Fig1

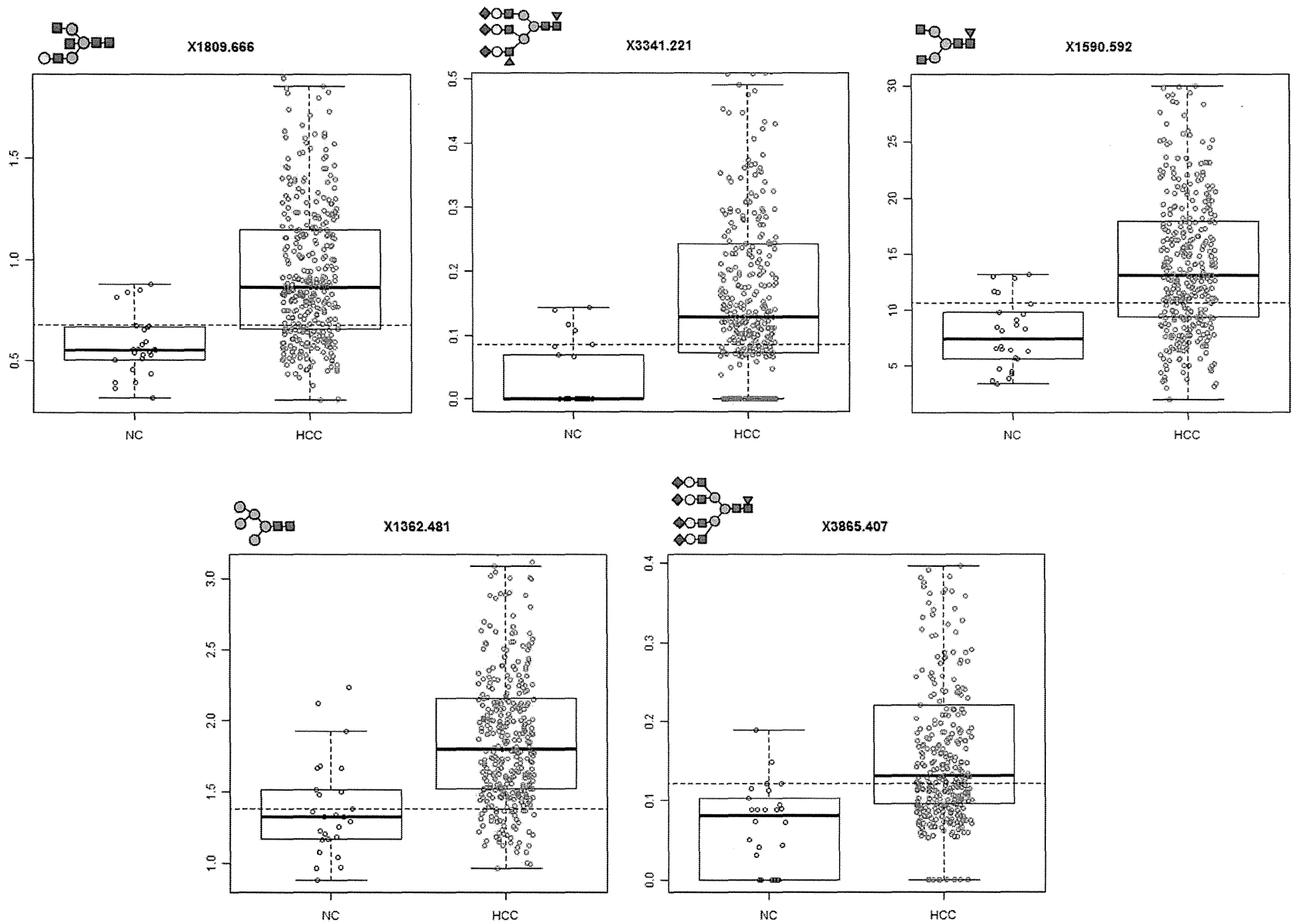
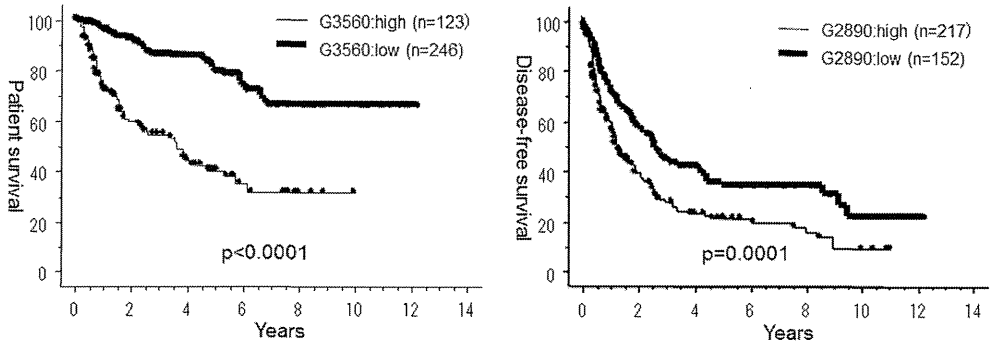


Fig1



248x93mm (300 x 300 DPI)

Intracellular localization of mesothelin predicts patient prognosis of extrahepatic bile duct cancer

FUTOSHI KAWAMATA[1], HIROFUMI KAMACHI[1], TAKAHIRO EINAMA[1], SHIGENORI HOMMA[1], MUNENORI TAHARA[1], MASAYA MIYAZAKI[3], SHINYA TANAKA[3], TOSHIYA KAMIYAMA[1], HIROSHI NISHIHARA[2], AKINOBU TAKETOMI[1] and SATORU TODO[1]

Departments of [1]General Surgery, [2]Translational Pathology and [3]Cancer Pathology, Hokkaido University School of Medicine, Sapporo 060-8638, Japan

Received July 30, 2012; Accepted September 14, 2012

DOI: 10.3892/ijo.2012.1662

Abstract. Mesothelin is expressed in various types of malignant tumors, and we recently reported that the expression of mesothelin was related to unfavorable patient outcome in pancreatic ductal adenocarcinoma and gastric adenocarcinoma. In this study, we examined the clinicopathological significance of mesothelin expression in extrahepatic bile duct cancer (EHBDCA), especially in terms of its association with the staining pattern. Tissue samples from 61 EHBDCA (9 hilar cholangiocarcinoma, 17 upper bile duct adenocarcinoma, 20 middle bile duct adenocarcinoma and 8 distal bile duct adenocarcinoma) were immunohistochemically examined. The expression levels of mesothelin in tumor cells was classified into the localization of mesothelin in luminal membrane and/or cytoplasm, in addition to high and low according to the staining intensity and proportion as a conventional analysis. 'High-level expression' of mesothelin (47.5%) was statistically correlated with liver metastasis (P=0.013) and poorer patient outcome (P=0.022), while 'luminal membrane positive' of mesothelin (52.5%) was more significantly correlated with liver metastasis (P=0.006), peritoneal metastasis (P=0.024) and unfavorable patient outcome (P=0.017). Moreover, we found that 'cytoplasmic expression' isolated from 'luminal membrane negative' of mesothelin represented the best patient prognosis throughout this study. We describe the expression pattern level of mesothelin, i.e., in luminal membrane or cytoplasm both high and low level, evidently indicate the patient prognosis of EHBDCA, suggesting the pivotal role of mesothelin in cancer promotion depending on its intracellular localization.

Correspondence to: Dr Hiroshi Nishihara, Department of Translational Pathology, Hokkaido University School of Medicine, Nishi-Ku, Kita 14, Nishi 7, Sapporo 060-8638, Japan
E-mail: hnishihara@s5.dion.ne.jp

Key words: mesothelin, intracellular localization, luminal membrane expression

Introduction

Extrahepatic bile duct cancer (EHBDCA), consisting of hilar cholangiocarcinoma and distal bile duct adenocarcinoma (excluding gallbladder cancer), is a rare disease in the United States with an incidence of 1-2/100,000/year (1). It occurs with great frequency in Asian countries, and is one of the common causes of cancer death in Japan, with near to 17,000 deaths annually (2). The 5-year survival rate of EHBDCA, even after the surgical resection is poor, ranging from 20 to 45% (3-5). The incidence of EHBDCA is increasing throughout the world with a high fatality rate; therefore, new prognostic markers and treatment for EHBDCA patients are urgently needed.

Mesothelin is expressed on normal mesothelial cells lining the pleura, pericardium and peritoneum (6,7). In addition, the overexpression of mesothelin has been found in several cancer types, including malignant mesothelioma, ovarian cancer and pancreatic cancer (8-11,12). The full length of human *mesothelin* gene codes the primary product, which is a 71-kDa precursor protein. This protein can be physiologically cleaved by certain furin-like proteases into a 40-kDa *C*-terminal fragment that remains membrane-bound and a 31-kDa *N*-terminal fragment, which is secreted into the blood (6). The *C*-terminal 40-kDa fragment is named mesothelin and is attached to the cell membrane through a glycosyl-phosphatidylinositol (GPI) anchor (13). The biological functions of mesothelin are not clearly understood, although recent studies have suggested that enforced expression of mesothelin increases cell proliferation and migration (14). In ovarian cancers, higher mesothelin expression was found to be associated with chemoresistance and shorter patient survival (15). In pancreatic cancer, mesothelin expression was immunohistochemically observed in all cases, while its absence was noted in non-cancerous pancreatic ductal epithelium, with or without pancreatitis (8,12,16,17). We recently found that the expression of mesothelin was related to an unfavorable patient outcome in pancreatic ductal adenocarcinoma (12), while the opposite result was reported in gastric cancer, in which the mesothelin expression was correlated with prolonged patients' survival (18). However, our consecutive investigation for mesothelin expression patterns in gastric cancer recently discovered that luminal membrane expression, not cytoplasmic expression

of mesothelin is a prominent negative prognostic factor for gastric cancer (19), suggesting the significance of expression pattern of mesothelin in clinicopathological analysis of cancer. In EHBDCA, Zhao *et al*, who first studied mesothelin expression in dysplasia and carcinoma of external bile duct, reported that mesothelin was expressed in 5 of 10 adenocarcinomas (50%) in cell membranes and cytoplasm (20); however, the detailed clinicopathological analysis of mesothelin expression in EHBDCA, especially with large number of the cases, has not yet been performed.

In this study, we investigated the mesothelin expression in 61 EHBDCA cases by immunohistochemistry, and its clinicopathological significance associated with patients' outcome was analyzed. Moreover, we focused on the intracellular localization of mesothelin, i.e., in luminal membrane and/or cytoplasm, and its clinicopathological significance associated with the patients' outcome.

Materials and methods

Patients' demography and tumor specimens. This study was performed with the approval of the Internal Review Board on Ethical Issues of Hokkaido University Hospital, Sapporo, Japan. The samples and the patient information were obtained under a blanket written informed consent. The subjects of this study were 61 patients who underwent radical surgery for bile duct adenocarcinoma between the years 2000 and 2008 at Hokkaido University Hospital by the Department of General Surgery, Hokkaido University, Graduate School of Medicine, Sapporo, Japan. The clinicopathological characteristics of these cases are summarized in Table I.

Mean age of patients was 67.5 years [±9.0 standard deviation (SD)]; 47 patients (77.0%) were male and 14 patients (23.0%) were female. The predominant sites of the cancer were the hilar bile duct in 16 cases (26.2%), upper bile duct in 17 cases (27.9%), middle bile duct in 20 cases (32.8%) and distal bile duct in 8 cases (13.1%). The surgical procedures consisted of the standard pancreatoduodenectomy in 21 (34.4%) cases, the pylorus-preserving pancreatoduodenecomy in 5 cases (8.2%), the extended right or left hemihepatectomy with extrahepatic bile duct resection in 28 cases (45.9%), and the extrahepatic bile duct resection in 7 cases (11.5%). Intraoperative diagnosis of the ductal resection margins was performed using frozen sections. When a positive margin was found, additional resection of marginal bile duct was performed to the maximum extent possible. R0 curative resection was achieved in 39 cases (63.9%), and R1 resection was achieved in 22 cases (36.1%). T-factor, N-factor, M-factor and clinical stage were assigned according to the TNM classification of the Union Internationale Contre le Cancer (UICC) (21). The median survival time of patients was 29.8 months (±3.5 SD).

Formalin-fixed paraffin-embedded tissue blocks were prepared from surgical specimens and sections were sliced and stained with hematoxylin and eosin (H&E) for routine histopathological examination. All specimens were diagnosed as EHBDCA.

