

Correlation of Clinicopathologic Features of Resected Hepatocellular Carcinoma with Hepatitis C Virus Genotype

Junya Murase,^{1,4} Shoji Kubo,¹ Shuhei Nishiguchi,² Kazuhiro Hirohashi,¹ Taichi Shuto,¹ Takashi Ikebe³ and Hiroaki Kinoshita¹

¹Second Department of Surgery, ²Third Department of Internal Medicine and ³Department of Pathology, Osaka City University Medical School, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585

Clinicopathologic findings in patients with hepatocellular carcinoma complicating hepatitis C virus and outcomes after liver resection were compared between different viral genotypes. One hundred and forty-seven patients with both anti-hepatitis C virus antibody and hepatitis C virus RNA in their sera underwent curative resection for hepatocellular carcinoma in our department between 1991 and 1997. Of these patients, 115 were infected with hepatitis C virus genotype 1b (group 1), and 32 were infected with 2a or 2b (group 2). Clinicopathologic findings and outcomes after operation were compared between the two groups. Alanine aminotransferase activity was significantly higher in group 2 than in group 1. Genotypes did not differ concomitantly with histopathologic features of the carcinoma or adjacent hepatic tissue. Although the tumor-free survival rate did not differ significantly between the two groups, recurrence was not detected during the period beyond 3 years following operation in group 2, while recurrences arose during that period in 16 group 1 patients, most of whom continued to manifest active hepatitis. In 7 of these 16 patients, the recurrent tumors were histologically multicentric in origin. The cumulative survival rate was significantly lower in group 1 than 2. Multivariate analysis indicated that genotype 1b was an independent risk factor for short survival. Patients infected with genotype 1b may have a relatively high risk of ongoing hepatocarcinogenesis and more aggressive progression of associated liver dysfunction, resulting in a poorer outcome than with other genotypes.

Key words: Liver resection — Hepatitis C virus — Viral genotype — Hepatocarcinogenesis — Multicentric carcinogenesis

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease, such as chronic hepatitis and cirrhosis, as well as hepatocellular carcinoma (HCC). Recent studies have demonstrated that HCV can be classified into several genotypes based on variations in nucleotide sequence.^{1,2} HCV genotype 1b is reportedly the most prevalent variant in the United States, Europe, and Japan.^{3–5} In Japan, among patients infected with HCV, about 70% of patients had the viral genotype 1b, 20% had genotype 2a, and 10% had genotype 2b. Patients with genotype 1a or 3 are rare.^{6,7} The pathogenicity of genotype 1b seems greater than that of other genotypes in terms of associated liver damage, progression of chronic hepatitis C, and development of HCC,^{5–17} although controversy persists.^{18–22} Another unfavorable association of genotype 1b is a relatively poor response to interferon.^{4,23,24} Additionally, after liver transplantation, patients infected with genotype 1b reportedly developed more aggressive liver disease than transplant recipients infected with other genotypes.^{16,25,26}

In spite of advances in medical imaging and perioperative management, outcome after resection of virally asso-

ciated HCC remains unsatisfactory, with a high recurrence rate as well as continued progression of associated liver disease.²⁷ Risk factors for recurrence and prognostic factors for other aspects of outcome after resection of HCC have been identified by several investigators.^{27–36} However, clinicopathologic findings in patients with HCC and outcome after resection of HCC have not been compared between different HCV genotypes, and we set out to do this in the present study.

PATIENTS AND METHODS

Patients Between 1991 and 1997, 152 patients with both anti-HCV antibody and HCV RNA in their sera, but lacking hepatitis B surface antigen, underwent curative resection for HCC in our department. Curative resection was defined as complete resection of all macroscopically detectable tumors, with absence of any histologically evident tumor along the parenchymal transection line. No tumors remained in the remnant liver according to computed tomography 3 to 4 weeks after resection. Among the 152 patients, 5 patients infected with more than one HCV genotype were excluded from this study. Remaining patients included 120 men and 27 women, with ages ranging from 46 to 77 years. In 4 of the 152 patients, HCC was

⁴ To whom correspondence should be addressed.
E-mail: m8784756@msic.med.osaka-cu.ac.jp

detected after interferon therapy. This study was conducted in accordance with the Helsinki Declaration and the guidelines of the ethics committee of our institution. Informed consent was obtained from each patient.

Detection of HCV RNA and HCV genotype analysis Serum HCV RNA was detected by polymerase chain reaction with reverse transcription and primers derived from a conserved 5'-untranslated region of the viral genome.³⁷⁾ HCV RNA was also detected using a branched DNA probe method (Quantiplex HCV-RNA, Chiron, Emeryville, CA). HCV genotypes were determined by amplification of the putative core gene described by Okamoto *et al.*³⁸⁾ and were classified according to Simmonds *et al.*²⁾

