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HEPATOLOGY

Adverse hepatic events caused by radiotherapy for advanced hepatocellular carcinoma

JUNJI FURUSE,* HIROSHI ISHII,* MICHITAKA NAGASE,* MITSUHIKO KAWASHIMA,† TAKASHI OGINO† AND MASAHIRO YOSHINO*

**Division of Hepatobiliary and Pancreatic Medical Oncology and †Department of Radiology, National Cancer Center Hospital East, Chiba, Japan*

Abstract

Background: Radiotherapy is often used to treat patients with unresectable advanced hepatocellular carcinoma (HCC). The present study examines the nature and frequency of adverse events with respect to liver function in such patients after radiotherapy.

Methods: Forty-six patients with HCC who underwent radiotherapy were retrospectively examined. Radiotherapy was applied using coplanar 2–3-beam arrangements to a target dose of 50 Gy/5 weeks. The adverse hepatic events were evaluated according to the National Cancer Institute *Common Toxicity Criteria* and the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer Late Radiation Morbidity Scoring Scheme during the acute phase and the late phase by following the patients for up to 1 year. The influence on survival by adverse hepatic events and other factors was analyzed.

Results: The full irradiation dose of 50 Gy was given to 40 patients (87.0%). Grade 3 or 4 toxicity was observed in 18 (39.1%) within 3 months after radiotherapy and in 11 (33.3%) of 33 thereafter, respectively. The most frequent and serious adverse events were hyperbilirubinemia, hypoalbuminemia, and ascites. The independent adverse prognostic factors for survival were portal vein tumor thrombus ($P = 0.0012$), tumor response ($P = 0.011$), acute adverse hepatic event ($P = 0.012$), and late adverse hepatic event ($P = 0.015$).

Conclusions: Hypoalbuminemia, hyperbilirubinemia, and ascites were important hepatic adverse events that developed after applying radiotherapy to treat advanced HCC. These adverse events seriously affected survival.

Key words: adverse event, external-beam radiotherapy, hepatocellular carcinoma.

INTRODUCTION

Unresectable advanced hepatocellular carcinoma (HCC) has been treated using radiotherapy with and without transcatheter arterial chemoembolization (TACE), and several institutions have reported positive results.[1–7] In contrast, irradiation also injures normal liver tissue and serious, radiation-induced liver disease can develop.[2,4] Most patients with HCC originally had liver dysfunction such as cirrhosis or chronic hepatitis due to hepatitis virus B or C. Therefore, hepatic adverse events that develop after radiotherapy for HCC must be defined.

Adverse events caused by irradiation have been evaluated according to the Radiation Therapy Oncology Group (RTOG) Acute Radiation Morbidity Scoring Criteria or RTOG/European Organization for Research and Treatment of Cancer (EORTC) Late Radiation Morbidity Scoring Scheme[8] and/or the late effects normal tissues (LENT) scoring system and the late effects toxicity scoring system (the SOMA scale).[9,10] However, the RTOG acute radiation morbidity scoring criteria do not mention liver dysfunction at the acute phase of irradiation. However, the National Cancer Institute *Common Toxicity Criteria* (NCI-CTC) can evaluate adverse events after cancer treatment.[11] Therefore, we

Correspondence: Junji Furuse, National Cancer Center Hospital East, 6-5-1, Kashiwanoha, Kashiwa, Chiba, 277-8577, Japan. Email: jfuruse@east.ncc.go.jp

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studied the adverse hepatic events using the NCI-CTC in patients with unresectable advanced HCC who underwent radiotherapy, and assessed their impact on survival.

METHODS

We retrospectively studied 46 patients with HCC who underwent radiotherapy for HCC between September 1992 and April 2000 at the National Cancer Center Hospital East. Patient characteristics are shown in Table 1. Hepatocellular carcinoma was clinically diagnosed using ultrasound, dynamic computed tomography (CT), angiography and serum α-fetoprotein concentrations. The main tumors were located in the right lobe in 27 patients, the left lobe in 18 and both lobes in one patient. The main tumor determined by the late phase of dynamic CT was 2.5–15.0 cm in diameter (mean ± SD, 7.3 ± 3.2 cm). Among 37 patients with tumor thrombus of a portal vein, the thrombus was located within the second branch of the portal vein in seven patients, within the first right or left portal vein in 17, and to the portal trunk in 13.

Table 1 Patient characteristics

Variable	No.patients (n = 46)
Gender	
Male	38
Female	8
Median age (years) (range)	61 (42–82)
Performance status†	
0	26
1	11
2	9
Child–Pugh classification (Pugh score)	
Grade A (5 or 6)	14
Grade B (7, 8, or 9)	27
Grade C (10–15)	5
Viral markers	
HBs Ag(+), HCV Ab(–)	7
HBs Ag(–), HCV Ab(+)	36
HBs Ag(–), HCV Ab(–)	3
Portal vein tumor thrombus	
(–)	9
(+)	7
(++)	30
Previous treatment	
None	24
TACE	11
Hepatectomy + TACE	5
PEI	3
PEI + TACE	3

PEI, percutaneous ethanol injection; TACE, transcatheter arterial chemoembolization.

†Eastern Cooperative Oncology Group score: 0, fully active; 1, restricted in physically strenuous activity; 2, ambulatory and fully capable of self-care; 3, capable of only limited self-care; and 4, completely disabled. Portal vein tumor thrombus: (–), none; (+), within second branches; (++), beyond second branches.

The target volume of radiotherapy was defined in order to exclude at least one-half of the non-cancerous liver volume outside of the irradiated volume. Accordingly, 34 of the 46 patients received radiotherapy only for their portal vein tumor thrombus and the other 12 patients received radiotherapy for the entire primary HCC and portal vein tumor thrombus. External beam radiotherapy (EBRT) to a target dose of 50 Gy/25 fractions per 5 weeks was planned using 6–21 MV (median 10 MV) of X-rays. The EBRT planning was performed using a CT-based 2-D planning system (CT Port, Toshiba, Tokyo, Japan). Gross tumor volume (GTV) was defined according to the aforementioned restriction regarding irradiated volume of non-cancerous liver, which was based on radiographic findings. Clinical target volume (CTV) encompassed gross tumor volume defined as a radiographically abnormal area with an additional 1–1.5 cm margin. Planning target volume (PTV), consisting of CTV with 1- and 2-cm margins in the cranial and caudal directions, respectively, was defined taking into account both the respiratory motion of the liver and daily set-up error. A 2- or 3-beam arrangement was used in order to minimize not only the irradiated volume of non-cancerous liver, but also the inhomogeneity of dose distribution within the PTV. Radiotherapy dose prescription was defined at the center of the GTV. The irradiation field ranged from 5.4 cm × 6.0 cm to 15.2 cm × 15.7 cm.