Immunohistochemical evaluation. Immunohistochemical staining against mesothelin was performed as described previously (12). In brief, the tissue sections were incubated with a mouse monoclonal antibody against mesothelin (clone 5B2 diluted 1:50; Novocastra, Newcastle Upon Tyne, UK) at a 1:50 dilution, and reacted with a dextran polymer reagent combined with secondary antibodies and peroxidase (Envision/HRP; Dako). All assessments were made in the tumor region of the specimen (x400). Each slide was evaluated independently by three pathologists (F. Kawamata, M. Miyazaki and H. Nishihara) who did not know the clinical outcomes. Immunostaining for mesothelin was evaluated for both the proportion and staining intensity of tumor cells in each case. The proportion of mesothelin expression was assessed according to the percentage of mesothelin-positive cells as follows: 0, 0%; +1, I<10%; +2, 10-50%; and +3, >50%. The staining intensity of mesothelin was evaluated as weak (+1) and moderate to strong (+2) (Table II). The final evaluation of mesothelin expression was assessed using the following scoring system: 'high-level expression' of mesothelin was defined as ≥+3 of the proportion score and/or +2 of the intensity score, while a 'low-level expression' of mesothelin was given when the total score was ≤+3 except in cases when the proportion score was +1 and the intensity score was +2 (Fig. 1). Furthermore, among the 61 cases of EHBDCA, the staining localization of mesothelin was evaluated in luminal membrane or cytoplasm. Cases in which the luminal membrane was stained even partially or faintly (Fig. 2A), or the entire circumference of the luminal membrane was explicitly stained

Table I. Clinicopathological characteristics of 61 patients with EHBDCA in this study.

Parameter	No. of cases
Age (years)	
<60	11
≥60	50
Mean ± SD	67.5±9.0
Gender	
Male	47
Female	14
Location	
Hilar	16
Upper	17
Middle	20
Distal	8
Surgical procedure	
Pancreatoduodenectomy	21
Pylorus-preserving pancreatoduodenectomy	5
Extended right or left hemihepatectomy with bile duct resection	28
Extrahepatic bile duct resection	7
Resection status	
R0	39
R1	22
T-factor	
T1	5
T2	27
T3	19
T4	10
N-factor	
N0	25
N1	36
M-factor	
M0	58
M1	3
Stage	
IA	4
IB	14
IIA	4
IIB	28
III	8
IV	3
Median survival (months)	29.8±3.5

SD, standard deviation.

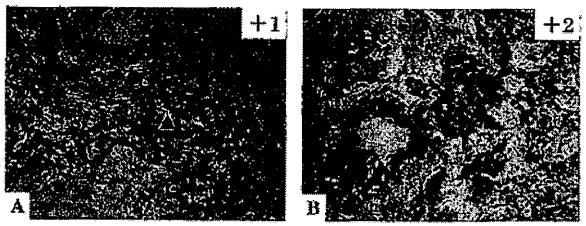


Figure 1. Representative cases of 'low-level expression' (A) and 'high-level expression' (B) of mesothelin in EHBDCA specimens by immunoistochemistly. (A) Partial luminal membrane staining (arrowhead; intensity, +1) and the weak cytoplasmic staining were observed in <50% area (proportion, +2). (B) Entire circumference of the luminal membrane was strongly positive in >50% tumor cells (intensity, +2; proportion, +3). (Magnification, x200).

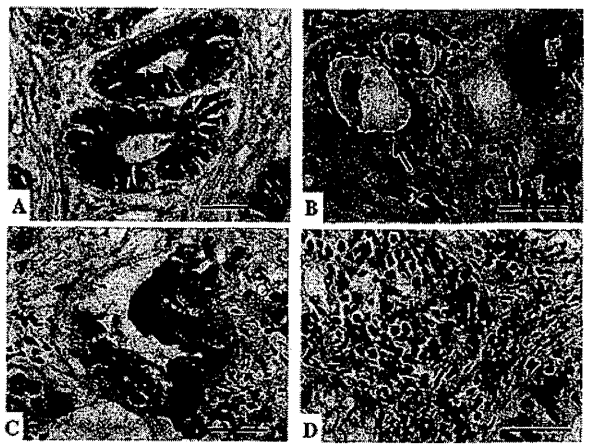


Figure 2. Representative cases of 'luminal membrane positive' (A, B) and 'luminal membrane negative' (C, D) of mesothelin in EHBDCA specimens by immunohistochemistry. (A) Granular cytoplasmic staining was observed (arrowheads; intensity, +2) and luminal membrane was also stained partially (arrows). (B) Entire circumference of the luminal membrane was explicitly stained (arrows). (C) Granular cytoplasmic, but no membranous staining in cancer cells was observed. (D) No expression of mesothelin was found in tumor cells, also designated 'mesothelin negative'. (Magnification, x400; scale bars, 50 μm).

Table II. Immunohistochemical findings of mesothelin expression.

Staining intensity on tumor cells	No. of cases (%)			
	Percentage of mesothelin-positive cells			
	0	1-10%	10-50%	>50%
Score 0	17 (27.9)	0 (0.0)	0 (0.0)	0 (0.0)
Score 1	0 (0.0)	13 (21.3)	2 (3.3)	1 (1.6)
Score 2	0 (0.0)	6 (9.8)	12 (19.7)	10 (16.4)

Table III. Correlation between mesothelin expression levels and clinicopathological features.

Parameter	Total	Mesothelin High-level (n=29)	Mesothelin Low-level (n=32)	P-value	Luminal membrane expression Positive (n=32)	Luminal membrane expression Negative (n=29)	P-value
Histopahological grade							
1 or 2	54	26	28	1.000	28	26	1.000
3	7	3	4		4	3	
pT-factor							
pT1-2	32	13	19	0.310	19	13	0.310
pT3-4	29	16	13		13	16	
pN-factor							
Negative	25	11	14	0.795	16	9	0.198
Positive	36	18	18		16	20	
pStage							
I-IIB	50	24	26	1.000	26	24	1.000
III-IV	11	5	6		6	5	
Lymphatic permeation							
Negative	23	10	13	0.792	12	11	1.000
Positive	38	19	19		20	18	
Blood vessel permeation							
Negative	26	11	15	0.606	11	15	0.200
Positive	35	18	17		21	14	
Perineural invasion							
Negative	9	3	6	0.478	3	6	0.287
Positive	52	26	26		29	23	
Resection margin							
pR0	39	20	19	0.594	24	15	0.069
pR1	22	9	13		8	14	
Recurrence							
No	18	6	12	0.172	6	12	0.090
Yes	43	23	20		26	17	
Liver metastasis							
No	47	18	29	0.013	20	27	0.006
Yes	14	11	3		12	2	
Local recurrence							
No	46	22	24	1.000	25	21	0.767
Yes	15	7	8		7	8	
Peritoneal metastasis							
No	49	20	29	0.052	22	27	0.024
Yes	12	9	3		10	2	

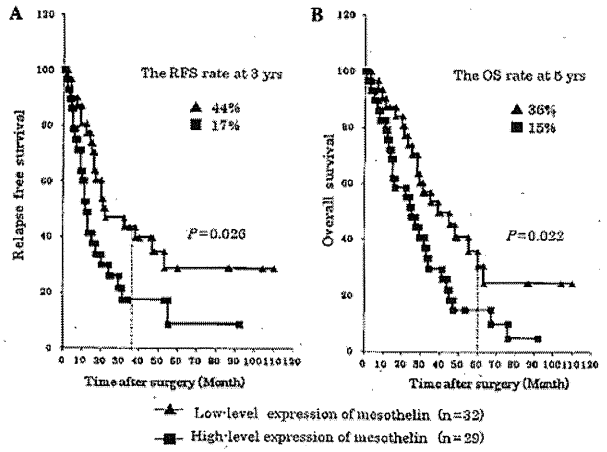


Figure 3. Relapse-free survival (RFS) and overall survival (OS) curves of EHBDCA patients according to the expression levels of mesothelin. The group of 'high-level expression' of mesothelin represented a statistically significantly unfavorable outcome compared to the group of 'low-level expression' (P=0.026 and 0.022, respectively).

(Fig. 2B) were judged as 'luminal membrane positive'. In cases with no membrane staining (Fig. 2D) and those in which only cytoplasmic staining (Fig. 2C) was observed in any intensity level, the term 'luminal membrane negative' was given.

Statistical analysis. We used the χ^2 test or Fisher's exact test to determine the correlation between mesothelin and clinicopathologic data. Survival curves for patients were drawn by the Kaplan-Meier method. Differences in survival curves were analyzed by the log-rank test. Prognostic implications of mesothelin expression and clinicopathologic parameters were analyzed by Cox univariate and multivariate proportional hazards models. All differences were considered significant at a P-value of <0.05. All statistical analyses were performed using the Ekuseru-Toukei 2010 software for Windows (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results

High-level expression of mesothelin was correlated with liver metastasis and poor patient outcome. The overexpression of mesothelin has been found in several cancer types, including malignant mesothelioma, ovarian cancer, and pancreatic cancer (11,12); thus, we first evaluated the comprehensive expression of mesothelin in EHBDCA. As described in Materials and Methods, 'high-level expression' and 'low-level expression' of mesothelin was attributed to all 61 cases of EHBDCA (Fig. 1). As summarized in Table II, 'high-level expression' was detected in 29 cases (47.5%), whereas 'low-level expression' was detected in 32 cases (52.5%). The statistical analysis for the clinicopathological parameters such as histological grade, T-factor and metastasis revealed that 'high-level expression' of mesothelin was significantly correlated with liver metastasis (P=0.013, Table III). Furthermore, recent studies reported that higher mesothelin expression was found to be associated with shorter patient survival; therefore, we examined the correlation of mesothelin overexpression with relapse-free survival (RFS) and overall survival (OS) in the EHBDCA patients. The group of 'high-level expression' of mesothelin had a significantly poorer RFS than the group of 'low-level expression' of mesothelin (P=0.026). In addition, the group of 'high-level expression' of mesothelin had a significantly poorer OS than the group of 'low-level expression' of mesothelin (P=0.022) (Fig. 3).

Luminal membrane expression of mesothelin is a prominent negative prognostic factor for the patients with EHBDCA. During our previous studies on pancreatic adenocarcinoma and gastric adenocarcinoma, we already noted that expression of mesothelin was found in the luminal membrane as well as in the cytoplasm (19). Mesothelin was reported to attach to the cell membrane through a glycosyl-phosphatidylinositol (GPI) anchor after being physiologically cleaved by some furin-like proteases (22), which are involved in the translocation of mesothelin, although the biological functions of mesothelin associated with its intracellular localization are not fully understood. Thus, we analyzed the intracellular localization of mesothelin by immunostaining to explore the clinicopathological significance of its translocation.

As shown in Table III, the group 'luminal membrane positive', which consisted of the cases with luminal membrane staining even partially, was 32 (52.5%) cases, while the group 'luminal membrane negative', which contained 17 cases which were completely mesothelin negative was comprised of 29 (47.5%) cases. The statistical analysis revealed that the incidence of luminal membrane positivity was significantly correlated with peritoneal metastasis (P=0.024) in addition to liver metastasis (P=0.006) (Table III). The analysis of the patients' overall survival showed that 'luminal membrane positive' of mesothelin indicated a significantly unfavorable RFS (P=0.012) and OS (P=0.017) compared to 'luminal membrane negative' of mesothelin (Fig. 4).

To clarify the mesothelin expression as an independent prognostic factor, we performed a univariate analysis of the 61 EHBDCA using the Cox proportional hazards model, the result indicated that resection margin, 'high-level expression' and 'luminal membrane positive' of mesothelin were significantly correlated with risks of cancer mortality. Multivariate analysis also confirmed that resection margin (RR 3.361, 95% CI, 1.670-6.763, P=0.0007) and 'luminal membrane positive' of mesothelin (RR 2.964, 95% CI, 1.401-6.296, P=0.0045) were independent predictors of the overall patient survival (Table IV).