Pathologic examination Resected liver specimens were sliced serially at a thickness of 5 mm and fixed in 10% formalin. Biopsy specimens were also fixed in 10% formalin. Tissue was then embedded in paraffin, followed by sectioning and staining with hematoxylin and eosin. Histopathologic diagnosis was based on the General Rules for the Clinical and Pathological Study of Primary Liver Cancer in Japan,³⁹⁾ with some modifications.^{40,41)} When a recurrent tumor(s) included a component of well-differentiated HCC, the tumor was also assumed to have a multicentric rather than metastatic origin according to previously reported criteria.^{35,42-45)} Noncancerous hepatic tissue was also examined pathologically according to the method described by Adachi *et al.*⁴⁶⁾ In a noncirrhotic liver, inactive hepatitis was defined as lymphocytic inflammation restricted to the widened portal tracts, leaving the parenchymal limiting plate preserved without necrosis of the periportal hepatocytes. Active hepatitis was defined as portal and periportal inflammation with erosion of the limiting plate, piecemeal necrosis, and fibrosis. In an inactive cirrhotic liver, the interface between the parenchyma and septa was sharply defined and cellular infiltration was either mild or confined to the septa. There was little or no focal necrosis or inflammation deep within the nodules. In an active cirrhosis, the interface between the nodules and septa was blurred by piecemeal necrosis and there were abundant inflammatory cells. A diagnosis was considered as confirmed when at least two pathologists agreed.

Operation and follow-up Bisegmentectomy was done in 17 patients, segmentectomy in 29, subsegmentectomy in 24, and partial resection in 77. After discharge from the hospital, patients were followed closely in the outpatient department. When tumor recurrence was suspected from abnormalities in tumor marker assays, ultrasonography, computed tomography, or some combination of these, angiography, an ultrasonographically guided biopsy, or both were performed urgently.

Factors affecting recurrence and outcome We studied putative risk factors for recurrence and unfavorable postoperative outcome as suggested by previous stud-

ies^{27-36,46,47)} and our own clinical experience. The variables chosen were age; sex; history of blood transfusion; history of alcohol abuse [at least 86 g of daily ethanol intake for at least 10 years, as defined by the Liver Cancer Study Group in Japan^{27,36)}]; results of laboratory tests such as serum concentrations of α -fetoprotein, albumin, and total bilirubin, activities of aspartate aminotransferase and alanine aminotransferase (ALT), platelet count, and indocyanine green retention rate at 15 min; observation of portal invasion and intrahepatic metastasis; cirrhosis and activity of hepatitis in noncancerous tissues; type of resection; surgical free margin; and operative blood loss. When the surgical free margin was less than 10 mm, the margin was defined as tumor wedge positive. When the changes in ALT activity after the operation were evaluated, the mean ALT activity obtained for each patient after the operation (laboratory tests were performed at least four times a year) was calculated.

Statistical analysis Student's *t* test was used to compare mean age between groups. The Mann-Whitney *U* test was used to compare the results of laboratory tests. The χ^2 test or Fisher's exact test was used to compare categorical data. Survival curves after the operation were calculated by the Kaplan-Meier method and the significance of differences was evaluated by means of the log-rank test. To estimate independent risk factors for recurrence and postoperative outcome, a Cox's proportional hazards regression analysis was performed. *P* values less than 0.05 were considered significant.

RESULTS

Clinicopathologic findings Of the 147 patients, 115 (78%) were infected with HCV genotype 1b (group 1), and 32 (22%) were infected with genotype 2a or 2b (group 2). There was no patient infected with a genotype other than 1b, 2a, and 2b. A comparison of the two groups revealed no significant difference of mean age, sex distribution, proportion of patients with a history of blood transfusion, or proportion of patients with a history of alcohol abuse (Table I). In 3 patients in group 1 and 1 patient in group 2, HCC was detected after interferon therapy ($P>0.999$). ALT activity was significantly higher in group 2 than in group 1 ($P=0.044$). Albumin concentration was significantly higher in group 2 than in group 1 ($P=0.016$), but that was within the normal range. No significant difference was seen between groups in the occurrence of an elevated α -fetoprotein concentration (>200 ng/ml), or in aspartate aminotransferase activity, total bilirubin concentration, platelet count, or indocyanine green retention rate at 15 min. No significant difference was apparent in tumor size, prevalences of portal invasion and intrahepatic metastasis, or degree of differentiation of the main tumor. No significant difference was noted in propor-

Table I. Comparison of Clinicopathologic Features in Two Groups Undergoing HCC Resection, with HCV Genotype

Variable	Group 1 (n=115)	Group 2 (n=32)	P
Sex (M/F) ^{a)}	93/22	27/5	0.651
Age (year) ^{b)}	62.2±5.4	61.4±6.5	0.881
Transfusion ^{a)} (%)	27 (24)	12 (40)	0.083
Alcohol abuse ^{a)} (%)	54 (48)	18 (58)	0.311
AST (IU/liter) ^{c)}	64 (37, 107)	62 (36, 122)	0.599
ALT (IU/liter) ^{c)}	71 (33, 124)	85 (36, 136)	0.044
Albumin (g/dl) ^{c)}	3.6 (3.2, 4.0)	3.8 (3.4, 4.1)	0.016
Total bilirubin (mg/dl) ^{c)}	0.8 (0.6, 1.4)	0.8 (0.5, 1.6)	0.829
Platelet (×10 ⁴ /mm ³) ^{c)}	12 (7, 20)	14 (7, 21)	0.329
ICGR15 (%) ^{c)}	18 (9, 33)	17 (9, 29)	0.332
AFP >200 ng/ml ^{a)} (%)	16 (14)	6 (19)	0.498
Tumor size (cm) ^{b)}	3.4±2.2	3.4±2.2	0.567
Differentiation of main tumor ^{a)} (%)			
Well-differentiated	16 (14)	2 (6)	0.185
Others	99 (86)	29 (94)	
Portal invasion ^{a)} (%)	22 (19)	10 (31)	0.142
Intrahepatic metastasis ^{a)} (%)	31 (27)	6 (19)	0.344
Noncancerous tissue			
Cirrhosis ^{a)} (%)			
Presence	60 (52)	13 (41)	0.248
Absence	55 (48)	19 (59)	
Inflammation ^{a)} (%)			
Active	79 (69)	24 (75)	0.663
Inactive	36 (31)	8 (25)	
Liver resection ^{a)} (%)			
Anatomic resection	52 (45)	18 (56)	0.269
Partial resection	63 (55)	14 (44)	
Tumor wedge positive ^{a)} (%)	63 (55)	17 (53)	0.868
Operative blood loss (g) ^{c)}	1170 (300, 3450)	1030 (190, 3200)	0.299

