18. Ogawa M, Hasegawa K, Naritomi T, Torii N, Hayashi N. Clinical features and viral sequences of various genotypes of hepatitis B virus compared among patients with acute hepatitis B. Hepatol Res **2002**; 23: 167–77.
19. Sugiyama M, Tanaka Y, Kurbanov F, et al. Direct cytopathic effects of particular hepatitis B virus genotypes in severe combined immunodeficiency transgenic with urokinase-type plasminogen activator mouse with human hepatocytes. Gastroenterology **2009**; 136:652–62. e3.
20. McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. Ann Intern Med **2001**; 135:759–68.
21. Chu CM. Natural history of chronic hepatitis B virus infection in adults with emphasis on the occurrence of cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol **2000**; 15(suppl):E25–30.
22. Kamatani Y, Wattanapokayakit S, Ochi H, et al. A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. Nat Genet **2009**; 41:591–5.
23. Kumar M, Satapathy S, Monga R, et al. A randomized controlled trial of lamivudine to treat acute hepatitis B. Hepatology **2007**; 45:97–101.
24. Tassopoulos NC, Koutelou MG, Polychronaki H, Paraloglou-Ioannides MHadziyannis SJ. Recombinant interferon-alpha therapy for acute hepatitis B: a randomized, double-blind, placebo-controlled trial. J Viral Hepat **1997**; 4:387–94.
25. Aldershvile J, Frösner GG, Nielsen JO, et al. Hepatitis B e antigen and antibody measured by radioimmunoassay in acute hepatitis B surface antigen-positive hepatitis. J Infect Dis **1980**; 141:293–8.
26. Aldershvile J, Nielsen JO. HBeAg, anti-HBe and anti-HBc IgM in patients with hepatitis B. J Virol Methods **1980**; 2:97–105.

Psycho-Oncology
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Depressive symptoms after treatment in hepatocellular carcinoma survivors: prevalence, determinants, and impact on health-related quality of life

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Abstract

***Objective*: The purposes of this study were to investigate the prevalence and determinants of depressive symptoms among hepatocellular carcinoma (HCC) survivors and to evaluate the impact of depressive symptoms on health-related quality of life (HRQOL).**

***Methods*: A cross-sectional study was conducted on 128 consecutive patients attending an outpatient clinic in Japan 1 year or more after curative treatment. To assess depressive symptoms and HRQOL, the participants were asked to complete the Center for Epidemiologic Studies Depressive Symptoms Scale, the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30, and EORTC QLQ-HCC18, respectively. Multiple logistic regression models were used to identify factors associated with depressive symptoms. EORTC QLQ-C30 and EORTC QLQ-HCC18 scores were compared between participants with and without depressive symptoms.**

***Results*: The prevalence of depressive symptoms among the HCC survivors was 28.3%. The multiple logistic regression analysis revealed that the determinants of depressive symptoms included poor Karnofsky performance status (odds ratio [OR] = 4.59, 95% CI = 1.03–20.55, $p = 0.04$), poor liver function (OR = 3.22, 95% CI = 1.11–10.0, $p = 0.03$), living alone (OR = 6.87, 95% CI = 2.53–18.63, $p = 0.0002$), and unemployment (OR = 5.18, 95% CI = 1.73–15.54, $p = 0.003$). Survivors with depressive symptoms had poorer HRQOL in almost all domains compared with survivors with no depressive symptoms.**

***Conclusions*: This study suggests that after treatment, many HCC survivors experience depressive symptoms that are strongly associated with poorer HRQOL.**

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Introduction

Hepatocellular carcinoma (HCC) is a major health problem worldwide [1]. It is the sixth most common malignancy in the world, with more than half a million new cases annually [1]. The HCC 5-year survival rate after liver resection or liver transplantation has reached over 50% because of improvements in diagnosis and treatment, and the number of HCC survivors has increased [2]. The HCC recurrence rate is very high because of chronic hepatitis, which is the predominant risk factor for HCC in China, Western countries, and Japan [2,3]. Therefore, it is becoming increasingly important to preserve health-related quality of life (HRQOL) of HCC patients during their prolonged life span.

It is known that many cancer survivors experience a number of symptoms and posttreatment effects, including depressive symptoms [4]. Although depressive symptom is a symptom that occurs during the course of cancer, it persists for years after the completion of treatment, and it is one of the most frequent symptoms experienced by cancer survivors [4,5]. It has been suggested that depressive symptoms strongly affect HRQOL [4,6] and can lead to a shorter survival of cancer patients [7,8]. Fortunately, depressive symptoms are treatable. Numerous randomized controlled trials show that psychological distress, including depressive symptoms, can be alleviated by pharmacologic and nonpharmacologic interventions [9]. Therefore, it is particularly important, for cancer survivors, to implement routine depressive symptoms screening and provide appropriate care and treatment.

Although research interest in depressive symptoms among cancer survivors has increased in recent decades, there have been no studies investigating depressive symptoms among HCC survivors. Therefore, little is known about the prevalence and causes of depressive symptoms among HCC survivors, or the characteristics of those most at risk of developing depressive symptoms. This situation makes it difficult to manage the problem. Thus, the aims of this study were to estimate the prevalence of depressive symptoms in HCC survivors more than 1-year posttreatment, to identify factors associated

with depressive symptoms, and to evaluate the impact of depressive symptoms on HRQOL.

Materials and methods

Data collection

We conducted a cross-sectional study of HCC survivors 1 year or more after HCC treatment (curative treatment). The HCC survivors were selected from patients who consecutively attended the Gastroenterology Outpatient Clinic of The University of Tokyo Hospital (a tertiary care teaching hospital). Patients went to see a doctor every 3 months to check for the recurrence of HCC. Patient medical records were reviewed prior to selecting potentially eligible patients. The eligibility criteria were as follows: (1) diagnosed with HCC more than 1 year prior to data collection and had curative treatment at The University of Tokyo Hospital; (2) able to communicate in Japanese; (3) able to participate in the study, as judged by an attending doctor; and (4) 20 years of age or older. Patients with evidence of metastatic or recurrent cancer, those with a history of other types of cancer, and those who were receiving cancer treatment were excluded from the study.

Data were collected after the patients' medical appointments from August 2008 to August 2009 by one of the investigators. Patients self-administered the questionnaires. Medical data were collected by reviewing the patients' medical care records. The investigator checked for absent responses after receiving the questionnaire and when possible, asked the patients to respond to missing items. The ethics committee of The University of Tokyo approved this study, and all participants provided their written informed consent.

Measurement of depressive symptoms

Depressive symptoms were measured using the Japanese version of the Center for Epidemiologic Studies Depressive Symptoms Scale (CES-D) [10]. The CES-D is a 20-item self-report questionnaire designed for the screening of depressive symptoms. Scores for each item are summed to give a range of total scores from 0 to 60. A higher score indicates a greater tendency toward depressive symptoms. A score of 16 points or higher suggests the presence of clinical depressive symptoms [10]. The reliability and validity of the Japanese version of the CES-D have been confirmed [10]. In the Japanese version, the cutoff value of 16 was also optimal, assessed by comparing the proportion of patients with CES-D score of 16 points or higher in a normal control group with that in a group of patients with mood disorders [10].

Measurement of health-related quality of life

The European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 (version 3.0) is a questionnaire for assessing HRQOL of cancer patients. The self-administered questionnaire includes a total of 30 items and includes six functioning scales: physical (five items), emotional (four items), role (two items), cognitive (two items), and social functioning (two items), as well as global health status (two items). The questionnaire also includes three symptom scales: vomiting (two items), fatigue (three items), and pain (two items). Six single items assess dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties. The global health status items are rated from 1 (very poor) to 7 (excellent), and the remaining items are rated 1 (not at all) to 4 (very much). All item response scores were converted into 0–100 scores according to the EORTC scoring guidelines. Higher scores mean a better function or a worse symptom. The reliability and validity of the Japanese version of the EORTC QLQ-C30 have been confirmed [11].

The EORTC QLQ-HCC18 is an HCC-specific supplemental module developed to augment QLQ-C30 and to enhance the sensitivity and specificity of HCC-related QOL issues [12,13]. The self-administered questionnaire includes a total of 18 items and includes six multi-item scales: fatigue (three items), body image (two items), jaundice (two items), nutrition (five items), pain (two items), and fever (two items). Two single items assess sexual life and abdominal swelling. The items are rated 1 (not at all) to 4 (very much). The scales and items are linearly transformed to a 0–100 score, where 100 represents the worst status. The reliability and validity of the Japanese version of the EORTC QLQ-HCC18 have been confirmed [14].

Sociodemographic characteristics

The following sociodemographic information was collected from the self-administered questionnaire: gender, age, employment status, educational level, and cohabitation status.

Clinical characteristics

The following clinical information was collected from the patients' medical records: Karnofsky performance status (KPS), etiology of liver disease, comorbidity other than chronic liver disease, liver function (Child–Pugh grade), history of HCC recurrence after initial treatment, and time since treatment. Higher scores in KPS signify better performance status. We placed cutoff value at 80 points, where patients begin to feel difficulties in normal activity or work. Liver function becomes worse in alphabetical order of Child–Pugh grades A, B, and C.

Statistical analysis

Descriptive statistics are used to present the prevalence of depressive symptoms and the characteristics of the participants. The prevalence of depressive symptoms was

Psycho-Oncology 22: 2347–2353 (2013)
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determined by calculating the proportion of patients exhibiting a score of 16 points or higher on the CES-D.

We used *t*-tests to compare the EORTC QLQ-C30 and EORTC QLQ-HCC18 domain scores between the HCC survivors with depressive symptoms and those with no depressive symptoms. The clinical relevance of the difference in the mean scores of HRQOL scales between groups was further measured by calculating the effect size using Cohen's *d* coefficient. As recommended [15], we considered *d* values less than 0.2, anything above 0.2 but less than 0.5, and anything at or above 0.5 as indicating small, moderate, and large effect sizes, respectively. Chi-squared tests, Fisher's exact tests, and *t*-tests were used to compare CES-D scores among sociodemographic and clinical variables, as appropriate. To identify the sociodemographic and clinical variables that were independently associated with depressive symptoms, multivariate logistic regression models were used. Variables with a p value of 0.2 or less were included in a backward variable selection. Odds ratios and 95% CIs were calculated for each variable in the final model. In all statistical tests, $p < 0.05$ (two-sided) was regarded as statistically significant. Statistical analyses were performed using SAS release 9.2 (SAS institute Inc., Cary NC, USA).

Results

Among 128 eligible patients, one refused to participate (because of a lack of time). Thus, data from 127 patients were included in this study, a response rate of 99.2%. There were no missing data at the item or scale level.

Table I. Sociodemographic and clinical characteristics of the study subjects

Variable	n (%)
Male gender	81 (63.7)
Age (years)[a]	69.0 ± 8.4
Employed full time or part-time	50 (39.4)
Education	
≤12 years	83 (65.3)
Living with family or other adults	85 (66.9)
Karnofsky performance status	
80–100	113 (88.9)
Etiology of liver disease	
Hepatitis C virus	75 (59.0)
Hepatitis B virus	43 (33.9)
Comorbidity other than chronic liver disease	
Yes	83 (65.4)
Child–Pugh grade	
A	96 (75.5)
History of HCC recurrence after initial treatment	
Yes	87 (68.5)
Time since treatment (months)[a]	24.7 ± 18.5

Values are expressed as numbers (%) unless otherwise specified.
HCC, hepatocellular carcinoma.
[a]Data are expressed as mean (standard deviation). Higher Karnofsky performance scores signify better performance status. Liver function becomes worse with increasing Child–Pugh grades A, B, and C.

Sociodemographic and clinical characteristics of the study subjects

Table 1 presents the sociodemographic and clinical characteristics of the study subjects. Most patients were men (63.7%), had good performance status (88.9%), and had good liver function (75.5%). The mean age of survivors was 69.0 years (standard deviation [SD] = 8.4), and the average time since treatment was 24.7 months (SD = 18.5).