Isolation of 'cytoplasmic expression' of mesothelin potentiates more exquisite prediction of prognosis in EHBDCA. To explore the clinicopathological value of the cytoplasmic expression of mesothelin, we performed a sub-analysis in 'luminal membrane negative', dividing the group into 17 cases of 'mesothelin negative' and 12 cases of 'cytoplasmic expression'. The P-value (OS, P=0.0085) between 'luminal membrane positive' and 'cytoplasmic expression' was minimum in these

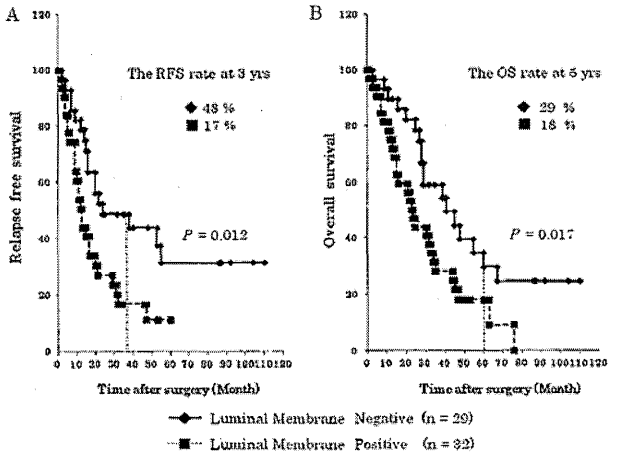


Figure 4. Relapse-free survival (RFS) and overall survival (OS) curves of EHBDCA patients according to the expression pattern of mesothelin. The group of 'luminal membrane positive' represented a statistically significantly unfavorable outcome compared to the group of 'luminal membrane negative' (P=0.012 and 0.017, respectively).

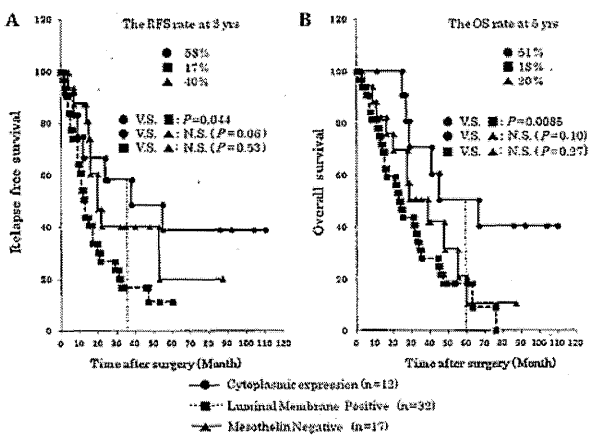


Figure 5. Relapse-free survival (RFS) and overall survival (OS) curves of EHBDCA patients among three groups of detailed expression patterns of mesothelin. 'Cytoplasmic expression' of mesothelin represented the best prognosis among the 3 groups.

survival analyses, suggesting the clinical benefit of isolation of 'cytoplasmic expression' of mesothelin (Fig. 5). Interestingly, 'cytoplasmic expression' of mesothelin represented relatively favorable patients' prognosis compared to 'mesothelin negative', although it was statistically not significant (RFS, P=0.06; OS, P=0.10).

Discussion

In this study, we confirmed that mesothelin expression is a prominent prognostic factor for EHBDCA patients as well as for other tumors such as pancreatic cancer and ovarian carcinoma described previously (12,15,23). Furthermore, we revealed that the expression pattern of mesothelin, in luminal membrane or cytoplasm, could be a more evident prediction factor for these patients. These results evidently support our recent report of mesothelin expression patterns in gastric cancer in which luminal membrane expression, not cytoplasmic expression of mesothelin is a prominent negative prognostic factor for gastric cancer (19).

The mechanism for the membranous localization of mesothelin should be explained as follows: the full length of the

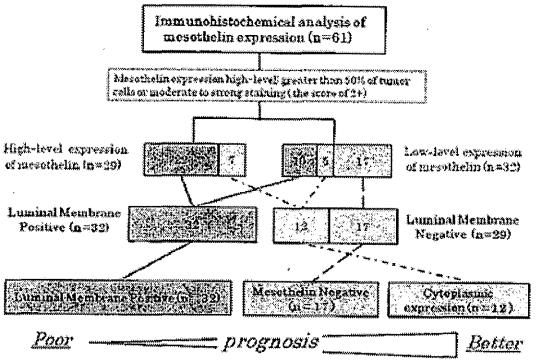


Figure 6. Flow chart of immunohistochemical evaluation of mesothelin expression and the prognostic aspect. The P-value (OS, P=0.0085) between 'luminal membrane positive' and 'cytoplasmic expression' was minimum in our survival analyses, suggesting the clinical benefit of isolation of 'cytoplasmic expression' of mesothelin.

Table IV. Univariate and multivariate analysis of patients' survival in EHBDCA.

		Univariate analysis		Multivariate analysis		
Factor	n=61	P-value	RR (95% CI)	RR (95% CI)	Hazard ratio	P-value
Histopahological grade						
1 or 2	54	0.3931	1		NC	
3	7		1.508 (0.588-3.871)			
pT-factor						
pT1-2	32	0.4264	1		NC	
pT3-4	29		1.266 (0.708-2.262)			
pN-factor						
Negative	25	0.3639	1		NC	
Positive	36		1.314 (0.729-2.368)			
pStage						
I-IIB	50	0.2026	1		NC	
III-IV	11		1.608 (0.774-3.339)			
Lymphatic permeation						
Negative	23	0.1908	1		NC	
Positive	38		1.537 (0.807-2.924)			
Blood vessel permeation						
Negative	26	0.2999	1		NC	
Positive	35		1.370 (0.756-2.482)			
Perineural invasion						
Negative	9	0.4733	1		NC	
Positive	52		0.728 (0.306-1.732)			
Resection margin						
pR0	39	**0.0398**	1	1.670-6.763	1	**0.0007**
pR1	22		1.859 (1.029-3.356)		3.361	
Mesothelin expression						
Low-level	32	**0.0236**	1	0.864-3.067	1	0.1317
High-level	29		1.968 (1.095-3.538)		1.621	
Luminal membrane expression of mesothelin						
Negative	29	**0.0175**	1	1.401-6.296	1	**0.0045**
Positive	32		2.078 (1.137-3.798)		2.964	

RR indicates relative risk/hazard ratio; CI, confidence interval. NC, not calculable.

Table V. Sub-analysis among three groups according to the intracellular expression pattern of mesothelin.

Parameter	Total (n=44)	Luminal membrane positive (n=32)	Cytoplasmic expression (n=12)	P-value	Total (n=49)	Luminal membrane positive (n=32)	Negative expression (n=17)	P-value	Total (n=29)	Cytoplasmic expression (n=12)	Negative expression (n=17)	P-value
Histopahological grade												
1 or 2	39	28	11	1.000	43	28	15	1.000	26	11	15	1.000
3	5	4	1		6	4	2		3	1	2	
pT-factor												
pT1-2	23	19	4	0.179	28	19	9	0.765	13	4	9	0.452
pT3-4	21	13	8		21	13	8		16	8	8	
pN-factor												
Negative	18	16	2	0.083	23	16	7	0.764	9	2	7	0.234
Positive	26	16	10		26	16	10		20	10	10	
pStage												
I-IIB	37	26	11	0.653	39	26	13	0.722	24	11	13	0.370
III-IV	7	6	1		10	6	4		5	1	4	
Lymphatic permeation												
Negative	14	12	2	0.282	21	12	9	0.370	11	2	9	0.064
Positive	30	20	10		28	20	8		18	10	8	
Blood vessel permeation												
Negative	16	11	5	0.732	21	11	10	0.134	15	5	10	0.462
Positive	28	21	7		28	21	7		14	7	7	
Perineural invasion												
Negative	3	3	0	0.551	9	3	6	**0.049**	6	0	6	**0.028**
Positive	41	29	12		40	29	11		23	12	11	
Resection margin												
pR0	30	24	6	0.152	32	24	8	0.065	14	6	8	1.000
pR1	14	8	6		17	8	9		15	6	9	
Recurrence												
No	11	6	5	0.139	13	6	7	0.172	12	5	7	1.000
Yes	33	26	7		36	26	10		17	7	10	
Liver metastasis												
No	30	20	10	0.282	36	20	16	**0.020**	26	10	16	0.553
Yes	14	12	2		13	12	1		3	2	1	
Local recurrence												
No	34	25	9	1.000	37	25	12	0.729	21	9	12	1.000
Yes	10	7	3		12	7	5		8	3	5	
Peritoneal metastasis												
No	34	22	12	**0.041**	37	22	15	0.175	27	12	15	0.498
Yes	10	10	0		12	10	2		2	0	2	

human *mesothelin* gene encodes a 71-kDa precursor protein that is proteolytically cleaved by some furin-like proteases into an *N*-terminal secreted form and a *C*-terminal fragment, the 40-kDa mesothelin, which is a glycosyl-phosphatidylinositol (GPI)-linked glycoprotein (6,13,15). Many researchers have investigated the role of the mesothelin expression in tumor biology and demonstrated the importance of mesothelin expression for tumor progression *in vitro* (14,24-26) and *in vivo* (27,28); however, the clinicopathological significance of the membrane localization of mesothelin has not been clarified. The SB2 anti-mesothelin antibody, which we employed here for IHC, can detect both the 71-kDa precursor protein and the 40-kDa *C*-terminal fragment, but not the 30-kDa *N*-terminal fragment. According to the reported molecular processing mechanism of mesothelin and specificity of antibody, luminal membrane staining probably indicates the 40-kDa membrane-bound form of mesothelin, while cytoplasmic staining would mean the 71-kDa precursor form of mesothelin. Our results support the idea that the 40-kDa membrane-bound form of mesothelin is an active form and promotes the aggressive features including increased cell motility, invasion or migration capabilities and growth of metastatic tumors (24,25,29).

The fact that 'cytoplasmic expression' of mesothelin paradoxically resulted in better OS than mesothelin with 'mesothelin negative' took us by surprise (Fig. 5B). The RFS rate at 3 years (58 and 40%, respectively) and OS at 5 years (61 and 20%, respectively) were demonstrably better in 'cytoplasmic expression' compared to 'mesothelin negative', although the final RFS and OS were not statistically significant (RFS, P=0.06; OS, P=0.10). As indicated above, the majority of mesothelin in cytoplasm must be the 71-kDa precursor form and might behave like a dominant negative form of mesothelin as a tumor suppressor. The conflicting results in some previous reports in which mesothelin expression was correlated with prolonged patient survival in gastric cancer (18) and in ovarian serous carcinoma (30), may be explained by confusing the luminal membrane and cytoplasmic expression of mesothelin. Isolation of 'mesothelin negative' might give us another disease entity, mesothelin-independent EHBDCA. The tumor cells in such a type of EHBDCA would obtain invasive ability without the association of mesothelin; therefore, this could indicate an alternative gene expression profiling. In fact, additional sub-analysis for clinicopathological parameters among the three groups showed interesting results. Frequent perineural invasion was observed in 'mesothelin negative' rather than in mesothelin positive cases even in luminal membrane or cytoplasm (P=0.049 and 0.028, respectively), while liver metastasis was abundantly found in 'luminal membrane positive' (Table V). Such conflicting results may suggest the distinct oncogenic process between mesothelin-associated and mesothelin-independent EHBDCA.

In terms of discovering the clinicopathological parameters, there are many previous studies demonstrating the prognostic significance of various molecules, such as epidermal growth factor receptor (EGFR) and c-erbB-2 (HER-2) in colorectal, breast and lung cancer (31). There are some other case reports describing a series of promising results targeting EGFR in patients with advanced biliary tract cancer (32-34); however, identification of useful prognostic markers for EHBDCA still needs investigation. In addition, lack of effective adjuvant therapy against advanced EHBDCA requires establishing new therapeutic methods based on reliable molecular targeting markers; thus, mesothelin could be one of the potential targets for cancer molecular targeting therapy. Recombinant anti-mesothelin immunotoxin SS1P (CAT-5001) and a high affinity chimeric anti-mesothelin monoclonal antibody MORAb-009 recently entered phase II clinical trials (35,36). To evaluate the therapeutic effect of such antibody-based medicine, pathological verification of membranous expression of the target molecule must be performed, because antibody-based drugs can usually access the molecules located on the cell membrane. We believe that luminal membrane expression of mesothelin in EHBDCA would be of clinical benefit not only as a prognostic factor but also as a predictive factor for the eligibility to mesothelin-targeting therapies (13,14,27,37,38).

In conclusion, we demonstrated the clinicopathological significance of the mesothelin expression as an independent prognostic factor. Moreover, identification of luminal membrane or cytoplasmic expression of mesothelin could be a reliable prognostic factor for EHBDCA and might offer a novel therapeutic strategy for patients with EHBDCA, including immunotherapy using peptide vaccine or monoclonal antibody therapy.