a) Numbers of patients.

b) Mean±SD.

c) Median with 10th and 90th percentiles.

Group 1 patients had HCV genotype 1b; group 2, genotype 2a or 2b; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICGR15, indocyanine green retention rate at 15 min; AFP, α -fetoprotein; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; Anatomic resection, bisegmentectomy, segmentectomy and subsegmentectomy.

tions of patients with cirrhosis or active hepatitis. Anatomic resections such as bisegmentectomy, segmentectomy, or subsegmentectomy were performed in 52 patients (45%) in group 1 and in 18 (56%) in group 2. No significant difference was found in the proportions of patients who were tumor wedge positive and who showed operative blood loss.

Risk factors for recurrence and overall postoperative outcome The overall tumor-free survival rate tended to be lower in group 1 than 2, but this difference was not statistically significant ($P=0.181$; Fig. 1, Table II). During the 3 years after operation, tumor-free survival rates decreased

along similar curves in both groups, but after 3 years following operation no group 2 patients developed a recurrence, whereas recurrence occurred in 16 group 1 patients. We next compared clinicopathologic features in patients with and without recurrences later than 3 years following operation. The proportions of patients infected with HCV genotype 1b and of patients with a high average of postoperative ALT activity (>45 IU/liter, indicating continuous active hepatitis) were significantly higher in patients with recurrence beyond 3 years following operation than in patients without recurrence (Table III). Pathologic examination of the recurrent tumor was possible in 8 of the 16

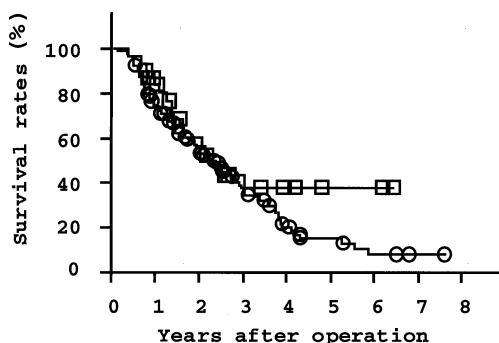


Fig. 1. Tumor-free survival rates after liver resection in two groups. Group 1 (○) consisted of patients infected with hepatitis C virus genotype 1b (n=115). Group 2 (□) consisted of patients infected with genotype 2a or 2b (n=32).

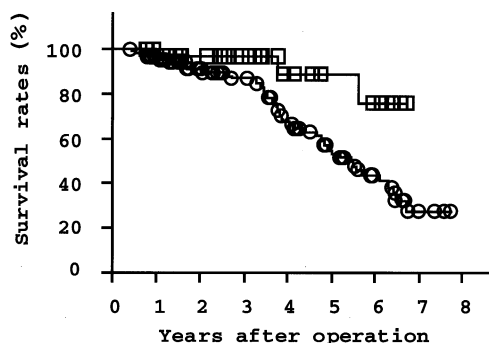


Fig. 2. Cumulative survival rates after liver resection in two groups. Group 1 (○) consisted of patients infected with hepatitis C virus genotype 1b. Group 2 (□) consisted of patients infected with genotype 2a or 2b. The survival rate was significantly lower in group 1 than in group 2 (P=0.019).

Table II. The Number of Patients at Risk for Recurrence over Follow-up Time

	Years after operation								
	0	1	2	3	4	5	6	7	8
Group 1	115	79	48	27	13	7	4	1	0
Group 2	32	23	14	6	4	2	2	0	0

Table III. Genotype and Hepatitis Activity in Patients with and without Recurrence More than 3 Years Following Operation

Variable	Recurrence more than 3 years following operation		P
	Yes (n=16)	No (n=17)	
Genotype (1b/other) ^{a)}	16/0	11/6	0.009
Postoperative ALT activity (>45 IU/liter) ^{a)} (%)	15 (94)	10 (59)	0.019

a) Numbers of patients. HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ALT, alanine aminotransferase.

group 1 patients with recurrence, and in 7 of the 8 patients the tumors showed histologic evidence of multicentric origin. The cumulative survival rate was significantly lower in group 1 than in group 2 (P=0.019; Fig. 2, Table IV). While 3 patients in group 2 died of HCC recurrence, 44 patients in group 1 died of recurrence and 3 others in the same group died of deteriorating liver function without tumor recurrence. We compared the types of treatments after recurrence between the two groups and no significant difference was found (Table V).