Characteristics of hepatocellular carcinoma survivors by depressive symptoms group

Using the dichotomous cutoff (CES-D score ≥ 16), 36 (28.3%) survivors were classified as having depressive symptoms. The average CES-D score was 21.9 (SD = 7.3, median = 20) and 8.5 (SD = 4.1, median = 9) for survivors with and without depressive symptoms, respectively. Table 2 presents the distribution of HCC survivors by depressive symptoms group. The mean age of survivors in the depressive symptoms group was 71.1 years (SD = 7.6). The mean age of survivors in the no-depressive symptoms group was 68.2 years (SD = 8.6).

There were significant differences in KPS scores, Child–Pugh grades, cohabitation, and employment between the two depressive symptoms groups. There were no differences between the depressive symptoms groups in terms of gender, age, etiology of liver disease, education, history of HCC recurrence after initial treatment, and time since treatment.

Multivariate logistic regression models of depressive symptoms

By using multivariate logistic regression procedures, four significant determinants of depressive symptoms were identified (Table 3). Having KPS scores less than 80, having Child–Pugh grade B or C, living alone, and being unemployed were associated with an increased likelihood of depressive symptoms. Multivariate logistic regression analysis with adjustment for age [16–19], KPS [16,20,21], and time since treatment [22,23], which are considered to be important factors related to depressive symptoms, yielded same results.

Depressive symptoms and health-related quality of life

The EORTC QLQ-C30 and EORTC QLQ-HCC18 scores by depressive symptoms groups are presented in Table 4. The HRQOL scores were significantly lower among HCC survivors with depressive symptoms than among survivors with no depressive symptoms in almost all domains, and the effect size was medium or large in all domains except for sexual interest. In addition to univariate analysis, we conducted a multivariate regression analysis with adjustment for age [24–26], gender [27], KPS [28], Child–Pugh grade [27,29,30], and history of

Psycho-Oncology **22**: 2347–2353 (2013)
DOI: 10.1002/pon

Table 2. Characteristics of hepatocellular carcinoma survivors by depressive symptoms group

Variables	CES-D score: Depressive symptoms ($n=36$)	CES-D score: No depressive symptoms ($n=91$)	p-value
Gender			0.05
Male	18 (50.0)	63 (69.2)	
Female	18 (50.0)	28 (30.8)	
Age (years)[a]	71.1 ± 7.6	68.2 ± 8.6	0.08
Employment status			0.001
Employed	6 (16.7)	44 (48.3)	
Unemployed	30 (83.3)	47 (51.7)	
Education			0.11
≤12 years	13 (36.1)	70 (76.9)	
>12 years	23 (63.9)	21 (23.1)	
Cohabitation status			<0.0001
Living with family or other adults	15 (41.6)	70 (76.9)	
Living alone	21 (58.4)	21 (23.1)	
Karnofsky performance status			<0.0001
80–100	26 (72.2)	87 (95.6)	
Less than 80	10 (27.8)	4 (4.4)	
Etiology of liver disease			
Hepatitis C virus			0.27
Yes	24 (66.7)	51 (56.0)	
No	12 (33.3)	40 (44.0)	
Hepatitis B virus			0.93
Yes	12 (33.3)	31 (34.1)	
No	24 (66.7)	60 (65.9)	
Comorbidity other than chronic liver disease			0.31
Yes	26 (72.2)	57 (62.6)	
No	10 (27.8)	34 (37.4)	
Child–Pugh grade			<0.0001
A	20 (55.6)	76 (83.5)	
B/C	16 (44.4)	15 (16.5)	
History of HCC recurrence after initial treatment			0.78
Yes	24 (66.7)	63 (69.2)	
No	12 (33.3)	28 (30.8)	
Time since treatment, months[a]	27.3 ± 18.7	27.6 ± 18.4	0.34

Values are expressed as numbers (%) unless otherwise specified. Higher Karnofsky performance scores signify better performance status. Liver function becomes worse with increasing Child–Pugh grades A, B, and C. CES-D, Center for Epidemiologic Studies Depressive symptoms Scale; HCC, hepatocellular carcinoma.
[a]Data are expressed as mean (standard deviation).

Table 3. Multivariate logistic regression model for depressive symptoms in hepatocellular carcinoma survivors

Variable	Adjusted OR	95% CI	p-value
KPS			
Less than 80	4.59	1.03–20.35	0.04
80–100 (ref)	1.00		
Child–Pugh grade			
B/C	3.22	1.11–10.0	0.03
A (ref)	1.00		
Cohabitation status			
Living alone	6.87	2.53–18.63	<0.001
Living with family or other adults (ref)	1.00		
Employment status			
Unemployed	5.18	1.73–15.54	0.003
Employed (ref)	1.00		

OR, odds ratio; KPS, Karnofsky performance status.

HCC recurrence after initial treatment [25,26,29], which are considered to be important factors related to HRQOL in HCC patients. As expected, depressive symptoms were independent factors related to almost all domains of HRQOL.

Discussion

To our knowledge, this is the first study that investigated the prevalence and determinants of depressive symptoms among the HCC survivors after their curative treatment. And this is the first study to investigate the impact of depressive symptoms on HRQOL precisely, using HCC-specific module. The prevalence of depressive symptoms among the HCC survivors was 28.3%. The multiple logistic regression

Psycho-Oncology **22**: 2347–2353 (2013)
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Table 4. Comparison of EORTC QLQ-C30 and EORTC QLQ-HCC18 scores between HCC survivor depressive symptoms groups

	Depressive symptoms ($n=36$)	No depressive symptoms ($n=91$)	Effect size[a]	p-value
EORTC QLQ-C30[b]				
Global health status/QOL[c]	50.9 ± 18.9	73.8 ± 17.7	1.25[d]	<0.0001
Functional scales[c]				
Physical function	72.0 ± 19.8	89.6 ± 11.6	1.08[d]	<0.0001
Role function	69.4 ± 28.3	91.0 ± 15.8	0.94[d]	<0.0001
Emotional function	71.5 ± 20.4	89.6 ± 12.0	1.08[d]	<0.0001
Cognitive function	64.8 ± 26.6	80.7 ± 17.0	0.71[d]	0.0007
Social function	75.5 ± 25.6	91.4 ± 15.8	0.75[d]	0.0002
Symptom scales[e]				
Fatigue	44.7 ± 23.1	24.3 ± 18.5	0.97[d]	<0.0001
Pain	26.8 ± 13.8	6.4 ± 29.6	0.88[d]	0.0003
Nausea/vomiting	3.7 ± 6.4	1.5 ± 8.1	0.30[f]	0.14
Dyspnea	25.9 ± 25.3	12.8 ± 19.0	0.59[d]	0.007
Appetite	25.9 ± 31.9	8.8 ± 19.1	0.65[d]	0.004
Insomnia	35.2 ± 34.7	14.3 ± 20.6	0.73[d]	0.001
Constipation	22.2 ± 20.2	12.1 ± 29.8	0.39[f]	0.06
Diarrhea	13.9 ± 23.1	6.9 ± 31.9	0.25[f]	0.10
Financial difficulties	22.2 ± 31.9	10.9 ± 31.9	0.35[f]	0.05
EORTC QLQ-HCC18[b]				
Symptom scales[e]				
Fatigue	39.5 ± 24.6	20.0 ± 18.0	0.90[d]	<0.0001
Body image	42.1 ± 28.8	22.9 ± 19.9	0.77[d]	0.0006
Jaundice	21.2 ± 16.9	10.4 ± 13.9	0.69[d]	0.006
Nutrition	22.4 ± 18.5	9.7 ± 9.8	0.86[d]	0.0004
Pain	22.2 ± 16.4	10.4 ± 13.1	0.79[d]	0.0003
Fever	9.7 ± 14.6	2.6 ± 7.8	0.60[d]	0.007
Abdominal swelling	34.3 ± 31.4	12.4 ± 18.4	0.85[d]	0.0003
Sexual interest	11.4 ± 22.8	8.4 ± 22.3	0.13[g]	0.51

Data are expressed as mean ± standard deviation.
QOL, quality of life; EORTC, European Organization for Research and Treatment of Cancer.
[a]Cohen's *d*.
[b]Scale scores range from 0 to 100.
[c]Higher score indicates higher QOL.
[d]Large effect size.
[e]Higher score indicates lower QOL.
[f]Medium effect size.
[g]Small effect size.

analysis revealed that the determinants of depressive symptoms included poor KPS, poor liver function, living alone, and unemployment. Survivors with depressive symptoms had poorer HRQOL in almost all domains compared with survivors with no depressive symptoms.

The prevalence of depressive symptoms (28.3%) among the HCC survivors was slightly higher than that reported for other liver diseases, such as chronic liver disease (23.6%) [31] and hepatitis C (20.0–28.0%) [32,33]. The patients in this study continued to suffer from hepatitis or cirrhosis even after being treated for HCC. Furthermore, specific problems, such as the burden of other symptoms, the uncertainty of treatment outcomes, the fear of recurrence, and the probable change in socioeconomic status, may contribute to depressive symptoms in cancer survivors [34]. These factors may be responsible for the observation of a higher prevalence of depressive symptoms in HCC survivors compared with that observed in chronic liver disease or hepatitis C patients.

The prevalence of depressive symptoms among the HCC survivors was higher than that reported among survivors of prostate cancer (17.0%) [35] or breast cancer (23.0%) [36] but lower than that reported among survivors of colorectal cancer (36.7%) [6]. Colorectal cancer survivors may have associated changes in bowel habit, and sexual or micturition problems after surgery, leading to a higher prevalence of depressive symptoms among them. Colorectal cancer patients undergo postoperative adjuvant therapy such as chemotherapy or radiotherapy. Although postoperative loss of function and the symptoms caused by adjuvant therapy are thought to contribute to depressive symptoms in other cancer survivors, HCC patients rarely undergo adjuvant therapy, and no functions are lost through treatment. Nevertheless, the fact that the prevalence of depressive symptoms among HCC survivors is similar to or higher than that among other cancer survivors indicates the need to take precautions against depressive symptoms in HCC patients.

Psycho-Oncology **22**: 2347–2353 (2013)
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To increase our knowledge of the factors associated with depressive symptoms among HCC survivors, we compared the depressive symptoms groups with a variety of sociodemographic and clinical variables. Our results indicate that sociodemographic and psychosocial variables such as living alone and being unemployed, in addition to physical variables such as poor KPS and decreased liver function, were associated with depressive symptoms. Previous studies with survivors of other cancers have identified physical [19,37], sociodemographic [17,38], and psychosocial variables [20,39–42] and modifiable health behaviors [43] to be important factors associated with depressive symptoms. We found these to be true for the survivors of HCC in our study and found that poor liver function was an HCC survivor-specific factor associated with depressive symptoms. HCC survivors continue to suffer from hepatitis or cirrhosis after curative treatment for HCC. With the progression of liver cirrhosis, they suffer from ascites, hepatic encephalopathy, and various physical symptoms, which may contribute to a higher psychological distress than other cancers. Healthcare professionals need to keep a close eye on the decrease of liver function after curative treatment.

Previous studies regarding the survivors of various types of cancer have indicated that depressive symptoms are associated to HRQOL [4]. In our study, we showed that depressive symptoms are strongly related to almost all domains of EORTC QLQ-C30 and HCC-specific module, EORTC QLQ-HCC18. The effect size was medium or large in all domains except for sexual interest, suggesting a big difference between individuals with depressive symptoms and those without. Thus, continuous screening for depressive symptoms of HCC survivors is warranted because it is a symptom that healthcare professionals tend to underestimate [44].

Our study was subject to some limitations. First, it was of cross-sectional design; therefore, no causal relations among the variables and depressive symptoms could be established. The study was conducted on a small number of HCC survivors at one hospital, and therefore, the findings may not be generalized to other populations. Second, we did not perform standardized psychiatric interviews; however, the CES-D has been shown to be a reliable and valid screening instrument for depressive symptoms. Third, we could not include age-matched and gender-matched noncancer control. This would be the subject for our further research. Fourth, we could not include variables such as mental disorder prior to cancer and health behavior. Future research should evaluate additional variables related to depressive symptoms following HCC treatment and their impact on HRQOL. Fifth, depressive symptoms and HRQOL based on the type of treatments received could not be explored in this study, as patients had varying treatment durations, types of treatment, and times between treatments.

