhara A, Sakamoto Y, et al. J Clin Microbiol 2002; 40: 439—445　4) 辻　邦彦，西森博幸，松居剛志，他．肝臓　2009；50：166—167　5) Kubo S, Hirohashi K, Tanaka H, et al. Cancer 2000; 88: 1016—1024

1) 虎の門病院肝臓センター
2) 肝臓研究室
*Corresponding author: hosa-p@toranomon.gr.jp

＜受付日2009年8月11日＞＜採択日2009年9月1日＞

Table　Factors associated with recurrence of HCC by univariate and multivariate analysis.

	Univariate		Multivariate	
factors	Hazard Ratio (95%CI)	P	Hazard Ratio (95%CI)	P
HBeAg (Positive)	1.53 (0.63-3.70)	0.343		
HBV DNA ($\geq$ 3.0 logcopies/mL)	2.49 (1.03-6.00)	0.042		
HBcrAg ($\geq$ 4.8 logU/mL)	10.4 (2.39-45.0)	0.002	8.50 (1.95-37.1)	0.004
AST ($\geq$ 50 IU/L)	2.47 (0.98-6.20)	0.055		
ALT ($\geq$ 40 IU/L)	2.37 (0.99-5.71)	0.054		
Platelets count ($< 10^5$ /mm^3)	2.20 (0.81-6.02)	0.123		
Serum Albumin ($<$ 3.5 g/dl)	1.39 (0.53-3.63)	0.505		
Serum bilirubin ($\geq$ 1.5 mg/dl)	1.11 (0.62-2.00)	0.713		
Prothorombin time ($<$ 80%)	2.23 (0.51-9.82)	0.286		
ICG-R 15 ($\geq$ 30%)	0.54 (0.16-1.87)	0.332		
AFP levels ($\geq$ 100 ng/mL)	1.81 (0.74-4.44)	0.194		
DCP levels ($\geq$ 100 mAU/mL)	2.09 (0.81-5.39)	0.129		
Tumor size ($\geq$ 21 mm)	2.02 (0.81-5.07)	0.133		
Tumor number (multiple)	4.03 (1.31-12.4)	0.015		
Presence of portal vein invasion	5.39 (1.69-17.2)	0.004	3.63 (1.15-11.5)	0.028

Abbreviation: AST, aspartate aminotransferase; ALT, alaine aminotransferase; ICG-R15: indocyanine green retention test at 15 min; AFP, alpha-fetoprotein; DCP, des-γ-carboxylprothorombin,

英文要旨

Low hepatitis B virus core-related antigen is a predictor of absence in post-treatment recurrence of hepatocellular carcinoma during antiviral therapy

Tetsuya Hosaka[1]*, Fumitaka Suzuki[1], Masahiro Kobayashi[1], Miharu Hirakawa[1], Yusuke Kawamura[1], Hiromi Yatsuji[1], Hitomi Sezaki[1], Norio Akuta[1], Yoshiyuki Suzuki[1], Satoshi Saitoh[1], Yasuji Arase[1], Kenji Ikeda[1], Mariko Kobayashi[2], Hiromitsu Kumada[1]

The tumor recurrence rate of hepatocellular carcinoma (HCC) is still high even in patients who receive a curative therapy. We analyzed predictive value of HBV-related viral markers, including HBcrAg, HBV DNA, and HBeAg, for HCC recurrence in the patients who developed HCC during antiviral nucleot(s)ide analogues therapy. By univariate analysis, HBV DNA, HBcrAg, tumor number and presence of portal vein invasion were significant predictive factors. By multivariate analysis, HBcrAg and presence of portal vein invasion were independent and significant predictive factors of recurrence after curative therapy for HCC. We conclude that HBcrAg is useful as a predictor of post-treatment recurrence of HCC after curative therapy in patients who received antiviral therapy.

Key words: HB core-related antigen, prediction of recurrence of HCC, nucleot(s)ide analogues

Kanzo 2009; 50: 588—589

1) Department of Hepatology, Toranomon Hospital, Tokyo
2) Department of Research Institute for Hepatology, Toranomon Branch Hospital, Kawasaki
*Corresponding author: hosa-p@toranomon.gr.jp

Cancer Letters 275 (2009) 27–34

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

A novel amplification target, *ARHGAP5*, promotes cell spreading and migration by negatively regulating RhoA in Huh-7 hepatocellular carcinoma cells