Multivariate analysis indicated that intrahepatic metastasis and portal invasion were independent risk factors for

Table IV. The Number of Patients at Risk for Survival over Follow-up Time

	Years after operation								
	0	1	2	3	4	5	6	7	8
Group 1	115	103	81	71	50	31	17	4	0
Group 2	32	27	23	17	10	7	5	0	0

Table V. Numbers of Patients Receiving Various Therapies for Recurrent Liver Tumor in Group 1 (n=81) and Group 2 (n=16)

	Group 1	Group 2
Second resection		
Alone	4	1
With TAE/HAI	2	0
With PEIT	2	1
With TAE/HAI and PEIT	9	0
TAE/HAI		
Alone	29	5
With PEIT	20	6
PEIT	4	2
No therapy	11 ^{a)}	1

a) Two patients who underwent radiation for bone metastasis. TAE, transarterial embolization; HAI, hepatic arterial injection; PEIT, percutaneous ethanol injection therapy.

recurrence (Table VI). The odds ratio for tumor recurrence with HCV genotype 1b relative to other genotypes was 1.619. Genotype 1b was an independent risk factor for short survival (P=0.044). Odds ratio for short survival with HCV genotype 1b relative to other genotypes was 3.346.

Table VI. Prognostic Factors for Recurrence and Short Survival in HCV-associated HCC, by Multivariate Analysis

Variable	Odds ratio	95%CI	P
Recurrence			
Intrahepatic metastasis	2.070	1.294–3.311	0.002
Portal invasion	1.898	1.093–3.289	0.023
HCV genotype 1b	1.619	0.928–2.825	0.090
Survival			
HCV genotype 1b	3.346	1.034–10.829	0.044
Intrahepatic metastasis	1.835	0.996–3.378	0.051

CI, confidence interval; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

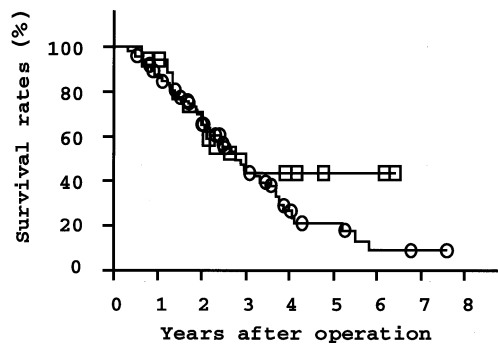


Fig. 3. Tumor-free survival rates after liver resection in the two groups without portal invasion or intrahepatic metastasis. ○ patients in group 1 without portal invasion or intrahepatic metastasis ($n=75$), □ patients in group 2 without them ($n=19$).

Seventy-five patients in group 1 and 19 patients in group 2 had HCC without portal invasion or intrahepatic metastasis, which are related to recurrence originating from the primary HCC.⁴⁷⁾ Although the tumor-free survival rates were not significantly different between the 75 group 1 patients and the 19 group 2 patients ($P=0.266$, Fig. 3), the cumulative survival rate was significantly lower in the 75 group 1 patients than in the 19 group 2 patients ($P=0.042$, Fig. 4).

DISCUSSION

Several studies have suggested a difference in pathogenicity of HCV genotypes with respect to development of HCC. In a prospective multivariate analysis of 163 consecutive patients, Bruno *et al.*¹⁷⁾ found that cirrhotic patients infected with HCV genotype 1b have a significantly higher risk of HCC than patients infected with other genotypes. Several case-control studies and cross-sectional studies^{4, 10–16)} have also shown a high prevalence of genotype 1b infection in patients with HCC. However, the relationship between HCV genotype and various forms of

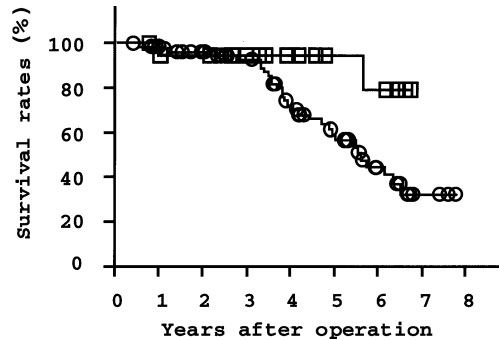


Fig. 4. Cumulative survival rates after liver resection in the two groups without portal invasion or intrahepatic metastasis. ○ patients in group 1 without portal invasion or intrahepatic metastasis ($n=75$), □ patients in group 2 without them ($n=19$). The survival rate was significantly lower in group 1 than in group 2 ($P=0.042$).

severe liver disease including HCC remains controversial.^{18–22)}

In the present study we found ALT activity to be significantly higher in group 2 (genotype 2a or 2b) than in group 1 (genotype 1b). The implication is that hepatitis was more active in group 2, although no significant difference in the proportion of patients with active hepatitis was found between the two groups. In group 2, HCC may develop in the context of more severe hepatitis than HCC occurring in group 1. In other words, HCC may have developed even in the presence of mild hepatitis in the patients with genotype 1b.