Acknowledgements

This research was supported by a Grant-in-Aid for Scientific Research (KAKENHI). The study sponsors had no involvement in the study design, in the collection, analysis and interpretation of data, in the writing of the manuscript, or in the decision to submit the manuscript for publication.

References

1. Ito K, Ito H, Allen PJ, *et al*: Adequate lymph node assessment for extrahepatic bile duct adenocarcinoma. Ann Surg 251: 675-681, 2010.
2. Ohashi M, Kusumi T, Sato F, *et al*: Expression of syndecan-1 and E-cadherin is inversely correlated with poor patient's prognosis and recurrent status of extrahepatic bile duct carcinoma. Biomed Res 30: 79-86, 2009.
3. Jarnagin WR, Fong Y, DeMatteo RP, *et al*: Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. Ann Surg 234: 507-519, 2001.
4. Akoad M and Jenkins R: Proximal biliary malignancy. Surg Clin North Am 88: 1409-1428, x-xi, 2008.
5. Veillette G and Castillo CF: Distal biliary malignancy. Surg Clin North Am 88: 1429-1447, xi, 2008.
6. Chang K and Pastan I: Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc Natl Acad Sci USA 93: 136-140, 1996.
7. Chang K, Pastan I and Willingham MC: Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. Int J Cancer 50: 373-381, 1992.
8. Argani P, Iacobuzio-Donahue C, Ryu B, *et al*: Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clin Cancer Res 7: 3862-3868, 2001.
9. Hassan R, Kreitman RJ, Pastan I and Willingham MC: Localization of mesothelin in epithelial ovarian cancer. Appl Immunohistochem Mol Morphol 13: 243-247, 2005.
10. Ordonez NG: Value of mesothelin immunostaining in the diagnosis of mesothelioma. Mod Pathol 16: 192-197, 2003.
11. Ordonez NG: Application of mesothelin immunostaining in tumor diagnosis. Am J Surg Pathol 27: 1418-1428, 2003.

12. Einama T, Kamachi H, Nishihara H, *et al*: Co-Expression of mesothelin and CA125 correlates with unfavorable patient outcome in pancreatic ductal adenocarcinoma. Pancreas 40: 1276-1282, 2011.
13. Hassan R, Bera T and Pastan I: Mesothelin: a new target for immunotherapy. Clin Cancer Res 10: 3937-3942, 2004.
14. Li M, Bharadwaj U, Zhang R, *et al*: Mesothelin is a malignant factor and therapeutic vaccine target for pancreatic cancer. Mol Cancer Ther 7: 286-296, 2008.
15. Cheng WF, Huang CY, Chang MC, *et al*: High mesothelin correlates with chemoresistance and poor survival in epithelial ovarian carcinoma. Br J Cancer 100: 1144-1153, 2009.
16. Hassan R, Laszik ZG, Lerner M, Raffeld M, Postier R and Brackett D: Mesothelin is overexpressed in pancreaticobiliary adenocarcinomas but not in normal pancreas and chronic pancreatitis. Am J Clin Pathol 124: 838-845, 2005.
17. Swierczynski SL, Maitra A, Abraham SC, *et al*: Analysis of novel tumor markers in pancreatic and biliary carcinomas using tissue microarrays. Hum Pathol 35: 357-366, 2004.
18. Baba K, Ishigami S, Arigami T, *et al*: Mesothelin expression correlates with prolonged patient survival in gastric cancer. J Surg Oncol 105: 195-199, 2012.
19. Einama T, Homma S, Kamachi H, *et al*: Luminal membrane expression of mesothelin is a prominent poor prognostic factor for gastric cancer. Br J Cancer 107: 137-142, 2012.
20. Zhao H, Davydova L, Mandich D, Cartun RW and Ligato S: S100A4 protein and mesothelin expression in dysplasia and carcinoma of the extrahepatic bile duct. Am J Clin Pathol 127: 374-379, 2007.
21. Sobin LH and Wittekind CW (eds): TNM Classification of Malignant Tumors. 6th edition. Wiley-Liss, New York, 2002.
22. Inami K, Kajino K, Abe M, *et al*: Secretion of N-ERC/mesothelin and expression of C-ERC/mesothelin in human pancreatic ductal carcinoma. Oncol Rep 20: 1375-1380, 2008.
23. Shimizu A, Hirono S, Tani M, *et al*: Coexpression of MUC16 and mesothelin is related to the invasion process in pancreatic ductal adenocarcinoma. Cancer Sci 103: 739-746, 2012.
24. Bharadwaj U, Marin-Muller C, Li M, Chen C and Yao Q: Mesothelin overexpression promotes autocrine IL-6/sIL-6R trans-signaling to stimulate pancreatic cancer cell proliferation. Carcinogenesis 32: 1013-1024, 2011.
25. Bharadwaj U, Marin-Muller C, Li M, Chen C and Yao Q: Mesothelin confers pancreatic cancer cell resistance to TNF-α-induced apoptosis through Akt/PI3K/NF-κB activation and IL-6/Mcl-1 overexpression. Mol Cancer 10: 106, 2011.
26. Chang MC, Chen CA, Hsieh CY, *et al*: Mesothelin inhibits paclitaxel-induced apoptosis through the PI3K pathway. Biochem J 424: 449-458, 2009.
27. Hassan R, Schweizer C, Lu KF, *et al*: Inhibition of mesothelin-CA-125 interaction in patients with mesothelioma by the anti-mesothelin monoclonal antibody MORAb-009: implications for cancer therapy. Lung Cancer 68: 455-459, 2010.
28. Bharadwaj U, Li M, Chen C and Yao Q: Mesothelin-induced pancreatic cancer cell proliferation involves alteration of cyclin E via activation of signal transducer and activator of transcription protein 3. Mol Cancer Res 6: 1755-1765, 2008.
29. Inami K, Abe M, Takeda K, *et al*: Antitumor activity of anti-C-ERC/mesothelin monoclonal antibody in vivo. Cancer Sci 101: 969-974, 2009.
30. Yen MJ, Hsu CY, Mao TL, *et al*: Diffuse mesothelin expression correlates with prolonged patient survival in ovarian serous carcinoma. Clin Cancer Res 12: 827-831, 2006.
31. Hudis CA: Trastuzumab - mechanism of action and use in clinical practice. N Engl J Med 357: 39-51, 2007.
32. Huang TW, Wang CH and Hsieh CB: Effects of the anti-epidermal growth factor receptor antibody cetuximab on cholangiocarcinoma of the liver. Onkologie 30: 129-131, 2007.
33. Sprinzl MF, Schimanski CC, Moehler M, Schadmand-Fischer S, Galle PR and Kanzler S: Gemcitabine in combination with EGF-Receptor antibody (Cetuximab) as a treatment of cholangiocarcinoma: a case report. BMC Cancer 6: 190, 2006.
34. Bralet MP, Bellin MF, Guettier C, Adam R and Paule B: Response to cetuximab and gemcitabine-oxaliplatin in an advanced case of intrahepatic cholangiocarcinoma. Clin Oncol (R Coll Radiol) 18: 426, 2006.
35. Kreitman RJ, Hassan R, Fitzgerald DJ and Pastan I: Phase I trial of continuous infusion anti-mesothelin recombinant immunotoxin SS1P. Clin Cancer Res 15: 5274-5279, 2009.
36. Hassan R, Cohen SJ, Phillips M, *et al*: Phase I clinical trial of the Chimeric anti-mesothelin monoclonal antibody MORAb-009 in patients with mesothelin expressing cancers. Clin Cancer Res 16: 6132-6138, 2010.
37. Hassan R, Bullock S, Premkumar A, *et al*: Phase I study of SS1P, a recombinant anti-mesothelin immunotoxin given as a bolus I.V. infusion to patients with mesothelin-expressing mesothelioma, ovarian, and pancreatic cancers. Clin Cancer Res 13: 5144-5149, 2007.
38. Hassan R, Ebel W, Routhier EL, *et al*: Preclinical evaluation of MORAb-009, a chimeric antibody targeting tumor-associated mesothelin. Cancer Immun 7: 20, 2007.

Surg Today (2013) 43:289–292
DOI 10.1007/s00595-012-0462-8

ORIGINAL ARTICLE

A rare point mutation in the Ras oncogene in hepatocellular carcinoma

Akinobu Taketomi · Ken Shirabe · Jun Muto · Shohei Yoshiya · Takashi Motomura · Yohei Mano · Tohru Ikegami · Tomoharu Yoshizumi · Kenji Sugio · Yoshihiko Maehara

Received: 16 May 2011 / Accepted: 17 May 2011 / Published online: 26 December 2012

Abstract
Purpose The Ras gene is one of the oncogenes most frequently detected in human cancers, and codes for three proteins (K-, N-, and H-Ras). The aim of this study was to examine the mutations in codons 12, 13 and 61 of the three Ras genes in cases of human hepatocellular carcinoma (HCC).
Methods Paired samples of HCC and corresponding non-malignant liver tissue were collected from 61 patients who underwent hepatectomy. A dot-blot analysis was used to analyze the products of the polymerase chain reaction (PCR) amplification of codons 12, 13, and 61 of K-, N- and H-Ras for mutations.
Results Only one mutation (K-Ras codon 13; Gly to Asp) was detected among the 61 patients. Interestingly, this patient had a medical history of surgery for both gastric cancer and right lung cancer. No mutations were found in codons 12 and 61 of K-Ras or codons 12, 13 and 61 of the N-Ras and H-Ras genes in any of the HCCs or corresponding non-malignant tissues.
Conclusions These findings indicated that the activation of Ras proto-oncogenes by mutations in codons 12, 13, and 61 does not play a major role in hepatocellular carcinogenesis.

Keywords Ras · Mutation · Hepatocellular carcinoma · Sorafenib

A. Taketomi (✉) · K. Shirabe · J. Muto · S. Yoshiya · T. Motomura · Y. Mano · T. Ikegami · T. Yoshizumi · K. Sugio · Y. Maehara
Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Japan
e-mail: taketomi@med.hokudai.ac.jp; taketomi@surg2.med.kyushu-u.ac.jp

Abbreviations

Asp	Asparagine
Glu	Glutamate
Gly	Glycine
HCC	Hepatocellular carcinoma
Lys	Lysine
PCR	Polymerase chain reaction
TTP	Time to progression
Val	Valine

Introduction

Hepatocellular carcinoma (HCC) is a global health problem, accounting for more than 80 % of all primary liver cancers, and is one of the most common malignancies worldwide [1]. Most patients with HCC also present with concomitant cirrhosis, which is the major clinical risk factor for hepatic cancer, and results from alcoholism or infection with the hepatitis B or hepatitis C virus. Primary liver malignancies (95 % of which are HCC) are the third and fifth leading causes of cancer death among males and females, respectively, in Japan [2]. Both liver resection and liver transplantation are potentially curative treatments for HCC [3–5]. Although other treatment options, including percutaneous radiofrequency ablation or chemolipiodolization are also available, there is no standard systemic therapy for advanced cases.

Sorafenib (BAY 43-9006, Nexavar) is a novel oral kinase inhibitor that targets multiple tyrosine kinases in vivo and in vitro, and is widely used for HCC [6]. The main targets of sorafenib are the receptor tyrosine kinase pathways which are frequently deregulated in cancer, such as the Ras pathway. The Ras pathway represents a dominant signaling network promoting cell proliferation and

survival. The binding of different growth factors (e.g. epidermal growth factor: EGF) to their receptors (e.g. epidermal growth factor receptor: EGFR) induces the activation of Ras, which in turn activates c-raf, MEK and ERK. Phosphorylated ERK in the nucleus activates transcription factors that regulate the expression of genes involved in cell proliferation and survival.

A phase II trial involving 137 patients with advanced HCC showed that sorafenib induced partial responses in less than 5 % of patients, but the observed median survival of 9.2 months with a median time to progression of 5.5 months was classified as evidence of potential clinical benefit, since the expected median survival of these patients is 6 months [7]. Consequently, a large phase III clinical trial (SHARP) was conducted in 602 patients with advanced HCC. The results showed a 31 % decrease in the risk of death, with a median survival of 10.6 months in the sorafenib arm versus 7.9 months for placebo [8]. In addition, sorafenib showed a significant benefit in terms of the time to progression (TTP) as assessed by independent radiological review, with a median TTP of 5.5 months for the sorafenib and 2.8 months for the placebo arm.

