Table 1 Basic characteristics

Age (yr)	67.0 (60.0-73.0)[1]
Gender (male/female)	647/271
HBsAg (-/+/ND)	582/196/140
anti-HCV (-/+/ND)	278/534/106
AIH/PBC/alcohol/NASH/BCS	10/6/85/16/2
Child-Pugh (A/B/C/ND)	704/172/36/6
Size (mm)	28.0 (19.0-45.0)[1]
Stage (Ⅰ/Ⅱ/Ⅲ/Ⅳ)[2]	163/306/287/162
Therapy (Loco/IVR/Cx/others/BSC)	459/332/78/11/38

[1]Median and interquartile range; [2]General rules for the clinical and pathological study of primary liver cancer from Liver Cancer Study Group of Japan. ND: Not determined; AIH: Autoimmune hepatitis; PBC: Primary biliary cirrhosis; NASH: Nonalcoholic steatohepatitis; BCS: Budd-Chiari syndrome; Loco: Logo-regional treatments, including resection, radiofrequency ablation, microwave coagulation and percutaneous ethanol injection; IVR: Interventional radiology including transcatheter arterial chemoembolization, and transarterial oily chemoembolization; Cx: Chemotherapy including hepatic arterial infusion chemotherapy, systemic chemotherapy and molecular targeting therapy; Others: Other therapies including stereotactic body radiation, proton beam and liver transplantation; BSC: Best supportive care; HBsAg: Hepatitis B surface antigen; HCV: Hepatitis C virus.

ter TACE or TACE following HAIC, with a clinical goal of striking the best balance between functional hepatic reserve and the volume of the targeted area. Stereotactic radiotherapy was considered when loco-regional treatments were indicated but not applicable, whereas liver transplantation was selected by an exclusive decision process. Treatments were classified into four groups: (1) loco-regional including resection, RFA, MWC and PEI; (2) interventional radiology (IVR) including TACE and TOCE; (3) chemotherapy (Cx) including HAIC and systemic chemotherapy; and (4) other, including stereotactic radiotherapy, proton beam and liver transplantation, which were applied to only 11 patients in total. If pleural treatments were added as an adjunct, the case was classified into a group according to the applied treatment with the highest regional control capability.

Measuring hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (HCV), anti-mitochondrial, anti-M2 and anti-nuclear antibodies serologically defined background liver diseases. A habitual daily alcohol intake of more than 60 g was considered alcohol abuse. Nonalcoholic steatohepatitis was diagnosed on the basis of histological findings, whereas Budd-Chiari syndrome was diagnosed angiographically. Patients who were negative for all of the above criteria were considered not definitive for a background liver disease. The institutional review board of our institution, which did not require informed consent for a retrospective study using medical records or imaging examinations, approved the present study, which conformed to the ethical guidelines of the 2008 Declaration of Helsinki.

Serum biochemistry and histological examination

HBsAg and anti-HCV antibodies were detected by a chemiluminescence immunoassay using the ARCHITECT HBsAg QT and ARCHITECT HCV kits (Abbott Japan Co. Ltd., Chiba, Japan), respectively. Serum anti-mitochondrial and anti-M2 antibodies were quantified using the commercial kits AMA FluoroAID-1 and Mesacup mitochondria M2 (MBL Co. Ltd., Nagoya, Japan), respectively. Total and *Lens culinaris* agglutinin A-reactive α-fetoprotein (AFP) serum concentrations were quantified with a liquid-phase binding assay system (LiBASys; Wako Pure Chemical Indus-tries Ltd., Osaka, Japan). L3 was calculated as a percentage of *Lens culinaris* agglutinin A-reactive species against total AFP. Serum des-γ-carboxy prothrombin (DCP) was measured using an electrochemiluminescence immunoassay (Wako Pure Chemical Industries Ltd, Osaka, Japan). Other blood biochemistries were routinely measured in the clinical laboratories of our hospital.

Two expert histologists independently rendered histological diagnoses based on microscopic observations of tissues stained with hematoxylin and eosin, silver, iron, periodic acid-Schiff, periodic acid-Schiff with diastase digestion, and azan. When there was any discordance between the two histologists, the specimen was reviewed to reach a consensus diagnosis.

Life expectancy and percent life expectancy

The Japanese life expectancy per year for each gender at a specific age is available for 1996 onwards and was downloaded from the Ministry of Health, Labour and Welfare[1]. The life expectancy for our cohort was plotted in three dimensions using O-Chart Standard software (ONO SOKKI Co., Ltd., Yokohama, Japan). The survival time for each case was divided by the life expectancy to obtain the percent life expectancy (%LE).

Statistical analysis

Patient ages were compared using the Kruskal-Wallis test, and Dunn's multiple comparison tests were used to compare the different periods in 5-year intervals. The influence of multiple factors on survival was evaluated using Cox regression analysis. The comparisons of categorical data were performed with the Fisher's exact test or the χ^2 test among three or two different age groups, respectively. Overall survival was demonstrated by calculating Kaplan-Meier survival fractions. All analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, United States), except for the multivariate analysis, which was performed using PASW statics 17.0 (SPSS Inc., Chicago, United States). A two-tailed *P* value less than 0.05 was considered statistically significant after Bonferroni correction.

RESULTS

Patients receiving active treatments for HCC are continuously aging in Japan

The median ages for each 5-year interval steadily increased from 61 (interquartile range: 55-66) years of age (from 1986 to 1990) to 71 (63-76) years of age (from 2006 to 2010), as shown in Figure 1A. The median age was sig-

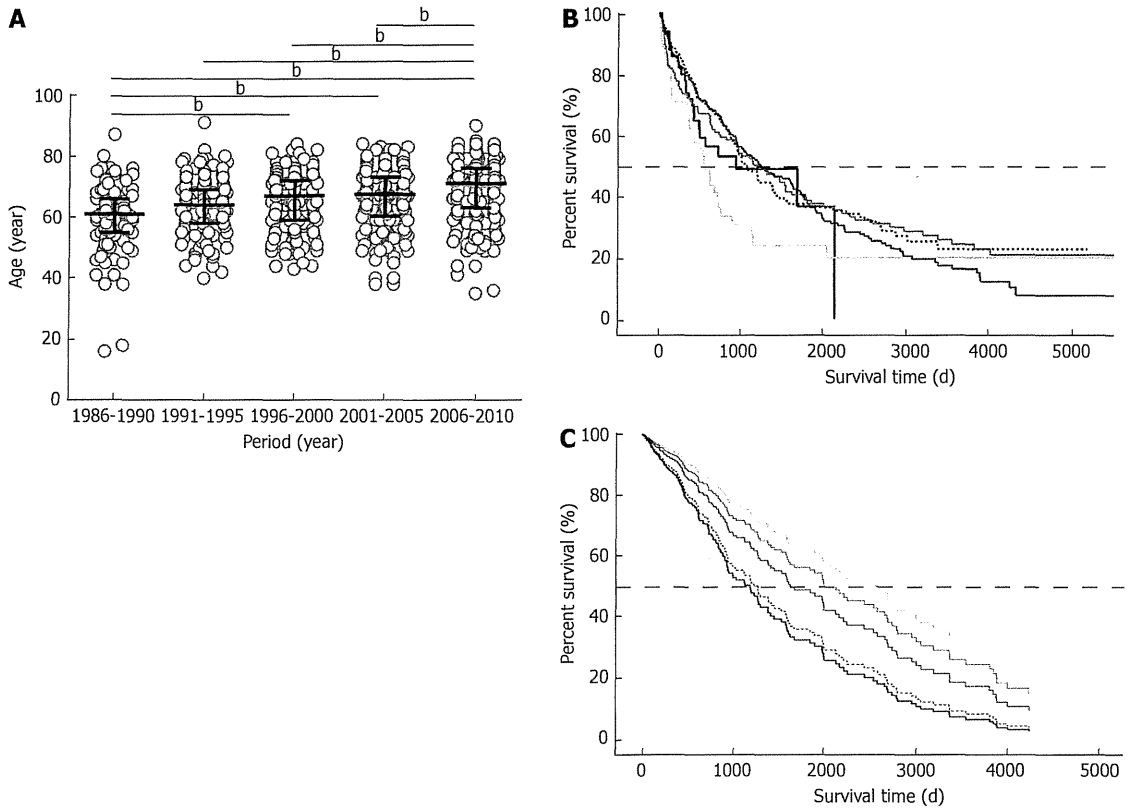

Figure 1 Age distribution in different periods and the survival of patients with hepatocellular carcinoma. A: The age of patients who were admitted for the management of hepatocellular carcinoma was plotted for each 5-year interval since 1986: the median ages were significantly different among the different periods ($P < 0.0001$); B: The overall survival of 740 patients in five age groups who have already died or have been followed for longer than 1 year was calculated on the basis of Kaplan-Meier survival fractions: the median survival time of all cases was 1094 d; C: Overall survival was compared among the different age groups after compensation for background characteristics using a Cox proportional hazard model and was significantly different among age groups ($P = 0.020$). The solid black and dotted lines are the survival curves of the 80 years of age or older and 70-79 years of age groups, respectively. The other lines are 60-69 years of age, 50-59 years of age and 49 years of age or younger groups, indicated in colors ranging from dark to pale. $^bP < 0.01$. The horizontal bars (A) indicate the median and interquartile range. The dotted horizontal lines (B and C) indicate a position of 50% survival.

nificantly different among the periods ($P < 0.0001$), and the median age of these patients increased by 10 years in the last 20 years. The patients admitted from 2006 to 2010 were significantly older than the patients who were hospitalized during any other periods ($P < 0.001$ *vs* 1986-1990, 1991-1995, 1996-2000; and $P < 0.01$ *vs* 2001-2005).

Aging is a significant factor affecting overall survival time in HCC

The median survival time for 740 patients who were deceased or were followed longer than 1 year was calculated from the Kaplan-Meier survival fractions as 1094 d. When the patients' ages were categorized into the five groups to test the dependence of survival on age, there was no significant survival difference among the groups ($P = 0.41$), and the least median survival time was 553 d in the -49 group, as shown in Figure 1B. For the Cox regression analysis, 379 cases were included because they were not missing values for the 10 explanatory variables: (1) age (years); (2) gender (male/female): (3) HBsAg (-/+); (4) anti-HCV (-/+);(5) AFP ($\log_{10}$); (6) L3 (%); (7) DCP ($\log_{10}$); (8) Child-Pugh class (A/B/C); (9) HCC stage (I / II / III / IV); and (10) therapy (loco-regional/IVR/Cx/other). Among these 10 variables, age, AFP, DCP, HBsAg, anti-HCV, Child-Pugh class, HCC stage and therapy were determined to be significant factors that influenced survival time (Table 2). The impact of age on survival time was found to be significant ($P = 0.020$), with a hazard ratio of 1.021, suggesting that a 10-year-older patient has a 1.23-fold higher risk of death. When the survival differences among the five age groups were estimated with the Cox proportional hazards model on the basis of the above 10 explanatory factors, overall survival was poorer with age and was the worst in the 80+ group, as shown in Figure 1C. The risk of death in the 80+ group was 2.41-times higher when compared with that of the -49 group.