The tumor-free survival rate was not significantly different between the two groups, although in group 1 tumors often recurred at more than 3 years following operation, while in group 2 all recurrences became evident within 3 years. The proportions of patients infected with HCV genotype 1b and of patients with continuing active hepatitis were significantly higher among patients with tumor recurrence more than 3 years following operation. Several other investigators have reported that active hepatitis is a risk factor for recurrence after operation.^{46–51)} We have found that the prevalence of multicentric carcinogenesis increases with advancing severity of active hepatitis.⁵²⁾ In this study, pathologic examination of recurrent tumors presenting more than 3 years following operation strongly suggested that at least 7 of 16 tumors were multicentric in origin, representing newly developed HCC. These results indicate that patients infected with HCV genotype 1b continue to harbor active hepatitis and continue to have a higher potential for carcinogenesis after HCC resection than patients infected with other genotypes.

In the patients we studied, the cumulative survival rate was significantly lower in group 1 than in group 2. As

regards the types of treatments for recurrent tumor(s) no significant difference was found between the two groups. As noted above, one factor was a higher incidence of recurrence, including the new development of HCC. Additionally, three patients in group 1 died of worsening liver function without recurrence of HCC. In contrast, no group 2 patient died of progressive liver failure. In patients without portal invasion or intrahepatic metastasis (those without risks for recurrence originating from the primary HCC), the cumulative survival rate was significantly lower in group 1 than in group 2 although the tumor-free survival rate was not significant between the two groups. More severe progression of chronic hepatitis C has been seen in patients infected with HCV genotype 1b than in those with genotype 2.⁵⁻⁹⁾ Therefore, deteriorating liver function may contribute to the lower cumulative survival rate in group 1, although the number of patients in this study was too small to allow a definitive conclusion regarding this issue.

The mechanisms responsible for greater pathogenicity of HCV genotype 1b remain unknown. HCV genotype 1b reportedly may have an enhanced capacity to escape host immunity, given that it has two hypervariable regions in

the envelope domain (HVR-1 and HVR-2) while other types have only HVR-1.⁵³⁾ As a result, type 1b may either undergo different regulation of gene expression or possess proteins producing more severe cytopathic effects in hepatocytes than proteins of other genotypes.^{17, 54)}

In conclusion, the results of this study suggest that the HCV genotype should be considered in the treatment of HCC, because different genotypes may be associated with differences in outcome following resection. Patients infected with HCV genotype 1b should be monitored closely even long term after the operation, because new HCCs may develop.

ACKNOWLEDGMENTS

We are very grateful to Professor T. Kuroki for his advice in the virological examinations, and we also thank Dr. H. Tanaka, Dr. T. Tsukamoto, Dr. H. Hamba and Dr. S. Takemura for their support in analysis. This work was supported in part by grants from the Ministry of Education, Science, Sports and Culture and the Ministry of Health and Welfare, Japan.

(Received August 12, 1999/Revised September 24, 1999/
Accepted September 30, 1999)

REFERENCES

- 1) Okamoto, H. and Mishiro, S. Genetic heterogeneity of hepatitis C virus. *Intervirology*, **37**, 68-76 (1994).
- 2) Simmonds, P., Alberti, A., Alter, H. J., Bonino, F., Bradley, D. W., Brechot, C., Brouwer, J. T., Chan, S.-W., Chayama, K., Chen, D.-S., Choo, Q.-L., Colombo, M., Cuypers, H. T. M., Date, T., Dusheiko, G. M., Esteban, J. I., Fay, O., Hadziyannis, S. J., Han, J., Hatzakis, A., Holmes, E. C., Hotta, H., Houghton, M., Irvine, B., Kohara, M., Kolberg, J. A., Kuo, G., Lau, J. Y. N., Lelie, P. N., Maertens, G., McOmish, F., Miyamura, T., Mizokami, M., Nomoto, A., Prince, A. M., Reesink, H. W., Rice, C., Roggendorf, M., Schalm, S. W., Shikata, T., Shimotohno, K., Stuyver, L., Treppe, C., Weiner, A., Yap, P. L. and Urdea, M. S. A proposed system for the nomenclature of hepatitis C viral genotypes. *Hepatology*, **19**, 1321-1324 (1994).
- 3) Dusheiko, G., Schmilovitz-Weiss, H., Brown, D., McOmish, F., Yap, P. L., Sherlock, S., McIntyre, N. and Simmonds, P. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology*, **19**, 13-18 (1994).
- 4) Nousbaum, J.-B., Pol, S., Nalpas, B., Landais, P., Berthelot, P., Bréchet, C. and the Collaborative Study Group. Hepatitis C virus types 1b (II) infection in France and Italy. *Ann. Intern. Med.*, **122**, 161-168 (1995).
- 5) Takada, N., Takase, S., Enomoto, N., Takada, A. and Date, T. Clinical backgrounds of the patients having different types of hepatitis C virus genomes. *J. Hepatol.*, **14**, 35-40 (1992).
- 6) Takada, A., Tsutsumi, M., Zhang, S.-C., Okanoue, T., Matsushima, T., Fujiyama, S. and Komatsu, M. Relationship between hepatocellular carcinoma and subtypes of hepatitis C virus: a nationwide analysis. *J. Gastroenterol. Hepatol.*, **11**, 166-169 (1996).
- 7) Tanaka, E., Kiyosawa, K., Matsushima, T., Ishikawa, K., Hino, K., Tanaka, S., Nose, H., Kumada, H., Iino, S., Kamimura, T., Unoura, M., Mizokami, M., Okanoue, T., Kuroki, T., Yamada, G., Miura, T., Yano, M., Tsubouchi, H., Kohara, M., Sato, S., Hattori, N. and Genotyping ELISA Study Group. Epidemiology of genotypes of hepatitis C virus in Japanese patients with type C chronic liver diseases: a multi-institution analysis. *J. Gastroenterol. Hepatol.*, **10**, 538-545 (1995).
- 8) Kobayashi, M., Tanaka, E., Sodeyama, T., Urushihara, A., Matsumoto, A. and Kiyosawa, K. The natural course of chronic hepatitis C: a comparison between patients with genotypes 1 and 2 hepatitis C viruses. *Hepatology*, **23**, 695-699 (1996).
- 9) Prati, D., Capelli, C., Zanella, A., Mozzi, F., Bosoni, P., Pappalettera, M., Zanuso, F., Vianello, L., Locatelli, E., Fazio, C. D., Ronchi, G., Ninno, E. D., Colombo, M. and Sirchia, G. Influence of different hepatitis C virus genotypes on the course of asymptomatic hepatitis C virus infection. *Gastroenterology*, **110**, 178-183 (1996).
- 10) Silini, E., Bottelli, R., Asti, M., Bruno, S., Candusso, M. E., Brambilla, S., Bono, F., Iamori, G., Tinelli, C., Mondelli, M. U. and Ideo, G. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a case-control study. *Gastroenterology*, **111**, 199-205 (1996).