Because Ras is one of the targets of sorafenib, it is important to determine whether mutations in the Ras gene result in the activation of the Ras/MAPK pathway in human HCCs. However, the relationship between Ras mutations and human HCC has not been fully evaluated. The present study was designed to investigate K-, N- and H-Ras (*KRAS, NRAS, HRAS*) somatic mutations in human HCC.

Materials and methods

Patients and tumor samples

Tumor tissue samples were obtained from 61 Japanese patients who underwent surgical resection for HCC during the period between December 1989 and April 1992 in the Department of Surgery and Science, Kyushu University Hospital, Fukuoka, Japan. Surgically resected tissue samples were frozen at −80 °C immediately after resection and were stored until use in this study. Written informed consent was obtained from all patients examined, and the current study was approved by the Kyushu University ethics committee.

DNA preparation and detection of Ras point mutations

High molecular weight DNA was isolated from frozen tumor samples, as described elsewhere [9]. Selective amplification of the Ras gene sequence was done using a PCR technique. The nucleotide sequences of the primers used are listed in Table 1. The PCR was performed at

Table 1 Ras gene primers used in this study

Gene/codon	Length (bp)	Sequence	
KRAS/12, 13	108	Forward	GACTGAATATAAACTTGTGG
		Reverse	CTATTGTTGGATCATATTCG
KRAS/61	128	Forward	TTCCTACAGGAAGCAAGTAG
		Reverse	CACAAAGAAAGCCCTCCCCA
HRAS/12, 13	63	Forward	GACGGAATATAAGCTGGTGG
		Reverse	TGGATGGTCAGCGCACTCTT
HRAS/61	73	Forward	AGACGTGCCTGTTGGACATC
		Reverse	CGCATGTACTGGTCCCGCAT
NRAS/12, 13	109	Forward	GACTGAGTACAAACTGGTGG
		Reverse	CTCTATGGTGGGATCATATT
NRAS/61	103	Forward	GGTGAAACCTGTTTGTTGGA
		Reverse	ATACACAGAGGAAGCCTTCG

bp base pairs

96 °C to denature the DNA (1 min), at 55 °C (*NRAS*), 57 °C (*KRAS*), 62 °C (*HRAS*) to anneal the primer (30 s), and at 72 °C to synthesize DNA (10 s to 1 min) using Taq DNA polymerase for 35–40 cycles in a DNA thermal cycler (Perkin-Elmer-Cetus). Amplified DNA samples were spotted onto nylon membranes (Hybond N+) for the hybridization analysis. All of the DNA isolated from the 61 tumor samples and the corresponding non-malignant liver tissues were screened for activated point mutations in codons 12, 13, and 61 of all three Ras genes using an oligonucleotide specific for the different sequences. The filters were prehybridized for 1 h at 55 °C in solution A (3.0 M tetramethylammonium chloride, 50 mM Tris-HCI, 2 HIMEDTA, 0.1 % SDS, 5× Denhardt's solution, 100 fg/ml denatured herring sperm DNA), and hybridized for 1 h at 55 °C in the same solution with 5 pmol ^{32}P-labeled probe. These filters were washed twice in 0.3 M NaCl, 0.02 M NaH2PO4, 2 mM EDTA and 0.1 % SDS at room temperature for 5 min, and in solution A without Denhardt's solution and herring sperm DNA, once for 5 min at room temperature and twice for 10 min at 60 °C. These filters were then exposed to Kodak XAR5 film. Human cancer cell lines carrying Ras genes mutations were used as positive controls. The colon cancer cell lines: SW620 (*KRAS* codon 12 GTT:Val), LSI80 (*KRAS* codon 12 GAT:Asp), and LOVO (*KRAS* codon 13 GAC:Asp) were obtained from the Japanese Cancer Research Resources Bank, and KMS4 (*KRASs* codon 12 TGT:Cys) was provided by Dr. Sugio (Institution?).

Results

The age of the 61 patients ranged from 43 to 79 years (average, 64.1 years), and 46 were males and 15 were

females. The positive rate of hepatitis surface B antigen was 12.9 %, and the positive rate of anti-hepatitis C virus antibody was 72.7 %. The mean tumor size was 4.47 cm.

One of the 61 HCCs (1.6 %) carried a point mutation, which was a G to A transition at codon 13 of the *KRAS* gene (Fig. 1). DNA extracted from the corresponding non-malignant liver tissue had the normal codon, suggesting that mutational activation of K-ras was involved in the malignant transformation in this case. This patient was positive for anti-hepatitis C virus antibodies, and was classified to have Child-Pugh A disease. The diameter of this patient's tumor was 12 cm, and the tumor was composed of well to moderately differentiated hepatocellular carcinoma. Interestingly, this patient had undergone surgery for gastric cancer 18 years before and lung cancer 12 years before the surgery for HCC.

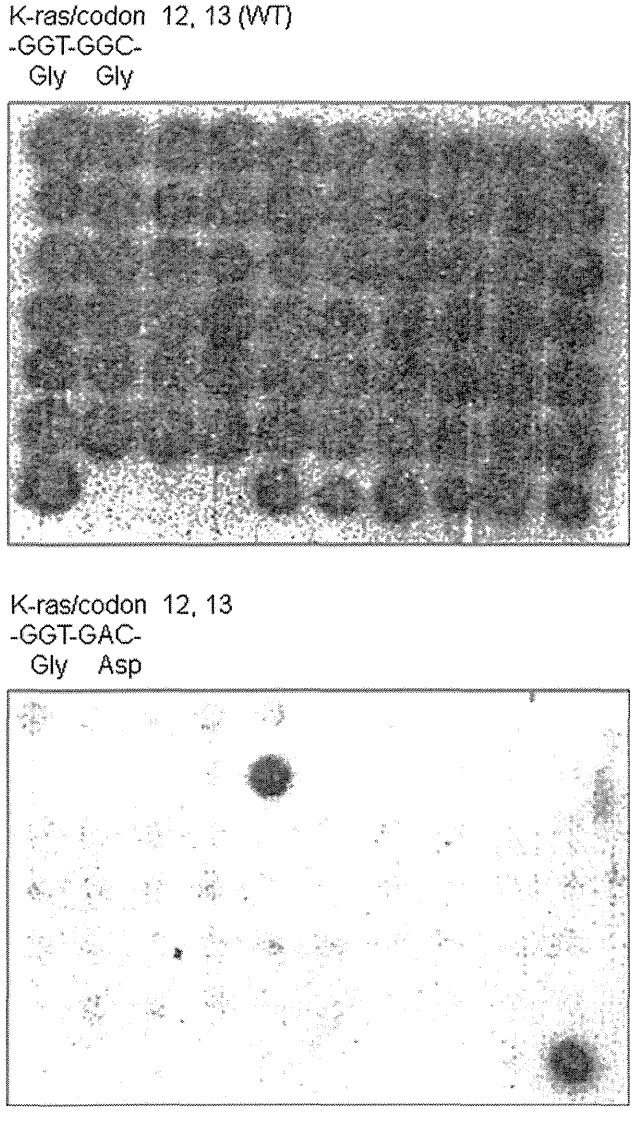


Fig. 1 Detection of a *KRAS* gene mutation in a patient with hepatocellular carcinoma. PCR-amplified DNA from 61 tumor samples was dotted onto nylon membranes and hybridized to a ^{32}P-labeled oligonucleotide probe. *WT* wild type *KRAS*

No mutational activation was found in codons 12 and 61 of *KRAS* or codons 12, 13 and 61 of the *NRAS* and *HRAS* genes in any of the HCCs or corresponding non-malignant tissue samples.

Discussion

This study examined 61 HCC tissues and their corresponding non-malignant liver tissues for a somatic mutation in codons 12, 13, and 61 of the *KRAS*, *HRAS*, or *NRAS* genes, which are known hot spots in various malignancies. However, the study showed the only one of the 61 HCCs (1.6 %) had a somatic mutation in codon 13 of the *KRAS* gene, indicating that Ras gene mutations do not appear to be related to the pathogenesis of most HCCs.

There have been several reports with small sample sizes regarding Ras gene mutations in HCC (Table 2). Most have reported that somatic mutations of the Ras gene in HCCs are uncommon, similar to the current study. Tsuda et al. [10] found only two tumors with Ras point mutations in surgically resected specimens from 30 HCC patients. In their patients, codon 12 of *KRAS* was altered from GGT, coding for Gly, to GTT, coding for Val in one case, and codon 61 of *NRAS* was altered from CAA, coding for Glu, to AAA, coding for Lys, in the other case. Tada et al. analyzed the mutations of the three Ras genes in 23 primary hepatic malignant tumors (12 hepatocellular carcinomas, nine cholangiocarcinomas, and two hepatoblastomas). Point mutations in *KRAS* codon 12 or *KRAS* codon 61 were found in 6 of the 9 cholangiocarcinomas. In contrast, there were no point mutations in any of 12 HCCs or two hepatoblastomas in codons 12, 13, or 61 of the Ras genes. The authors concluded that Ras gene mutations are not related to the pathogenesis of HCC, but play an important role in pathogenesis of cholangiocarcinoma.

Sorafenib is the first molecule with specific targets involved in the pathogenesis of HCC that has become available for routine clinical use. It is an orally applicable

Table 2 Reported Ras gene mutations in HCC patients

Author [references]	No. of patients	Ras gene mutation		
		KRAS	*NRAS*	*HRAS*
Tsuda et al. [10]	30	1 (codon 12)	1 (codon 61)	0
Tada et al. [14]	12	0	0	0
Ogata et al. [15]	19			2
Challen et al. [16]	19	1 (codon 61)	3 (codon 61)	0
Leon et al. [17]	12	1 (codon 61)	0	0
This study	61	1 (codon 13)	0	0

multi-kinase inhibitor that acts by blocking tumor cell proliferation and angiogenesis through the inhibition of serine/threonine kinases [11]. Sorafenib can increase survival by up to 3 months in patients with advanced HCC and acceptable liver function [8]. On the other hand, severe side effects have been reported with sorafenib, including hand-foot skin reactions or liver dysfunction [7, 8]. Therefore, it is important to identify prognostic markers and to establish the proper selection criteria for using sorafenib. Mutations of the Ras genes in cases of HCCs were systemically evaluated in this study because the Ras signaling pathway is the main target of sorafenib. The results indicated that mutational activation of Ras genes is uncommon in the pathogenesis of HCCs. Caraglia et al. [12] reported that the presence of phosphorylated ERK activity in peripheral blood mononuclear cells is valuable for predicting the response to sorafenib therapy in HCC patients. An in vitro study confirmed that phosphorylated ERK was a potential biomarker predicting the sensitivity of HCC to sorafenib [13]. Therefore, a mutation in the RAF/MEK/ERK pathway may be involved in the drug resistance to sorafenib, rather than a Ras mutation.

In summary, only one of 61 HCCs (1.6 %) in the present study carried a point mutation, which was a G to A transition in codon 13 of the *KRAS* gene. No mutational activation was found in codons 12 and 61 of *KRAS* or in codons 12, 13 and 61 of the *NRAS* or *HRAS* genes in any of the HCCs or corresponding non-malignant tissue samples. These findings suggested that Ras gene mutations are not related to the pathogenesis of most HCCs. The signaling pathways downstream of Ras should be examined to identify markers to predict a response to sorafenib.

Acknowledgments We thank Professor Brian Quinn for his review of this manuscript. No financial support was received for this work from any company. This study was supported in part by a grant from the Scientific Research Fund of the Ministry of Education of Japan.

Conflict of interest None of the authors has any conflict of interest.