Fractional life expectancy is an indicator of survival benefit adjusted for age

It may be reasonable to assume that older patients will have shorter survival times irrespective of effective treatments, preserved functional hepatic reserve, or other factors, simply because of their shorter residual length of life. To compare the survival from the point of aging, survival was normalized by life expectancy. A ratio

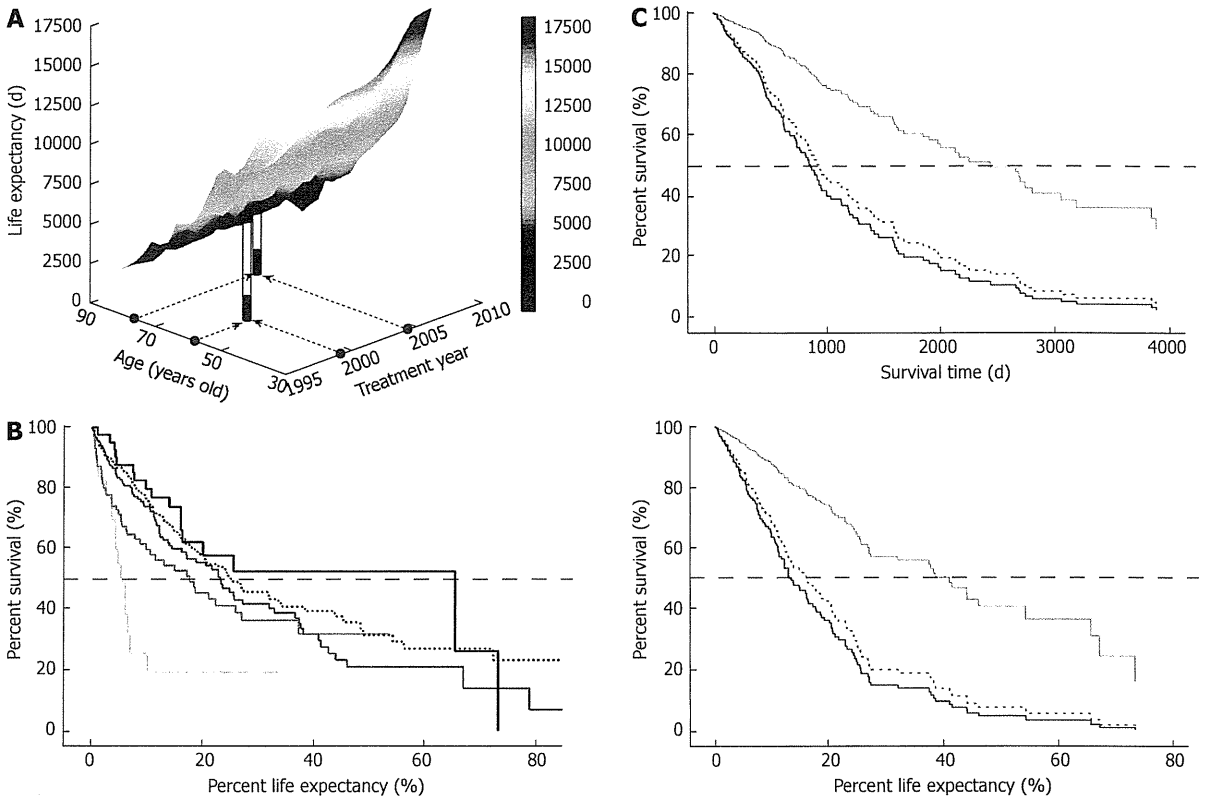

Figure 2 Life expectancy and percent life expectancy of patients with hepatocellular carcinoma. A: Life expectancy (LE) for each case was plotted in a three-dimensional space. The percent LE (%LE) was defined as the ratio between survival time and LE and is shown for representative cases. The LE of a male at 59 years of age in the year 1999 is 7928 d, whereas the LE is 3760 d for a 77-year-old male in the year 2004 (white piles). Both patients survived for 1779 d, as indicated by the black piles, with %LE values of 22.4% and 48.6%, respectively; B: A survival proportion was expressed in %LE in the five different age groups, and the median %LE of all 504 cases was 22.9%. The solid black and dotted lines are the survival curves of 80 years of age or older and 70-79 years of age group, respectively. The other lines represent 60-69 years of age, 50-59 years of age and 49 years of age or younger groups, in colors ranging from dark to pale, respectively; C: In a cohort of 328 patients for whom LE is available, the survival among patients receiving loco-regional, interventional radiology (IVR), or chemotherapy (Cx) treatments was evaluated on the basis of absolute time (upper panel) or %LE (lower panel). The solid black and dotted lines are survival curves for Cx and IVR, respectively, and the gray line represents the loco-regional group. The dotted horizontal lines indicate a position of 50% survival.

of survival days to the expected residual life length is defined as the %LE. Life expectancy data for each age and gender are available from 1996 onward in Japan; therefore, life expectancy was plotted for the 504 cases in our cohort (Figure 2A). Overall survival based on %LE revealed a median survival percentage of 22.9%. When the survival based on %LE was compared among the five different age groups, the median survival was significantly different among the groups (Figure 2B, $P < 0.0001$), ranging from 5.4% in the -49 group to the best rate of 65.7% in the 80+ group.

Among 504 patients, 174 cases were excluded from further analyses because a therapeutic intervention was never performed or because one or more of the 10 explanatory candidate factors was not measured. Among the remaining 330 cases, only two patients received therapies that were categorized in "other". Finally, 328 cases were subjected to Cox regression analysis to investigate the survival differences associated with different therapeutic modalities. As shown in Figure 2C, the survival curves were very similar to the evaluations based on survival time (upper) and %LE (lower). Both analyses showed that loco-regional therapies far surpassed IVR and Cx in terms of survival benefit.

When the relationship between survival time and %LE was evaluated in each case, however, it became clear that the two survival indicators were not consistent. For example, the same survivals of 1779 d for males at 59 and 77 years of age in 1999 and 2004 gave rise to very different %LE values of 22.4% and 48.6%, respectively, as indicated in Figure 2A. In another example, a 69-year-old female in 2002 and a male of the same age in 1999 survived 2693 and 1980 d, respectively. Because their life expectancies were 7125 and 5168 d, respectively, the shorter absolute survival value for the male surpassed the female's longer survival in terms of %LE at 37.8% and 38.3%, respectively. Taken together, %LE is a potential alternative for evaluating survival benefit in HCC patients among different age groups.

Elderly patients survive for the highest percentage of their life expectancy after receiving active treatments for HCC

Although the overall survival trends and survival benefits

Table 2 Cox regression analysis for survival time

Variable	Significance	HR	95%CI for HR	
			Lower	Upper
Age	0.020	1.021	1.003	1.040
Gender	0.652	1.079	0.775	1.503
HBsAg	0.029	1.598	1.051	2.432
anti-HCV	0.036	1.503	1.027	2.198
AFP	0.000	1.314	1.135	1.521
L3	0.288	1.004	0.997	1.012
DCP	0.005	1.216	1.061	1.395
Child-Pugh class				
A	0.000			
B	0.000	2.307	1.562	3.408
C	0.001	3.373	1.617	7.035
Tumor stage[1]				
I	0.000			
II	0.148	1.504	0.865	2.616
III	0.051	1.751	0.997	3.074
IV	0.000	5.715	2.985	10.939
Therapy category				
Loco-regional	0.000			
IVR	0.000	2.567	1.800	3.661
Chemotherapy	0.000	2.861	1.675	4.889
Others	0.000	6.151	2.505	15.107

[1]General rules for the clinical and pathological study of primary liver cancer from Liver Cancer Study Group of Japan. HR: Hazard ratio; AFP: α-fetoprotein; L3: Percentage of fucosylated fraction in AFP; DCP: Des-γ-carboxy prothrombin; Loco-regional: Therapies including resection, radiofrequency ablation, microwave coagulation and percutaneous ethanol injection; IVR: Interventional radiology including transcatheter arterial chemoembolization and transarterial oily chemoembolization; Chemotherapy: Therapies including hepatic arterial infusion chemotherapy, systemic chemotherapy and molecular targeting therapy; Others: Other therapies including stereotactic body radiation, proton beam and liver transplantation; HCV: Hepatitis C virus; HBsAg: Hepatitis B surface antigen.

Table 3 Cox regression analysis for percent life expectancy

Variable	Significance	HR	95%CI for HR	
			Lower	Upper
Gender	0.072	0.717	0.499	1.030
HBsAg	0.043	1.709	1.018	2.869
anti-HCV	0.043	1.597	1.015	2.511
AFP	0.000	1.348	1.145	1.587
L3	0.421	1.004	0.995	1.012
DCP	0.015	1.243	1.043	1.482
Child-Pugh class				
A	0.001			
B	0.001	2.144	1.393	3.300
C	0.084	2.375	0.891	6.332
Tumor stage[1]				
I	0.000			
II	0.554	1.216	0.637	2.320
III	0.180	1.554	0.816	2.960
IV	0.000	3.879	1.871	8.045
Therapy category				
Loco-regional	0.000			
IVR	0.000	2.755	1.816	4.179
Chemotherapy	0.000	3.365	1.884	6.010
Others	0.246	3.359	0.435	25.971
Age group				
49 years/younger	0.240			
50s	0.242	0.598	0.253	1.415
60s	0.107	0.496	0.211	1.164
70s	0.047	0.436	0.192	0.990
80 years/older	0.041	0.348	0.126	0.958

[1]General rules for the clinical and pathological study of primary liver cancer from Liver Cancer Study Group of Japan; HR: Hazard ratio; AFP:α-fetoprotein; L3: Percentage of fucosylated fraction in AFP; DCP: Des-γ-carboxy prothrombin; Loco-regional: Therapies including resection, radiofrequency ablation, microwave coagulation and percutaneous ethanol injection; IVR: Interventional radiology including transcatheter arterial chemoembolization, and transarterial oily chemoembolization; Chemotherapy: Therapies including hepatic arterial infusion chemotherapy, systemic chemotherapy and molecular targeting therapy; Others: Other therapies including stereotactic body radiation, proton beam and liver transplantation; HCV: Hepatitis C virus; HBsAg: Hepatitis B surface antigen.

of different therapies were consistent between the analyses using dependent variables of survival time and %LE, the two analyses indicated substantially different survival benefits when the survival was compared among the different age groups. A comparison based on survival time in 330 cases revealed that the 80+ group had the worst survival (Figure 3A upper panel), consistent with a similar finding in the initial cohort of 760 patients (Figure 1C). However, when the survival benefit was evaluated on the basis of %LE, age was again found to be a significant explanatory factor (P = 0.022), but the 80+ group showed the best survival among the five different age groups, as shown in the lower panel of Figure 3A. Intriguingly, the -49 group revealed the worst outcomes in this analysis. The hazard ratio of the 80+ group against the -49 group was 0.35 for death (Table 3). The other significant explanatory factors for %LE were HBsAg and anti-HCV, AFP, DCP, Child-Pugh score, tumor-node-metastasis (TNM) stage, and therapeutic options, and the maximal hazard ratios for each variable were 1.71, 1.60, 1.35, 1.24, 2.14, 3.88 and 3.37, respectively, suggesting that age is one of the most powerful determinants of %LE.