Despite these limitations, this study contributes to highlight a potential target group for the intervention to prevent and treat depressive symptoms in HCC survivors.

Conclusion

This study found that many HCC survivors experienced depressive symptoms after their curative treatment. Depressive symptoms were influenced by sociodemographic and clinical factors and had a negative impact on HRQOL, with poorer scores in almost all domains among patients with depressive symptoms. Healthcare professionals should pay more attention to the possibility of depressive symptoms among HCC survivors with poor KPS, poor liver function, who live alone, and/or are unemployed. Interventions for depressive symptoms among patients with cancer have been shown to be effective; therefore, we believe that implementing a program geared toward HCC patients and survivors would be beneficial. Because multiple physical and social factors were associated with depressive symptoms among the HCC survivors, it is important to provide comprehensive interdisciplinary interventions in addition to normal treatment for depressive symptoms. Future research should evaluate additional variables related to depressive symptoms following HCC treatment and their impact on HRQOL over time.

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Conflict of interest

The authors of this study did not receive any financial support for this study and declare no conflict of interest.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;**55**:74–108.
2. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003;**362**:1907–1917.
3. Ikeda K, Saitoh S, Tsubota A, *et al.* Risk factors for tumor recurrence and prognosis after curative resection of hepatocellular carcinoma. *Cancer* 1993; **71**:19–25.
4. Shi Q, Smith TG, Michonski JD, Stein KD, Kaw C, Cleeland CS. Symptom burden in cancer survivors 1 year after diagnosis: a report from the American Cancer Society's Studies of Cancer Survivors. *Cancer* 2011;**117**:2779–2790.
5. Newport DJ, Nemeroff CB. Assessment and treatment of depression in the cancer patient. *J Psychosom Res* 1998;**45**:215–237.
6. Tsunoda A, Nakao K, Hiratsuka K, Yasuda N, Shibusawa M, Kusano M. Anxiety, depression

Psycho-Oncology **22**: 2347–2353 (2013)
DOI: 10.1002/pon

and quality of life in colorectal cancer patients. *Int J Clin Oncol* 2005;**10**:411–417.
7. Satin JR, Linden W, Phillips MJ. Depression as a predictor of disease progression and mortality in cancer patients: a meta-analysis. *Cancer* 2009;**115**:5349–5361.
8. Hamer M, Chida Y, Molloy GJ. Psychological distress and cancer mortality. *J Psychosom Res* 2009;**66**:255–258.
9. Jacobsen PBDK, Swaine ZN, Watson IS. Management of anxiety and depression in adult cancer patients: toward and evidence-based approach. In: Oncology: An Evidence-Based Approach. Springer-Verlag: New York, 2006.
10. Shima SST, Kitamura T, Asai M. A new self-rating scale for depression. *Clin Psychiatry* 1985;**27**:717–723.
11. Kobayashi K, Takeda F, Teramukai S, *et al.* A cross-validation of the European Organization for Research and Treatment of Cancer QLQ-C30 (EORTC QLQ-C30) for Japanese with lung cancer. *Eur J Cancer* 1998;**34**:810–815.
12. Blazeby JM, Currie E, Zee BC, Chie WC, Poon RT, Garden OJ. Development of a questionnaire module to supplement the EORTC QLQ-C30 to assess quality of life in patients with hepatocellular carcinoma, the EORTC QLQ-HCC18. *Eur J Cancer* 2004;**40**:2439–2444.
13. Chie WC, Blazeby JM, Hsiao CF, *et al.* International cross-cultural field validation of an European Organization for Research and Treatment of Cancer questionnaire module for patients with primary liver cancer, the European Organization for Research and Treatment of Cancer quality-of-life questionnaire HCC18. *Hepatology* 2012;**55**:1122–1129.
14. Mikoshiba N, Tateishi R, Tanaka M, *et al.* Validation of the Japanese version of the EORTC hepatocellular carcinoma-specific quality of life questionnaire module (QLQ-HCC18). *Health Qual Life Outcomes* 2012;**10**:58.
15. Cohen J A power primer. *Psychol Bull* 1992;**112**:155–159.
16. Jehn CF, Flath B, Strux A, *et al.* Influence of age, performance status, cancer activity, and IL-6 on anxiety and depression in patients with metastatic breast cancer. *Breast Cancer Res Treat* 2012;**136**:789–794.
17. Cella DF, Orofiamma B, Holland JC, *et al.* The relationship of psychological distress, extent of disease, and performance status in patients with lung cancer. *Cancer* 1987;**60**:1661–1667.
18. Dugan W, McDonald MV, Passik SD, Rosenfeld BD, Theobald D, Edgerton S. Use of the Zung Self-Rating Depression Scale in cancer patients: feasibility as a screening tool. *Psycho-Oncology* 1998;**7**:483–493.
19. Kaasa S, Malt U, Hagen S, Wist E, Moum T, Kvikstad A. Psychological distress in cancer patients with advanced disease. *Radiother Oncol* 1993;**27**:193–197.
20. Wenzel LB, Fairclough DL, Brady MJ, *et al.* Age-related differences in the quality of life of breast carcinoma patients after treatment. *Cancer* 1999;**86**:1768–1774.
21. Linden W, Vodermaier A, Mackenzie R, Greig D. Anxiety and depression after cancer diagnosis: prevalence rates by cancer type, gender, and age. *J Affect Disord* 2012;**141**:343–351.
22. Mehnert A, Lehmann C, Graefen M, Huland H, Koch U. Depression, anxiety, post-traumatic stress disorder and health-related quality of life and its association with social support in ambulatory prostate cancer patients. *Eur J Cancer Care (Engl)* 2010;**19**:736–745.
23. Uchitomi Y, Mikami I, Kugaya A, *et al.* Depression after successful treatment for nonsmall cell lung carcinoma. *Cancer* 2000;**89**:1172–1179.
24. Kondo Y, Yoshida H, Tateishi R, *et al.* Health-related quality of life of chronic liver disease patients with and without hepatocellular carcinoma. *J Gastroenterol Hepatol* 2007; **22**:197–203.
25. Chen L, Liu Y, Li GG, Tao SF, Xu Y, Tian H. Quality of life in patients with liver cancer after operation: a 2-year follow-up study. *Hepatobiliary Pancreat Dis Int* 2004;**3**:530–533.
26. Ueno S, Tanabe G, Nuruki K, *et al.* Quality of life after hepatectomy in patients with hepatocellular carcinoma: implication of change in hepatic protein synthesis. *Hepatogastroenterology* 2002;**49**:492–496.
27. Lai HL, Lin SY, Yeh SH. [Exploring uncertainty, quality of life and related factors in patients with liver cancer]. *Hu Li Za Zhi* 2007;**54**:41–52.
28. Shun SC, Chiou JF, Lai YH, *et al.* Changes in quality of life and its related factors in liver cancer patients receiving stereotactic radiation therapy. *Support Care Cancer* 2008;**16**:1059–1065.
29. Wang YB, Chen MH, Yan K, Yang W, Dai Y, Yin SS. Quality of life after radiofrequency ablation combined with transcatheter arterial chemoembolization for hepatocellular carcinoma: comparison with transcatheter arterial chemoembolization alone. *Qual Life Res* 2007;**16**:389–597.
30. Steel JL, Chopra K, Olek MC, Carr BI. Health-related quality of life: hepatocellular carcinoma, chronic liver disease, and the general population. *Qual Life Res* 2007;**16**:203–215.
31. Weinstein AA, Kallman Price J, Stepanova M, *et al.* Depression in patients with nonalcoholic fatty liver disease and chronic viral hepatitis B and C. *Psychosomatics* 2011;**52**:127–132.
32. Lee DHJH, Regenstein FG, Perrillo RP. Morbidity of chronic hepatitis C as seen in a tertiary care medical center. *Dig Dis Sci* 1997;**42**:186–191.
33. Dwight MM, Kowdley KV, Russo JE, Ciechanowski PS, Larson AM, Katon WJ. Depression, fatigue, and functional disability in patients with chronic hepatitis C. *J Psychosom Res* 2000;**49**:311–317.
34. Reich M, Lesur A, Perdrizet-Chevallier C. Depression, quality of life and breast cancer: a review of the literature. *Breast Cancer Res Treat* 2008;**110**:9–17.
35. Shinn EH, Basen-Engquist K, Thornton B, Spiess PE, Pisters L. Health behaviors and depressive symptoms in testicular cancer survivors. *Urology* 2007;**69**:748–753.
36. Akechi TOT, Imoto S, Yamawaki S, Uchitomi Y. Biomedical and psychosocial determinants of psychiatric morbidity among postoperative ambulatory breast cancer patients. *Breast Cancer Res Treat* 2001;**65**:195–202.
37. Akechi T, Okamura H, Nishiwaki Y, Uchitomi Y. Psychiatric disorders and associated and predictive factors in patients with unresectable nonsmall cell lung carcinoma: a longitudinal study. *Cancer* 2001;**92**:2609–2622.
38. Dugan WMM, Passik SD, Rosenfeld BD, Theobald D, Edgerton S. Use of the Zung Self-Rating Depression Scale in cancer patients: feasibility as a screening tool. *Psycho-Oncology* 1998;**7**:483–493.
39. Epping-Jordan JE, Compas BE, Osowiecki DM, *et al.* Psychological adjustment in breast cancer: processes of emotional distress. *Health Psychol* 1999;**18**:315–326.
40. Revenson TAWC, Felton BJ. Social supports as stress buffers for adult cancer patients. *Psychosomatic Med* 1983;**45**:321–331.
41. Taniguchi K, Akechi T, Suzuki S, Mihara M, Uchitomi Y. Lack of marital support and poor psychological responses in male cancer patients. *Support Care Cancer* 2003; **11**:604–610.
42. Demakakos P, Nazroo J, Breeze E, Marmot M. Socioeconomic status and health: the role of subjective social status. *Soc Sci Med* 2008;**67**:330–340.
43. Boyes AW, Girgis A, D'Este C, Zucca AC. Flourishing or floundering? Prevalence and correlates of anxiety and depression among a population-based sample of adult cancer survivors 6months after diagnosis. *J Affect Disord* 2011;**135**:184–192.
44. Fallowfield L, Ratcliffe D, Jenkins V, Saul J. Psychiatric morbidity and its recognition by doctors in patients with cancer. *Br J Cancer* 2001;**84**:1011–105.

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CT With Hepatic Arterioportography as a Pretreatment Examination for Hepatocellular Carcinoma Patients: A Randomized Controlled Trial

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OBJECTIVES: **The combination of computed tomography with hepatic arteriography and arterial portography (CTHA/CTAP) can detect additional hepatocellular carcinoma (HCC) nodules undetected by conventional dynamic CT.**

METHODS: **In this single-center, randomized, open-label, controlled trial, we randomly assigned 280 patients who were diagnosed as having HCC by conventional dynamic CT, and eligible for radiofrequency ablation (RFA), to undergo CTHA/CTAP before treatment, or to the control group. Newly detected HCC nodules by CTHA/CTAP were intended to be ablated completely. The primary end point was recurrence-free survival and the key secondary end point was overall survival. The analysis was conducted on an intention-to-treat basis. Those with nonablated nodules were treated as for recurrence.**

RESULTS: **A total of 75 nodules were newly diagnosed as HCC by CTHA/CTAP in 45 patients. Three patients (one in the CTHA/CTAP group and two in the control group) who refused treatment were excluded from all analyses. The cumulative recurrence-free survival rates at 1, 2, and 3 years were 60.1, 29.0, and 18.9% in the CTHA/CTAP group and 52.2, 29.7, and 23.1% in the control group, respectively ($P=0.66$ by log-rank test; hazard ratio, 0.94 for CTHA/CTAP vs. control; 95% confidence interval (CI), 0.73–1.22). The cumulative overall survival rates at 3 and 5 years were 79.7 and 56.4% in the CTHA/CTAP group and 86.8 and 60.1% in the control group, respectively ($P=0.50$; hazard ratio, 1.15, 95% CI, 0.77–1.71).**

CONCLUSIONS: **CTHA/CTAP may detect recurrent lesions earlier. However, CTHA/CTAP before RFA did not improve cumulative recurrence-free survival or overall survival.**

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INTRODUCTION

Hepatocellular carcinoma (HCC) ranks as the fifth most common cancer worldwide (1). In Japan, ~35,000 patients die from HCC every year (2), and the main cause of HCC is hepatitis C virus infection. In chronic hepatitis patients, screening of HCC is usually performed by ultrasonography, and the diagnosis is confirmed by contrast-enhanced dynamic computed tomography (CT). Hyperattenuation in the arterial phase and hypoattenuation in the equilibrium phase are considered to be definitive signs of HCC (3–7). Hyperattenuation in the arterial phase is more emphasized when contrast material is injected from the hepatic artery through a catheter, because dilution of contrast material in the systemic circulation is avoided, thus keeping a high concentration of contrast material in the liver. This technique is called CT during hepatic arteriography (CTHA) (6,8–10). Similarly, hypoattenuation in the equilibrium phase is accentuated after injection of contrast material into the superior mesenteric artery, which is referred to as CT during arterial portography (CTAP) (11–14). The combination of CTHA and CTAP gives higher sensitivity and specificity for HCC detection than conventional dynamic enhanced CT (8).