References

1. El Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132:2557–76.
2. Okita K. Clinical aspects of hepatocellular carcinoma in Japan. Intern Med. 2006;45:229–33.
3. Taketomi A, Kitagawa D, Itoh S, Harimoto N, Yamashita Y, Gion T, et al. Trends in morbidity and mortality after hepatic resection for hepatocellular carcinoma: an institute's experience with 625 patients. J Am Coll Surg. 2007;204:580–7.
4. Taketomi A, Sanefuji K, Soejima Y, Yoshizumi T, Uhciyama H, Ikegami T, et al. Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. Transplantation. 2009;87:531–7.
5. Llovet JM, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. Semin Liver Dis. 2005;25:181–200.
6. Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. Hepatology. 2008;48:1312–27.
7. Abou Alfa GK, Schwartz L, Ricci S, Amadori D, Santoro A, Figer A, et al. Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. J Clin Oncol. 2006;24:4293–300.
8. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359:378–90.
9. Sugio K, Ishida T, Yokoyama H, Inoue T, Sugimachi K, Sasazuki T. Ras gene mutations as a prognostic marker in adenocarcinoma of the human lung without lymph node metastasis. Cancer Res. 1992;52:2903–6.
10. Tsuda H, Hirohashi S, Shimosato Y, Ino Y, Yoshida T, Terada M. Low incidence of point mutation of c-Ki-ras and N-ras oncogenes in human hepatocellular carcinoma. Jpn J Cancer Res. 1989;80:196–9.
11. Tanaka S, Arii S. Current status of molecularly targeted therapy for hepatocellular carcinoma: basic science. Int J Clin Oncol. 2010;15:235–41.
12. Caraglia M, Giuberti G, Marra M, Addeo R, Montella L, Murolo M, et al. Oxidative stress and ERK1/2 phosphorylation as predictors of outcome in hepatocellular carcinoma patients treated with sorafenib plus octreotide LAR. Cell Death Dis. 2011;2:e150.
13. Zhang Z, Zhou X, Shen H, Wang D, Wang Y. Phosphorylated ERK is a potential predictor of sensitivity to sorafenib when treating hepatocellular carcinoma: evidence from an in vitro study. BMC Med. 2009;7:41.
14. Tada M, Omata M, Ohto M. Analysis of ras gene mutations in human hepatic malignant tumors by polymerase chain reaction and direct sequencing. Cancer Res. 1990;50:1121–4.
15. Ogata N, Kamimura T, Asakura H. Point mutation, allelic loss and increased methylation of c-Ha-ras gene in human hepatocellular carcinoma. Hepatology. 1991;13:31–7.
16. Challen C, Guo K, Collier JD, Cavanagh D, Bassendine MF. Infrequent point mutations in codons 12 and 61 of ras oncogenes in human hepatocellular carcinomas. J Hepatol. 1992;14:342–6.
17. Leon M, Kew MC. Analysis of ras gene mutations in hepatocellular carcinoma in southern African blacks. Anticancer Res. 1995;15:859–61.

Kamiyama et al. World Journal of Surgical Oncology 2012, 10:107
http://www.wjso.com/content/10/1/107

WORLD JOURNAL OF SURGICAL ONCOLOGY

RESEARCH Open Access

Analysis of the risk factors for early death due to disease recurrence or progression within 1 year after hepatectomy in patients with hepatocellular carcinoma

Toshiya Kamiyama[1*], Kazuaki Nakanishi[2], Hideki Yokoo[1], Hirofumi Kamachi[2], Munenori Tahara[1], Tatsuhiko Kakisaka[1], Yosuke Tsuruga[1], Satoru Todo[2] and Akinobu Taketomi[1]

Abstract

Background: Liver resection for hepatocellular carcinoma (HCC) has the highest local controllability among all local treatments and results in a good survival rate. However, the recurrence rates of HCC continue to remain high even after curative hepatectomy Moreover, it has been reported that some patients with HCC have an early death due to recurrence. We analyzed the preoperative risk factors for early cancer death.

Methods: Between 1997 and 2009, 521 consecutive patients who underwent hepatectomy for HCC at our center were assigned to group ED (death due to HCC recurrence or progression within 1 year after hepatectomy) and group NED (alive over 1 year after hepatectomy). Risk factors for early cancer death were analyzed.

Results: Group ED included 48 patients, and group NED included 473 patients. The cause of death included cancer progression (150; 78.1%), operation-related (1; 0.5%), hepatic failure (15; 7.8%), and other (26; 13.5%). Between the ED and NED groups, there were significant differences in albumin levels, Child-Pugh classifications, anatomical resections, curability, tumor numbers, tumor sizes, macroscopic vascular invasion (portal vein and hepatic vein), alpha-fetoprotein (AFP) levels, AFP-L3 levels, protein induced by vitamin K absence or antagonism factor II (PIVKA-II) levels, differentiation, microscopic portal vein invasion, microscopic hepatic vein invasion, and distant metastasis by univariate analysis. Multivariate analysis identified specific risk factors, such as AFP level > 1,000 ng/ml, tumor number ≥ 4, tumor size ≥ 5 cm, poor differentiation, and portal vein invasion. With respect to the preoperative risk factors such as AFP level, tumor number, and tumor size, 3 (1.1%) of 280 patients with no risk factors, 12 (7.8%) of 153 patients with 1 risk factor, 24 (32.9%) of 73 patients with 2 factors, and 9 (60.0%) of 15 patients with 3 risk factors died within 1 year of hepatectomy ($p < 0.0001$).

Conclusions: Hepatectomy should be judiciously selected for patients with AFP level > 1,000 ng/ml, tumor number ≥ 4, and tumor size ≥ 5 cm, because patients with these preoperative risk factors tend to die within 1 year after hepatectomy; these patients might be better treated with other therapy.

Keywords: Hepatocellular carcinoma, Hepatectomy, Early death

* Correspondence: t-kamiya@med.hokudai.ac.jp
[1]The Department of General Surgery, Hokkaido University Graduate School of Medicine, North 15, West 7, Kita-ku, Sapporo 060-8638, Japan
Full list of author information is available at the end of the article

Background

Liver resection for the treatment of hepatocellular carcinoma (HCC) has the highest controllability among all local treatments and results in a good survival rate [1,2]. However, recurrence rates remain high and are the main cause of early death even after curative hepatectomy [3]. Moreover, it has been reported that some patients with HCC have an early death due to recurrence [4]. In the remnant liver after hepatectomy, tumor recurrence is recognized as intrahepatic metastasis caused by dissemination of cells in the portal vein or metachronous multicentric hepatocarcinogenesis [5]. The risk factors for early recurrence are reported to be related to tumor cell dissemination due to tumor characteristics such as vascular invasion [6,7] and intrahepatic metastasis [8]. Though the two algorithms that were proposed from the Barcelona Clinic Liver Cancer (BCLC) classification [9] and Japanese guideline [10] recommend that multiple HCCs be treated by transcatheter arterial chemoembolization with lipiodol (TACE) or sorafenib, hepatectomy beyond these algorithms was actually performed in the clinical scene. However, the risk factors for early death due to HCC recurrence or progression within 1 year after hepatectomy have not been clearly evaluated [11].

On the other hand, the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) trial [12] recently reported the effectiveness of sorafenib in the treatment of advanced HCC. In this report, median overall survival was 10.7 months in the sorafenib group and 7.9 months in the placebo group. If patients have an early death within 1 year due to recurrence after hepatectomy, there might be no benefit of hepatectomy compared to sorafenib. Therefore, the risk factors for early death within 1 year after hepatectomy due to HCC recurrence or progression should be evaluated, and the appropriateness of hepatectomy for patients with advanced HCC should be investigated.

To identify the risk factors related to early death after hepatectomy, we analyzed the outcomes of 521 consecutive patients who underwent primary hepatectomy for HCC at our center.

Methods

Patients

Between January 1997 and May 2009, 521 consecutive patients underwent hepatectomy for HCC at our center. These patients were followed for at least 1 year, and then assigned to group ED (death due to HCC recurrence or progression within 1 year after hepatectomy) or group NED (alive over 1 year after hepatectomy). The resulting ED group included 48 (9.2%) patients, and the resulting NED group included 465 (89.3%) patients. Of all 521 patients, 8 (1.5%) patients who died of liver failure, other causes, and postoperative complications within 1 year after hepatectomy were excluded from group ED and NED. The mean age of 513 patients of group ED and NED was 61.3 years, with a range of 18–87 years. Of the 513 patients, 427 (83.2%) were male and 86 (16.8%) were female, 221 (43.1%) were hepatitis B virus surface antigen-positive, 189 (36.8%) were hepatitis C virus antibody-positive, and 175 (34.1%) had cirrhosis. At least 2 weeks before hepatectomy, imaging studies were performed and preoperative serum alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3), and protein induced by vitamin K absence or antagonism factor II (PIVKA-II) levels were simultaneously measured using standard methods. Among the 513 patients, 499 (97.3%) were categorized as Child-Pugh class A (Table 1). The patients were followed up for a median of 84.2 months (range, 12.5–165.0 months). This study was approved by the Institutional Review Board of the Hokkaido University, School of Advanced Medicine.

Hepatectomy

Anatomical resection is defined as a resection in which lesion(s) are completely removed anatomically on the basis of Couinaud's classification (segmentectomy, sectionectomy, and hemihepatectomy or extended hemihepatectomy) in patients with sufficient functional reserve. Non-anatomical partial resection was performed as a limited resection or tumor enucleation. When R0 resections were performed, the resection surface was found to be histologically free of HCC. Indocyanine green retention rates at 15 min (ICGR15) were measured to evaluate liver function reserve, regardless of the presence or absence of cirrhosis.

HCC recurrence

Every 3 months for the first 2 years after hepatectomy, the patients underwent follow-up evaluations comprising liver function tests, measurements of tumor markers AFP and PIVKA-II, ultrasonography (US), and dynamic computed tomography (CT). After 2 years, routine CT was performed only once every 4 months. If recurrence was suspected, CT and magnetic resonance imaging (MRI) were performed; if necessary, CT during angiography and bone scintigraphy were also performed. This enabled precise diagnoses of the site, number, size, and invasiveness of the recurrent lesions.

Statistical analysis

Patient survival (PS) rates were determined via the Kaplan-Meier method. Univariate analysis was performed; then multivariate analysis and logistic regression were performed only on significant variables. Statistical analyses (StatView 5.0 for Windows: SAS Institute Inc., Cary, NC) were performed using standard tests (X^2, *t*-test) where appropriate. Significance was defined as $p < 0.05$.

Table 1 Univariate analysis of the risk factors of death from cancer progression within 1 year after hepatectomy

		Group ED ($n=48$)	Group NED ($n=465$)	*p*-value
Sex	Male	40	387	
	Female	8	78	0.9849
Age	<60	24	211	
	60 ≤	24	254	0.5405
HBV	+	26	195	
	-	22	270	0.1033
HCV	+	14	175	
	-	34	290	0.2469
Albumin	<4	33	214	
(g/dl)	4 ≤	15	251	0.0027
Total bilirubin	<0.8	32	283	
(mg/dl)	0.8 ≤	16	182	0.4314
ICGR15 (%)	<15	27	250	
	15 ≤	21	215	0.7421
Child-Pugh	A	42	457	
	B	6	8	<0.0001
AFP (ng/ml)	≤200	15	355	
	200<, ≤1,000	5	37	
	1,000<	28	73	<0.0001
AFP-L3 (%)	≤15	23	334	
	15% < 40<	5	49	
	40<	20	83	0.0002
PIVKA-II (mAU/ml)	≤100	10	258	
	100<, ≤1,000	8	93	
	1,000<	30	114	<0.0001
Liver cirrhosis	Present	17	158	
	Absent	31	307	0.8414
Curability	R0 R1	40	443	
	R2	8	22	0.0008
Anatomical resection	Yes	42	326	
	No	6	139	0.0108
Tumor number	1	16	321	
	2, 3	11	113	
	4 ≤	21	31	<0.0001
Tumor size	≤2 cm	4	64	
	2-5 cm	6	254	
	5 cm ≤	38	147	<0.0001
Macroscopic vascular invasion	Absent	28	440	
(portal vein, hepatic vein)	Present	20	25	<0.0001
Differentiation	Well	0	50	
	Moderate	19	308	
	Poor	29	92	
	Necrosis	0	15	<0.0001
Microscopic portal vein invasion	vp0	10	369	
	vp1	13	60	
	vp2	7	15	
	vp3	12	15	
	vp4	6	6	<0.0001
Microscopic hepatic vein invasion	vv0	28	439	
	vv1	8	12	
	vv2	9	10	
	vv3	3	4	<0.0001
Distant metastasis	Absent	43	459	
	Present	5	6	<0.0001

HCC: hepatocellular carcinoma.
NED: alive 1 year after hepatectomy.
ED: death due to HCC recurrence or progression within 1 year after hepatectomy.
HBV: hepatitis B virus s antigen.
HCV: anti-hepatitis C virus antibody.
ICGR15: indocyanin green retention rate at 15 min.
AFP: alpha-fetoprotein.
AFP-L3: *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein.
PIVKA-II: protein induced by vitamin K absence or antagonism factor II.
vp0: no tumor thrombus in the portal vein.
vp1: tumor thrombus distal to the second branches of the portal vein.
vp2: tumor thrombus in the second branches of the portal vein.
vp3: tumor thrombus in the first branch of the portal vein.
vp4: tumor thrombus extension to the trunk or the opposite side branch of the portal vein.
vv0: no tumor thrombus in the hepatic vein.
vv1: tumor thrombus in a branch of the hepatic vein.
vv2: tumor thrombus in the right, middle, or left hepatic vein trunk or the short hepatic vein.
vv3: tumor thrombus to the inferior vena cava.