Tumors were more advanced in the young

Functional hepatic reserve and anatomical tumor extent were compared among three groups: a younger group of -49 and 50s, a middle-aged group consisting of 60s and 70s, and an elderly group of 80+. As shown in the upper panel of Figure 3B, the functional hepatic reserve, as assessed by the Child-Pugh class, did not differ among the three groups (P = 0.34), while the TNM stage tended to be more advanced in the younger group (Figure 3B middle panel, P = 0.060). Advanced disease was significantly more frequent in the younger group as compared to the middle-aged group (P = 0.010), whereas there was no difference between the middle-aged and elderly groups (P = 0.75). A similar trend was observed for HBsAg positivity (P < 0.0001). In the younger group, HBsAg was positive in 45.7% of patients, while it was positive in only 14.8% and 6.7% of patients in the middle-aged and elderly groups, respectively, which led to a significant difference between the younger and middle-aged groups (P < 0.0001), but no significant difference between the middle-aged and elderly groups (P = 0.23).

Case

This case is an example of an 85-year-old Japanese male

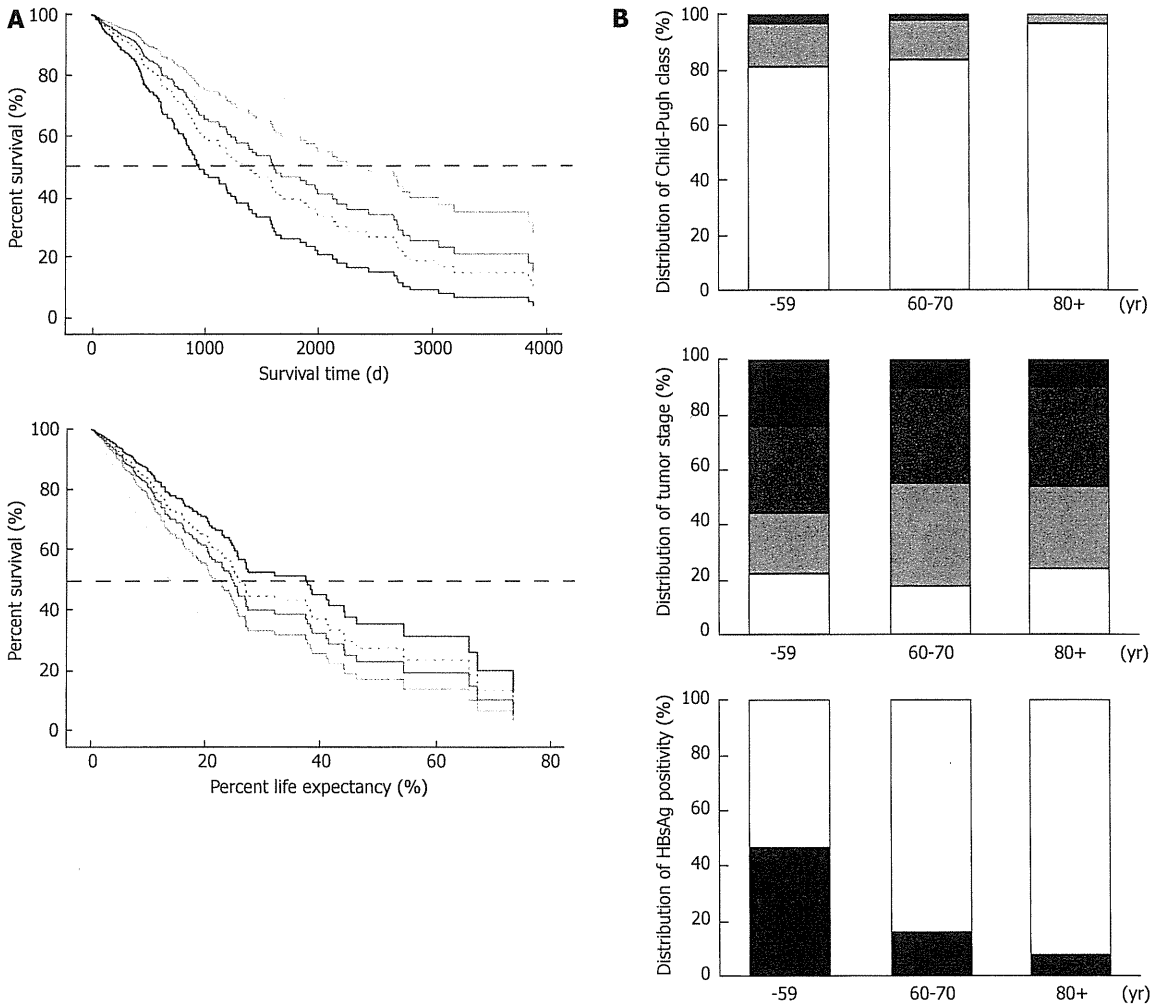

Figure 3 Differences in survival and background characteristics by age groups. A: In a cohort of 330 patients for whom life expectancy (LE) data are available, the survival of five different age groups was evaluated on the basis of absolute time (upper panel) or percent LE (lower panel). The solid black and dotted lines are the survival curves of the 80 years of age or older and 70-79 years of age groups, respectively. The other lines are 60-69 years of age, 50-59 years of age and 49 years of age or younger groups, indicated in colors ranging from dark to pale. The oldest group showed the worst survival in days but the best in percent LE; significantly better than that of the youngest group ($P = 0.041$); B: The distributions of Child-Pugh class (upper), tumor stage (middle), and hepatitis B surface antigen (HBsAg) positivity (lower) among three age groups: 59 years of age or younger, 60-79 years of age, and 80 years of age or older. For the hepatic reserve, the white, grey and black columns indicate Child-Pugh A, B and C classes, respectively, whereas tumor stages from I to IV are represented in order from white to black. The black column reveals that HBsAg was positive in the lower graph. There was no significant difference in terms of functional hepatic reserve among the three groups, although anatomical tumor extent and frequency of positive reaction for HBsAg were significantly higher in the youngest group as compared with the middle-aged group ($P = 0.010$ and $P < 0.0001$, respectively). The dotted horizontal lines indicate a position of 50% survival.

who was admitted to our hospital on April 2009 for the treatment of an HCC that was approximately 20 mm in diameter and located in segment 6 (Figure 4A). He suffered from HCV infection and diabetes mellitus, for which he had used insulin by injection for more than a decade, and he had one prior hepatic resection for HCC. His life expectancy upon admission was 6.27 years. RFA was completed after TACE through the right intercostal space under ultrasound guidance. Twenty-five months after the ablation, however, a follow-up CT revealed that the HCC had spread to wide areas of segments 6 and 7 with portal vein tumor thrombus that extended up to the right main trunk (Figure 4B). Relying on the Child A-class preserved hepatic functional reserve, HAIC was our recommendation at this stage, delivered through a catheter that was implemented and connected to a port under the skin. After receiving written informed consent from the patient, 125 mg of 5-fluorouracil and 5 mg of cis-diamminedichloroplatinum were infused over 23 h and 60 min, respectively, through the common hepatic artery, and repeated for 5 consecutive days. After 2 d of no drug administration, the same schedule was performed for the following 2 wk. The 5-d HAIC was then repeated every 2-3 mo. The tumor and portal vein tumor thrombi gradually disappeared to reveal an enormous tumor reduction by July 2012 (Figure 4C). A new lesion appeared in segment 5 and gradually enlarged to 15 mm in diameter; therefore, RFA was performed again on August 2012 (Figure 4D). In September 2012, the patient turned 89 years old and has survived for 42 mo (55.8% of LE)