Yasuyuki Gen [a], Kohichiroh Yasui [a,*], Keika Zen [a], Tomoaki Nakajima [a], Kazuhiro Tsuji [a], Mio Endo [a], Hironori Mitsuyoshi [a], Masahito Minami [a], Yoshito Itoh [a], Shinji Tanaka [b], Masafumi Taniwaki [c], Shigeki Arii [b], Takeshi Okanoue [a,d], Toshikazu Yoshikawa [a]

[a] *Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kamigyo-ku, Kyoto 602-8566, Japan*
[b] *Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University, Tokyo, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan*
[c] *Molecular Hematology and Oncology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, 465 Kajii-cho, Kamigyo-ku, Kyoto 602-8566, Japan*
[d] *Department of Hepatology, Saiseikai Suita Hospital, Suita, Osaka 564-0013, Japan*

ARTICLE INFO

Article history:
Received 19 August 2008
Received in revised form 23 September 2008
Accepted 30 September 2008

Keywords:
ARHGAP5
p190-B RhoGAP
RhoA
Amplification
Hepatocellular carcinoma

ABSTRACT

RhoA, a member of the Rho family of small GTPases, directs the organization of the actin cytoskeleton and is involved in regulating cell shape and movement. Its activity is negatively regulated by p190-B RhoGAP (GTPase-activating protein). We investigated DNA copy number aberrations in human hepatocellular carcinoma and esophageal squamous cell carcinoma cell lines using a high-density oligonucleotide microarray and found a novel amplification at chromosomal region 14q12. We identified *ARHGAP5* (the gene encoding p190-B RhoGAP) as a probable target for the amplification at 14q12, and our results showed that p190-B RhoGAP promotes cells spreading and migration by negatively regulating RhoA activity in Huh-7 hepatocellular carcinoma cells.

1. Introduction

Members of the Rho family of small GTPases act as molecular switches. In response to extracellular signals, they direct the organization of the actin cytoskeleton and alter gene expression [1]. Rho proteins, which include the much-studied Cdc42, Rac1 and RhoA, are involved in regulating cell shape, polarity and movement and establishing cell-cell junctional complexes. Accordingly, their activity is tightly controlled by regulatory proteins that determine whether GTP or GDP is bound. Rho proteins are activated by guanine nucleotide exchange factors, which catalyze the release of GDP and thus allow GTP to bind the proteins. Rho proteins in turn are inactivated by Rho GTPase-activating proteins (GAPs), which bind to the Rho proteins and induce them to hydrolyze their bound GTP to GDP. p190-B RhoGAP, a member of the RhoGAP family, negatively regulates RhoA activity [2,3].

Amplification of DNA in certain regions of chromosomes plays a crucial role in the development and progression of human malignancies, specifically when proto-oncogenic target genes within those amplicons are overexpressed. Oncogenes that are often amplified in cancers include *MYC*, *ERBB2* and *CCND1*.

In the present study, we investigated DNA copy number aberrations in human hepatocellular carcinoma (HCC) and

* Corresponding author. Tel.: +81 75 251 5519; fax: +81 75 251 0710.
E-mail address: yasuik@koto.kpu-m.ac.jp (K. Yasui).

0304-3835/$ - see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2008.09.036

esophageal squamous cell carcinoma (ESCC) cell lines and found a novel amplification at chromosomal region 14q12. Because the region may harbor one or more proto-oncogenes whose overexpression following amplification contributes to the initiation or progression of HCC and ESCC, we carried out molecular definition of the amplicon. We show here that the p190-B RhoGAP gene (*ARHGAP5*) within the 14q12 amplicon is amplified and overexpressed, and that p190-B RhoGAP promotes cell spreading and migration in Huh-7 hepatocellular carcinoma cells.

2. Materials and methods

2.1. Cell lines

A total of 10 HCC cell lines (JHH-6, JHH-7, SNU354, SNU398, SNU423, SNU475, Huh-1, Huh-7, HLE and PLC/PRF/5) and 10 ESCC cell lines (T.T, EC-GI-10, KYSE140, KYSE220, TE-4, TE-5, TE-6, TE-10, TE-14 and TE-15) were examined. All cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS). Genomic DNA was isolated from each cell line using the Puregene DNA isolation kit (Gentra, Minneapolis, MN, USA).

2.2. Array analysis

Array analyses were performed using the GeneChip Mapping 250K Sty array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions. In brief, 250 ng of genomic DNA was digested with a restriction enzyme (StyI), ligated to an adaptor and amplified by PCR. Amplified products were fragmented, labeled by biotinylation and hybridized to the microarrays. Hybridization was detected by incubation with streptavidin–phycoerythrin conjugate, and the array was scanned. Analysis was performed as previously described [4]. Copy number changes were calculated using the Copy Number Analyzer for Affymetrix GeneChip Mapping Arrays (CNAG; http://www.genome.umin.jp) [5].