- 11) Yamauchi, M., Nakahara, M., Nakajima, H., Sakamoto, K., Hirakawa, J. and Toda, G. Different prevalence of hepatocellular carcinoma between patients with liver cirrhosis due to genotype II and III of hepatitis C virus. *Int. Hepatol. Commun.*, **2**, 328–332 (1994).
- 12) De Mitri, M. S., Poussin, K., Baccarini, P., Pontisso, P., D'Errico, A., Simon, N., Grigioni, W., Alberti, A., Beaugrand, M., Pisi, E., Brechot, C. and Paterlini, P. HCV-associated liver cancer without cirrhosis. *Lancet*, **345**, 413–415 (1995).
- 13) El-Refaie, A., Savage, K., Bhattacharya, S., Khakoo, S., Harrison, T. J., El-Batanony, M., Soliman, E.-S., Nasr, S., Mokhtar, N., Amer, K., Scheuer, P. J. and Dhillon, A. P. HCV-associated hepatocellular carcinoma without cirrhosis. *J. Hepatol.*, **24**, 277–285 (1996).
- 14) Tanaka, K., Ikematsu, H., Hirohata, T. and Kashiwagi, S. Hepatitis C virus infection and risk of hepatocellular carcinoma among Japanese: possible role of type 1b (II) infection. *J. Natl. Cancer Inst.*, **88**, 742–746 (1996).
- 15) Hatzakis, A., Katsoulidou, A., Kaklamani, E., Touloumi, G., Koumantaki, Y., Tassopoulos, N. C., Karvountzis, G., Gioustozi, A., Hadziyannis, S. and Trichopoulos, D. Hepatitis C virus 1b is the dominant genotype in HCV-related carcinogenesis: a case-control study. *Int. J. Cancer*, **68**, 51–53 (1996).
- 16) Zein, N. N., Poterucha, J. J., Gross, J. B., Jr., Wiesner, R. H., Therneau, T. M., Gossard, A. A., Wendt, N. K., Mitchell, P. S., Germer, J. J. and Persing, D. H. Increased risk of hepatocellular carcinoma in patients infected with hepatitis C genotype 1b. *Am. J. Gastroenterol.*, **91**, 2560–2562 (1996).
- 17) Bruno, S., Silini, E., Crosignani, A., Borzio, F., Leandro, G., Bono, F., Asti, M., Rossi, S., Larghi, A., Cerino, A., Podda, M. and Mondelli, M. U. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. *Hepatology*, **5**, 754–758 (1997).
- 18) Yamada, M., Kakumu, S., Yoshioka, K., Higashi, Y., Tanaka, K., Ishikawa, T. and Takayanagi, M. Hepatitis C virus genotypes are not responsible for development of serious liver disease. *Dig. Dis. Sci.*, **39**, 234–239 (1994).
- 19) Yotsuyanagi, H., Koike, K., Yasuda, K., Moriya, K., Hino, K., Kurokawa, K. and Iino, S. Hepatitis C virus genotypes and development of hepatocellular carcinoma. *Cancer*, **76**, 1352–1355 (1995).
- 20) Takano, S., Yokosuka, O., Imazeki, F., Tagawa, M. and Omata, M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. *Hepatology*, **21**, 650–655 (1995).
- 21) Zeuzem, S., Franke, A., Lee, J.-H., Herrmann, G., Rüter, B. and Roth, W. K. Phylogenetic analysis of hepatitis C virus isolates and their correlation to viremia, liver function tests, and histology. *Hepatology*, **24**, 1003–1009 (1996).
- 22) Benvegnù, L., Pontisso, P., Cavalletto, D., Noventa, F., Chemello, L. and Alberti, A. Lack of correlation between hepatitis C virus genotypes and clinical course of hepatitis C virus-related cirrhosis. *Hepatology*, **25**, 211–215 (1997).