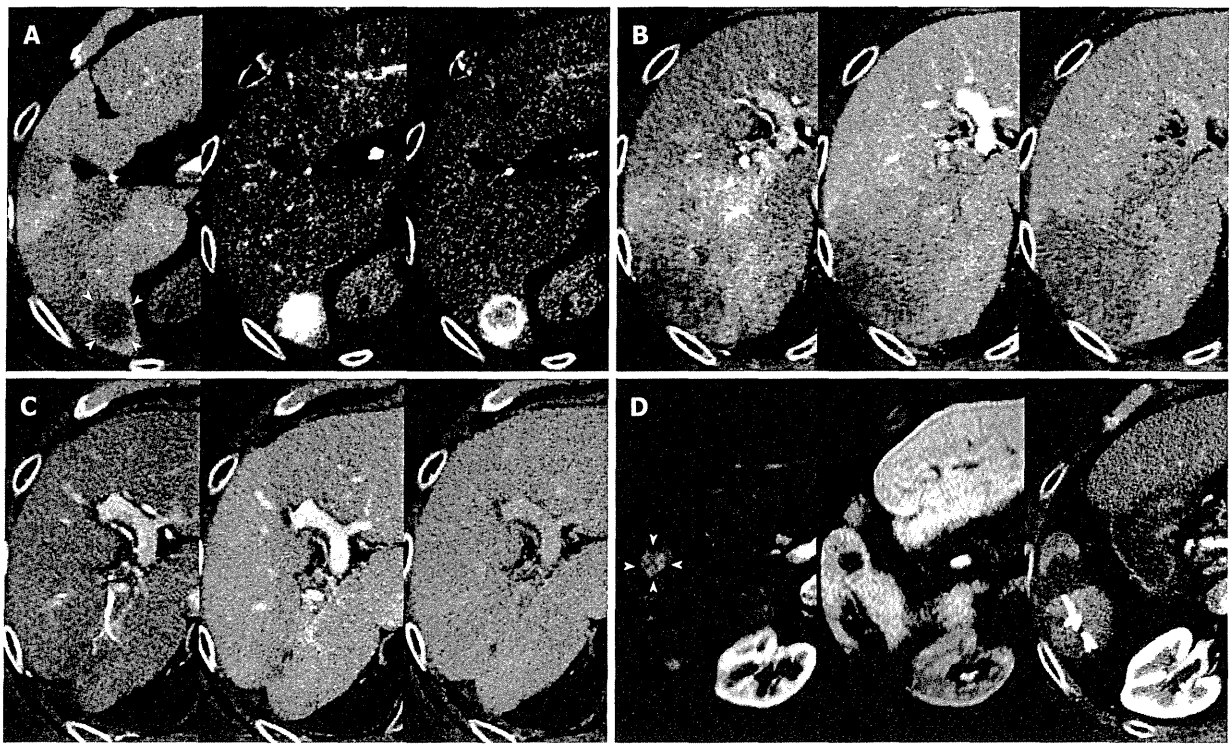

Figure 4 Representative follow-up images of successfully treated hepatocellular carcinoma in an elderly patient. A: A classical hepatocellular carcinoma (HCC) was detected in segment 6 of the liver on April 12, 2009 as demonstrated by (1) a lower intensity up on computed tomography (CT) during arterial portography, indicated by arrowheads (left); (2) vigorous staining during the arterial phase of the CT during hepatic arteriography (middle); and (3) washout with a corona-like peripheral enhancement during the equilibrium phase of the CT during hepatic arteriography (right); B: A dynamic CT 25 mo after the initial radiofrequency ablation revealing recurrent HCCs, which had spread to large areas of segments 6 and 7 and extended to the main trunk of the right portal vein. The images were obtained during arterial, portal and equilibrium phases of the dynamic CT study, shown in order from left to right; C: After hepatic arterial infusion chemotherapy *via* a catheter using 5-fluorouracil and cis-diamminedichloroplatinum for 15 mo, an enormous tumor reduction, including the portal vein tumor thrombus, was achieved. The images were obtained during arterial, portal, and equilibrium phases of the dynamic CT study, shown in order from left to right; D: A new 10-mm lesion appeared in segment 6 during hepatic arterial infusion chemotherapy treatment, and a second radiofrequency ablation (RFA) was applied 41 mo after the initial RFA. Magnetic resonance imaging study using a contrast medium of gadolinium ethoxybenzyl diethylene-triamine-pentaacetic-acid showing the arterial supply (left, arrowheads) and a defect in the hepatobiliary phase (middle) of the study. An arterial phase image of the dynamic CT (right) obtained one day after RFA revealing that the ablated area of lower intensity included the target.

since the initial RFA. He is in good shape with a PS of 0 and without severe complaints.

DISCUSSION

In this study, we introduced a new indicator, the %LE, to evaluate whether active treatments for HCC are beneficial for patients over 80 years of age. Considering that morbidity, mortality and other health-related outcomes are generally compared in populations after adjusting for age structures, the age should also be normalized when survival is compared among different age groups. Based on the assumption of a community downscaling to an individual, the life expectancy adjustment among individuals should correspond to age adjustment among communities. As shown in Figure 1B and C, there is a large difference in survival curves between the Kaplan-Meier fractions and the Cox hazards after compensation using 10 explanatory factors. The worst survival of the -49 group in the Kaplan-Meier analysis was the best survival in the Cox regression. In contrast, when %LE was used, the order of survival was consistent between the Kaplan-Meier and Cox regression analyses (Figures 2B and 3A lower panel). The explanatory factors for survival time and %LE in the Cox regression analyses were consistent, and the analyses using survival time or %LE revealed similar survival curves among the different therapeutic approaches (Figure 2C); therefore, it is suggested that the factor that explains the large difference between the Kaplan-Meier and Cox regression analyses using survival time is age. Therefore, it is assumed that the impact of age on survival irrespective of liver pathophysiology can be normalized using %LE instead of survival time. The worst survival of the -49 group in the %LE analysis concurs with the common clinical experience in Japan of higher HBsAg positivity in the younger generation of HCC, leading to the onset of HCC at advanced stages[10]. Taken together, these data suggest that the %LE can be a useful factor to compare survival benefit, especially between cohorts of patients with large differences in age.

It is controversial whether active intervention is beneficial to elderly patients, and inconsistent recommendations have been reported for various diseases, including HCC. Studies that demonstrate an adverse influence of

aging on survival in HCC were all reported more than 20 years ago[11-14], whereas recent reports emphasized the benefit of active treatments in the elderly[15-18], suggesting improvements of the medical and social environments over the past decades. Unfortunately, however, all recent reports discussed survival benefits in the elderly principally based on Kaplan-Meier survival fractions. As shown in Figure 1B, a simple comparison of survival time does not reveal survival differences among various age groups. However, one should not conclude that age is not a significant factor for survival on the basis of Kaplan-Meier analysis, because survival varies widely among the different age groups in the Cox regression analysis (Figure 1C). Although our conclusion is consistent with previous reports that a therapeutic scheme should not be changed because of a patient's age, this study explains the rationale of active intervention for HCC in the elderly more theoretically. However, our conclusions are based on the study from a single institution and a limited number of patients. To establish the best approach for the elderly patients, a multicenter study should be conducted using a larger cohort.

Generally, our institution applies the same process to decide a treatment strategy irrespective of the patient's age, and this approach led to the best survival in the elderly in terms of %LE, as shown in this study. We also presented the case of a patient over 80 years of age, for whom active interventions such as RFA, TACE or HAIC were safely applied and provided survival benefit. Although it is important to thoroughly assess the risks and to obtain written informed consent, because aging is associated with a progressive deterioration of organ function that reduces the functional reserve to recover from stress/complications[19], our data suggested that age itself should not be a reason to change a treatment strategy. The number of elderly patients in one institution is generally limited, as is the case in this study; therefore, it is difficult to clarify risk determinants that are specific for elderly patients. Future trials should include efforts to enroll the elderly and to clarify factors specific for them that determine outcomes, not only for physical function, but also for quality of life and independence. Facing the world's highest proportion of elderly people, Japanese society should play a primary role in the application of guidelines for the elderly subset by achieving maximal benefits while minimizing risks.

COMMENTS

Background

Increasing life expectancy and a falling birth rate have lead to population aging worldwide, especially in developed countries. As a result, the proportion of elderly patients is steadily rising in many diseases, including hepatocellular carcinoma (HCC). There is much controversy concerning medical interventions for elderly patients; therefore, it is important to clarify a treatment strategy specific for the elderly in terms of both survival benefit and medical resources.

Research frontiers

Aging itself has a significant impact on survival days; therefore, a simple comparison of survival days among different age groups may not be suitable for evaluating survival benefit with regard to aging. Another approach is required to compensate for the effect of aging on survival.

Innovations and breakthroughs

To avoid the confounding effect between survival days and aging, the research team led by Takeshi Suda from Division of Gastroenterology and Hepatology, Niigata University introduced percent life expectancy instead of survival days as a novel indicator for the evaluation of survival benefit. Using percent life expectancy, the authors finally concluded that a therapeutic approach for HCC should not be restricted because of patient age.

Applications

Facing progressive population aging, medical society should play a primary role in the application of guidelines for the elderly subset by achieving maximal benefits while minimizing risks. In future trials with larger cohorts, percent life expectancy should play an important role in evaluating survival benefits associated with aging.

Terminology

Computed tomography (CT) during hepatic arteriography and CT during arterial portography are one of the best ways to evaluate perfusion characteristics of a nodular lesion in the liver, and provide the diagnostic rationale for HCC. Radiofrequency ablation therapy and percutaneous ethanol injection are puncture-based locoregional approaches for HCC. Both are usually applied percutaneously under ultrasound guidance, and utilize radiofrequency waves and anhydrous ethanol, respectively, to degenerate cancer cells. Transcatheter arterial chemoembolization and transarterial oily chemoembolization are interventional radiology including the embolization of hepatic arteries feeding HCC. These techniques employ gelatin and oil for embolization, respectively, and gelatin achieves a prolonged obstruction. The tumor markers of α-fetoprotein, L3, and des-γ-carboxy prothrombin are commonly measured as useful independent indicators for the biological malignant potential of HCC.

Peer review

Facing the world highest elderly ratio in Japan, it is necessary to clarify whether a medical decision process that is applied for the general population provides similar benefits for elderly patients in terms of survival. For this purpose, the authors introduced a new indicator, percent life expectancy, to normalize aging effects, and evaluated the survival benefit among different age groups. Many developed countries, such as United States and China, are also facing population aging; therefore, it is important to clarify risk determinants specific for the elderly in terms of individual aspects and medical economy. This manuscript will have a significant impact and the hepatology community will recognize the importance of the gerontological aspect.

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Low fat intake is associated with pathological manifestations and poor recovery in patients with hepatocellular carcinoma

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Abstract

Background: This study aimed to clarify whether dietary deviation is associated with pathological manifestations in hepatocellular carcinoma (HCC) patients.

Methods: Dietary intake was estimated in 35 HCC cases before and after hospitalization by referencing digital camera images of each meal. Pathological conditions were evaluated in nitrogen balance, non-protein respiratory quotient (npRQ), neuropsychiatric testing and recovery speed from HCC treatment.

Results: On admission, nitrogen balance and npRQ were negative and less than 0.85, respectively. Five patients were judged to have suffered from minimal hepatic encephalopathy that tended to be associated with a lowered value of npRQ ($p = 0.082$). The energy from fat intake showed a tendency of positive correlation with npRQ ($p = 0.11$), and the patients with minimal hepatic encephalopathy took significantly fewer energy from fat ($p = 0.024$). The energy difference from fat between diets at home versus those in the hospital showed a significant positive correlation with npRQ change after admission ($p = 0.014$). The recovery speed from invasive treatments for HCC showed a significant negative correlation with npRQ alteration after admission ($p = 0.0002$, $r = -0.73$).

Conclusions: These results suggest the lower fat intake leads to deterioration of energy state in HCC patients, which associates with poor recovery from invasive treatments and various pathological manifestations.

Keywords: Hepatocellular carcinoma, Protein-energy malnutrition, Minimal hepatic encephalopathy, Non-protein respiratory quotient

Background

Hepatocellular carcinoma (HCC) is the third most common cause of cancer death in the world and accounts for over 500,000 deaths a year [1]. HCC is a unique type of cancer, which mostly arises in livers with chronic necroinflammatory damage due to various etiologies such as hepatitis C virus infection and non-alcoholic steatohepatitis [2]. Because long lasting necroinflammation leads to reductions of functional liver reserves, the patient's survival cannot be predicted from cancer stage alone, which is done in the other types of cancer. It has been reported that a system integrating both cancer stage and functional liver reserve can accurately stratify patient survival rates [3]. For example, the Japan integrating scoring indicates that the functional liver reserve as assessed by the Child-Pugh scoring system has an impact on a patients' survival equivalent to anatomical cancer extension [4]. Thus, it is advisable to manage functional liver reserves in HCC patients in parallel with their cancer treatment in order to improve survival.