2.3. Fluorescence in situ hybridization (FISH)

We performed FISH using three bacterial artificial chromosomes (BACs), RP11-113E19, RP11-431H16 and RP11-54H22 as probes (Invitrogen, Carlsbad, CA, USA), as described previously [6]. The BACs were selected based on homology with locations in the human genome according to the database provided by the UCSC (http://genome.ucsc.edu/).

2.4. Real-time quantitative PCR

We quantified genomic DNA and mRNA using a real-time fluorescence detection method, as described previously [6]. The primers used were as follows: *ARHGAP5* mRNA (forward, 5′-CATCTGTTTTTGGCCAACCT-3′; reverse, 5′-gtggaggagccacaatgttt-3′); *HEATR5A* mRNA (forward, 5′-TGTGCCTCCTACTCATGCTG-3′; reverse, 5′-gagatggcctgagct tgaac-3′); *c14orf126* mRNA (forward, 5′-gtgctttttcaaggga gctg-3′; reverse, 5′-ttcctccaagggtagcttga-3′); *NUBPL* mRNA (forward, 5′-cttggccttgtccaaaacat-3′; reverse, 5′-acaattggc tggcctgtatc-3′). These primers were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) based on sequence data obtained from the NCBI database (http://www.ncbi.nlm.nih.gov/). *GAPDH* and long interspersed nuclear element 1 (LINE-1) were used as endogenous controls for mRNA and genomic DNA levels, respectively.

2.5. RNA interference (RNAi)

For RNAi, small interfering RNA (siRNA) duplex oligoribonucleotides targeting *ARHGAP5* (5′-CAAGATCATAATATCAATCTA-3′) and control (non-silencing) siRNA duplexes were synthesized by QIAGEN (Valencia, CA, USA). The siRNAs were delivered into Huh-7 cells using HiPerfect Transfection Reagent (QIAGEN), according to the manufacturer's protocol.

2.6. Immunoblotting

Immunoblots were prepared according to previously reported methods [7]. Cell lysates (20 μg protein per sample) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 10% acrylamide gels. Anti-p190-B RhoGAP monoclonal antibody was obtained from BD Transduction Laboratories (Lexington, KY, USA); anti-RhoA monoclonal antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA); and anti-β-actin monoclonal antibody was from Sigma–Aldrich (Tokyo, Japan). For immunoblotting, we used anti-p190-B RhoGAP, anti-RhoA and anti-β-actin at dilutions of 1:250, 1:100 and 1:5000, respectively. For secondary immunodetection, we used anti-mouse IgG (Amersham, Tokyo, Japan) diluted 1:5000. Protein binding was detected using the ECL system (Amersham).

2.7. RhoA activity assay

Active RhoA levels were measured using the enzyme-linked immunosorbent assay (ELISA)-based G-LISA RhoA activation assay Biochem Kit (Cytoskeleton, Denver, CO, USA) according to the manufacturer's instructions. In brief, Huh-7 cells were transfected with siRNA targeting *ARHGAP5* or negative control siRNA, or were left untreated. Cells were then cultured under the standard conditions in DMEM containing 10% FCS. After 48 h, cells were harvested for the RhoA activity assay or trypsinized and held in suspension for 1 h in DMEM containing 1% FCS. The suspended cells were then plated on 6-well plates coated with 5 μg/ml fibronectin (BD Transduction Laboratories) and harvested for the RhoA activity assay at the indicated time points. For the RhoA activity assay, cells were lysed in 70 μl of G-LISA lysis buffer, scraped into tubes and snap frozen in liquid nitrogen. Cell lysates were subsequently thawed, clarified for 2 min at 10,000g, and protein concentrations were normalized between the various time points. Equal amounts of total protein were added to a 96-well plate coated with the Rho-binding domain of Rho effector pro-

teins (which bind active GTP-bound Rho) in triplicate and incubated at 4 °C for 30 min with vigorous shaking. Active Rho levels were determined by subsequent incubations with anti-Rho antibody and secondary horseradish peroxidase-conjugated antibody for 45 min each at room temperature. After adding developing solution, the level of active Rho was determined by measuring absorbance at 490 nm using an ELISA plate reader. Equal loading of total RhoA protein at each time point was determined via immunoblotting using anti-RhoA antibody as described above. Experiments were repeated at least three times.