Transcatheter arterial chemoembolization was performed in 35 patients with HCC lesions outside the field of irradiation. A mixture of iodized oil (Lipiodol; Andre Guerbet, Aulnay-sous-Bois, France) and epirubicin followed by gelatin sponge particles was injected from the right or left hepatic artery. The TACE was repeated every 2–3 months. It was performed once in 14 patients, twice for 12, three times for three patients, four times for three patients, and 5–10 times for five patients, respectively.

We evaluated hepatic function during the acute phase starting from irradiation until 90 days after, according to the NCI-CTC, and the late phase (from 90 days to 1 year later) according to the RTOG/EORTC Late Radiation Morbidity Scoring Scheme. Adverse events affecting liver function were evaluated by measuring serum bilirubin, serum albumin, serum glutamic oxaloacetic transaminase (SGOT); serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), prothrombin time, ascites, portal vein flow and hepatic enlargement. We evaluated an abnormality that worsened one or more grades in each parameter in patients with liver dysfunction before irradiation as an adverse event. Patients underwent dynamic CT to evaluate the response at 2–3-month intervals after therapy. Computed tomography was performed to obtain contiguous transverse sections using the helical scanning method at a section thickness of 5 mm. Tumor response was assessed according to the WHO criteria.[12]

We statistically analyzed the results using the Kruskal–Wallis Exact test to compare hepatic adverse events among grades determined by the Child–Pugh

classification, between patients with and without TACE, and among grades of portal vein tumor thrombus. Survival was calculated using the Kaplan–Meier method from the start of irradiation. The statistical significance of differences between survival curves was determined according to the log–rank test. The Cox proportional hazards model was used for multivariate analysis of prognostic factors. Differences with $P < 0.05$ were considered significant.

RESULTS

The full 50-Gy irradiation dose was feasible in 40 of the 46 patients (87.0%). Irradiation was stopped because of general deterioration in two patients at 46 and 44 Gy, massive ascites in two at 42 and 36 Gy, bleeding from esophageal varices in one at 36 Gy, and hepatic encephalopathy in one at 18 Gy. Histological examination of the ascites confirmed the absence of malignant cells.

Adverse hepatic events

We identified 28 (60.9%) adverse hepatic events among 46 patients during the acute phase. The most common adverse events were hyperbilirubinemia, hypoalbuminemia, and ascites. Grade 3 or 4 toxicity developed in 18 (39.1%) with hyperbilirubinemia, hypoalbuminemia, ascites, or elevation of SGOT, SGPT, ALP, and/or γ-GTP. SGOT and/or SGPT values were temporarily elevated to grade 3 in three patients and one patient started to bleed from esophageal varices, which elevated the SGOT grade to 4. The ALT and γ-GTP values also transiently increased to grade 3 in one patient.

We evaluated adverse events during the late phase in 33 patients. Eight patients died within 3 months of starting irradiation, and five patients who died within 4 months were excluded because they were terminal during the last month and adverse effects of irradiation could not be accurately evaluated. Adverse events developed in 19 (57.6%) of the 33 patients, the most frequent being ascites, hyperbilirubinemia and hypoalbuminemia. Grade 3 or 4 toxicity appeared in 11 (33.3%) with hyperbilirubinemia, ascites, elevation of SGOT, SGPT, ALP, and/or γ-GTP. Serum glutamic oxaloacetic transaminase, SGPT, ALT, and/or γ-GTP increased to grade 3 in 4 patients, but this was also accompanied by hyperbilirubinemia or ascites.

Relationship between adverse hepatic events and tumor response

Fourteen of the 46 patients achieved a partial response, but no complete responses were observed. The overall response rate was 30.4% (95% confidence interval [CI], 17.7–45.8%). Table 2 shows the relationship between acute adverse hepatic events and tumor response. The frequency of adverse events that developed during the acute phase tended to be higher in patients without tumor response than with tumor response ($P = 0.087$).

Table 2 Relationship between local response and acute hepatic adverse event

		Grade of acute hepatic adverse event Adverse hepatic event		
	n	0–1	≥2	
PR	14	10	4	$P = 0.087$
NC	22	9	13	
PD	10	3	7	

NC, no change; PD, progressive disease; PR, partial response.

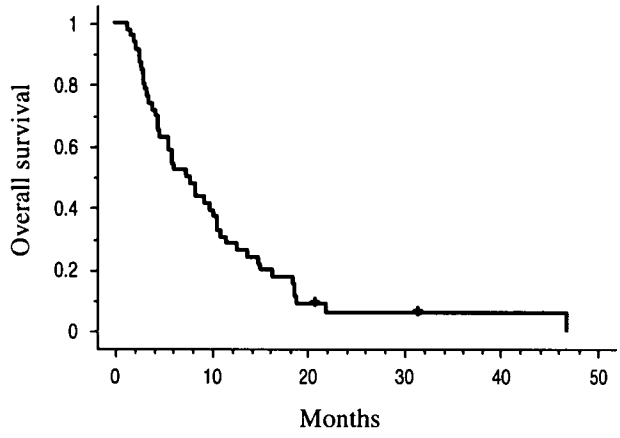

Figure 1 Overall survival rates of all patients ($n = 46$). The median survival time was 7.5 months, and the 1- and 2-year survival rates were 28.3% and 5.8%, respectively.

Relationship between adverse hepatic events and other factors

To determine the influence on adverse hepatic events by other factors, we examined the relationship between the adverse events and these factors: liver function before irradiation, using the Child–Pugh classification (Pugh score) and combined treatment of TACE, and the presence of portal vein tumor thrombi. The frequency of adverse events that developed during the acute phase tended to be higher in patients with more serious liver dysfunction (score 7, 8, or 9 and 10–15) than with mild liver dysfunction (score 5 or 6; $P = 0.070$). There were statistically no differences between patients treated with and without TACE during any period ($P = 0.61$). Regarding portal vein tumor thrombi, adverse events developed more often in patients with than without portal vein tumor thrombi; in particular, the difference during the acute phase was statistically significant ($P = 0.012$).