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If new HCC nodules are detected with CTHA/CTAP, in addition to those detected with dynamic CT, the treatment of choice may be changed (15,16). For example, surgical resection and liver transplantation are usually contraindicated for multinodular HCC; that is, exceeding three nodules. Percutaneous tumor ablation methods, such as ethanol injection and microwave coagulation, have played an important role as nonsurgical treatments that can achieve high local cure rates without affecting background liver function (17–20). Radiofrequency ablation (RFA) is currently considered to be the most effective first-line percutaneous ablation protocol because of its greater efficacy in terms of local cure as compared with ethanol injection (21–24). However, even after complete ablation, patients frequently encounter intrahepatic tumor recurrence at a rate of 50% in 2 years, the majority of which occurs at locations distant from the primary ablated site (25). Considering the tumor doubling time, many nodules diagnosed as recurrent within 2 years were probably present at the time of first ablation. If nodules that are undetectable by conventional dynamic CT could be detected and ablated, the recurrence rate would be decreased.

Although CTHA/CTAP is one of the most sensitive techniques available for detection of small HCC, its disadvantages include invasiveness, high cost, and a high false-positive rate (26). The indication for CTHA/CTAP can be justified only when the expected benefits exceed the risk and cost of the procedure. We conducted a single-center, randomized, open-label, controlled trial to assess the utility of CTHA/CTAP before RFA in patients with early-stage HCC by comparing recurrence-free and overall survival.

METHODS

Patients

The study population consisted of patients with early-stage HCC with an indication for RFA. Those who met the following criteria were enrolled between September 2004 and February 2009: (i) diagnosis of typical HCC on dynamic CT performed within 2 weeks, i.e., hyperattenuation during the arterial phase and hypoattenuation during the equilibrium phase (5,6); (ii) tumor size ≤3.0 cm and no more than three tumor nodules; (iii) Child–Pugh class A liver function; and (iv) age >20 years. Exclusion criteria were: allergy to contrast media; portal or hepatic vein tumor thrombosis; extrahepatic metastasis; diffuse and infiltrative tumors; renal failure (serum creatinine >2.0 mg/dl, or serum urea nitrogen >30 mg/dl); impaired coagulation (e.g., platelet count $<50\times10^3/\mu l$, or prothrombin activity <50%); pregnancy; or past history of choledochojejunostomy. We included those with previous treatments as well as treatment-naive cases provided that there was no local recurrence at enrollment. These inclusion criteria and the study design did not change till the study completely ended. The study design conformed to the Declaration of Helsinki Principles and was approved by the ethics committee of our institution. The study was registered at the University Hospital Medical Information Network (UMIN) Clinical Trial Registry (UMIN-CTR000000070). Written informed consent was obtained from each patient. This study complied with the CONSORT guidelines for reporting of clinical trials (27).

Study design

Before receiving RFA, patients were randomly assigned to undergo CTHA/CTAP or not in equal numbers. Patient registration and randomization were performed by computer-generated allocation at a web-based data center (Internet Data and Information Center for Medical Research) administered by UMIN. At the time of randomization, patients were stratified either as treatment naive, for whom RFA was planned as an initial treatment for HCC, or recurrent, for whom RFA was planned for recurrent HCC. The randomization was based on the Efron's biased-coin design (28). In principal, the assignment was not blinded to the investigators and the participants. The interval between random assignment and implementation of treatment for HCC was <4 weeks. CTHA/CTAP was performed on the assigned patients on the second day of admission, and RFA was performed 2 or 3 days later, given that the total number of HCC nodules remained <4. When ≥4 HCC nodules were detected on CTHA/CTAP, patients first received transarterial chemoembolization (TACE) immediately after CTHA/CTAP, followed later by RFA to achieve complete ablation of the tumor nodules.

Radiographic procedures

For the diagnosis of HCC at study entry, intravenous contrast-enhanced dynamic CT was performed on an outpatient basis using an X-ray CT device with 4, 8, or 16 detector rows (Aquilion 4/16; Toshiba, Tokyo, Japan; LightSpeed Qx/I, LightSpeed Ultra; GE Healthcare, Milwaukee, WI). Images were obtained during the early arterial, late arterial, and equilibrium phases at 28, 40, and 120 s after starting the intravenous bolus injection of iopamidol (Iopamiron; Nihon Schering, Osaka, Japan) or iohexol (Omnipaque; Daiichi Sankyo, Tokyo, Japan) at a rate of 2.3–3.3 ml/s with a power injector. The total dose of iodine was 0.7 g/kg body weight, with an upper limit of 37 g iodine. The injection time for the contrast material was 30 s. Images were reconstructed with a section thickness of 2.5 mm and a reconstruction interval of 1.5 mm, and were reviewed by experienced radiologists.

CTHA/CTAP was performed on an inpatient basis. First, a 4-Fr modified Shepherd-hook catheter and a 4-Fr hepatic-curve catheter were placed in the celiac artery and superior mesenteric artery, respectively, through bilateral femoral arteries, according to Seldinger's method. Digital subtraction angiography was performed from the celiac artery to evaluate hepatic artery anatomy. A microcatheter was inserted through the 4-Fr catheter and placed in the proper or common hepatic artery for hepatic arteriography.

The CTAP catheter was placed in the superior mesenteric artery in all cases. In the case of a replaced or accessory right hepatic artery, the catheter was inserted well beyond the origin of the hepatic artery to prevent contrast medium overflow into the hepatic artery. Less than 30 ml of contrast agent, which was diluted to 100 mg I/ml, was used before the CTHA/CTAP study. First, CTAP was performed using 90 ml nonionic contrast medium diluted to 100 mg I/ml, and then CT scanning was performed 30 s after the start of the injection at a rate of 3.0 ml/s. Multidetector-row CT images were obtained during a single breath hold in a longitudinal direction with collimation of 1 mm, table speed of 30 mm/s, 120 kVp, and

300 mAs. CTHA was performed at least 5 min after CTAP, using the same parameters. CT scanning was performed at 10 and 45 s after the start of contrast medium injection into the microcatheter at a rate of 2.0–2.5 ml/s. A total of 30–50 ml contrast agent diluted to 100 mg I/ml was used. When the liver was perfused by two or more hepatic arteries such as a replaced right hepatic artery, accessory right hepatic artery, or left hepatic artery downstream of the left gastric artery, CTHA was performed from each of the respective arteries. A diagnosis of typical HCC on CTHA/CTAP was defined as a round hypervascular nodule on CTHA with a defect on CTAP, accompanied by corona enhancement during the second phase of CTHA or hypoattenuation during the equilibrium phase of prior dynamic CT (10,29).

TACE was additionally performed when ≥4 HCC nodules were detected on CTHA/CTAP, as evaluated at the time by the operating radiologist. The procedure used 3.0 ml contrast medium, 30 mg doxorubicin (Adriacin; Kyowahakko Kirin, Tokyo, Japan), and 3.0 ml iodized oil (Lipiodol Ultra-Fluid; Guerbet Japan, Tokyo, Japan). The amounts of contrast medium and iodized oil in this suspension were arbitrarily adjusted according to tumor size. This agent was injected into each feeder of the HCC, followed by infusion of 2-mm-diameter gelatin sponge particles (Gelpart; Nihonkayaku, Tokyo, Japan).

CTHA/CTAP images were scrutinized by two experienced radiologists, who made the final diagnosis. The radiologists were not blinded to information regarding the preceding conventional dynamic CT. Preceding intravenous contrast-enhanced dynamic CT was retrospectively reviewed for nodules newly diagnosed by CTHA/CTAP to determine whether the nodules could have been detected on dynamic CT.

Radiofrequency ablation

RFA was performed on an inpatient basis. The precise procedure of RFA is described elsewhere (30). All RFA procedures were performed percutaneously under ultrasonographic guidance. We used a 17-gauge cooled-tip electrode (Cool-Tip; RF Ablation System, Covidien, Boulder, Colombia, CO) for RFA. Radiofrequency energy was delivered for 6–12 min for each application. For large tumors, the electrode was repeatedly inserted into different sites, such that the entire tumor could be enveloped by assumed necrotic volumes. A CT scan with a 5-mm section thickness was performed 1–3 days after RFA to evaluate technical effectiveness. Complete ablation was defined as hypoattenuation of the entire tumor. We intended to ablate not only the tumor but also some of the liver parenchyma surrounding it. When we suspected that some portion of tumor remained nonablated, RFA was repeated. We did not predefine the procedure number in a treatment: treatment was generally continued until CT imaging demonstrated necrosis of the entire tumor.

Follow-up

The follow-up regimen after RFA consisted of blood tests and monitoring of tumor markers in an outpatient setting. Ultrasonography and dynamic CT were performed every 4 months. Tumor recurrence was defined as a newly developed lesion on a dynamic CT that showed hyperattenuation in the arterial phase with washout in the late phase. Recurrent site was categorized as intrahepatic recurrence distant from ablated nodules, local tumor progression defined as the appearance of viable cancer tissue touching the ablated nodules, and extrahepatic metastasis (31). The follow-up was censored in February 2011 when 2 years had passed after the enrollment of patient 280. No interim analysis was specified in the protocol.

End points

The primary end point was recurrence-free survival, where both recurrence and death were treated as an event. We intended to ablate all detected nodules in both groups. When additional nodules were detected by CTHA/CTAP, the newly detected nodules were also ablated. When >3 nodules were diagnosed as HCC by CTHA/CTAP, we performed TACE and subsequently intended to ablate all of the nodules. When nonablated viable tumors were detected by CT for treatment evaluation, those cases were treated as an event 120 days after randomization. Even when newly detected nodules showed dense Lipiodol deposits after TACE, the nodules were considered as viable if the nodules were nonablated.

Secondary end points were the number of additional nodules detected by CTHA/CTAP, the proportion of patients with complete ablation, overall survival, and safety of CTHA/CTAP and RFA. Complications were defined according to the guidelines of the Society of Interventional Radiology (32). According to the guidelines, major complications were defined as those that required therapy or prolonged hospitalization, or left permanent adverse sequelae, or death.

Statistical analysis

This study was designed to detect a 15% increase in 2-year recurrence-free survival in the CTHA/CTAP group from an anticipated 35% in the control group. To detect this difference with a power of 80% and type I error of 5% (two-sided test), we needed 280 patients (140 for each arm). Differences between groups for each characteristic were tested for significance with Fisher's exact test for categorical variables and *t*-test for continuous variables. All data necessary for analysis was corrected in the main computer server system of University of Tokyo, Department of Gastroenterology.