2.8. Immunofluorescence

Huh-7 cells were transfected with siRNA targeting *ARHGAP5* or negative control siRNA or were left untreated. Cells were harvested 48 h after transfection, suspended for 1 h in DMEM containing 1% FCS and then plated on glass slides coated with fibronectin for 10, 20, 40, 60 or 180 min. Cells were fixed for 10 min in 3.7% formaldehyde, permeabilized for 2 min in 1% Triton X-100 and incubated for 1 h with a blocking buffer (phosphate-buffered saline containing 3% bovine serum albumin). The cells were then incubated for 1 h at room temperature with anti-p190-B RhoGAP monoclonal antibody diluted 1:200 in blocking buffer. Fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Cappel, Aurora, OH, USA) was used to detect the primary antibody. Actin filaments and nuclei were counterstained with rhodamine-phalloidin (Molecular Probes, Eugene, OR, USA) and 4',6-diamidino-2-phenylindole (DAPI; Sigma–Aldrich), respectively.

2.9. Monolayer wound healing assay

Huh-7 cells were transfected with siRNA targeting *ARHGAP5* or negative control siRNA or left untreated. After 24 h, cells in DMEM with 1% FCS were seeded on glass slides coated with fibronectin and allowed to adhere overnight.

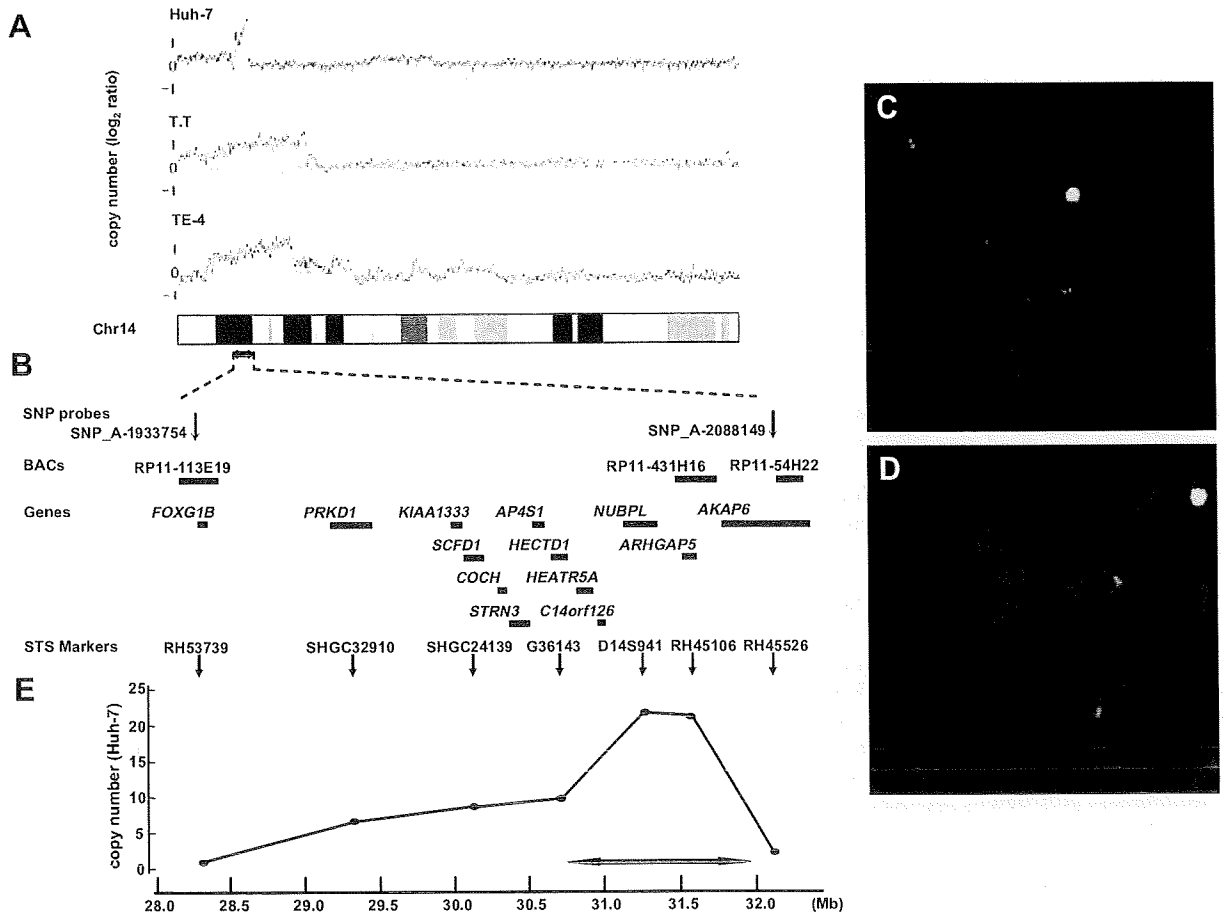

Fig. 1. Map of the amplicon at 14q12. (A) Copy number profiles for chromosome 14 in Huh-7, T.T and TE-4 cells. Copy number values were determined by GeneChip Mapping 250 K array analyses. (B) The positions of the Affymetrix SNP probes, three BACs used as probes for FISH experiments, the 13 genes within the 14q12 amplicon, and the seven STS markers used for real-time quantitative PCR on genomic DNA are shown according to the UCSC genome database (http://genome.ucsc.edu/). (C and D) Representative images of two-color FISH on metaphase chromosomes from Huh-7 cells using BACs: paired RP11-431H16 (green; C) and RP11-113E19 (red; C), or paired RP11-431H16 (green; D) and RP11-54H22 (red; D). (E) Copy numbers at the seven STS marker loci in Huh-7 cells as measured by real-time quantitative PCR with reference to LINE-1 controls. Values are normalized such that the copy number in genomic DNA derived from normal lymphocytes has a value of 2. The smallest region of amplification is indicated (arrow).

We scratched wounds in the cell monolayer using a sterile 200-μl pipet tip, rinsed the cells with phosphate-buffered saline and added DMEM containing 10% FCS with or without mitomycin C (10 μg/ml, Nacalai Tesque, Kyoto, Japan). Cells were allowed to migrate into the wound for 0, 12, or 24 h before fixation. Cells were stained with Giemsa stain (Nacalai Tesque) or were triple-labeled with anti-p190-B RhoGAP, rhodamine-phalloidin and DAPI as described above. Wound widths were measured in three randomly chosen regions. Experiments were repeated at least three times.