In terms of nutritional state, a characteristic feature of patients suffering from liver cirrhosis is protein-energy malnutrition (PEM) [5,6]. An insufficient energy intake of less than 30 kcal/kg has been reported to be associated with a poor prognosis in cases of liver cirrhosis [7]. Randomized prospective case control studies have revealed that nutritional intervention in order to support sufficient energy intake significantly improves patient

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survival [8-10]. Unfortunately, it is common for cirrhotic patients to present with comorbidities such as hypermetabolism, inefficient digestion and anorexia [11], which counteract the beneficial effects of sufficient energy intake. The occurrence of hepatic encephalopathy due to an improper protein diet makes it even more difficult to practically maintain nutritional-energy balance during cirrhosis.

Along with energy intake, the body aims to maintain energy balance adopting various ways. In patients with anorexia nervosa, the physiological adaptation to malnutrition is expressed as the refeeding syndrome, when the extra energy is administered even with an appropriate amount to body size [12,13]. A daily consumption of a high-fat diet alters the homeostatic regulation [14-16]. Lean people consuming a high-fat diet are associated with increased energy expenditure at rest and a relatively higher fat oxidation to avoid weight gain [17]. These facts suggest that a nutritional intervention should be adjusted not statically but dynamically in association with personal daily life. In this report, we evaluated dietary intake both at home and in hospital among patients with HCC from the points of PEM, minimal hepatic encephalopathy (MHE) and recovery from cancer treatment, and show that dietary deviation is an important consideration when invasive treatments are planned. In addition, the impact of nutritional intervention is discussed for the optimal management of HCC.

Methods

Patients

Thirty-five consecutive cases suffering from HCC with various histories of liver disease were enrolled in this study (Table 1, Group 1). When hospital admission was primarily to treat HCC, a digital camera and questionnaires were provided to record the diet at home several days before admission. A computer-aided neuropsychiatric test (NP test) and assessment of body composition based on a bioelectrical impedance analysis using InBody system (BIOSPACE, Tokyo, Japan) were performed upon admission. In addition, a dietician calculated energy intake based on Japanese dietary allowance according to home photo images, which were obtained at least three consecutive days prior to the admission both before and after each meal including snacks, and descriptions from the questionnaires. In patients without any special comorbidity such as diabetes mellitus, a regular hospital diet of 1800 kcal/day was served. Any nutritional support including branched-chain amino acids (BCAA) was kept as it was in the outpatient clinic. On the next day, day 1, nitrogen balance and non-protein respiratory quotient (npRQ) were evaluated. On day 4 after admission, one day before the application of invasive therapy, the NP test, nitrogen balance and npRQ were assessed again. The questionnaires and recording of digital photos before and after each meal were continued until day 4 in order to calculate actual energy intake for the hospital and non-hospital provided diet. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Niigata University Graduate School of Medical and Dental Sciences Human Research Committee. Written informed consent was obtained from all patients for publication of individual clinical details.

Table 1 Summary of patients' characteristics

Items	Group 1 (*n* = 35)	Group 2 (*n* = 20)
Age	68.5 ± 8.2	71.0 ± 8.2
Gender (male/female)	28/7	14/6
Body mass index	24.3 ± 3.2	24.6 ± 3.5
Background (HBV/HCV/Alcohol/NASH/PBC/AIH)	12/14/6/3/1/0	4/11/3/1/0/1
Child-Pugh score (5/6/7/8/9)	20/7/6/0/2	11/5/3/0/1
TNM stage (I/II/III/IV)	18/9/3/5	6/5/4/5
Treatment (RFA/TACE/TOCE/HAIC/Sorafenib/No)	8/6/3/11/1/6	4/6/3/7/0/0
BCAA supplementation (+/−)	21/14	11/9
PT-INR on admission	1.12 ± 0.09	1.09 ± 0.08

HBV hepatitis B virus, *HCV* hepatitis C virus, *NASH* nonalcoholic steatohepatitis, *PBC* primary biliary cirrhosis, *AIH* autoimmune hepatitis, *TNM* tumor-node-metastasis, *RFA* radiofrequency ablation, *TACE* transcatheter hepatic arterial chemoembolization, *TOCE* transcatheter oily chemoembolization, *HAIC* hepatic arterial infusion chemotherapy, *BCAA* branched-chain amino acids, *PT-INR* prothrombin time-international normalized ratio.

Neuropsychiatric test

When a patient received an abnormal value for the revised version of the Hasegawa dementia scale [18], he/she was excluded from this study. MHE was evaluated using a computer-aided quantitative NP test [19], which consisted of the eight following categorical tests: number connection tests A and B, a figure position test, a digit symbol test, a block design test, and reaction time tests A, B and C. Because the test results were affected by age, abnormality in each category was originally defined as values beyond the 90th percentile for an age-matched value over a 5-year interval, which was obtained from 542 healthy Japanese volunteers from 40 to 69-years old. Unfortunately, there were many patients who were 70-years old or older. To make an assessment for a patient over 69-years old, a linear regression curve was deduced that excluded reaction time tests, which were fit by second-order polynomial non-linear regression. Each regression analysis showed a significant correlation giving r squares of 0.95, 0.93, 0.77, 0.99, 0.91, 0.94, 0.93 and 0.82, respectively. Thus, in this study, value for each test was decided to be abnormal when it exceeded the 90th

percentile, which was calculated from the regression curve for the same age. Because many elderly patients do not react properly to the test due to a lack of experience using a computer, MHE was not diagnosed if the abnormal value appeared in one category but normal during the second evaluation. Instead, MHE was diagnosed when an abnormal value was reproducibly recorded even in one category or when abnormal values were obtained at least once in multiple categories.

Measurements of non-protein respiratory quotient and estimations of energy metabolism

Energy metabolism was analyzed using an indirect calorimeter, AERO MONITOR AE300S (Minato Medical Science Co., Ltd. Osaka, Japan), on day 1 and day 4 after overnight bed rest and fasting. Every five seconds, oxygen consumption (VO2) and carbon dioxide production (VCO2) were measured until steady-state values were obtained over two consecutive minutes. A steady state was defined by a variation between 5% and 10% in the average value for oxygen consumption and carbon dioxide production over 3 minutes. npRQ was calculated as an average of the following ratio: VCO2/VO2. Urine urea nitrogen (UUN) was measured by urease indophenol method in urine that was collected throughout the day without any intravenous infusion. Resting energy expenditure (REE) was calculated according to the following formula [20]: ((15.913 × VO2 + 5.207 × VCO2) × 1.44 - 4.646 × TUN) × 0.239. TUN (total urea nitrogen) was calculated in the urine, which was collected over 24 hours, as UUN + 4 if UUN exceeded 15 g/day; otherwise, TUN was calculated as UUN × 1.17 + 0.7. Basal energy expenditure (BEE) was estimated using a Harris-Benedict equation, in which BEE was 66.5 + (13.75 × body weight in kg) + (5.003 × body height in cm) - (6.775 × age) for males and was 655.1 + (9.563 × body weight in kg) + (1.850 × body height in cm) - (4.676 × age) for females. The daily energy requirement was estimated from REE or BEE by multiplying by stress and activity coefficients of 1.1 and 1.3, respectively. The stress coefficient was selected between no stress; 1.0 and suffering from advanced cancer; 1.2, while the activity coefficient was decided between simple walking; 1.2 and light labour; 1.4, respectively. The protein and energy malnutrition was diagnosed in the case that npRQ was less than 0.85 [21] as well as nitrogen balance was negative.

Normalization of therapeutic invasiveness using prothrombin time

In order to standardize a recovery speed form HCC treatments based on therapeutic intensity, a reduction rate of prothrombin time (PT-INR) was used as an indicator for the intensity. Given nadir as a day showing minimum value of PT-INR after finishing an entire series of treatment against HCC in one admission, a reduction rate of PT-INR was defined as a reduction percentage of PT-INR at nadir against PT-INR on admission. The formula calculating PT-INR reduction rate is as follows where PT-INRad and PT-INRnad indicate PT-INR on admission and at the nadir after a series of treatment, respectively.

PT-INR reduction rate (%) = (PT-INRad − PT-INRnad)/PT-INRad × 100

Then, a recovery speed from treatments for HCC was evaluated based on a length of hospital satay (day) after nadir that was normalized by dividing with the PT-INR reduction rate as follows.

Recovery speed = hospital stay after PT-INRnad/PT-INR reduction rate × 100

Statistical analysis

Categorical data were compared between two groups using paired or unpaired t tests when groups were matched or unmatched, respectively. Correlation between npRQ and various factors were analyzed by calculating the Pearson correlation coefficient. All analyses were performed using GraphPad Prism 6 software (GraphPad Software, Inc. La Jolla, USA) except for a multivariate linear regression analysis, for which PASW statics 17.0 (SPSS Inc., Chicago, USA) was used, and a two-sided P-value less than 0.05 was considered statistically significant.

Results

Patients hospitalized for hepatocellular carcinoma treatments frequently suffered from protein-energy malnutrition.

Patients who were admitted for active treatment of HCC had a home diet with an average of 1977 ± 513 (mean ± SD) kcal/day leading to a negative nitrogen balance of −2.1 ± 4.5 g/dl (Figure 1A and 1B). Because among 35 cases, a case could not properly collect urine sample for a day on admission causing loss of UUN data, nitrogen balance and REE were evaluated for 34 cases. The energy malnutrition was also observed in the average npRQ value of 0.83 ± 0.061 on day 1, which was estimated to reflect the energy status at home (Figure 1C). npRQ on day 1 was not significantly correlated with BMI of 24.3 ± 3.2 kg/m^2 ($p = 0.35$) nor other representative body composition markers such as intra and extra cellular water, percent body fat or soft lean mass (data not shown). The average energy intake in the hospital was 1834 ± 290 kcal/day and tended to be lower than that at home ($p = 0.061$, Figure 1A). However, the negative nitrogen balance of −3.0 ± 2.8 g/dl was not significantly different ($p = 0.31$, Figure 1B), and the energy state was even significantly improved to a normal range of npRQ on day 4 at 0.86 ± 0.075 ($p = 0.0032$, Figure 1C). The energy requirements were calculated by multiplying 1.1 and 1.3 as stress and activity coefficients, respectively, to REE or BEE (Materials & Methods), which