Recurrence-free survival and overall survival were calculated using the Kaplan–Meier method and were compared by the log-rank test. Cox proportional hazard regression was used to calculate hazard ratios with 95% confidence interval (CI) between the groups in univariate and multivariate settings. The primary end point was evaluated in subgroups according to the following characteristics: age, sex, body mass index, treatment naivety, hepatitis B surface antigen (HBsAg) positivity, hepatitis C virus antibody positivity, tumor size, tumor number, platelet count, tumor marker positivity for α-fetoprotein (AFP), lens culinaris agglutinin-reactive fraction of AFP, and des-γ-carboxy prothrombin. An adjusted hazard ratio comparing the groups was calculated using multivariate Cox regression with factors that showed significance

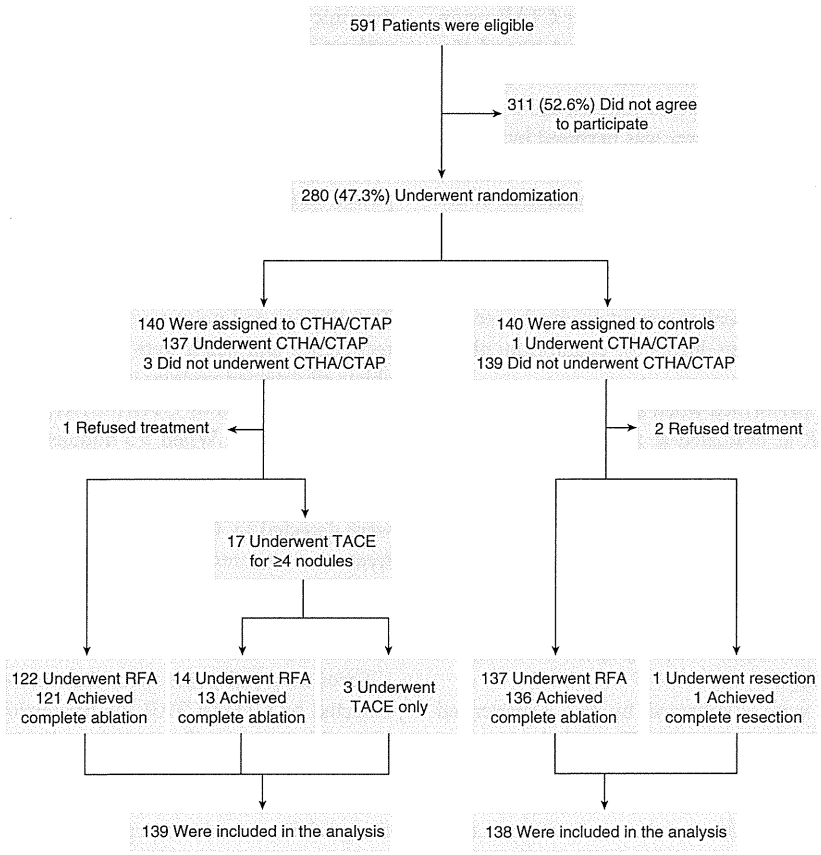

Figure 1. Patient enrollment and outcomes. CTAP, computed tomography during arterial portography; CTHA, computed tomography during hepatic arteriography; RFA, radiofrequency ablation; TACE, transarterial chemoembolization.

in univariate analysis. Data at entry were used for the analyses. A *post hoc* analysis comparing the recurrence-free survival of those with and without newly diagnosed HCC in the CTHA/CTAP group was performed.

All analyses were performed on an intention-to-treat basis. Differences with a two-sided P value of <0.05 were considered statistically significant. Data processing and analysis were performed with S-PLUS ver. 7 (TIBCO Software, Palo Alto, CA). Finally, all authors had access to the study data and had reviewed and approved the final manuscript.

RESULTS

Patient enrollment

According to the study protocol, the registration started from September 2004 for 5 years and the follow-up was censored in February 2011 when 2 years had passed after the enrollment of patient 280. During the study period, 280 of 591 (47.4%) eligible patients agreed to participate in the trial, and 140 of these were randomly assigned to undergo CTHA/CTAP before RFA. Three patients declined to undergo CTHA/CTAP after assignment. A total of 140 patients were randomly assigned to the control group. One patient assigned to the control group received CTHA/CTAP because of strong preference (**Figure 1**).

Treatment

In 45 (32.4%) patients, 75 nodules with a median diameter of 8 mm (range, 2–20) were additionally diagnosed by experienced radiologists as definite HCC on CTHA/CTAP. The detailed characteristics of newly diagnosed nodules have been reported previously (33). In 17 patients, the number of HCC nodules exceeded 3 after CTHA/CTAP, and TACE was performed subsequently. We intended to ablate all nodules by RFA including additionally detected nodules. In 122 patients, there were ≤3 HCC nodules, and complete ablation was obtained in 121 patients (99.2%). Among 17 patients treated with TACE, 14 (82.4%) subsequently underwent RFA and complete ablation was obtained in 13 (92.9%) patients. The remaining 3 patients (17.6%) did not undergo RFA because of tumor nodule multiplicity in 2 patients and simultaneously diagnosed malignant B-cell lymphoma in the third patient. Among 140 patients who were assigned to the control group, 137 (97.9%) were treated with RFA, and complete ablation was obtained in 136 (99.3%) patients. One patient withdrew consent and underwent hepatic resection. Two patients refused to receive

Table 1. Baseline characteristics of the patients[a]

Characteristics	CTHA/CTAP (*N*=139)	Control (*N*=138)	*P* value
Age, years	70 (63–74)	70 (64–75)	0.43
Male, *n* (%)	93 (67)	86 (62)	0.42
Alcohol >80 g/day, *n* (%)	23 (17)	20 (15)	0.82
BMI (kg/m^2)	23.1 (21.4–25.1)	23.4 (21.2–25.3)	0.48
Viral markers			
HCVAb positive, *n* (%)	104 (75)	99 (72)	0.59
HBsAg positive, *n* (%)	21 (15)	20 (14)	1
Serum albumin (g/dl)	3.8 (3.6–4.1)	3.9 (3.6–4.1)	0.20
Total bilirubin (mg/dl)	0.8 (0.6–1.0)	0.8 (0.6–1.0)	0.31
AST (IU/l)	56 (34–69)	57 (33–70)	0.84
ALT (IU/l)	54 (29–63)	57 (27–73)	0.61
Platelet count (×10^3/μl)	128 (89–163)	130 (91–159)	0.88
Prothrombin activity (%)	80 (72–90)	81 (74–87)	0.39
Treatment-naive case, *n* (%)	77 (55)	74 (54)	0.81
Previously treated case, n *(%)*	62 (45)	64 (46)	
Resection, *n* (%)[b]	15 (24)	16 (25)	0.27
RFA, *n* (%)[b]	46 (74)	45 (70)	
Ethanol injection, *n* (%)[b]	10 (16)	3 (4.6)	
TACE, *n* (%)[b]	11 (18)	7 (11)	
Tumor size (cm)	1.6 (1.2–2.0)	1.7 (1.2–2.0)	0.91
Single nodule, *n* (%)	101 (73)	98 (71)	0.76
AFP >100 ng/ml, *n* (%)	23 (17)	24 (17)	0.85
DCP >100 mAU/ml, *n* (%)	16 (12)	22 (16)	0.28
AFP-L3 >15%, *n* (%)	16 (12)	15 (11)	0.86

AFP, α-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of AFP; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CTHA/CTAP, computed tomography during hepatic arteriography and arterial portography; DCP, des-γ-carboxy prothrombin; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; RFA, radiofrequency ablation, TACE, transarterial chemoembolization.

[a]Data are expressed as median (25th–75th percentiles) or number (percent).

[b]Including overlap.

any treatment and were lost to follow-up. Finally, 139 (99.3%) patients in the CTHA/CTAP group and 138 (98.6%) patients in the control group were included in the analysis.

Patient characteristics

There was no statistically significant difference in patient characteristics between the groups (**Table 1**). Median age at enrollment was 70 years, and approximately two-thirds of patients were male. Approximately 55% of patients were treatment-naive cases and the remaining patients had a history of previous treatment. Among those previously treated patients, the median interval between the initial treatment and the study enrollment was 42 (interquartile range, 22–65) months in the CTHA/CTAP group and 30 (20–61) months in the control group. There was no statistically significant difference between the two groups ($P=0.72$). The total number of HCC nodules detected in original contrast-enhanced dynamic CT was 197 (101 patients were uninodular and the rest were multinodular) in the CTHA/CTAP group and 196 (98 patients were uninodular and the rest were multinodular) in the control group.

Recurrence

By the end of the follow-up, tumor recurrence was identified in 109 patients (78.4%) in the CTHA/CTAP group and 112 patients (81.2%) in the control group. The distribution of recurrent site was intrahepatic distant recurrence ($N=98$), local tumor progression ($N=7$), both ($N=1$), and extrahepatic metastasis ($N=3$) in the CTHA/CTAP group and intrahepatic distant recurrence ($N=103$), local tumor progression ($N=4$), both ($N=2$), and extrahepatic metastasis ($N=3$) in the control group. Five patients (3.6%) in the CTHA/CTAP group and 1 patient (0.7%) in the control group in whom complete ablation could not be obtained by RFA were treated as recurrence on 120 days after randomization when the first follow-up CT would have been scheduled. In each group, four patients died without recurrence. The cumulative recurrence-free survival rates at 1, 2, and 3 years were 60.1, 29.0, and 18.9% in the CTHA/CTAP group and 52.2, 29.7, and 23.1% in the control group, respectively (**Figure 2a**). The difference between the two groups was not statistically significant ($P=0.66$ by log-rank test; hazard ratio, 0.94 for CTHA/CTAP vs. control; 95% CI, 0.73–1.22). The CTHA/CTAP group showed better recurrence-free survival with marginal statistical significance in the subgroups with higher AFP or AFP-L3 values (**Figure 3**).

Univariate Cox regression analysis identified older age ($P=0.01$), hepatitis C virus antibody positivity ($P=0.001$), lower albumin level ($P=0.04$), recurrent cases ($P<0.001$), multinodular HCC ($P<0.001$), and higher AFP level ($P=0.02$) as significant predictors for recurrence-free survival (**Table 2**). Adjusted hazard ratio of the CTHA/CTAP group vs. the control group by multivariate Cox regression analysis was 0.86 (95% CI, 0.67–1.12; $P=0.27$, **Table 3**).

Overall survival

By the end of the follow-up, 51 patients (36.7%) in the CTHA/CTAP group and 45 patients (32.6%) in the control group died. The cumulative overall survival rates at 3 and 5 years were 79.7 and 56.4% in the CTHA/CTAP group and 86.8 and 60.1% in the control group, respectively (**Figure 2b**). There was no statistically significant difference between the groups ($P=0.50$ by log-rank test; hazard ratio, 1.15, 95% CI, 0.77–1.71).

Safety

No procedural complications attributable to CTHA/CTAP or TACE were observed. Major complications related to RFA were observed in 2 patients (1.4%) in the CTHA/CTAP group (2 with neoplastic seeding) and in 3 patients (2.2%) in the control group (1 each with hepatic infarction, hemothorax, and neoplastic seeding). There was no procedure-related death.