- 23) Martinot-Peignoux, M., Marcellin, P., Pouteau, M., Castelnau, C., Boyer, N., Poliquin, M., Degott, C., Descombes, I., Breton, V. L., Milotova, V., Benhamou, J. P. and Erlinger, S. Pretreatment serum hepatitis C virus RNA levels and hepatitis C virus genotype are the main and independent prognostic factors of sustained response to interferon alpha therapy in chronic hepatitis C. *Hepatology*, **22**, 1050–1056 (1995).
- 24) Chemello, L., Bonetti, P., Cavalletto, L., Talato, F., Donadon, V., Casarin, P., Belussi, F., Frezza, M., Noventa, F., Pontisso, P., Benvegnù, L., Casarin, C., Alberti, A. and the TriVeneto Viral Hepatitis Group. Randomized trial comparing three different regimens of alpha-2a-interferon in chronic hepatitis C. *Hepatology*, **22**, 700–706 (1995).
- 25) Fèray, C., Gigou, M., Samuel, D., Paradis, V., Mishiro, S., Maertens, G., Reynes, M., Okamoto, H., Bismuth, H. and Brechot, C. Influence of the genotypes of hepatitis C virus on the severity of recurrent liver disease after liver transplantation. *Gastroenterology*, **108**, 1088–1096 (1995).
- 26) Gane, E. J., Portmann, B. C., Naoumov, N. V., Smith, H. M., Underhill, J. A., Donaldson, P. T., Maertens, G. and Williams, R. Long-term outcome of hepatitis C infection after liver transplantation. *N. Engl. J. Med.*, **334**, 815–820 (1996).
- 27) Liver Cancer Study Group of Japan. Primary liver cancer in Japan: clinicopathologic features and results of surgical treatment. *Ann. Surg.*, **211**, 277–287 (1990).
- 28) Belghiti, J., Panis, Y., Farges, O., Benhamou, J. P. and Fekete, F. Intrahepatic recurrence after resection of hepatocellular carcinoma complicating cirrhosis. *Ann. Surg.*, **214**, 114–117 (1991).
- 29) Jwo, S.-C., Chiu, J.-H., Chau, G.-Y., Loong, C.-C. and Lui, W.-Y. Risk factors linked to tumor recurrence of human hepatocellular carcinoma after hepatic resection. *Hepatology*, **16**, 1367–1371 (1992).
- 30) Ikeda, K., Saitoh, S., Tsubota, A., Arase, Y., Chayama, K., Kumada, H., Watanabe, G. and Tsurumaru, M. Risk factors for tumor recurrence and prognosis after curative resection of hepatocellular carcinoma. *Cancer*, **71**, 19–25 (1993).
- 31) Kosuge, T., Makuuchi, M., Takayama, T., Yamamoto, J., Shimada, K. and Yamasaki, S. Long-term results after resection of hepatocellular carcinoma: experience of 480 cases. *Hepatogastroenterology*, **40**, 328–332 (1993).
- 32) Nagasue, N., Uchida, M., Makino, Y., Takemoto, Y., Yamanoi, A., Hayashi, T., Chang, Y.-C., Kohno, H., Nakamura, T. and Yukata, H. Incidence and factors associated with intrahepatic recurrence following resection of hepatocellular carcinoma. *Gastroenterology*, **105**, 488–494 (1993).
- 33) Okada, S., Shimada, K., Yamamoto, J., Takayama, T., Kosuge, T., Yamasaki, S., Sakamoto, M. and Hirohashi, S. Predictive factors for postoperative recurrence of hepatocellular carcinoma. *Gastroenterology*, **106**, 1618–1624 (1994).
- 34) Di Carlo, V., Ferrari, G., Castoldi, R., Nadalin, S., Marengi, C., Molteni, B., Taccagni, G. and Castrucci, M.