Survival

The overall survival curve is shown in Figure 1. Of all the 46 patients, 45 have now died except for one patient

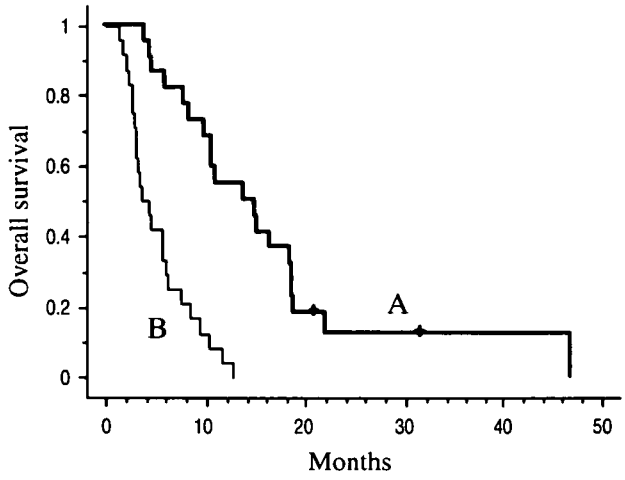

Figure 2 Survival curves according to grades of adverse hepatic event during the acute phase. Curve A, survival of patients with grade 0 or 1 ($n = 24$), median survival time is 13.8 months; curve B, patients with grade ≥2 ($n = 22$), median survival time is 3.5 months. One-year survival rate was 54.5% in grade 0 or 1, 4.2% in grade ≥2. $P < 0.0001$.

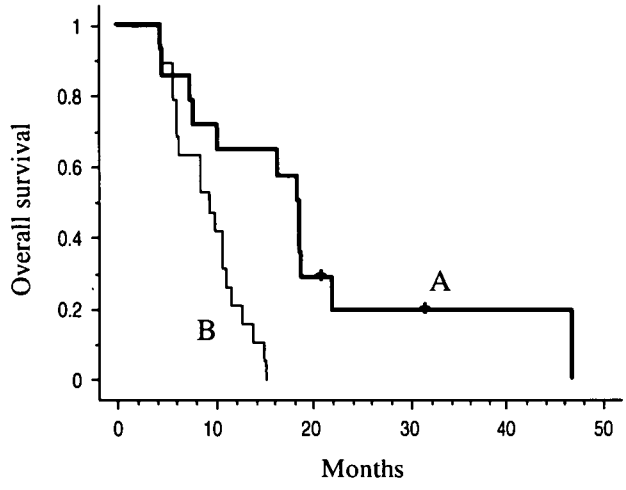

Figure 3 Survival curves according to grades of adverse hepatic event during the late phase. Curve A, survival of patients with grade 0 or 1 ($n = 14$), median survival time 18.5 momths; curve B, patients with grade ≥2 ($n = 19$), median survival time 9.3 months. One-year survival rate was 64.3% in grade 0 or 1, 15.8% in grade ≥2, respectively. $P < 0.0007$.

with whom we have lost contact. The causes of death were tumor progression in 31, liver failure in seven, variceal bleeding in two, pneumonitis due to irradiation in one, and other diseases in four (heart disease in two, renal dysfunction in one, and lung edema in one). Pneumonitis developed 2 months after irradiation. The median survival time (MST) was 7.5 months, and the 1- and 2-year survival rates were 28.3% and 5.8%, respectively. We divided patients with adverse hepatic events during the acute and late phase into the following groups: A, grade 0 or 1; and B, grades 2 or more. We compared survival between the two groups (Figs 2,3), and the survival rates of the two groups statistically differed ($P < 0.0001$, 0.0007).

Prognostic factors related to survival

Performance status, the Child–Pugh classification, the presence of portal vein tumor thrombi, tumor response, and adverse hepatic events during the acute phase and late phase had a prognostic significance for survival (Table 3). Significant prognostic factors identified by univariate analysis were entered into multivariate analysis (Table 4). The independent adverse prognostic factors for survival in patients with HCC treated with radiotherapy were portal vein tumor thrombus ($P = 0.0012$), tumor response ($P = 0.011$), acute adverse hepatic event ($P = 0.012$), and late adverse hepatic event ($P = 0.015$).

DISCUSSION

Although radiotherapy has long been applied to patients with unresectable HCC, it has never been recognized as totally satisfactory. However, local radiation with a limited-field high dose of 48–72.6 Gy is effective for treating HCC.[1-5] In contrast, to achieve positive treatment effects against HCC using radiotherapy, attention should be paid to liver tolerance, because irradiation also injures the normal liver and serious radiation-induced liver disease can develop.[2] Cheng *et al.* reported that radiation-induced liver disease and gastrointestinal bleeding are the most frequent treatment-related toxicities and that six of 25 patients developed radiation-induced liver disease evidenced by elevated ALP and transaminases and non-malignant ascites.[5] Guo *et al.* reported that two patients died of liver failure or variceal bleeding associated with the therapy, and that serum bilirubin, serum transaminase and ascites increased by 13%, 27% and11%, respectively.[6] Although radiation-induced liver dysfunction should be assessed in detail, to our knowledge there are few efforts that have been directed towards this issue.

The present study examined adverse hepatic events caused by radiotherapy in two periods: acute phase within 90 days and the late phase from 90 days to 1 year later. Hypoalbuminemia, hyperbilirubinemia, and ascites were the most frequently seen in grade 3 or 4 of adverse events during both periods. Elevation of SGOT, GPT, ALP, and γ-GTP were almost always accompanied by hyperbilirubinemia, hypoalbuminemia, and/or ascites and increased temporarily. Thus, hyperbilirubinemia, hypoalbuminemia and ascites seem to be important factors when evaluating liver dysfunction with respect to radiotherapy as a treatment for HCC.

Variceal rupture and hepatic encephalopathy were important complications of HCC and/or liver cirrhosis. Variceal bleeding developed during and after irradiation in two of the present patients. Hepatic encephalopathy developed during irradiation in one patient. Each of these patients developed serious liver dysfunction and died early. We therefore believe that the development of

Table 3 Univariate analysis of prognostic factors for survival in patients with advanced hepatocellular carcinoma treated by radiotherapy

Variable	*n*	Median survival (months)	Survival rate (%) 1 year	2 year	*P*
Gender					
Male	38	7.5	26.3	3.9	0.59
Female	8	7.3	37.5	12.5	
Age (years)					
<60	20	5.7	40.0	0	0.98
≥60	26	7.9	19.2	7.7	
Performance status					
0	26	9.8	38.5	10.3	0.016
1, 2	20	5.8	15.0	0	
Child–Pugh classification					
Grade A	14	14.3	57.1	7.1	0.049
Grade B, C	32	5.9	15.6	6.3	
HCV Ab					
Positive	36	7.9	27.8	7.4	0.29
Negative	10	5.0	30.0	0	
Portal vein tumor thrombus					
(–) or (+)	16	12.1	50.0	18.8	0.002
(++)	30	4.6	16.7	0	
TACE					
With	11	11.1	36.4	9.1	0.53
Without	35	6.0	25.7	4.3	
Tumor response					
PR	14	13.8	64.3	7.1	0.007
NC + PD	32	4.5	12.5	6.3	
Adverse hepatic event during acute phase					
Grade 0 or 1	24	13.8	54.5	12.1	<0.0001
Grade ≥2	22	3.5	4.2	0	
Adverse hepatic events during late phase					
Grade 0 or 1	14	18.5	64.3	12.7	0.0007
Grade ≥2	19	9.3	15.8	0	

NC, no change; PD, progressive disease; PR, partial response; TACE, transcatheter arterial chemoembolization.
Portal vein tumor thrombus: (–), none; (+), within second branches; (++), beyond second branches.