2.10. Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). *P* values of <0.05 were considered significant.

3. Results

3.1. Detection of 14q12 amplicon in HCC and ESCC cell lines by array analyses

We screened for DNA copy number aberrations in 10 HCC cell lines and 10 ESCC cell lines using GeneChip Mapping 250 K array analysis. Of the 20 cell lines, one HCC cell line, Huh-7, and two ESCC cell lines, T.T and TE-4, commonly exhibited copy number gains at chromosomal region 14q12 (Fig. 1A). In particular, Huh-7 cells showed a high-level gain indicative of amplification in a narrow region on 14q12 between the positions recognized by the Affymetrix SNP_A-1933754 and SNP_A-2088149 probes. To confirm amplification in Huh-7 cells, we performed FISH analyses using BACs RP11-113E19, RP11-431H16 and RP11-54H22 as probes (Fig. 1B–D). BAC RP11-431H16 generated strong signals as a small homogeneously staining region (HSR), indicating amplification (Figs. 1C, D). In contrast, BACs RP11-113E19 or RP11-54H22 did not show a HSR pattern, indicating their positions outside the amplicon (Fig. 1C and D). Furthermore, we determined gene dosages in Huh-7 cells at the STS markers RH53739, SHGC32910, SHGC24139, G36143, D14S941, RH45106, and RH45526 loci by real time quantitative PCR. (Fig.1B and E). The highest copy number was observed at the D14S941 and RH45106 loci. Taken together, we defined the smallest region of amplification between markers G36143 and RH45526. The extent of the amplicon was estimated to be 1.2 Mb. This region includes four known or predicted protein-coding genes, *HEATR5A*, *c14orf126*, *NUBPL*, and *ARHGAP5*.

3.2. Identification of candidate target genes in the 14q12 amplicon

The 14q12 region may harbor one or more genes (henceforth called 'target genes') that, when activated by amplification, play a role in carcinogenesis. A common criterion for designating a gene as a putative target is that amplification leads to its overexpression [8]. Using real-time quantitative PCR, we determined mRNA levels of all four genes within the amplicon in the 10 HCC cell lines and 10 ESCC cell lines. Among the four genes, *HEATR5A* and *ARHGAP5* were commonly overexpressed in Huh-7, T.T and TE-4 cells, the cell lines that were found to have copy number gains at 14q12 (Fig. 2A). These findings identified *ARHGAP5*, which encodes p190-B RhoGAP, as one of candidate target genes for the 14q12 amplicon.