Results

Causes of death

PS rates ($n=521$) at 1, 2, 5, and 10 years were 90.5%, 84.6%, 69.0%, and 54.2%, respectively (Figure 1), with 192 deaths (36.9%). The causes of death, whether within 1 year post-hepatectomy or later, included HCC recurrence or progression ($n=150$; 78.1%), liver failure ($n=15$; 7.8%), other causes ($n=26$; 13.5%), and postoperative complications ($n=1$; 0.5%). Of the 150 patients who died of HCC recurrence or progression, 48 (32.0%) died within 1 year after hepatectomy (Figure 2). The patients who died of liver failure ($n=4$), other causes ($n=3$), and postoperative complications ($n=1$) within 1 year after hepatectomy were excluded from group ED and NED.

Clinicopathological characteristics and operative variables

Patient characteristics and perioperative outcomes are shown in Table 1. Between the ED and NED groups,

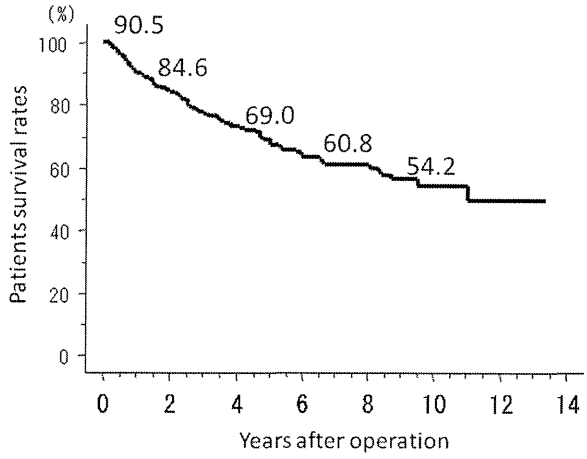


Figure 1 Survivals of all 521 patients at 1, 2, 5, 7, and 10 years were 90.5%, 84.6%, 69.0%, 60.8%, and 54.2%, respectively.

there were significant differences in albumin levels, Child-Pugh classifications, anatomical resections, curability, tumor numbers, tumor sizes, macroscopic vascular invasion (portal vein and hepatic vein), AFP levels, AFP-L3 levels, PIVKA-II levels, differentiation, microscopic portal vein invasion, microscopic hepatic vein invasion, and distant metastasis. Tumor-related factors are also shown in Table 1. When the risk factors that were identified as significant by univariate analysis were included in a multivariate analysis via logistic regression, it was found that AFP level, tumor number, tumor size, differentiation, and microscopic portal vein invasion were independent risk factors for early death due to HCC recurrence or progression within 1 year after hepatectomy (Table 2).

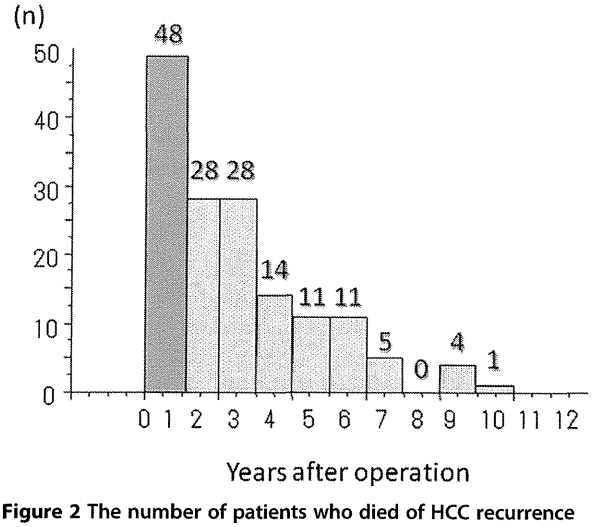


Figure 2 The number of patients who died of HCC recurrence or progression after hepatectomy. Of the 150 patients who died of HCC recurrence or progression, 48 patients (32.0%) died within 1 year after hepatectomy.

Table 2 Logistic regression analysis based on univariate analysis of the risk factors of death from cancer progression within 1 year after hepatectomy

Risk factor	*p*	Risk ratio	95% CI
AFP(ng/ml):>1,000(vs. ≤ 200)	0.0079	4.098	1.447-11.628
Tumor number 4 ≤ (vs. 1)	0.0208	3.535	1.206-10.361
Tumor size (cm) 5 ≤ (vs. 2–5)	0.0295	3.687	1.139-11.936
Differentiation poor (vs. moderately)	0.0179	2.8	1.194-6.565
vp1(vs. vp0)	0.0037	5.02	1.691-14.909
vp2(vs. vp0)	0.0034	8.507	2.029-35.667

AFP: alpha-fetoprotein.
vp0: no tumor thrombus in the portal vein.
vp1: tumor thrombus distal to the second branches of the portal vein.
vp2: tumor thrombus in the second branches of the portal vein.

Risk factors for early death

Independent, preoperatively evaluable risk factors for early death were identified by multivariate analysis as AFP > 1,000 ng/ml, tumor number ≥ 4, and tumor size ≥ 5 cm. The patients of group ED and NED ($n = 513$) were categorized into three levels of risk: risk 0 if they had no risk factors ($n = 276$), risk 1 if they had any one risk factor ($n = 151$), risk 2 if they had any two risk factors ($n = 71$), and risk 3 if they had all three risk factors ($n = 15$). In risk 0, 3 patients (1.1%), in risk 1, 12 patients (7.9%), in risk 2, and 24 patients (33.8%); in risk 3, 9 patients (60.0%) died within 1 year after hepatectomy ($p < 0.0001$) (Table 3). PS rates for risk 0, risk 1, risk 2, and risk 3 at 1 year were 98.9%, 91.7%, 66.1%, and 40.0%, respectively (Figure 3). Multivariate analysis showed that the risk ratio of risk 1 vs. risk 0 was 7.856, that of risk 2 vs. risk 0 was 46.468, and that of risk 3 vs. risk 0 was 136.5 (Table 3).

Discussion

When the patients were categorized by the number of independent, preoperatively evaluable risk factors, the

Table 3 Logistic regression analysis of three risk levels of death from cancer progression within 1 year after hepatectomy

	No. of patients	No. of ED (%)	Risk ratio	95% CI
Risk 0	276	3 (1.1)	1	
Risk 1	151	12 (7.9)	7.856	2.181-28.302
Risk 2	71	24 (33.8)	46.468	13.452-160.514
Risk 3	15	9 (60.0)	136.5	29.354-634.752

HCC: hepatocellular carcinoma.
ED: death due to HCC recurrence or progression within 1 year after hepatectomy.
Risk 0: they had no risk factors.
Risk 1: they had any 1 risk factor.
Risk 2: they had any 2 risk factors.
Risk 3: they had all 3 risk factors.
Risk factors: AFP > 1,000 ng/ml, tumor number ≥ 4, and tumor size ≥ 5 cm.

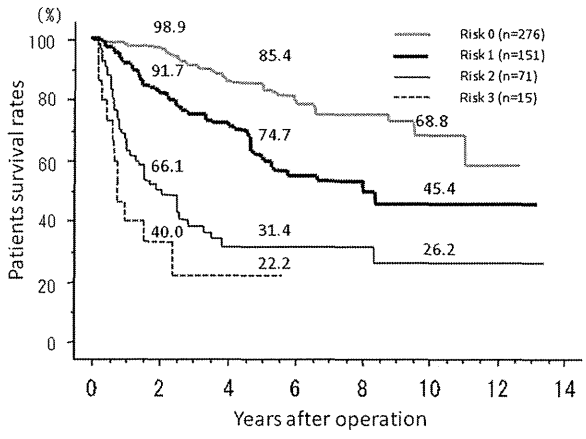


Figure 3 PS rates for risk 0, risk 1, risk 2, and risk 3 at 1 year were 98.9%, 91.7%, 66.1%, and 40.0%, respectively. PS rates for risk 0, risk 1, risk 2, and risk 3 at 1 year were 85.4%, 61.5%, 31.4%, and 22.2%, respectively.

early death rate within 1 year was 60.0% for patients with three risk factors: AFP > 1,000 ng/ml, tumor number ≥ 4, and tumor size ≥ 5 cm, while the early death rate was 1.1% for patients with no risk factors. Therefore, the appropriateness of hepatectomy for HCC should be carefully examined for patients who have large and multiple HCC with high AFP levels; these patients might be better treated with other therapeutic options, such as TACE or sorafenib.

Early recurrence is the main cause of early death within 1 year after hepatectomy. The risk factors for early recurrence are reported to be related to tumor cell dissemination due to tumor characteristics such as vascular invasion [6,7] and intrahepatic metastasis [8]. Because these factors are diagnosed only by postoperative pathological examination, preoperatively evaluable factors are necessary to decide the appropriateness of hepatectomy in advanced HCC. Among preoperative risk factors, an HCC tumor larger than 5 cm is reported to be an important indicator of a high risk of recurrence after resection [13] and has a higher incidence of intrahepatic metastasis and portal venous invasion [14,15]. Therefore, it is believed that an HCC tumor larger than 5 cm has high malignant potential. In this study and another report [11], tumor size ≥ 5 cm reflected this high malignant potential and was selected as an independent risk factor for early death due to HCC recurrence or progression within 1 year after hepatectomy.

Multivariate analysis also shows that tumor number is an important predictor of recurrence. Lai et al. [16] reported that the presence of multiple nodules was the most powerful predictor of both long-term survival and tumor recurrence. Because multiple HCC originates from disseminated cancer cells and not from multicentric carcinogenesis, multiple HCC is a more aggressive phenotype than solitary HCC. Yang reported that, after resection of solitary large HCC, the clinical and pathological characteristics and outcome are similar to those of small HCC, but are significantly better than those of nodular HCC (node number ≥ 2) [17]. It has also been reported that the expression levels of some human genes closely related to invasion and metastasis were significantly lower in solitary large HCC than in nodular HCC [17,18]. They proposed solitary large HCC as a specific subtype, less malignant than nodular HCC. Moreover, in multiple HCC, it was speculated that latent tumors, intrahepatic micrometastases that might be subsequently found to produce early recurrent tumors, could already be present in the remnant liver at the time of surgery. Therefore, tumor number ≥ 4 was selected in the current study as a significant factor predicting early death after hepatectomy.

In our study, multivariate analysis showed that an AFP level over 1,000 ng/ml was an independent factor related to early death. Previous reports have shown that AFP is an independent predictor of prognosis [19], even in patients who had undergone hepatectomy [20]. High levels of AFP in fully developed HCC or in the serum of the host are associated with more aggressive behavior and increased anaplasis [21]. On the other hand, it is well known that AFP levels may increase in some patients with acute and chronic hepatitis without HCC [22,23] and that elevation of AFP levels correlates with inflammation caused by background diseases and hepatocyte regeneration [24]. However, because the elevation of AFP levels by hepatitis or regeneration is usually not so high, only 200 ng/ml [25], AFP levels over 1,000 ng/ml might specifically indicate tumor malignancy. Yamanaka et al. [26] also reported that the serum AFP value per tumor diameter was the most significant risk factor for early death within 1 year after resection in patients with stage II–III HCC by multivariate analysis.

Given these preoperatively evaluable risk factors, the probability of early death after hepatectomy can be estimated by the number of risk factors. In risk 0, 3 patients (1.1%), in risk 1, 12 patients (7.9%), in risk 2, 24 patients (33.8%), and in risk 3, 9 patients (60.0%) died within 1 year after hepatectomy. The risk ratio of risk 1 vs. risk 0 was 7.856, that of risk 2 vs. risk 0 was 46.468, and that of risk 3 vs. risk 0 was 136.5 by multivariate analysis. PS rates for risk 3 at 1 year were 40.0%, while in the SHARP trial, survival rates at 1 year were 44% in the sorafenib group [12]. Moreover, Takayasu et al. reported that the survival rate at 1 year of patients with ≥ 4 tumors, ≥5.1 cm in diameter was 74% [27]. In this way, because the surgical outcome of patients with all three risk factors was worse than that of the patients treated with sorafenib or TACE, these patients might be better treated with other therapeutic options than hepatectomy for the first

line treatment. However, selected patients with risk 1 and 2 who might be beyond BCLC and Japanese algorithms should not be excluded from hepatectomy because of their good outcome: 91.7%, 66.1% at 1 year of PS.