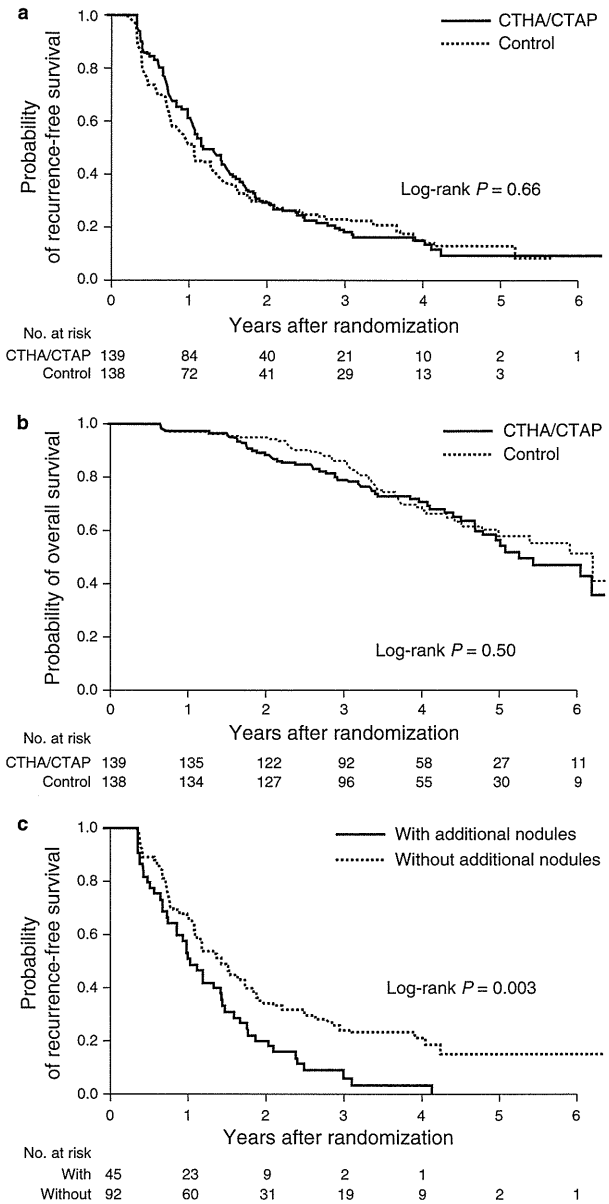

Figure 2. Kaplan–Meier estimate of the recurrence-free survival and overall survival. (**a**) The cumulative recurrence-free survival rates at 1, 2, and 3 years were 60.1, 29.0, and 18.9% in the CTHA/CTAP group and 52.2, 29.7, and 23.1% in the control group, respectively. (**b**) The cumulative overall survival rates at 3 and 5 years were 79.7 and 56.4% in CTHA/CTAP group and 86.8 and 60.1% in the control group, respectively. (**c**) Patients with an additional nodule detected by CTHA/CTAP showed significantly poorer recurrence-free survival than those without an additional nodule. CTAP, computed tomography during arterial portography; CTHA, computed tomography during hepatic arteriography.

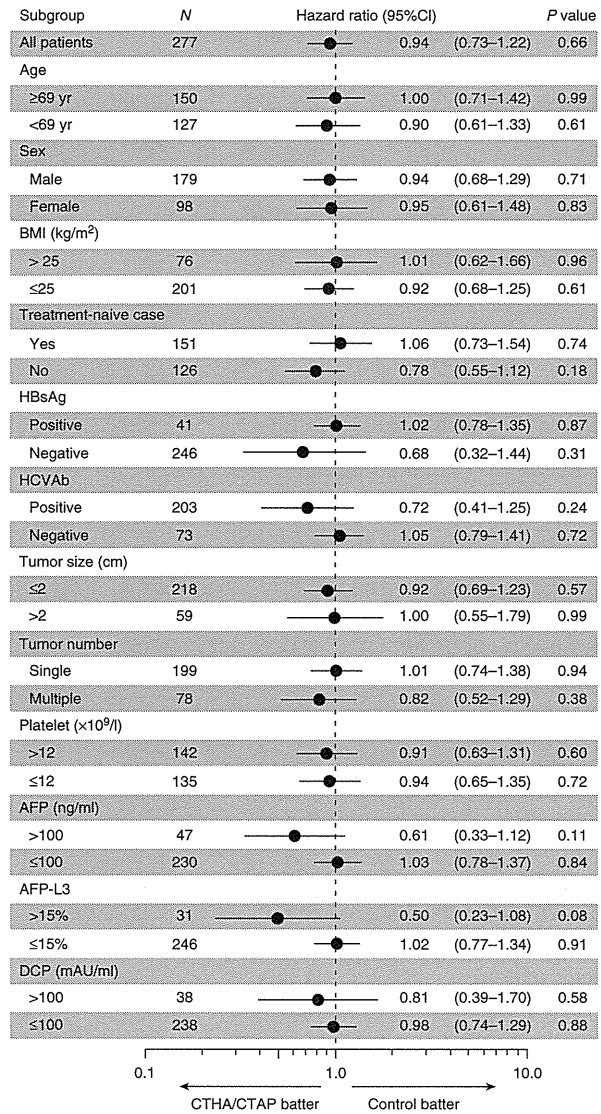

Figure 3. Recurrence-free survival of subgroups by Cox proportional hazard regression according to clinical characteristics at study entry. AFP, α-fetoprotein; BMI, body mass index; CI, confidence interval; CT, computed tomography; CTAP, computed tomography during arterial portography; CTHA, computed tomography during hepatic arteriography; DCP, des-γ-carboxy prothrombin; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; yr, year.

Recurrence-free survival between those with and without additional nodules in CTHA/CTAP group

As a *post hoc* analysis, we compared the recurrence-free survival between those with ($N=45$) and without ($N=92$) additional HCC nodules diagnosed by CTHA/CTAP. As compared with those in whom additional HCC nodules were not detected by CTHA/CTAP, those with additional nodules included more HBsAg-negative patients (97.7 vs. 78.3%, $P=0.002$), previously treated patients (62.2 vs. 23.9%, $P=0.006$), and patients with multiple HCC nodules on dynamic CT (44.4 vs. 17.4%, $P=0.002$). Patients with additional nodule by CTHA/CTAP showed significantly poorer

Table 2. Univariate Cox's proportional hazard regression analysis of the risk for recurrence-free survival

Variable	Hazard ratio (95% CI)	*P* value
CTHA/CTAP vs. control	0.94 (0.73–1.22)	0.66
Age (per year)	1.02 (1.00–1.04)	0.01
Female vs. male	1.02 (0.78–1.34)	0.88
Alcohol >80 g/day	1.02 (0.88–1.17)	0.81
HCVAb positive	1.69 (1.23–2.31)	0.001
BMI (per 1.0 kg/m^2)	1.02 (0.98–1.06)	0.35
Albumin (per 1.0 g/dl)	0.72 (0.52–0.98)	0.04
Total bilirubin (per 1.0 mg/dl)	1.02 (0.97–1.07)	0.51
AST >40 IU/l	1.14 (0.99–1.31)	0.07
ALT >40 IU/l	1.05 (0.92–1.20)	0.45
Platelet count >10×10^3/μl	0.89 (0.78–1.01)	0.08
Recurrent case	2.33 (1.79–3.02)	<0.001
Tumor size of maximal nodule >2.0 cm	0.97 (0.85–1.10)	0.62
Multinodular	1.38 (1.20–1.59)	<0.001
AFP >100 ng/ml	1.21 (1.03–1.43)	0.02
DCP >100 mAU/ml	0.99 (0.82–1.20)	0.93
AFP-L3 >15%	1.20 (0.99–1.46)	0.07

AFP, α-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of AFP; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; CTHA/CTAP, computed tomography during hepatic arteriography and arterial portography; DCP, des-γ-carboxy prothrombin; HCVAb, hepatitis C virus antibody.

Table 3. Multivariate Cox's proportional hazard regression analysis of the risk for recurrence-free survival

Variable	Hazard ratio (95% CI)	*P* value
CTHA/CTAP vs. control	0.86 (0.0.67–1.12)	0.27
Age (per year).	1.01 (0.99–1.02)	0.36
HCVAb positive	1.36 (0.98–1.89)	0.07
Albumin (per 1.0 g/dl)	0.75 (0.53–1.07)	0.11
Recurrent case	2.21 (1.69–2.89)	<0.001
Multinodular	1.69 (1.27–2.25)	<0.001
AFP >100 ng/ml	1.41 (0.996–1.98)	0.052

AFP, α-fetoprotein; CI, confidence interval; CTHA/CTAP, computed tomography during hepatic arteriography and arterial portography; HCVAb, hepatitis C virus antibody.

recurrence-free survival than those without additional nodules ($P=0.003$, **Figure 2c**).

DISCUSSION

An advance in diagnostic technology generally indicates improved sensitivity or specificity, which corresponds to the detection of smaller lesions with a clearer view in imaging modalities. In our previous study, we showed that 75 nodules with a mean diameter of 8.7 mm (range, 2–20 mm) in 45 (33%) of 139 patients who underwent CTHA/CTAP were additionally diagnosed as definite HCC, compared with dynamic CT examination (33). However, no significant difference was observed in terms of recurrence-free survival between those who did and did not undergo CTHA/CTAP before RFA.

One reason for this discrepancy may be that the impact of CTHA/CTAP on recurrence reduction was diluted by a long-term follow-up of >2 years. It is unlikely that CTHA/CTAP could detect small nodules that would be detected ≥2 years later by conventional dynamic CT. In fact, the number of recurrences identified within 1 year after enrollment was lower in the CTHA/CTAP group than the control group (54 vs. 65, data not shown).

Another reason could be that fewer patients achieved complete ablation of target nodules in the CTHA/CTAP group than in the control group. The additionally diagnosed HCC nodules were small, and detection of these nodules by ultrasonography was difficult. Recent technologies such as contrast ultrasonography or fusion imaging, which can improve the accuracy of ablation techniques (34–36), may increase the probability of detection of smaller nodules before RFA.

Precise evaluation of the stage of progression is important for deciding on treatment procedures in HCC management. Seventeen patients in the CTHA/CTAP group were diagnosed with ≥4 nodules by CTHA/CTAP, which is not considered suitable for RFA according to widely used criteria.

In our previous study, we showed that recurrence as opposed to initial occurrence, multinodularity on dynamic CT, and HBsAg negativity were significant predictors for finding additional HCC by CTHA/CTAP (33). In fact, the CTHA/CTAP group showed better outcomes in the subgroups with HBsAg-negative cases, previously treated patients, and multinodular HCC. However, *post hoc* analysis comparing recurrence-free survival of those with and without additional nodules detected by CTHA/CTAP showed that those with a higher probability of additional nodules were also at a higher risk of recurrence. The advantage of CTHA/CTAP in finding more HCC nodules might be counter balanced by the higher risk of recurrence.

This study has several limitations. First, the additional nodules detected by CTHA/CTAP were not confirmed histologically. Therefore, we cannot exclude the possibility of overdiagnosis. Second, 45% of the patients had a history of previous treatment including resection, RFA, and TACE. Those previous treatments might substantially alter the hemodynamic status in the liver and affect the accuracy of CTHA/CTAP. On the other hand, in the previously treated cases, the radiologists could refer to the past series of dynamic CT during performing CTHA/CTAP, which might improve the accuracy of CTHA/CTAP as compared with treatment-naive cases. Third, 17 patients in the CTHA/CTAP group underwent TACE as a salvage treatment because total number of HCC nodules exceeded 3 after CTHA/CTAP. This might affect the recurrence-free and overall survival in the CTHA/CTAP group.

Our results may be extrapolated to other imaging modalities including gadoxetic acid–enhanced magnetic resonance imaging and second-generation contrast ultrasonography (37,38). These newly developed modalities also make possible the detection of small nodules that are invisible by dynamic CT. However, better diagnosis does not necessarily lead to better primary outcome.

In conclusion, CTHA/CTAP before RFA resulted in improved HCC diagnosis and detection of additional nodules in one-third of the study participants. However, it did not improve recurrence-free survival. The indications for CTHA/CTAP should be evaluated carefully.

Study protocol URL: https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&recptno=R000000117&type=summary&language=E.

CONFLICT OF INTEREST

Guarantor of the article: Ryosuke Tateishi, MD, PhD.

Specific author contributions: Conception and design: R.T., M.A., N.Y., T.G., S.S., H.Y., Y.M., and M.O.; analysis: R.T. and Y.M.; treatment and data collection: T.O., R.T., M.A., S.M., M.S., K.U., T.A., K.E., Y.K., T.G., and S.S.; drafting article: T.O.; critical revision: R.T., M.A., H.Y., K.O., and K.K.

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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Computed tomography with hepatic arteriography and arterial portography (CTHA/CTAP) give higher hepatocellular carcinoma (HCC) detection sensitivity than conventional dynamic enhanced CT.
- ✓ CTHA/CTAP is an invasive procedure requiring the insertion of an intraarterial catheter through a femoral puncture.
- ✓ The indication for CTHA/CTAP can be justified only when the expected benefits exceed the risks and cost of the procedure.