Table 4 Independent prognostic factors for survival in patients with advanced HCC treated by radiotherapy

Variable	Category	Relative risk (95%CI)	*P*
Performance status	0	1	0.69
	1 or 2	1.20 (0.50–2.98)	
Child–Pugh classification	A	1	0.95
	B or C	1.03 (0.43–2.50)	
Portal vein tumor thrombus	(–) or (+)	1	0.0012
	(++)	4.91 (1.88–12.84)	
Tumor response	PR	1	0.011
	NC + PD	3.68 (1.35–10.06)	
Adverse hepatic event during acute phase	Grade 0 or 1	1	0.012
	Grade ≥2	3.59 (1.32–9.79)	
Adverse hepatic event during late phase	Grade 0 or 1	1	0.015
	Grade ≥2	3.96 (1.30–12.01)	

CI, confidence interval; HCC, hepatocellular carcinoma; NC, no change; PD, progressive disease; PR, partial response; TACE, transcatheter arterial chemoembolization.
Multivariate analysis using the Cox proportional hazards model.
Portal vein tumor thrombus: (–), none; (+), within second branches; (++), beyond second branches.

the esophageal varices and hepatic encephalopathy, including during the irradiation therapy, should be carefully considered.

We examined the relationship between adverse hepatic events and survival. We confirmed that survival rates worsened according to the grade of adverse events (grades 0 or 1, and 2 or more) and differences between the two groups in each period were statistically significant. On the other hand, tumor characteristics such as α-fetoprotein level, tumor size, number of tumors, and portal involvement have been reported as prognostic factors for HCC.[13–15] Therefore, we examined the independent adverse prognostic factors for survival by multivariate analysis with the Cox proportional hazards model. As for the results, the independent adverse prognostic factors for survival in patients with HCC treated by radiotherapy were portal vein tumor thrombus ($P = 0.0012$), tumor response ($P = 0.011$), acute adverse hepatic events ($P = 0.012$), and late adverse hepatic events ($P = 0.015$). The present results indicated that adverse hepatic event can be a significant prognostic factor in addition to other conventional factors such as the presence of a portal vein tumor thrombus. Particularly, once patients develop grade 2 or more adverse events, the prognosis is potentially very poor, so they require careful follow up.

The present study found that grade 3 or 4 adverse hepatic events developed at a very high rate compared with other reports.[4–6] Thirty-seven (80.4%) of the present patients had tumor thrombi of the portal vein, which may explain why the frequency of adverse hepatic events was so high. We examined the relationship between the grade of the tumor thrombus and adverse hepatic events. Adverse events developed more often in patients with, than without portal vein tumor thrombi, particularly during the acute phase. Acute adverse effects of radiotherapy are generally reversible. However, those adverse effects, particularly hepatic ones, can often be irreversible in patients with advanced HCC. From this point of the view, we considered that hepatic adverse events during the acute phase affected the survival. When it is to be applied to patients who have advanced HCC such as those with portal vein tumor thrombi, radiotherapy should be carefully considered and patients should be closely observed.

The present study has some limitations that should be recognized with respect to the evaluation of the hepatic adverse effects. The present study was a retrospective analysis, and it included two groups of patients who had the different irradiation targets: mainly the portal vein tumor thrombus and the entire HCC lesion including the tumor thrombus. It was difficult to evaluate exactly the hepatic adverse effects, if the volume of the normal liver tissue irradiated was calculated. Recently, 3-D planning has been applied to determine the CTV in patients with HCC.[16,17] In the radiotherapy using this 3-D planning, the dose–volume histogram can be used for calculation of the volume of the normal liver tissue irradiated in radiotherapy for HCC. Because EBRT planning was performed using a 2-D planning system in the present study and the patients were heterogeneous in the radiation targets, we could not examine the volume of the normal liver tissue irradiated. The results of the present study must be understood under these limitations, and a prospective analysis of the hepatic adverse effects should be performed using factors that were important in this study under consideration of the volume of the normal liver tissue irradiated.

In conclusion, we confirm that hyperbilirubinemia, hypoalbuminemia and ascites are important factors that reflect survival. Furthermore, bleeding of an esophageal varix and hepatic encephalopathy also negatively affect prognosis. The evaluation of adverse hepatic events seems to be valuable in predicting prognosis.

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＜短　報＞

高度進行肝細胞癌に対するシスプラチン動注化学療法：重篤な有害事象は稀ではない

石井　浩　古瀬　純司　仲地　耕平　吉野　正曠

緒言：進行・再発肝細胞癌に対し，本邦では血管塞栓療法(TAE)が広く行われているが，高度脈管浸襲によるTAE非適応例やTAE無効例は少なくない．このような症例に対しては各種化学療法が試みられてきたが，肝細胞癌に対し確実な抗腫瘍効果を有する薬剤は未だない．この中で，シスプラチンは単剤全身投与で15%の抗腫瘍効果[1]を有し，多剤併用でより高い奏効率[2]が報告されている．一方，腫瘍内薬剤濃度を高め抗腫瘍効果増強を期待する動注化学療法は，全身投与に比較して概ね抗腫瘍効果が高く，シスプラチンを含むレジメンで奏効率41～51%[3,4]が報告されている．

動注用アイエーコール(日本化薬株式会社)は，シスプラチンを動脈内投与に適した微粉末にした新規剤型の抗癌剤であり，静注用シスプラチン同様に癌細胞内DNA鎖と結合，DNA合成とそれに続く癌細胞分裂を阻害することで殺細胞効果を示す．本剤の調整濃度(1.5 mg/ml)は静注用シスプラチン(0.5 mg/ml)と比較して高い．これにより，シスプラチンの濃度依存性殺細胞効果は高まることが期待される．また，製剤総量の減量により動注所要時間の短縮が可能となった．国内後期第II相試験(治験)では，100 mgを70 ml生理食塩水に溶解，60 mg/m^{2}を20～40分かけて投与することを4～6週間隔で繰り返す方法で行われ，奏効率33.8%と単剤では従来の承認薬剤を上回る良好な成績が示され，2004年1月29日に製造が承認された．