On the other hand, in this study, macroscopic vascular invasion (portal and hepatic veins) was not indicated by multivariate analysis as an independent risk factor related to early death. It has been reported that the prognosis of patients with portal vein tumor thrombus (PVTT) in the main trunk or first branch is very poor; the median survival period of patients with portal thrombosis is only 2.7 months without appropriate treatment [28]. However, recently reported patients showed long-term survival rates when hepatectomy was combined with pre- or postoperative treatment. We reported the efficacy of a combination of hepatectomy and preoperative radiotherapy for PVTT in the main trunk or first branch. The 1-, 3-, and 5-year survival rates in hepatectomized patients with preoperative radiotherapy for PVTT were 100%, 53.3%, and 40.0%, respectively [29]. Minagawa [30] reported that the survival rate of patients with PVTT, including those who underwent hepatic resection with preoperative transcatheter arterial chemoembolization, was 42% at 5 years. Nagano [31] reported that 15 patients with HCC with PVTT were treated with FU arterial infusion and interferon therapy (FAIT) and surgery, and that all the patients (100%) survived over 1 year; without FAIT and surgery, 10 patients (67%) died within 1 year. Therefore, even if patients have HCC with macroscopic vascular invasion, particularly PVTT in the main trunk or first branch, hepatectomy is not contraindicated in these patients when combined with pre- or postoperative treatment. In the patients with risk 0, 1, of 45 patients 19 had macroscopic vascular invasion. Of these 19 patients, only 5 (26.3%) died within 1 year after hepatectomy. In the 26 patients with risk 2, 3, 15 patients (57.7%) died within 1 year after hepatectomy. Concerning Child-Pugh B cirrhosis, the high-risk patients could be also indentified. From these data, though macroscopic vascular invasion and Child-Pugh B cirrhosis were poor prognostic factors, the patients who had these factors did not always die in 1 year after hepatectomy. Using our risk levels, the patients with extremely poor prognosis could be identified from the patients who had poor prognostic factors such as macroscopic vascular invasion or Child-Pugh B. Therefore, concerning risk levels, risk 0 to 3 was very important and useful for predicting the prognosis of patients with HCC who underwent hepatectomy.

Conclusions

In conclusion, the appropriateness of hepatectomy in the treatment of HCC should be carefully considered when patients have the following preoperative risk factors: AFP > 1,000 ng/ml, tumor number ≥ 4, and/or tumor size ≥ 5 cm; these patients might be better treated with other therapeutic options, i.e., sorafenib and TACE. However, even if patients have HCC with PVTT in the main trunk or first branch, hepatectomy is not contraindicated when combined with pre- or postoperative treatment.

Abbreviations

HCC: Hepatocellular carcinoma; PS: Patient survival; ICGR15: Indocyanine green retention rate at 15 min; AFP: Alpha-fetoprotein; AFP-L3: *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein-L3 fraction; PIVKA-II: Protein induced by vitamin K absence or antagonism factor II; US: Ultrasonography; CT: Computed tomography; MRI: Magnetic resonance imaging; TACE: Transcatheter arterial chemoembolization.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

The authors wish to thank the staff of General Surgery, Graduate School of Medicine, Hokkaido University, for their kind cooperation.

Author details

[1]The Department of General Surgery, Hokkaido University Graduate School of Medicine, North 15, West 7, Kita-ku, Sapporo 060-8638, Japan. [2]The Department of Transplantation Surgery, Hokkaido University Graduate School of Medicine, North 15, West 7, Kita-ku, Sapporo060–8638, Japan.

Authors' contributions

TK designed the research; TK, KN, and HY acquired of the data; TK, KN, HY, HK, TK, YT, ST, and AT analyzed the data; TK wrote the paper. All authors read and approved the final manuscript.

Received: 7 March 2012 Accepted: 14 June 2012
Published: 14 June 2012

References

1. Arii S, Yamaoka Y, Futagawa S, Inoue K, Kobayashi K, Kojiro M, Makuuchi M, Nakamura Y, Okita K, Yamada R: **Results of surgical and nonsurgical treatment for small-sized hepatocellular carcinomas: a retrospective and nationwide survey in Japan. The Liver Cancer Study Group of Japan.** *Hepatology* 2000, **32**:1224–1229.
2. Hasegawa K, Kokudo N, Imamura H, Matsuyama Y, Aoki T, Minagawa M, Sano K, Sugawara Y, Takayama T, Makuuchi M: **Prognostic impact of anatomic resection for hepatocellular carcinoma.** *Ann Surg* 2005, **242**:252–259.
3. Kamiyama T, Nakanishi K, Yokoo H, Kamachi H, Tahara M, Suzuki T, Shimamura T, Furukawa H, Matsushita M, Todo S: **Recurrence patterns after hepatectomy of hepatocellular carcinoma: implication of Milan criteria utilization.** *Ann Surg Oncol* 2009, **16**:1560–1571.
4. Kondo K, Chijiiwa K, Makino I, Kai M, Maehara N, Ohuchida J, Naganuma S: **Risk factors for early death after liver resection in patients with solitary hepatocellular carcinoma.** *J Hepatobiliary Pancreat Surg* 2005, **12**:399–404.
5. Adachi E, Maeda T, Matsumata T, Shirabe K, Kinukawa N, Sugimachi K, Tsuneyoshi M: **Risk factors for intrahepatic recurrence in human small hepatocellular carcinoma.** *Gastroenterology* 1995, **108**:768–775.
6. Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M: **Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy.** *J Hepatol* 2003, **38**:200–207.
7. Poon RT, Fan ST, Ng IO, Lo CM, Liu CL, Wong J: **Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma.** *Cancer* 2000, **89**:500–507.
8. Ikeda Y, Kajiyama K, Adachi E, Yamagata M, Shimada M, Yanaga K: **Early recurrence after surgery of hepatocellular carcinoma.** *Hepatogastroenterology* 1995, **42**:469–472.

9. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodes J: **Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver.** *J Hepato* 2001, **35**:421–430.
10. Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, Matsuyama Y, Okazaki M, Okita K, Omata M, Saida Y, Takayama T, Yamaoka Y: **Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan.** *Hepatol Res* 2008, **38**:37–51.
11. Regimbeau JM, Abdalla EK, Vauthey JN, Lauwers GY, Durand F, Nagorney DM, Ikai I, Yamaoka Y, Belghiti J: **Risk factors for early death due torecurrence after liver resection for hepatocellular carcinoma: results of a multicenter study.** *J Surg Oncol* 2004, **85**:36–41.
12. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Haussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J: **Sorafenib in advanced hepatocellular carcinoma.** *N Engl J Med* 2008, **359**:378–390.
13. Shah SA, Greig PD, Gallinger S, Cattral MS, Dixon E, Kim RD, Taylor BR, Grant DR, Vollmer CM: **Factors associated with early recurrence after resection for hepatocellular carcinoma and outcomes.** *J Am Coll Surg* 2006, **202**:275–283.
14. Adachi E, Maeda T, Kajiyama K, Kinukawa N, Matsumata T, Sugimachi K, Tsuneyoshi M: **Factors correlated with portal venous invasion by hepatocellular carcinoma: univariate and multivariate analyses of 232 resected cases without preoperative treatments.** *Cancer* 1996, **77**:2022–2031.
15. Kosuge T, Makuuchi M, Takayama T, Yamamoto J, Shimada K, Yamasaki S: **Long-term results after resection of hepatocellular carcinoma: experience of 480 cases.** *Hepatogastroenterology* 1993, **40**:328–332.
16. Lai EC, You KT, Ng IO, Shek TW: **The pathological basis of resection margin for hepatocellular carcinoma.** *World J Surg* 1993, **17**:790. discussion 91.
17. Yang LY, Wang W, Peng JX, Yang JQ, Huang GW: **Differentially expressed genes between solitary large hepatocellular carcinoma and nodular hepatocellular carcinoma.** *World J Gastroenterol* 2004, **10**:3569–3573.
18. Wang W, Yang LY, Huang GW, Lu WQ, Yang ZL, Yang JQ, Liu HL: **Genomic analysis reveals RhoC as a potential marker in hepatocellular carcinoma with poor prognosis.** *Br J Cancer* 2004, **90**:2349–2355.
19. Nomura F, Ohnishi K, Tanabe Y: **Clinical features and prognosis of hepatocellular carcinoma with reference to serum alpha-fetoprotein levels. Analysis of 606 patients.** *Cancer* 1989, **64**:1700–1707.
20. Hanazaki K, Kajikawa S, Koide N, Adachi W, Amano J: **Prognostic factors after hepatic resection for hepatocellular carcinoma with hepatitis C viral infection: univariate and multivariate analysis.** *Am J Gastroenterol* 2001, **96**:1243–1250.
21. Matsumoto Y, Suzuki T, Asada I, Ozawa K, Tobe T, Honjo I: **Clinical classification of hepatoma in Japan according to serial changes in serum alpha-fetoprotein levels.** *Cancer* 1982, **49**:354–360.
22. Smith JB: **Occurrence of alpha-fetoprotein in acute viral hepatitis.** *Int J Cancer* 1971, **8**:421–424.
23. Silver HK, Gold P, Shuster J, Javitt NB, Freedman SO, Finlayson ND: **Alpha(1)-fetoprotein in chronic liver disease.** *N Engl J Med* 1974, **291**:506–508.
24. Fujiyama S, Tanaka M, Maeda S, Ashihara H, Hirata R, Tomita K: **Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma.** *Oncology* 2002, **62**(Suppl 1):57–63.
25. Lok AS, Lai CL: **Alpha-Fetoprotein monitoring in Chinese patients with chronic hepatitis B virus infection: role in the early detection of hepatocellular carcinoma.** *Hepatology* 1989, **9**:110–115.
26. Yamanaka J, Yamanaka N, Nakasho K, Tanaka T, Ando T, Yasui C, Kuroda N, Takata M, Maeda S, Matsushita K, Uematsu K, Okamoto E: **Clinicopathologic analysis of stage II-III hepatocellular carcinoma showing early massive recurrence after liver resection.** *Gastroenterol Hepatol* 2000, **15**:1192–1198.
27. Takayasu K, Arii S, Kudo M, Ichida T, Matsui O, Izumi N, Matsuyama Y, Sakamoto M, Nakashima O, Ku Y, Kokudo N, Makuuchi M: **Superselective transarterial chemoembolization for hepatocellular carcinoma. Validation of treatment algorithm proposed by Japanese guidelines.** *J Hepatol* 2012, **56**:886–892.
28. Llovet JM, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, Bru C, Rodes J, Bruix J: **Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials.** *Hepatology* 1999, **29**:62–67.
29. Kamiyama T, Nakanishi K, Yokoo H, Tahara M, Nakagawa T, Kamachi H, Taguchi H, Shirato H, Matsushita M, Todo S: **Efficacy of preoperative radiotherapy to portal vein tumor thrombus in the main trunk or first branch in patients with hepatocellular carcinoma.** *Int J Clin Oncol* 2007, **12**:363–368.
30. Minagawa M, Makuuchi M, Takayama T, Ohtomo K: **Selection criteria for hepatectomy in patients with hepatocellular carcinoma and portal vein tumor thrombus.** *Ann Surg* 2001, **233**:379–384.
31. Nagano H, Sakon M, Eguchi H, Kondo M, Yamamoto T, Ota H, Nakamura M, Wada H, Damdinsuren B, Marubashi S, Miyamoto A, Takeda Y, Dono K, Umeshit K, Nakamori S, Monden M: **Hepatic resection followed by IFN-alpha and 5-FU for advanced hepatocellular carcinoma with tumor thrombus in the major portal branch.** *Hepatogastroenterology* 2007, **54**:172–179.

doi:10.1186/1477-7819-10-107
Cite this article as: Kamiyama *et al.*: Analysis of the risk factors for early death due to disease recurrence or progression within 1 year after hepatectomy in patients with hepatocellular carcinoma. *World Journal of Surgical Oncology* 2012 **10**:107.