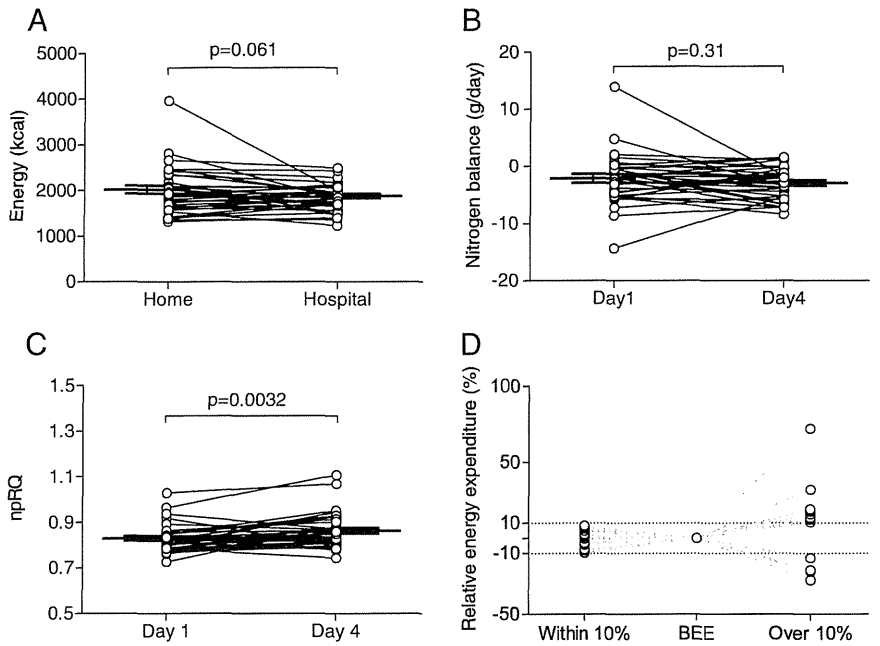

Figure 1 Energy intake and protein-energy malnutrition in patients with hepatocellular carcinoma. (**A**) The average energy intake of 35 HCC patients at home was 1977 ± 513 kcal/day (mean ± SD) and tended to be higher than the 1834 ± 290 kcal/day in the hospital (p = 0.061). (**B**) Nitrogen balance was measured from a 24 hr specimen of urine and was −2.1 ± 4.5 g/dl and −3.0 ± 2.8 g/dl on days 1 and 4 after admission, respectively. There was no significant difference between these values (p = 0.31). (**C**) Non-protein respiratory quotient on day 1 was 0.83 ± 0.061 and was significantly lower than 0.86 ± 0.075 on day 4 (p = 0.0032). (**D**) Daily energy requirement was calculated based on resting energy expenditure (REE) or the basal energy expenditure (BEE) estimated from the Harris-Benedict equation. When stress and activity indices were set at 1.1 and 1.3, respectively, 15 out of 34 cases showed more than 10% difference between REE-based and BEE-based estimations.

resulted into 1940 ± 385 kcal or 1860 ± 281 kcal/day, respectively. Fifteen out of 34 cases showed more than 10% difference between REE-based and BEE-based estimations (Figure 1D). Although protein-energy malnutrition was observed on day 1, there was no significant difference between total energy intake at home, 1977 ± 513 kcal/day, and the energy requirement calculated from REE, 1940 ± 385 kcal/day, (p = 0.60). There were 21 cases that received the nutritional support of BCAA in 35 cases (Table 1, Group 1), and no significant difference was observed between cases with and without BCAA supplementation in nitrogen balance or npRQ day 1 (p = 0.99 and p = 0.53, respectively).

Minimal hepatic encephalopathy is associated with energy malnutrition

MHE was diagnosed in 5 cases on the basis of the computer-aided NP test. MHE-positive cases consisted of elderly patients with an average age of 77.2 ± 1.9 years, which was significantly older than the 67.0 ± 8.0 years for the cases that exhibited a negative NP test (p = 0.0081, Figure 2A). The energy state in MHE-positive cases was critically impaired on admission, which was indicated by the npRQ day 1 of 0.78 ± 0.027. These patients tended to be malnourished in comparison to MHE-negative cases with an npRQ of 0.84 ± 0.062 (Figure 2B, p = 0.082). In contrast, there were no significant differences between MHE-positive and -negative groups in regard to energy intake and body composition of skeletal muscle amount as shown in Figure 2C and 2D, respectively (p = 0.51 and p = 0.18, respectively).

Poor energy intake from fat is associated with minimal hepatic encephalopathy and starvation in patients with protein-energy malnutrition

The deviation of diet in relation to energy malnutrition and MHE was observed as a reduced energy intake from fat. The relative energy intake from fat at home tended to be correlated with npRQ day 1 as shown in Figure 3A (p = 0.11, r = 0.28). Moreover, the difference in energy from fat between home and hospital diets showed a significant positive correlation with npRQ change between day 1 and day 4 (p = 0.014, r = 0.41, Figure 3B).

In accordance with the relationship between MHE and npRQ as described above, MHE-positive cases relied on significantly fewer energy from fat at home with 18.9 ± 3.8% in comparison to 23.6 ± 4.2% in MHE-negative cases (p = 0.024, Figure 3C). Because MHE-positive cases consisted of significantly older people (70-years old or older) as shown in Figure 2A, the lower intake of energy

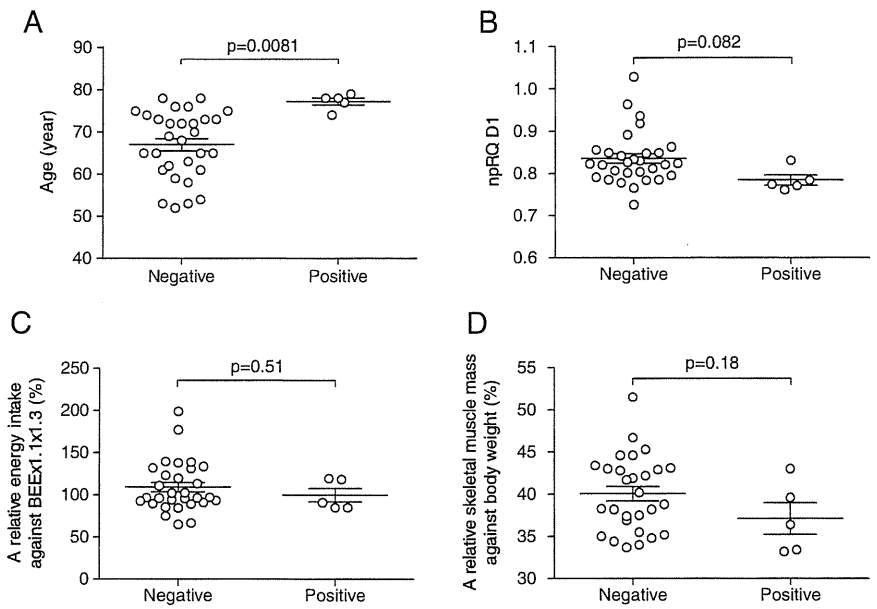

Figure 2 Relationship between minimal hepatic encephalopathy (MHE) and various clinicopathological factors. (**A**) Five patients who were diagnosed with MHE using a computer-aided neuropsychiatric test were significantly older in age (77.2 ± 1.9) than the MHE-negative cases (67.0 ± 8.0) (p = 0.0081). (**B**) npRQ on day 1 in MHE-positive and MHE-negative cases were 0.78 ± 0.027 and 0.84 ± 0.062, respectively, and tended to be lower in MHE-positive cases (p = 0.082). (**C**) Relative energy intakes at home expressed as a percentage to basal energy expenditure multiplied by stress and activity indices of 1.1 and 1.3, respectively, were 109.0 ± 30.0% and 99.7 ± 17.7% in MHE-negative and -positive cases, and were not significantly different each other (p = 0.51). (**D**) Similarly, body compositions of skeletal muscle mass relative to body weight were 40.1 ± 4.5% and 37.1 ± 4.2% in MHE-negative and -positive cases, and were not significantly different each other (p = 0.18).

from fat in MHE-positive cases may be confounded by age differences between MHE-positive and MHE–negative cases. However, given that these patients were 70-years old or older, there was still a significant difference in terms of energy intake from fat. Specifically, an average energy intake from fat of 24.0 ± 4.6% for 14 MHE-negative cases was significantly higher than that for 5 MHE-positive cases (p = 0.040, Figure 3D). Moreover, the difference of energy intake from fat was compared between general population and our cohort using 2010 national surveillance data from Japan. In people with age of 70 or older, there was no significant difference of energy percentage from fat between MHE-negative patients in this study and general population (22.9 ± 10.5% (n = 562), p = 0.69). There was a significant correlation of percent body fat neither with total energy intake nor relative energy intake from fat (p = 0.28 or p = 0.43, respectively).

Deterioration of npRQ over hospitalization is associated with poor recovery from invasive therapies for hepatocellular carcinoma

The effects of transition from home to hospital diets on energy malnutrition and recovery from invasive therapies were evaluated. Because these patients were treated using various therapeutic options such as transcatheter hepatic arterial chemoembolization or radiofrequency ablation, the invasiveness affected each case differently. To compare the recovery speed among cases, the invasiveness has to be standardized. For this purpose, we employed PT-INR reduction rate (Materials and Methods).

If no treatment was conducted or PT-INR was not reduced after treatments, those cases were excluded from the analysis in order to specifically examine the deterioration-recovery sequence. Furthermore, cases that showed the minimal PT-INR value amid a multiple treatment course were also excluded. Because only 11 cases were qualified for a further analysis from the original 35 cases, additional 9 cases were subjected to analysis of the PT-INR reduction rate and npRQ day 1 and day 4. Finally, a total of 20 cases (Table 1, Group 2) were subjected to further analyses. After these therapies, PT-INR was reduced 5.8 ± 3.8% on its nadir of 5.8 ± 5.1 days after each treatment.

There was no significant correlation between the PT-INR reduction rate and the difference in npRQ between day 1 and day 4 (p = 0.065, r = 0.42, Figure 4A), thereby suggesting that therapeutic option was not selected on the basis of the change in energy state after hospitalization. On the other hand, when the length of hospital stay after the nadir of PT-INR was normalized with the PT-INR reduction rate and used as an indicator of the recovery speed after invasive treatments, the recovery speed