- Surgical treatment and prognostic variables of hepatocellular carcinoma in 122 cirrhotics. *Hepatogastroenterology*, **42**, 222–229 (1995).
- 35) Kumada, T., Nakano, S., Takeda, I., Sugiyama, K., Osada, T., Kiriya, S., Sone, Y., Toyoda, H., Shimada, S., Takahashi, M. and Sassa, T. Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. *Hepatology*, **25**, 87–92 (1997).
- 36) Kubo, S., Kinoshita, H., Hirohashi, K., Tanaka, H., Tsukamoto, T., Shuto, T. and Kuroki, T. High malignancy of hepatocellular carcinoma in alcoholic patients with hepatitis C virus. *Surgery*, **121**, 425–429 (1997).
- 37) Nishiguchi, S., Kuroki, T., Nakatani, S., Morimoto, H., Takeda, T., Nakajima, S., Shiomi, S., Seki, S., Kobayashi, K. and Otani, S. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet*, **346**, 1051–1055 (1995).
- 38) Okamoto, H., Sugiyama, Y., Okada, S., Kurai, K., Akahane, Y., Sugai, Y., Tanaka, T., Sato, K., Tsuda, F., Miyakawa, Y. and Mayumi, M. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. *J. Gen. Virol.*, **73**, 673–679 (1992).
- 39) Liver Cancer Study Group of Japan. “The General Rules for the Clinical and Pathological Study of Primary Liver Cancer,” 3rd Ed. (1992). Kanehara Co., Tokyo.
- 40) Edmondson, H. A. and Steiner, P. E. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer*, **7**, 462–503 (1954).
- 41) Kondo, F., Wada, K., Nagato, Y., Nakajima, T., Kondo, Y., Hirooka, N., Ebara, M., Ohto, M. and Okuda, K. Biopsy diagnosis of well-differentiated hepatocellular carcinoma based on new morphologic criteria. *Hepatology*, **9**, 751–755 (1989).
- 42) Tsuda, H., Oda, T., Sakamoto, M. and Hirohashi, S. Different pattern of chromosomal allele loss in multiple hepatocellular carcinomas as evidence of their multifocal origin. *Cancer Res.*, **52**, 1504–1509 (1992).
- 43) Okuda, K., Tanaka, M., Nakayama, K., Saito, H., Tanikawa, K., Nakashima, O. and Kojiro, M. Clinicopathologic comparison between resected hepatocellular carcinomas (HCC) and recurrent tumors: a special reference to multicentric carcinogenesis of HCC. *Int. Hepatol. Commun.*, **1**, 65–71 (1993).
- 44) Miyagawa, S., Kawasaki, S. and Makuuchi, M. Comparison of the characteristics of hepatocellular carcinoma between hepatitis B and C viral infection: tumor multicentricity in cirrhotic liver with hepatitis C. *Hepatology*, **24**, 307–310 (1996).
- 45) Kubo, S., Kinoshita, H., Hirohashi, K., Tanaka, H., Tsukamoto, T., Hamba, H., Shuto, T., Yamamoto, T., Ikebe, T. and Wakasa, K. Patterns of and risk factors for recurrence after liver resection for well-differentiated hepatocellular carcinoma: a special reference to multicentric carcinogenesis after operation. *Hepatogastroenterology* (1999), in press.
- 46) Adachi, E., Maeda, T., Matsumata, T., Shirabe, K., Kinukawa, N., Sugimachi, K. and Tsuneyoshi, M. Risk factors for intrahepatic recurrence in human small hepatocellular carcinoma. *Gastroenterology*, **108**, 768–775 (1995).
- 47) Shimada, M., Hasegawa, H., Gion, T., Shirabe, K., Taguchi, K., Takenaka, K., Tanaka, S. and Sugimachi, K. Risk factors of the recurrence of hepatocellular carcinoma originating from residual cancer cells after hepatectomy. *Hepatogastroenterology* (1999), in press.
- 48) Shirabe, K., Takenaka, K., Taketomi, A., Kawahara, N., Yamamoto, K., Shimada, M. and Sugimachi, K. Postoperative hepatitis status as a significant risk factor for recurrence in cirrhotic patients with small hepatocellular carcinoma. *Cancer*, **77**, 1050–1055 (1996).
- 49) Ko, S., Nakajima, Y., Kanehiro, H., Hisanaga, M., Aomatsu, Y., Kin, T., Yagura, K., Ohyama, T., Nishio, K., Ohashi, K., Sho, M., Yamada, T. and Nakano, H. Significant influence of accompanying chronic hepatitis status on recurrence of hepatocellular carcinoma after hepatectomy: result of multivariate analysis. *Ann. Surg.*, **224**, 591–595 (1996).
- 50) Tarao, K., Takemiya, S., Tamai, S., Sugimasa, Y., Ohkawa, S., Akaike, M., Tanabe, H., Shimizu, A., Yoshida, M. and Kakita, A. Relationship between the recurrence of hepatocellular carcinoma (HCC) and serum alanine aminotransferase levels in hepatectomized patients with hepatitis C virus-associated cirrhosis and HCC. *Cancer*, **79**, 688–694 (1997).
- 51) Kubo, S., Nishiguchi, S., Shuto, T., Tanaka, H., Tsukamoto, T., Hirohashi, K., Ikebe, T., Wakasa, K., Kuroki, T. and Kinoshita, H. Effects of continuous hepatitis with persistent hepatitis C viremia on outcome after resection of hepatocellular carcinoma. *Jpn. J. Cancer Res.*, **90**, 162–170 (1999).
- 52) Kubo, S., Nishiguchi, S., Hirohashi, K., Shuto, T., Kuroki, T., Minamitani, S., Ikebe, T., Yamamoto, T., Wakasa, K. and Kinoshita, H. Clinicopathological criteria for multicentricity of hepatocellular carcinoma and risk factors for such carcinogenesis. *Jpn. J. Cancer Res.*, **89**, 419–426 (1998).
- 53) Choo, Q.-L., Richman, K. H., Han, J. H., Berger, K., Lee, C., Dong, C., Gallegos, C., Coit, D., Medina-Selby, A., Barr, P. J., Weiner, A. J., Bradley, D. W., Kuo, G. and Houghton, M. Genetic organization and diversity of the hepatitis C virus. *Proc. Natl. Acad. Sci. USA*, **88**, 2451–2455 (1991).
- 54) Tsukiyama-Kohara, K., Iizuka, N., Kohara, M. and Nomoto, A. Internal ribosome entry site within hepatitis C virus RNA. *J. Virol.*, **66**, 1476–1483 (1992).