We determined copy numbers of *ARHGAP5* in the 10 HCC and 10 ESCC cell lines by real-time quantitative PCR (Fig. 2B). Copy number changes were counted as gains if the results of the analysis for a given tumor cell type exceeded the twofold levels of the gene in normal cells. A copy number gain of *ARHGAP5* was observed in six (30%) of the 20 cell lines: Huh-7, T.T, KYSE140, TE-4, TE-6 and TE-10.

We examined the expression of p190-B RhoGAP protein in 4 HCC and 4 ESCC cell lines by immunoblot analysis. As shown in Fig. 2C, expression levels of p190-B RhoGAP were higher in cell lines exhibiting copy number gains of *ARHGAP5* (Huh-7, T.T, KYSE140, TE-4 and TE-10) than other cell lines that did not show gains (SNU354, Huh-1 and PLC/PRF/5).

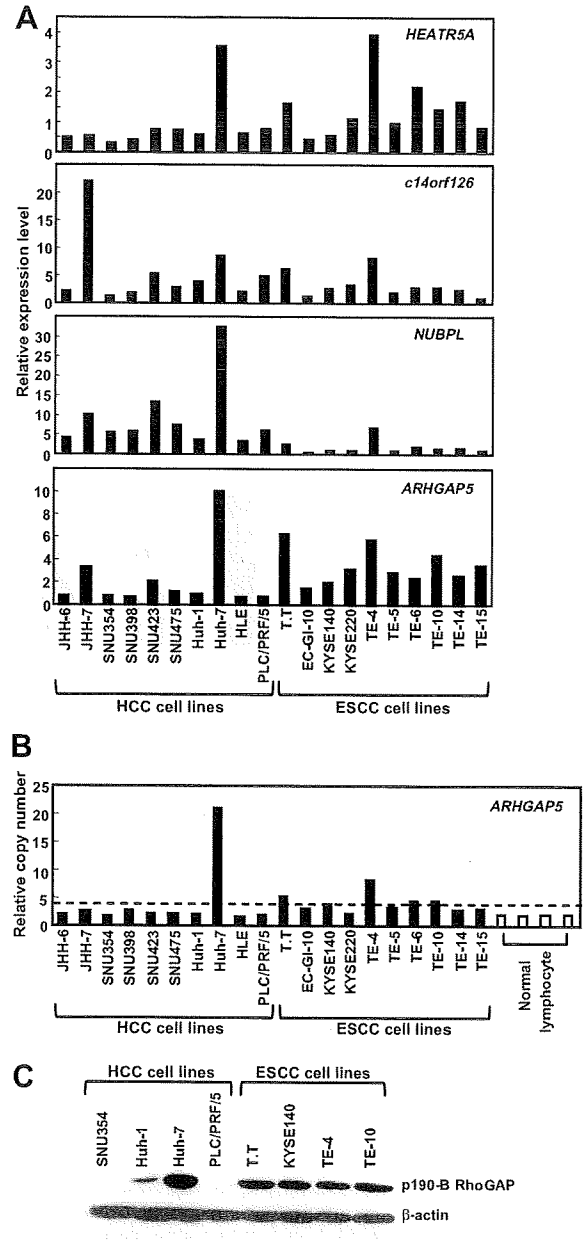

Fig. 2. Amplification and overexpression of *ARHGAP5* in Huh-7, T.T and TE-4 cell lines. (A) Relative expression levels of four genes (*HEATR5A*, *c14orf126*, *NUBPL* and *ARHGAP5*) within the 14q12 amplicon in 10 HCC and 10 ESCC cell lines as evaluated by real-time quantitative PCR. Results are presented as expression levels of each gene relative to a reference gene (*GAPDH*) to correct for variations in the amount of RNA. (B) Copy numbers at the *ARHGAP5* locus (the STS marker RH45106) in 10 HCC cell lines, 10 ESCC cell lines and four normal peripheral blood lymphocytes as measured by real-time quantitative PCR with reference to LINE-1 controls. Values are normalized such that the average copy number in genomic DNA derived from four normal lymphocytes has a value of 2. A value of 4, which is a twofold increase in copy number of normal lymphocytes, was used to determine the cut-off value for copy number gain, shown as a dotted line. (C) Levels of p190-B RhoGAP and β-actin, an internal control, determined by immunoblotting in 4 HCC and 4 ESCC cell lines.