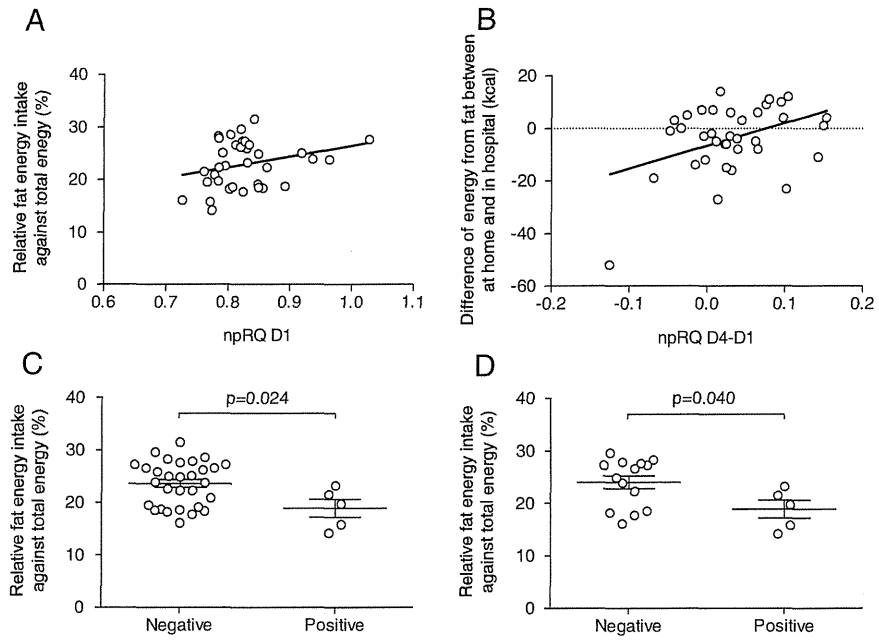

Figure 3 Effects of energy intake from fat on non-protein respiratory quotient and minimal hepatic encephalopathy. (**A**) There was a tendency of correlation between energy intake from fat relative to total energy intake at home and non-protein respiratory quotient (npRQ) day 1 ($p = 0.11$, $r = 0.28$). (**B**) The difference in energy intake from fat between at home and in hospital diets was significantly correlated with the alteration of npRQ day 1 and day 4 ($p = 0.014$, $r = 0.41$). (**C**) and (**D**). The relative energy intake from fat to total energy intake was 18.9 ± 3.8% in minimal hepatic encephalopathy (MHE)-positive cases and was significantly lower than 23.6 ± 4.2% in MHE-negative cases ($p = 0.024$). Even for the cases that were limited to 70-years old or older people, the energy intake from fat was 24.0 ± 4.6% in MHE-negative cases, which was significantly higher than MHE-positive cases ($p = 0.040$).

showed a significant negative correlation with the difference in npRQ between day 1 and day 4 ($p = 0.0002$, $r = -0.73$, Figure 4B). Consistently, the length of hospital stay after the nadir of PT-INR was significantly longer in 5 cases with deteriorated npRQ after admission comparing with that in 15 cases, in which npRQ was improved after admission (175 ± 76 days *vs* 40 ± 59 days, respectively, $p = 0.0006$, Figure 4C).

In order to confirm that npRQ difference between day 1 and day 4 is a significant determinant for the length of hospital stay after treatment of HCC, a multiple linear regression analysis was performed by employing the recovery speed as a dependent variable. Independent variables were consisted of 8 factors; age, gender, background liver diseases, TNM stage, Child-Pugh score, body mass index, BCAA supplementation, and npRQ difference. In the results, npRQ difference was selected as an only significant explanatory ($p = 0.001$, Table 2).

Discussion

Several lines of evidence strongly indicate that a functional hepatic reserve possesses similar impacts on the survival of HCC cases with anatomical cancer extension [3,4,22-24]. On the other hand, several randomized prospective case control studies have revealed that active nutritional intervention significantly improves the prognosis in patients with liver cirrhosis [8-10]. Taken together with the evidence that energy intake lower than 30 kcal/kg leads to a poor prognosis in cirrhotic patients [7], it is reasonable to assume that a strategy to preserve functional hepatic reserves should be incorporated into a treatment scheme for HCC. In this report, we first evaluated protein-energy status in patients who were facing active interventional treatments for HCC. In these cases, PEM was clearly present on admission as a negative nitrogen balance and an npRQ less than 0.85 in association with MHE that was diagnosed in 5 out of 35 cases. These results strongly suggest that nutritional intervention should be started before hospitalization in patients with HCC. In terms of nutritional support, BCAA supplementation was reported to elongate event-free survival by improving PEM in cirrhotic patients [25], while its efficacy was equivocal in cases receiving radiofrequency ablation as the form of HCC treatment [26]. Although the nitrogen balance and npRQ were not significantly different between cases with and without BCAA supplementation in this study, the limited number of cases does not provide a conclusive result. The significance of BCAA supplementation under active treatments of HCC should be further evaluated in a larger cohort.

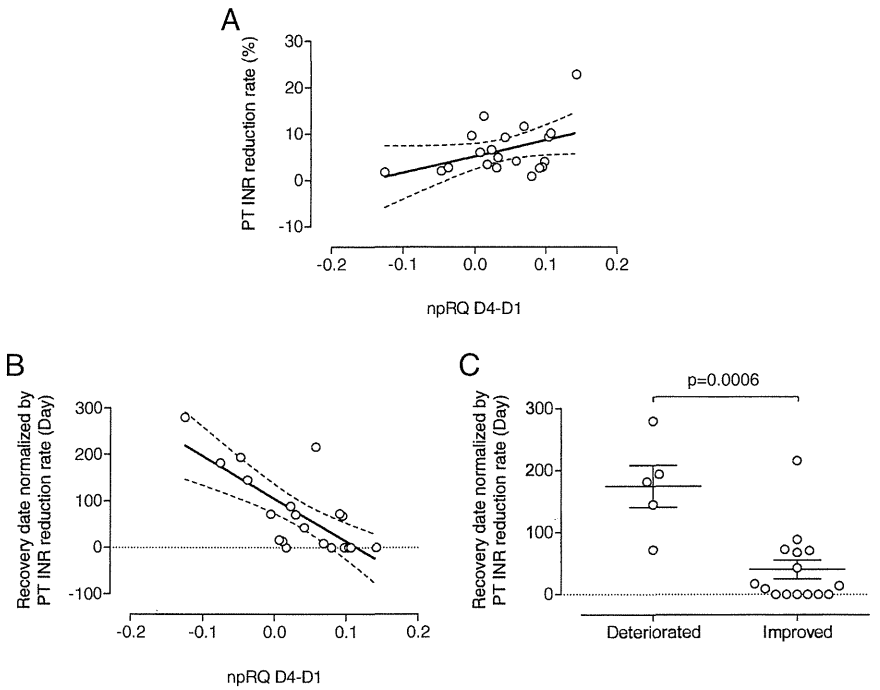

Figure 4 Association of recovery speed from invasive treatment and non-protein respiratory quotient. (**A**) Prothrombin time (PT-INR) reduction rate, which was the maximal reduction of PT-INR after treatment against the level at admission, was estimated as one of indicators for the magnitude of invasiveness and was not correlated with the non-protein respiratory quotient (npRQ) difference between days 1 and 4 ($p = 0.065$, $r = 0.42$). (**B**) The length of hospital stay after the nadir of PT-INR due to a treatment exhibited a significant negative correlation with the npRQ difference between days 1 and 4 after normalization by PT-INR reduction rate ($p = 0.0002$, $r = -0.73$). (**C**) The length of hospital stay after the nadir of PT-INR was 175 ± 76 days and 40 ± 59 days in npRQ-deteriorated and npRQ-improved cases after admission, respectively, and significantly different each other ($p = 0.0006$).

It was reported that BEE underestimates energy requirements in patients with liver cirrhosis, which leads to a hypermetabolic state [27]. Consistently, the BEE-based energy requirement calculated from the Harris-Benedict equation was different from the REE-based estimation more than 10% in more than 44% cases in this study. On the other hand, the total energy intake at home was not significantly different from the daily energy requirement, which was estimated from REE by multiplying 1.1 and 1.3 as the stress and activity coefficients, respectively. Although energy equivalents are considered in the REE-based calculation, these PEM suffering patients suggest that liver cirrhosis affected energy state not only by inducing hypermetabolism but also by hampering the absorption and/or efficient usage of nutrients. Furthermore, the increase of npRQ above 0.85 after admission even with consumption of less energy suggests that it is practically difficult to select appropriate activity and stress coefficients. Taken together, it is strongly recommended that the protein-energy state should be used to define an appropriate daily energy intake in cirrhotic patients using indicators such as npRQ.

Table 2 Result of multivariate linear regression analysis for recovery speed

Variables	Unstandardized coefficients	Significance
(Constant)	−27.102	0.908
Age	1.612	0.557
Gender	−33.052	0.368
Background liver disease	−34.672	0.223
TNM Stage	6.114	0.663
Child-Pugh score	−23.669	0.192
Body mass index	7.926	0.174
BCAA supplementation	46.270	0.194
npRQ Day4 - Day1	−1161.490	0.001

TNM tumor-node-metastasis, *BCAA* branched-chain amino acids, *npRQ* non-protein respiratory quotient, Day1, the first morning after admission, Day4, the forth morning after admission.

The computer-aided NP test is one of the few quantitative approaches for the diagnosis of MHE that were recommended in the guidelines provided by the World Congress of Gastroenterology-commissioned Working Party [28] due to their high specificity for diagnosing hepatic encephalopathy [29]. Currently, however, the diagnosis and clinical significance of MHE have not been

well defined [30]. While abnormal values at least in two tests among eight subsets were reported to be required achieving 80% of sensitivity [31], the same setting were also employed for diagnostic criteria in NP test consisting of four subsets instead of eight [32]. Another problem for NP test is age dependency [19]. There are no available control data for patients over the age of 69. Hence, it may be difficult to distinguish an early stage of dementia from MHE. In addition, there is a concern that these results may be affected by unfamiliarity with using a computer device, especially when elderly patients are the subjects. In this study, the 90th percentile for normal controls was estimated in each test from a regression line deduced from the values of controls between ages 40 to 69. A high Pearson's coefficient value for this regression line demonstrated the goodness of fit for all eight categories. Patients who were diagnosed with dementia were excluded from the study on the basis of a revised Hasegawa dementia scale [18]. Although senile decay in reaction time and/or cognition may not be completely excluded from our MHE diagnostic criteria, the lower values of npRQ in all 5 MHE-positive patients strongly suggested that MHE diagnosed by our criteria was associated with functional hepatic reserve. Easy and reliable diagnostic criteria for MHE should be further explored through extensive studies using a larger cohort to prove the clinical significance of MHE in association with the energy malnutrition.

This study suggested that an insufficient fat source impaired the recovery from invasive treatments for HCC in cirrhotic patients. An indicator of energy state, npRQ, was significantly changed after admission in association with the energy difference for fat consumed between home and the hospital. Consistent with the association between MHE cases and lower npRQ, the relative energy from fat was significantly lower in the cases that were diagnosed with MHE. Taken together, it is suggested that energy state should be improved before invasive treatments to promote a rapid recovery, and specifically, energy from fat should be provided at a dose recommended for the regular dietary allowance, which is between 20% to 30% of total energy intake [33,34]. In terms of normalization of therapeutic invasiveness, PT-INR was employed in this study. Although serum concentrations of NH_3 and total bilirubin were tested for this purpose, these values were prerequisitely altered due to extrahepatic circumstances such as constipation or constitutive jaundice. Because a single criterion of PT-INR was employed, the relationship between fat intake and recovery from HCC treatments should be confirmed using other measures in the future.