WHAT IS NEW HERE

- ✓ Our study is the first randomized controlled trial (RCT) to evaluate the utility of CTHA/CTAP before radiofrequency ablation (RFA) in patients with HCC in the whole world.
- ✓ The best candidates for CTHA/CTAP were patients with multinodular HCC, and recurrent cases after previous treatment.
- ✓ However, CTHA/CTAP before RFA did not improve cumulative recurrence-free survival or overall survival.
- ✓ These observations are clinically important as the technique had limited utility and highlights the observation that patient outcomes are probably not related to the presence of small liver nodules.
- ✓ These findings reinforce the notion of genetic determinants of HCC recurrence.

REFERENCES

1. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745–50.
2. Kiyosawa K, Umemura T, Ichijo T *et al.* Hepatocellular carcinoma: recent trends in Japan. Gastroenterology 2004;127:S17–26.
3. Itai Y, Matsui O. Blood flow and liver imaging. Radiology 1997;202:306–14.
4. Matsui O. Detection and characterization of hepatocellular carcinoma by imaging. Clin Gastroenterol Hepatol 2005;3:S136–40.
5. Teratani T, Yoshida H, Shiina S *et al.* A novel display of reconstruction computed tomography for the detection of small hepatocellular carcinoma. Liver Int 2004;24:619–24.
6. Fujishima T, Yoshida H, Obi S *et al.* Analysis of factors influencing hepatocellular carcinoma detection: efficient use of computed tomography during arterial portography and during hepatic arteriography. J Gastroenterol 2005;40:266–73.
7. Sangiovanni A, Manini MA, Iavarone M *et al.* The diagnostic and economic impact of contrast imaging techniques in the diagnosis of small hepatocellular carcinoma in cirrhosis. Gut 2010;59:638–44.
8. Murakami T, Oi H, Hori M *et al.* Helical CT during arterial portography and hepatic arteriography for detecting hypervascular hepatocellular carcinoma. AJR Am J Roentgenol 1997;169:131–5.
9. Kanematsu M, Hoshi H, Imaeda T *et al.* Detection and characterization of hepatic tumors: value of combined helical CT hepatic arteriography and CT during arterial portography. AJR Am J Roentgenol 1997;168:1193–8.
10. Irie T, Takeshita K, Wada Y *et al.* CT evaluation of hepatic tumors: comparison of CT with arterial portography, CT with infusion hepatic arteriography, and simultaneous use of both techniques. AJR Am J Roentgenol 1995;164:1407–12.
11. Small WC, Mehard WB, Langmo LS *et al.* Preoperative determination of the resectability of hepatic tumors: efficacy of CT during arterial portography. AJR Am J Roentgenol 1993;161:319–22.
12. Soyer P, Levesque M, Elias D *et al.* Detection of liver metastases from colorectal cancer: comparison of intraoperative US and CT during arterial portography. Radiology 1992;183:541–4.
13. Soyer P, Levesque M, Elias D *et al.* Preoperative assessment of resectability of hepatic metastases from colonic carcinoma: CT portography vs sonography and dynamic CT. AJR Am J Roentgenol 1992;159:741–4.
14. Matsui O, Kadoya M, Suzuki M *et al.* Work in progress: dynamic sequential computed tomography during arterial portography in the detection of hepatic neoplasms. Radiology 1983;146:721–7.
15. Sherman M, Klein A. AASLD single-topic research conference on hepatocellular carcinoma: conference proceedings. Hepatology 2004;40:1465–73.
16. Tateishi R, Shiina S, Ohki T *et al.* Treatment strategy for hepatocellular carcinoma: expanding the indications for radiofrequency ablation. J Gastroenterol 2009;44 (Suppl 19): 142–6.
17. Ebara M, Ohto M, Sugiura N *et al.* Percutaneous ethanol injection for the treatment of small hepatocellular carcinoma. Study of 95 patients. J Gastroenterol Hepatol 1990;5:616–26.
18. Livraghi T, Giorgio A, Marin G *et al.* Hepatocellular carcinoma and cirrhosis in 746 patients: long-term results of percutaneous ethanol injection. Radiology 1995;197:101–8.
19. Shiina S, Tagawa K, Niwa Y *et al.* Percutaneous ethanol injection therapy for hepatocellular carcinoma: results in 146 patients. AJR Am J Roentgenol 1993;160:1023–8.
20. Seki T, Wakabayashi M, Nakagawa T *et al.* Percutaneous microwave coagulation therapy for patients with small hepatocellular carcinoma: comparison with percutaneous ethanol injection therapy. Cancer 1999;85:1694–702.
21. Rossi S, Di Stasi M, Buscarini E *et al.* Percutaneous RF interstitial thermal ablation in the treatment of hepatic cancer. AJR Am J Roentgenol 1996;167:759–68.
22. Allgaier HP, Deibert P, Zuber I *et al.* Percutaneous radiofrequency interstitial thermal ablation of small hepatocellular carcinoma. Lancet 1999;353:1676–7.
23. Livraghi T, Goldberg SN, Lazzaroni S *et al.* Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. Radiology 1999;210:655–61.
24. Curley SA, Izzo F, Ellis LM *et al.* Radiofrequency ablation of hepatocellular cancer in 110 patients with cirrhosis. Ann Surg 2000;232:381–91.
25. Shiina S, Tateishi R, Arano T *et al.* Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. Am J Gastroenterol 2012;107:569–77; quiz 578.
26. Jang HJ, Lim JH, Lee SJ *et al.* Hepatocellular carcinoma: are combined CT during arterial portography and CT hepatic arteriography in addition to

triple-phase helical CT all necessary for preoperative evaluation? Radiology 2000;215:373–80.
27. Rennie D. CONSORT revised--improving the reporting of randomized trials. JAMA 2001;285:2006–7.
28. Efron B. Forcing a sequential experiment to be balanced. Biometrika 1971;58:403.
29. Kitao A, Zen Y, Matsui O *et al.* Hepatocarcinogenesis: multistep changes of drainage vessels at CT during arterial portography and hepatic arteriography--radiologic-pathologic correlation. Radiology 2009;252:605–14.
30. Tateishi R, Shiina S, Teratani T *et al.* Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. Cancer 2005;103:1201–9.
31. Goldberg SN, Grassi CJ, Cardella JF *et al.* Image-guided tumor ablation: standardization of terminology and reporting criteria. J Vasc Interv Radiol 2009;20:S377–90.
32. Sacks D, McClenny TE, Cardella JF *et al.* Society of Interventional Radiology clinical practice guidelines. J Vasc Interv Radiol 2003;14:S199–202.
33. Ohki T, Tateishi R, Akahane M *et al.* Characteristics of hepatocellular carcinoma nodules newly detected by computed tomography during arteriography and arterial portography: preliminary report of a randomized controlled trial. Hepatol Int 2011 (e-pub ahead of print).
34. Minami Y, Kudo M. Review of dynamic contrast-enhanced ultrasound guidance in ablation therapy for hepatocellular carcinoma. World J Gastroenterol 2011;17:4952–9.
35. Numata K, Fukuda H, Morimoto M *et al.* Use of fusion imaging combining contrast-enhanced ultrasonography with a perflubutane-based contrast agent and contrast-enhanced computed tomography for the evaluation of percutaneous radiofrequency ablation of hypervascular hepatocellular carcinoma. Eur J Radiol 2012;81:2746–53.
36. Masuzaki R, Shiina S, Tateishi R *et al.* Utility of contrast-enhanced ultrasonography with Sonazoid in radiofrequency ablation for hepatocellular carcinoma. J Gastroenterol Hepatol 2011;26:759–64.
37. Ichikawa T, Saito K, Yoshioka N *et al.* Detection and characterization of focal liver lesions: a Japanese phase III, multicenter comparison between gadoxetic acid disodium-enhanced magnetic resonance imaging and contrast-enhanced computed tomography predominantly in patients with hepatocellular carcinoma and chronic liver disease. Invest Radiol 2010;45:133–41.
38. Hatanaka K, Kudo M, Minami Y *et al.* Differential diagnosis of hepatic tumors: value of contrast-enhanced harmonic sonography using the newly developed contrast agent, Sonazoid. Intervirology 2008; 51 (Suppl 1): 61–9.

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Induction of p53-Dependent p21 Limits Proliferative Activity of Rat Hepatocytes in the Presence of Hepatocyte Growth Factor

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Abstract

Background: Hepatocyte growth factor (HGF), a potent mitogen for hepatocytes, enhances hepatocyte function without stimulating proliferation, depending on the physiological conditions. p53, a transcription factor, suppresses the cell proliferation by expressing $p21^{WAF1/CIP1}$ in various tissues.

Aim: To investigate the mechanism through which the hepatocytes maintain mitotically quiescent even in the presence of HGF.

Methods: We studied the relationship between p53 and p21 expression and the effect of p53-p21 axis on hepatocyte proliferation in primary cultured rat hepatocytes stimulated by HGF. Hepatic p21 levels are determined serially after partial hepatectomy or sham operation in rats.

Results: DNA synthesis was markedly increased by HGF addition in rat hepatocytes cultured at low density but not at high density. Cellular p53 levels increased in the hepatocytes cultured at both the densities. p21 levels were increased and correlated with cellular p53 levels in hepatocytes cultured at high density but not at low density. When the activity of p53 was suppressed by a chemical inhibitor for p53, cellular p21 levels were reduced, and DNA synthesis was increased. Similarly, p21 antisense oligonucleotide increased the DNA synthesis. In rats after partial hepatectomy, transient elevation of hepatic p21 levels was observed. In contrast, in sham-operated rats, hepatic p21 levels were increased on sustained time scales.

Conclusion: p53-related induction of p21 may suppress hepatocyte proliferation in the presence of HGF in the setting that mitogenic activity of HGF is not elicitable.

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Introduction

Proliferation of hepatocytes occurs following the loss of parenchymal cells, while hepatocytes are usually mitotically quiescent. Hepatocyte growth factor (HGF), originally identified as a potent mitogen for hepatocytes in culture, has a pluripotent effect on various types of cells [1–6]. Previous reports indicate that circulating HGF levels in humans are increased with various degrees in physiological and pathological conditions such as acute hepatitis, fulminant hepatic failure, chronic hepatitis, liver cirrhosis, renal failure, post-partial hepatectomy and post-non-hepatectomized abdominal surgery [3,4,7–10]. In the liver of experimental animal models, mitogenic, anti-inflammatory, anti-apoptotic and anti-fibrogenetic activities of HGF have been observed [3,7]. In primary cultured hepatocytes, HGF addition has been shown to facilitate proliferation or function of the cells depending on the culture condition [2,11]. The mechanism is still under investigation if the specific activities of HGF are selectively expressed. Previously, we reported that HGF exerted mitogenic activity on hepatocytes through the induction of p53, a transcription factor, which increased production of transforming growth factor α (TGF-α), a complete mitogen for hepatocytes [12–14]. However, the mechanism is unknown through which hepatocytes maintain mitotically quiescent when HGF exerts other activities.

Though recent several reports including ours indicate that p53 can stimulate cell proliferation by the specific induction of promoters for growth-associated factors such as TGF-α, p53 is generally recognized as a 'tumor suppressor gene', because, in some pathophysiological conditions, it up-regulates p21, which arrests cell cycle at G1 phase, and inhibits cell proliferation both in vitro and in vivo [14–17]. While p21 expression can be induced by growth-inhibitory stimuli such as DNA damage [18–22], recent

reports indicate the possibility that addition of some growth factors induces p21, and suppresses DNA synthesis especially in malignant cell lines[18,23–28]. However, relationship between p53 and p21 and its significance in non-malignant cells including hepatocytes in the presence of growth factors is still under investigation.

In this paper, we showed that p21 was up-regulated by HGF stimulation through the induction of p53, and suppressed hepatocyte proliferation in the setting that mitogenic activity was not elicitable.