当院では本剤が市販された7月12日からTAE非適応・無効例に対し，積極的にシスプラチン肝動注化学療法を施行してきた．しかし，この間，重篤な有害事象をしばしば経験したことから，本療法が本邦に広く普及する前に注意喚起を促すことを目的として自験例の有害事象の実態を調査した．

方法：対象は2004年7月12日から11月17日までに本剤を用いた肝動注化学療法を施行したすべての肝細胞癌例とした．

有害事象(治療との因果関係を問わない全ての好ましくない事象)および薬物有害反応(有害事象のうち薬物との因果関係が否定できないもの)は治療開始日から治療終了日まで，治療継続中の場合は2004年12月28日直近の最終診察日までの間の最悪のgradeをNCI-CTC version 2.0日本語訳JCOG版第2版[5]に従い評価した．抗腫瘍効果は固形がん治療効果のための新ガイドライン(RECIST)に従い評価した．

治療は動注用アイエーコール100 mgを70 mlの生理食塩水に加えて溶解し，腫瘍状況に応じて固有肝動脈もしくはそれより抹消より挿入したカテーテルから40分間で投与した．投与後は4～6週間休薬，これを1コースとし，明らかな病態悪化が見られない場合は投与を繰り返した．治療開始にあたり，患者本人に本療法を説明し文書による同意を得た．

成績：対象は18例(男性17例)，年齢中央値63歳，ウィルスマーカーはHBs抗原陽性4例，HCV抗体陽性10例，双方陰性4例であった．全例，腫瘍径2 cm以上の多発例であり，7例が門脈浸襲(本幹5例，右一次分枝2例)を有した．また，5例は巨大腫瘍に伴う疼痛を有した．本療法選択の理由は，TAE無効10例，高度脈管浸襲によるTAE非適応5例，巨大腫瘍3例であった．肝予備能はChild-Pugh grade A：5例，同B：13例であった．6例が初回治療例，12例が既治療(肝切除9回，経皮的局所壊死療法12回，TAE 37回，動注化学療法19回，体外照射放射線療法1回，全身性化学療法1回)後の再発例であり，初回治療から本療法開始までの期間は2.4～92.4カ月(中央値42.3)であった．

全例1コースまでの評価が終了，7例は治療継続中(1～4コース)，11例は治療終了(1～3コース)であった．治療終了の理由は，進行4例，肝不全6例，早期死亡1例であった．治療後2カ月以内の早期死亡例が4例みられ，死因は進行2例，肝不全に伴う代謝性アシドーシス(Table 1：acidosis)及び心血管障害(Table 1：cardiovascular)各1例であった．抗腫瘍効果は部分奏効1例，安定10例，進行4例，評価不能3例であった．Grade 3/4の血液学的，非血液学的有害事象は各々7例(39%)，14例(78%)にみられた．有害事象の中で，同一grade内での変動を除いた薬物有害反応をTable 1に示した．Grade 3/4の薬物有害反応は血液毒性6例(33%)，非血液毒性12例(67%)にみられた．Grade 3/4の非血液毒性は①Child-Pugh grade B：13例中10例，②複数回の経動脈的治療後の再発：9例中7例にみられ，①かつ②の6例では全例に出現した．

考察：肝切除や局所壊死療法の適応とならない進行肝細胞癌に対する第一選択の治療法はTAEであり，肝動注化学療法がTAEに代替し得るエビデンスはない．従って肝動注化学療法の対象は，今回のようなTAE非適応・無効である高度進行例が中心となることが予想される．このような高度進行例ではChild-Pugh grade C例を除いても，背景肝障害，及びこれによる汎血球減少を有し，化学療法を安全に行うための臓器機能が確保されていないことが少なくない．これが重篤な有害事象割合が高率である理由であるが，同一grade内での変動を除いた薬物有害反応でみても一般的な他癌腫の化学療法に比較し高率といえる．

本療法はTAEが適応とならない高度門脈浸潤例に対する第一選択治療，TAE無効例における第二選択治療として期待される．しかし，今回のように肝予備能良好例でも重篤な有害反応を来すことが稀ではなく，Child-Pugh grade B例

国立がんセンター東病院肝胆膵内科

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Table 1　The worst adverse drug reaction per patient (N=18)

Grade	1	2	3	4
Hemoglobin	3 (17%)	4 (22%)	3 (17%)	1 (6%)
Leukocytes	5 (28%)	3 (17%)	1 (6%)	0
Neutrophils	3 (17%)	1 (6%)	1 (6%)	0
Platelets	2 (11%)	2 (11%)	3 (17%)	0
Hamatological (total)	3 (17%)	7 (39%)	5 (28%)	1 (6%)
Cardiovascular	0	0	0	1 (6%)
Prothrombin time	1 (6%)	1 (6%)	1 (6%)	0
Fever	4 (22%)	0	0	0
Nausea	5 (28%)	1 (6%)	0	0
Epistaxis	0	1 (6%)	0	0
Hematemesis	0	0	1 (6%)	0
Alkaline phosphatase	0	1 (6%)	1 (6%)	0
Bilirubin	8 (44%)	6 (33%)	1 (6%)	1 (6%)
Hypoalbuminemia	1 (6%)	7 (39%)	0	0
SGOT	1 (6%)	3 (17%)	9 (50%)	0
SGPT	3 (17%)		0	0
Hyponatremia	5 (28%)	-	3 (17%)	0
Hyperkalemia	0	1 (6%)	0	0
Hypokalemia	4 (22%)	-	1 (6%)	0
Acidosis	0	0	0	1 (6%)
Hyperglycemia	5 (28%)	4 (22%)	1 (6%)	0
Creatinine	2 (11%)	1 (6%)	0	0
Non-hematological (total)	0	6 (33%)	9 (50%)	3 (17%)

や濃厚な既治療後の再発例では特に慎重な症例選択及び経過観察が必要である．

索引用語：有害反応，経動脈的治療，血管塞栓療法

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英文要旨

The frequency of severe adverse effect in hepatic arterial cisplatin infusion chemotherapy was not rare for far advanced hepatocellular carcinoma patients

Hiroshi ISHII, Junji FURUSE, Kouhei NAKACHI, Masahiro YOSHINO

National Cancer Center Hospital East, Kashiwa

The aim of this study was to clarify the frequency of severe toxicity in hepatic arterial cisplatin infusion chemotherapy for far advanced hepatocellular carcinoma patients. Study subjects were consecutive 18 patients with hepatocellular carcinoma no longer amenable to transcatheter arterial chemoembolization. Hepatic arterial infusion using cisplatin 100 mg alone was repeated at 4 to 6-week intervals until disease progression. According to National Cancer Institute Common Toxicity Criteria version 2, the grade 3 or worse hematological and non-hematological adverse events (reactions) were observed in 7 (6) and 14 (12) of the 18 patients, respectively. Two patients died within 2 months due to possible toxicities of the treatment. This treatment was so toxic that candidates should be selected among patients with good hepatic reserve.