Although the limited case numbers in this study may have resulted in an inadequate assessment of the biological variability, as neither npRQ nor MHE was associated with body compositions such as BMI, extra cellular water, percent body fat, soft lean mass, or skeletal muscle amount, it is assumed that orally taken fat was directly consumed as an energy source. Nonesterified fatty acid (NEFA) suppresses gluconeogenesis in the liver through insulin secretion. At the same time, however, NEFA desensitizes the liver to insulin *via* insulin receptor substrates, which surpass insulin induction and lead to net elevation of gluconeogenesis [35-40]. Furthermore, fat from diet is absorbed in the form of chylomicrons and is taken up by hepatocytes as a remnant after digestion at the capillary endothelium by lipoprotein lipase [41], promoting gluconeogenesis as a source of energy and substrates such as acetyl-CoA, NADH and ATP. Through β-oxidation processes, acetyl-CoA is subjected to not only gluconeogenesis but also the generation of ketone bodies, which are major energy sources in the brain [42]. In a whole body, npRQ could increase as long as peripheral tissues have glucose and/or ketones to oxidize even under the situation where npRQ decreased in the liver due to gluconeogenesis and ketogenesis [43]. Under PEM, it is teleological for the liver that NEFA uptake is increased in association with up-regulation of gluconeogenesis and ketogenesis [44]. Recently, it was reported that p38 mitogen-activated protein kinase plays a crucial role in the activation of gluconeogenic genes by NEFA [45]. Although the results presented here should be confirmed by a large scale study, our notion is in line with the guideline from the European Society for Clinical Nutrition and Metabolism, which recommends 40% to 50% of non-protein energy requirements (more than 30% of total energy requirements) should be provided by lipid in parenteral nutrition in patients with liver diseases [46]. An appropriate amount of fat intake may have the potential to improve PEM and MHE under the condition such as cirrhosis, in which sugar and protein metabolisms cannot work properly [47,48].

Conclusions

This study suggested that PEM is a common feature in patients with HCC, and energy state can quickly change based on dietary deviation, which affects various clinical manifestations and recovery from invasive treatments. These findings strongly suggest that nutritional intervention especially for fat intake should be involved in the HCC treatment scheme both at home and in the hospital. Because a hypermetabolic state and inappropriate nutritional usage may hamper the calculation of an exact energy requirement in cirrhotic patients, nutritional supports should be conducted based on a nutritional assessment, which includes nitrogen balance, npRQ and MHE.

Abbreviations

BCAA: Branched-chain amino acids; BEE: Basal energy expenditure; HCC: Hepatocellular carcinoma; NEFA: Nonesterified fatty acid; npRQ:

Non-protein respiratory quotient; NP- test: Neuropsychiatric test; PEM: Protein-energy malnutrition; PT-INR: Prothrombin time; REE: Resting energy expenditure; TUN: Total urea nitrogen; UUN: Urine urea nitrogen; VCO2: Carbon dioxide production; VO2: Oxygen consumption.

Competing interests
All authors declare that they have no competing interest to disclose.

Authors' contributions
TS designed the research, analyzed data and wrote the manuscript. TK, TK, KY, YSK performed npRQ, MHE, body composition and dietary intake analyses. HN and TM evaluated the results of npRQ and nutritional aspect, respectively, and made disciplinary advice. YA made critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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AWARD REVIEW

Clinical significance of cell cycle inhibitors in hepatocellular carcinoma

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Abstract It is well accepted that cell cycle regulators are strongly implicated in the progression of cancer development. p16 and p27 are potent cyclin-dependent kinase (CDK) inhibitors involved in G1 phase progression, and are regarded as adverse prognostic biomarkers for various types of cancers. It has been reported that the main mechanism for p16 inactivation is aberrant DNA methylation, while p27 is exclusively inactivated by proteasome-mediated protein degradation. We have found that p27 is decreased in around half of hepatocellular carcinomas (HCCs), and in some cases p27 is inactivated by inappropriate interaction with cyclin D1/CDK4 complexes. In such cases, p16 is concomitantly inactivated through DNA methylation. Taking into consideration the complex interaction between p16 and p27, a comprehensive analysis including p16 and p27 would be useful for predicting the prognosis of HCC patients.

Keywords Cell cycle · p16 · Id-1 · p27 · Liver cirrhosis · Hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and its incidence is still increasing in Africa, China, and South-East Asia. The development of HCC is related to various types of etiologic factors including hepatitis B virus (HBV) and C virus (HCV) infection, alcohol abuse, obesity, and Aflatoxin B1 mycotoxin [1–3]. Currently, the prognosis of HCC remains poor, because the majority of these patients suffer from liver cirrhosis and are unsuitable for curative surgical treatment [4, 5]. To improve the prognosis of these patients, elucidation of the molecular mechanism of hepatocarcinogenesis is invaluable. It is well known that many types of molecular processes are deregulated in hepatoma cells, including genetic abnormalities, epigenetic alteration of oncogenes, and micro-RNA alterations [6–8]. Above all, deregulation of the cell cycle regulators is regarded as one of the important causalities of the early steps of hepatocarcinogenesis [9–11], because increased cell proliferation has been proven to be tightly correlated with cancer risk in liver cirrhosis [12, 13].

The cell cycle mainly comprises periods of DNA synthesis (S phase) and mitosis (M phase), and these two phases are segregated by interphases termed Gap1 (G1) and Gap2 (G2) phases. During cell cycle progression, multistep cellular events including DNA replication, chromosome

segregation, protein synthesis, and protein phosphorylation are sequentially regulated by cell cycle regulators, including cyclins, cyclin-dependent kinases (CDKs), and cyclin-dependent kinase inhibitors (CDKIs) [14]. Of note, many studies have highlighted that G1 phase-associated cell cycle regulators might be closely implicated in the early steps of carcinogenesis [11, 15, 16]. During cell cycle progression, G1 phase is the first step in which a cell decides to arrest in the quiescent state, transit to mitosis, or undergo the next cell cycle. Two major cyclin–CDK complexes are implicated in the progression of G1: cyclin D/CDK4 or 6 complexes for early G1 phase and cyclin E/CDK2 complex for late G1 to S phase. These cyclin/CDK complexes are required for hyperphosphorylation of retinoblastoma protein (Rb) and dissociation of transcription factor E2F from Rb, leading to the transcription of genes implicated in S phase progression. Conversely, G1 phase-related cyclin–CDK complexes are negatively regulated by two main CDKIs: $p16^{INK4A}$ (abbreviated to p16), which specifically inhibits cyclin D-Cdk4 activity, and $p27^{Kip1}$ (abbreviated to p27), which associates with and inactivates cyclin E [11, 17]. It is generally considered that p16 is inactivated via DNA methylation [18], while p27 is degraded via the ubiquitin–proteasome system in cancer cells [19]. Intriguingly, however, since our first report demonstrating that p16 is inactivated in around half of HCC by DNA methylation at multiple sites of the promoter region [20], an accumulating body of evidence has revealed that the regulatory mechanism of CDK inhibitors are more complex than previously thought. In this Award Lecture Review, we discuss recent studies of the functional mechanism of G1-phase cell cycle inhibitors p16 and p27, particularly focusing on their clinical significance in HCC (Fig. 1).

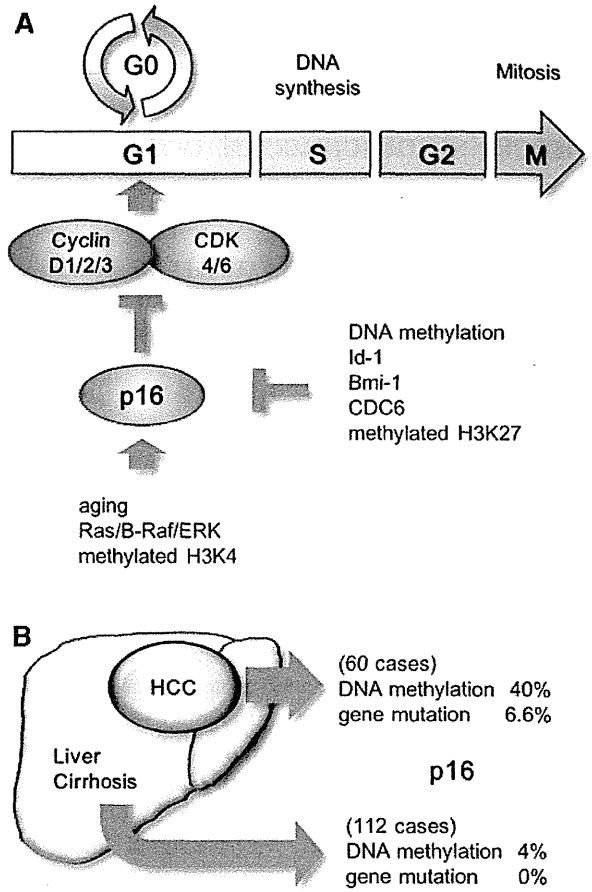

Fig. 1 **a** Key regulatory mechanisms controlling p16 activity. p16 inhibits cyclin D/CDK4 or 6 complexes at the G1–S phase. p16 is activated by RAS/B-RAF/ERK signaling and ageing, but is inhibited by DNA methylation, or by ID-1, BMI-1, CDC6, or methylated histones. **b** p16 is mainly inactivated by DNA methylation in half of HCC cases. DNA methylation of the p16 gene is also observed in some cases of liver cirrhosis

p16 as a determinant of age-related cellular senescence

p16 (also called MTS1 and INK4a) is a member of the INK4 family, which also comprises p15 (INK4b), p18 (INK4c), and p19 (INK4d). Both p16 and p15 genes are located at the same locus, INK4a/ARF/INK4b (also called CDKN2a and CDKN2b), on chromosome 9p21. They bind to CDK4 and CDK6 to block the formation of cyclin D–CDK4 or 6 complexes, leading to G1-cell cycle arrest through inhibiting phosphorylation of Rb. The INK4a/ARF/INK4b locus has been identified as encoding a tumor suppressor, because it exhibits frequent deletion in a wide variety of human cancers including melanoma, pancreatic adenocarcinoma, glioblastoma, non-small cell lung cancer, and HCC [17, 18]. This locus also produces p19ARF by an alternative reading frame shift, which induces apoptosis and cell cycle arrest through p53 activity. Interestingly, Li et al. [21] reported that the INK4a/ARF/INK4b locus is completely silenced in induced pluripotent stem (iPS) cells and embryonic stem (ES) cells, and reactivated during reprogramming, suggesting that this locus might be a gatekeeper of cell proliferation and reprogramming. Several studies have reported that p16 is involved in the senescence and proliferation of several types of progenitor cells and is even implicated in the promotion of ageing. Zindy et al. [22]. observed that the levels of p16 and $p18^{INK4c}$, but not $p15^{INK4b}$ or $p19^{INK4d}$, were increased in senescent cultured mouse embryo fibroblasts. Furthermore, Krishnamurthy et al. [23]. described that, of all the cell cycle inhibitors, p16 and p19ARF were only significantly increased in old-aged rodent tissues, suggesting that the INK4a/ARF locus might be a possible effector of age-related disorders. Their findings have been extended to further studies of a large number of aged human tissues, and it has now been