Materials and Methods

Assay for p53 of cultured hepatocytes

Hepatocyte extracts were prepared according to the protocol from the manufacturer of the p53 enzyme-linked immunosorbent assay (ELISA) kit (Roche Molecular Biochemicals, Germany) [14].

Preparation of liver and cultured hepatocytes for p21 assay

Liver tissues and cultured hepatocytes were homogenized in the low salt resuspension buffer (pH 7.4, 50 mmol/L tris (hydroxymethyl) aminomethane, 5 mmol/L ethylenediaminetetraacetic acid, 0.2 mmol/L phenylmethylsulfonyl fluoride 1 μg/mL pepstatin and 0.5 μg/mL leupeptin). The suspensions were incubated with p21 antigen extraction agent (1.0 M potassium chloride, 6% zwittergent (Calbiochem, CA)), and centrifuged. The resultant supernatants were applied to ELISA described below.

Assay for p21 in liver extracts and cultured hepatocytes

The sandwich ELISA for p21 was developed using polyclonal anti p21 IgG (Santa Cruz, CA) and monoclonal p21 antibody (Santa Cruz, CA) as capture and detector antibodies, respectively. Horseradish peroxidase conjugated goat anti-mouse IgG (Zymed, CA) was used to detect the antibody-p21 complex.

The standard curve for p21 (1–164, full length amino acids, Santa Cruz Biotechnology, CA) of this assay made with the buffer showed the lower limit at 1.25 ng/mL. When the sample of rat liver prepared for p21 assay was diluted with the buffer, the dilution curve was similar to the standard curve. When p21 protein was diluted with sample of rat liver or hepatocytes prepared for p21 assay, the dilution curve was similar to the standard curve.

Determination of 5-bromo-2′-deoxy-uridine (BrdU) incorporation and total protein content of cultured hepatocytes

Incorporated BrdU was determined by ELISA, using BrdU labeling and the detection kit III (Roche Molecular Biochemicals, Germany). The total cellular protein was measured by Bradford's method [29].

Experiments with cultured hepatocytes

Hepatocytes were isolated from rat livers according to Seglen's method [30]. The isolated cells were cultured at densities of either 1.2×10^5 cells/cm^2 (high density culture) or 2.5×10^4 cells/cm^2 (low density culture) in the medium and incubated for 27 hours as we previously reported [14]. The medium was changed to Williams' medium E (WE) containing 10% fetal calf serum (FCS), various concentrations of HGF and 0.1 mmol/L BrdU. The cells were harvested serially for the determination of both the cellular p53 and p21 levels and, the BrdU incorporation into cellular DNA.

To study the effect of inhibition of p53 function on p21 levels and DNA synthesis in hepatocytes, the hepatocytes were cultured in WE containing 10% FCS, with or without 10 ng/mL HGF, 1 mmol/L BrdU and various concentrations of pifithrin-α (Alexis Biochemicals, CA) dissolved in dimethyl sulfoxide (DMSO) or the same concentrations of DMSO [31]. The cells were harvested 18 hours later to determine the cellular p21 levels and 24 hours later to determine the BrdU incorporation into cellular DNA.

To examine the effect of inhibition of p21 production on hepatocyte DNA synthesis, the hepatocytes were cultured in WE containing 10% FCS, 10 ng/mL HGF, 1 mmol/L BrdU and various concentrations of either p21 antisense oligonucleotide (5′-GACATCACCAGGATCGGACAT-3′), complementary to position 85–105 of rat p21 mRNA, or nonsense oligonucleotide (5′-GCAACGCTACTACGCAAGTAG-3′), containing the same numbers of G, C, A, and T as the p21 antisense oligonucleotide [32]. The cells were harvested as above, to determine the cellular p21 levels and the BrdU incorporation into cellular DNA.

Animal experiments

Five to six-weeks-old Male Sprague-Dawley rats (Japan SLC, Japan) were subjected to either of two-thirds partial hepatectomy (PH) or sham operation under diethyl ether anesthesia. In sham-operated rats, the abdomen was cut open under similar anesthesia, and the liver was briefly exposed outside the peritoneal cavity. The rats were serially anesthetized with diethyl ether. The liver was perfused through the portal vein with saline. After a near total exsanguination, the liver was excised and used for the p21 assays.

All animal study protocols conformed to and approved by the guideline of the Faculty of Medicine, University of Tokyo for humane care.

Statistical analyses

The differences between two unpaired samples were defined as significant when the p-values by both the Student's *t*-test and the Mann-Whitney *U* test were less than 0.05. The dose related effects were tested by one-way analysis of variance followed by Spearman's correlation test.

Results

Changes in DNA synthesis of cultured rat hepatocytes after HGF treatment

We determined the effect of cell density on DNA synthesis of cultured hepatocytes simulated by HGF. The addition of 10 ng/mL HGF to the medium caused only minor increase on DNA synthesis in hepatocytes cultured at high density. In hepatocytes cultured at low density, DNA synthesis increased after 12 hours of incubation, peaked at 24–30 hours and decreased thereafter by HGF addition (Figure 1). DNA synthesis was not induced significantly in high density cultured hepatocytes by HGF treatment.

Cellular p53 and p21 levels in cultured rat hepatocytes treated with HGF

To investigate the effect of HGF on p53 and p21 expressions and their relationship in proliferating and non-proliferating rat hepatocytes, we determined p53 and p21 protein levels in cultured hepatocytes at low and high density in the presence of various concentration of HGF.

As shown in Figure 2, when rat hepatocytes were cultured at high density with HGF, p53 levels increased at 10 ng/mL or 20 ng/mL of HGF addition ($F = 32.5$, $p<0.01$; $r = 0.84$, $p<0.01$).

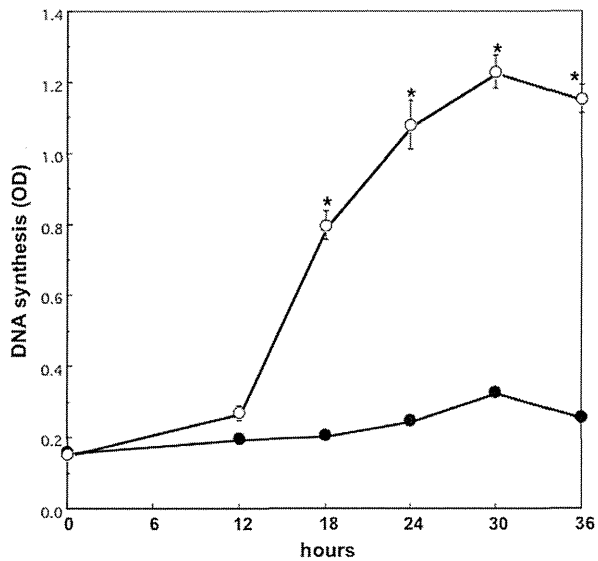

Figure 1. Changes in DNA synthesis of hepatocytes after HGF treatment. Rat hepatocytes were cultured in WE containing 10% FCS, 10 ng/mL HGF and 0.1 mmol/L BrdU, and were harvested serially. Closed circles denote hepatocytes cultured at high density. Open circles denote hepatocytes cultured at low density. Data are mean ± SEM of eight dishes. *p<0.01 compared with the values cultured for 0 hours.
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In hepatocytes cultured at low density, HGF addition also increased cellular p53 levels significantly in a dose-related manner up to 10 ng/mL of HGF ($F = 23.4$, $p<0.01$; $r = 0.90$, $p<0.01$) (Figure 2).

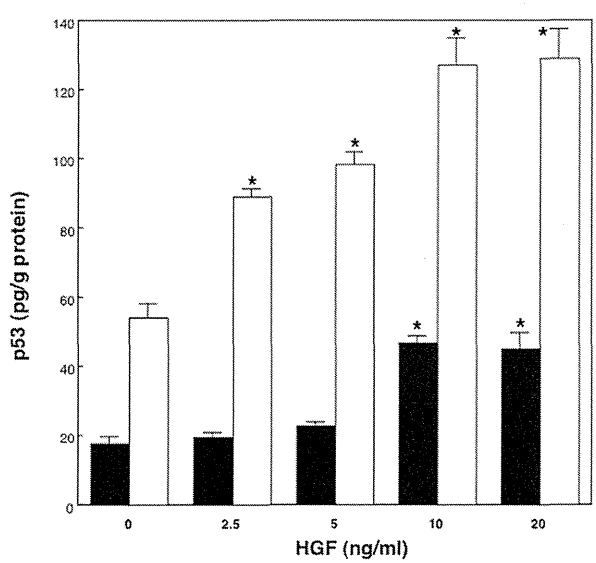

Figure 2. Cellular p53 levels in hepatocytes treated with HGF. Rat hepatocytes were cultured in WE containing 10% FCS and various concentration of HGF for 18 hours. Closed bars denote hepatocytes cultured at high density. Open bars denote hepatocytes cultured at low density. Data are mean ± SEM of four dishes. *p<0.01 compared with the values in the absence of HGF.
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The levels of p53 in high density cultured hepatocytes treated with 10 ng/mL HGF increased after 6 hours and reached a maximum at 24 hours (data not shown), while, in low density cultured hepatocytes treated with 10 ng/mL HGF, p53 levels significantly increased from 6 hours and peaked at 18 to 24 hours of incubation, similar to our previous report [14].

The levels of p21 protein in high density cultured hepatocytes treated with 10 ng/mL HGF increased in a time dependent manner (Figure 3). When hepatocytes cultured at high density were treated with HGF at increasing concentrations, p21 levels at 18 hours after HGF addition increased in a dose-related manner ($F = 73.0$, $p<0.01$; $r = 0.88$, $p<0.01$), and correlated with the cellular p53 levels ($r = 0.69$; $p<0.01$) (Figure 4 and 5A). p21 levels in low density cultured hepatocytes were not increased by HGF addition, nor correlated with p53 levels (Figure 3, 4 and 5B).

p21 levels were increased in a dose related manner by HGF and correlated with p53 levels at high density cultured hepatocytes, while, at low density cultured hepatocytes, p53, but not p21, levels were increased by HGF and there was no correlation between p53 and p21 levels.

The effect of pifithrin-α on p21 levels and BrdU incorporation in rat hepatocytes cultured at high and low density in the presence or absence of HGF

To elucidate the relationship between p53 expression and p21 expression as well as DNA synthesis in hepatocytes at non-proliferating condition even in the presence of HGF, we determined the effect of pifithrin-α, a chemical inhibitor of p53, on p21 levels and BrdU incorporation of hepatocytes cultured at high density in the presence of HGF.

The levels of p21 treated with 10 ng/mL HGF for 18 hours in hepatocytes at high density were reduced by the addition of

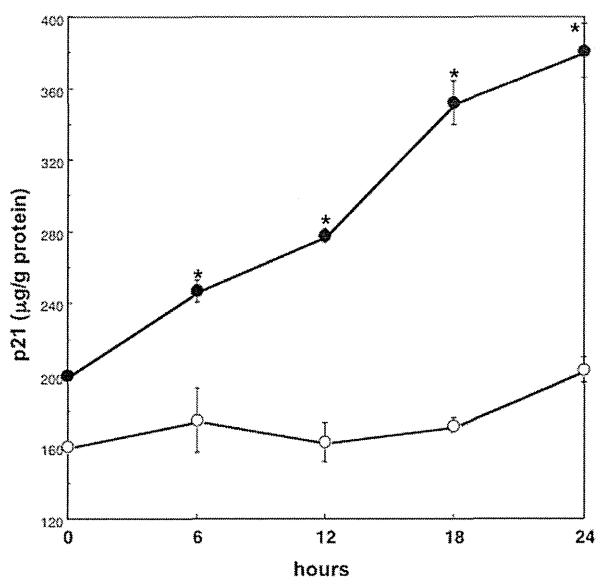

Figure 3. Serial changes in p21 protein levels of hepatocytes treated with HGF. Rat hepatocytes were cultured at high density in WE containing 10% FCS and 10 ng/mL HGF, and were harvested serially. Closed circles denote hepatocytes cultured at high density. Open circles denote hepatocytes cultured at low density. Data are mean ± SEM of four dishes. *p<0.01 compared with the values cultured for 0 hours.
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