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特集 臨床腫瘍学の現状と展望

V．がん薬物療法の実際

5．肝胆膵癌

Furuse Junji 古瀬 純司　Ishii Hiroshi 石井 浩　Nakachi Kohei 仲地 耕平　Suzuki Eiichiro 鈴木英一郎
Yoshino Masahiro 吉野 正曠*
*国立がんセンター東病院肝胆膵内科

はじめに

1997年，進行膵がんにおいてgemcitabine(ジェムザール®)と5-fluorouracil(5-FU®)との無作為化比較試験によりgemcitabineの有効性が証明され，以降gemcitabineが進行膵がんに対する標準化学療法として広く用いられている．しかし，その治療成績は満足のいくものではなく，多くの新しい治療法の開発が行われている．一方，肝・胆道がんにおいては依然標準といえる化学療法はなく，その確立が急務である．

肝がんにおける化学療法

原発性肝がんは，肝細胞がんや肝内胆管がんなど多くの組織型がみられ，極めて多彩である[1]．ここでは，原発性肝がんの約90%を占める肝細胞がんについて述べる．

1．化学療法の適応

肝細胞がんにおいては，がんの進行度と肝障害度に応じて治療法が選択され，肝切除，ラジオ波やエタノール注入による局所壊死療法，動脈化学塞栓療法(TACE)が標準治療として行われている．化学療法は，高度の門脈腫瘍塞栓を伴う例，上記の標準治療が適応困難な例，および遠隔転移例が適応となる．

肝細胞がんに対する化学療法は，肝動脈から注入する経動脈性化学療法(動注化学療法)と，経静脈あるいは経口による全身化学療法に分けられる．肝細胞がんでは，肝硬変など慢性肝障害を背景にもつ例が多いことから，肝障害を助長するリスクも大きく，その適応は慎重に考慮する必要がある．肝障害度評価としてChild-Pugh分類が汎用されているが[2]，Child-Pugh Cの肝機能不良例では化学療法は原則として禁忌である．

2．動注化学療法

TACE無効例やTACEの適応とならない巨大腫瘍，高度門脈腫瘍塞栓例が適応となる．ここではリピオドールを併用しない抗がん剤のみを注入する治療を，動注化学療法として取り上げる．わが国では動注化学療法剤としてepirubicin(ファルモルビシン®)，5-fluorouracilが主に用いられてきたが，2004年7月，cisplatin(アイエーコール®)の保険適応が承認された．**表1**に主な薬剤の治療成績を示す．

Epirubicinは一般に奏効率15%程度と報告されている[3]．門脈1次分枝以上に及ぶ高度門脈腫瘍塞栓例を対象に，同治療の有効性を検討したところ，19%の奏効率が得られ，2年以上の長期生存例も認めている[9]．Cisplatinはepirubicinより高い抗腫瘍効果が認められ，動注化学療法でも有効性が期待されているが，骨髄毒性や肝障害の増悪など，重篤な有害事象も少なくないことから，適応は慎重に行うべきである[10]．5-fluorouracilの動注化学療法は，原則的にリザーバーシステムの留置が必要である．最近では5-fluorouracilを基本薬剤とした併用治療が試みられ，5-fluorouracil＋cisplatinや5-fluorouracil＋interferon(IFN)で高い奏効率が報告されている[7,8]．しかし，これらの動注化学療法の有効性や位置づけは確立しておらず，比較試験など多数例での検討が必要である．

0287-3648/05/¥500/論文/JCLS

表1　肝細胞がんに対する主な動注化学療法

抗がん剤	奏効率	MST(月)	1年生存率	報告者(報告年)
Epirubicin	15%(8/53)	6.8	28%	Epirubicin Study Group in Japan(1987)[3]
Floxuridine/LV/DXR/CDDP	41%(12/29)	15.0	54%	Patt(1994)[4]
MTX/5-FU/CDDP/IFN	47%(7/15)*	7.0	27%	Urabe(1998)[5]
CDDP/IFN	33%(6/18)	4.9	27%	Chung(2000)[6]
CDDP/5-FU	48%(23/48)*	10.2	45%	Ando(2002)[7]
5-FU/IFN	52%(12/23)*	—	—	左近(2003)[8]
Epirubicin	19%(3/16)*	6.0	21%	光永(2004)[9]

MST：median survival time, LV：leucovorin, DXR：doxorubicin, CDDP：cisplatin, MTX：methotrexate, 5-FU：5-fluorouracil, IFN：interferon, *：門脈腫瘍塞栓例のみが対象.

表2　肝細胞がんに対する主な全身化学療法

抗がん剤	奏効率	MST(月)	1年生存率		報告者(報告年)
Mitoxantrone	23%(4/17)	5.0	—		Colleoni(1992)[11]
CDDP	15%(4/26)	—	—		Okada(1993)[12]
Gemcitabine	18%(5/28)	4.4	20%		Yang(2000)[13]
Gemcitabine	0%(0/30)	6.9	40%		Fuchs(2002)[14]
Epirubicin/etoposide	39%(14/36)	10.0	28%		Bobbio-Pallavicini(1997)[15]
CDDP/DXR/5-FU/IFN	26%(13/50)	8.9	36%		Leung(1999)[16]
5-FU/Mit/CDDP(FMP)	27%(14/51)	11.6	44%		Ikeda(2005)[17]
EPI/CDDP/5-FU(ECF)	14%(3/21)	10.0	70%		Boucher(2002)[18]
5-FU/IFN-α-2b	14%(4/28)	15.5	62%		Patt(2003)[19]
*Doxorubicin	3%(2/60)	2.5	5%	p=0.036	Lai(1988)[20]
BSC	n=46	1.8	5%		
*IFN-α	31%(11/35)	2.6	18%	p=0.047	Lai(1993)[21]
BSC	n=36	1.8	3%		
*Tamoxifen	0%(0/58)	14.3	51%	p=0.75	Castells(1995)[22]
Placebo	n=62	6.0	43%		
*IFN-α	7%(2/30)	15.2	58%	p=0.19	Llovet(2000)[23]
BSC	n=30	7.2	38%		

MST：median survival time, CDDP：cisplatin, DXR：doxorubicin, 5-FU：5-fluorouracil, IFN：interferon, Mit：mitoxantrone, EPI：epirubicin, BSC：best supportive care, *：無作為化比較試験.

3．全身化学療法

動注化学療法と同様，標準治療の適応とならない例，TACEの無効例，および遠隔転移例に適応される．門脈腫瘍塞栓例は極めて予後が不良であり，現在のところ全身化学療法では有効例は極めて少なく，適応は難しい．

単剤で15%以上の奏効率が報告されている薬剤は，cisplatin，mitoxantrone(ノバントロン®)など，わずかである．多剤併用療法では，5-fluorouracil/mitoxantrone/cisplatin(FMP)，cisplatin/doxorubicin/5-fluorouracil/IFN-αで25%を超える高い奏効率が報告されている(表2)．これまで肝細胞がん患者を対象とした無作為化比較試験がいくつか行われている．Doxorubicinでは，無治療群に比べ有意に生存期間の延長が得られているが[20]，tamoxifenやIFN-αでは生存期間の改善は明らかではない[21-23]．これまで肝細胞がんでは，有効な全身化学療法は確立しておらず，生存期間を評価項目とした大規模試験が必要である．また，肝細胞がんは血管新生が豊富な腫瘍であり，VEGFR阻害薬などの分子標的薬の開発が期待されている．

胆道がん

1．全身化学療法の適応

胆道がんにおける化学療法は，奏効例は認めるもの

表3　胆道がんに対する主な全身化学療法

抗がん剤	奏効率	MST(月)	1年生存率	報告者(報告年)
Gemcitabine	22%(7/32)	11.5	44%	Penz(2001)[25]
Gemcitabine	36%(9/25)	7.0	17%	Gallardo(2001)[26]
LV/5-FU/CDDP	34%(10/29)	9.5	50%	Taieb(2002)[27]
5-FU/LV/oxaliplatin	19%(3/16)	9.5	−	Nehls(2002)[28]
Gemcitabine/docetaxel	9%(4/43)	11.0	42%	Kuhn(2002)[29]
CDDP/EPI/5-FU	19%(7/37)	5.9	24%	Morizane(2003)[30]
Gemcitabine/oxaliplatin	33%(11/33)	15.4	57%	Andre(2004)[31]
Gemcitabine/5-FU/LV	12%(5/42)	9.7	36%	Alberts(2005)[32]
EPI/CDDP/UFT/LV	23%(9/40)	7.9	32%	Park(2005)[33]
Gemcitabine/CDDP	28%(11/40)	8.4	−	Thongprasert(2005)[34]
Gemcitabine/capecitabine	31%(14/45)	14.0	49%	Knox(2005)[35]
*MMC/gemcitabine	20%(5/25)	6.7	−	Kornek(2004)[36]
MMC/capecitabine	31%(8/26)	9.3	−	

MST：median survival time, LV：leucovorin, 5-FU：5-fluorouracil, CDDP：cisplatin, EPI：epirubicin, UFT：tegafur-uracil, MMC：mitomycin C. *：無作為化比較試験.

の根治例はなく，切除不能の進行例が治療対象となる．また，胆嚢がんの化学療法の治療成績を検討したところ，全身化学療法は全身状態が不良な例(PS 2)に比べ，良好な例(PS 0または1)で予後の改善に寄与することが確認されており[24]，現状では全身状態を十分考慮した上で適応を考慮すべきである．

2．全身化学療法の現状

表3に最近の主な化学療法の治療成績を示す．多剤併用による化学療法が多く試みられ，最近ではgemcitabineを基本薬剤とした多剤併用療法により，比較的高い奏効率が報告されつつある．わが国では現在，胆道がんに保険適応が承認されている薬剤は，tegafur-uracil(UFT®)，doxorubicin(アドリアシン®)，cytarabine(キロサイド®；ただし他の抗腫瘍薬と併用)に限られている．しかし，これらの薬剤では有効性は期待できず，胆道がんに有効な薬剤の開発と標準治療の確立が急務である．最近，わが国ではgemcitabineやS-1による臨床試験が行われ，保険適応の承認が期待されている．

膵がん

1．全身化学療法の現状

1997年，5-fluorouracilとの無作為化比較試験によりgemcitabineの有用性が明らかとなり，gemcitabineが進行膵がんにおける新たな標準治療薬として各国に広がった[37]．Gemcitabineはcytarabineと構造的に類似した代謝拮抗薬に分類される抗がん剤であり，細胞内で三リン酸化物に代謝され，DNA合成を阻害することにより，強い殺細胞作用を示す．米国で行われた5-fluorouracilとの無作為化比較試験では，gemcitabine群において疼痛，performance status，体重減少など症状緩和効果が高率に認められ，生存期間においても有意差がみられた．わが国でも第1相試験が行われ，同様の投与法が可能であることが確認され，治療効果においても同等以上の成績が得られたことから，保険適応が承認された[38]．現在，gemcitabine 1,000 mg/m^2，週1回，3週投与，1週休薬を1コースとして繰り返す投与が推奨投与法として行われている．

一方，gemcitabineの投与法を変えることにより，その治療効果を高める試みが行われている．Temperoらは，gemcitabineの活性体である三リン酸化物の形成が投与量と投与時間に依存しており，10 mg/m^2/minが最適な投与速度であるという基礎実験から，1,500 mg/m^{2}を150分で投与する定速静注法(10 mg/m^2/min)を試みている．2,200 mg/m^{2}を30分で投与する方法との無作為化比較第2相試験では，奏効率は両者で差がみられなかったものの，生存期間中央値(MST)は8.0カ月と5.0カ月，1年生存率は28.8%と9.0%と，定速静注法で有意に良好であることが示された[39]．現在，米国のstudy groupであるECOGにおいて，gemcitabine 1,000 mg/m^{2}による標準投与法，gemcitabine定速静注法，gemcitabine 1,000 mg/m^{2}の定速静注投与とoxaliplatin(エルプラット®)の併用療法(GEMOX)の3群による大規模な無作為化比較試験が行われている．

Gemcitabineが標準治療薬として確立したとはいえ，

表4　進行膵がんにおけるgemcitabine単独と他レジメンの無作為化比較試験

抗がん剤	n	奏効率	MST(月)	1年生存率		報告者(報告年)
Gemcitabine	63	5.4%	5.7	18%	p=0.0025	Burris(1997)[37]
5-FU	63	0%	4.4	2%		
Gemcitabine	162	5.6%	5.4	20%	p=0.09	Berlin(2002)[40]
Gem/5-FU	160	6.9%	6.7	18%		
Gemcitabine	99	8.0%	6.0	—	p=0.12	Heinemann(2003)[41]
Gem/CDDP	96	10.2%	8.3	—		
Gemcitabine	173	4.4%	6.6	20%	p=0.79	Rocha Lima(2004)[42]
Gem/irinotecan	169	16.1%	6.3	20%		
Gemcitabine	156	16.7%	7.1	28%	p=0.13	Louvet(2005)[43]
Gem/oxaliplatin	157	28.7%	9.0	38%		
Gemcitabine	174	6.3%	6.7	23%	p=0.52	O'Reilly(2004)[44]
Gem/exatecan	175	8.2%	6.2	21%		
Gemcitabine	282	9.1%	6.2	20%	p=0.85	Richards(2004)[45]
Gem/pemetrexed	283	18.3%	6.3	21%		
Gemcitabine	159	8.0%	5.9	17%	p=0.025	Moore(2005)[46]
Gem/erlotinib	160	8.6%	6.4	24%		
Gemcitabine	285	7.9%	7.3	28%	p=0.31	Herrmann(2005)[47]
Gem/capecitabine	284	10.1%	8.4	30%		

MST：median survival time, 5-FU：5-fluorouracil, Gem：gemcitabine, CDDP：cisplatin.

その治療成績は依然満足できるものではない．現在，gemcitabineと他の薬剤との併用治療が盛んに試みられている．これまで5-fluorouracil, cisplatin, irinotecan（カンプト®），oxaliplatinなどとの併用療法による第3相臨床試験が報告されている（表4）[40-47]．多くの併用療法においてgemcitabine単独に比べ高い奏効率が得られるものの，明らかな生存期間の延長は認められなかった．2005年ASCO会議では，EGFRを阻害する分子標的薬erlotinibとgemcitabineの併用療法が，gemcitabine単独との無作為化比較試験において，有意に生存期間を改善したと報告され，注目を集めた．しかし，その差はわずかであり，同併用療法で得られる利点をさらに詳細に検討する必要がある．

Gemcitabine以外の薬剤として，わが国ではirinotecanやS-1（ティーエスワン®）などの臨床試験が行われ，高い奏効率が認められている[48,49]．また，S-1とgemcitabineとの併用療法による第1相試験が行われ，30～40%と高い奏効率が期待されることから[50]，多数例による臨床試験が行われつつある．

2．分子標的薬

がんの分子生物学，分子遺伝学の急速な進歩により，がん細胞に特徴的な遺伝子発現が明らかになり，その変異した分子を標的にした治療薬，いわゆる分子標的治療（molecular targeting therapy）が開発されている．膵がんにおいても，EGFR（epidermal growth factor receptor），VEGFR（vascular endothelial growth factor receptor），MMP（matrix metalloproteinase），K-ras遺伝子の異常など，様々な分子標的が明らかとなっている．膵がんにおける分子標的薬としては，先に述べたerlotinibのほかにもVEGFR阻害薬bevacizumab，EGFR阻害薬cetuximabなどが注目され，gemcitabineとの併用治療による臨床試験が行われている[51,52]．

3．全身化学療法の適応

切除不能の進行膵がんは，遠隔転移を認めない局所進行例と遠隔転移を有する例に分けられる．UICC第6版による進行度分類では，局所進行がんは腹腔動脈，あるいは上腸間膜動脈浸潤を認めるT4NxM0（Stage Ⅲ）に当たり，遠隔転移はTxNxM1（Stage Ⅳ）になる．遠隔転移例では，現在gemcitabineによる全身化学療法が標準治療として行われている．局所進行例においては，5-fluorouracilによる同時併用放射線化学療法が標準治療として位置づけられているが，消化管毒性などが少なくないことから，gemcitabineによる全身化学療法も多く行われている．Gemcitabineを中心とした全

身化学療法の臨床試験では，遠隔転移例とともに局所進行例も対象に含めたものが少なくない．Gemcitabineは強い放射線増感作用も認めており，gemcitabineによる放射線化学療法も試みられているが，際立った治療成績は得られていない[53]．現在，局所進行膵がんにおける標準治療は，むしろ混乱しているといってもいい状況であり，より有効な治療法の開発とともに，放射線化学療法とgemcitabineを用いた全身化学療法による大規模な比較試験も必要である．

おわりに

肝・胆道・膵がんにおいては，切除不能進行がんに対する治療戦略を考える上で，化学療法は重要な役割を果たしている．今後，より良好な抗腫瘍効果を有する治療法の開発が期待されるとともに，質の高い臨床試験の実施が必要である．

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Current Status of Systemic Chemotherapy for Hepatobiliary and Pancreatic Cancer

Junji Furuse, Hiroshi Ishii, Kohei Nakachi,
Eiichiro Suzuki and Masahiro Yoshino*

*Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital East

Gemcitabine has shown promise in advanced pancreatic cancer in a randomized trial comparing it with 5-FU. Gemcitabine is more effective than 5-FU in alleviating some disease-related symptoms, and it also offers an advantage in terms of survival rate. Gemcitabine has been accepted as the standard agent for the treatment of advanced pancreatic cancer, and despite numerous trials comparing gemcitabine-based combination chemotherapy and gemcitabine alone, no regimen has been found to have a definite survival advantage over gemcitabine alone. Molecular targeting agents, such as erlotinib, bevacizumab, and cetuximab, have recently been used in gemcitabine-based combination chemotherapy for pancreatic cancer. No regimen of systemic chemotherapy has shown a definite clinical benefit for hepatobiliary cancer. Most chemotherapy regimens have been performed as clinical trials, and although no usefulness of systemic chemotherapy for hepatocellular carcinoma or biliary tract cancer has been demonstrated in any of them, some promising agents are being developed, e.g., hepatic arterial infusion chemotherapy with 5-FU and CDDP or 5-FU and interferon for hepatocellular carcinoma, and gemcitabine or S-1 for biliary tract cancer. Well-designed clinical trials are needed to establish a standard regimen for advanced hepatobiliary cancer.

Regression of intestinal adenomas by vaccination with heat shock protein 105-pulsed bone marrow-derived dendritic cells in $Apc^{Min/+}$ mice

Kazunori Yokomine,[1,2] Tetsuya Nakatsura,[1,3] Satoru Senju,[1] Naomi Nakagata,[4] Motozumi Minohara,[5] Jun-ichi Kira,[5] Yutaka Motomura,[1,3] Tatsuko Kubo,[6] Yutaka Sasaki[2] and Yasuharu Nishimura[1,7]

Departments of [1]Immunogenetics and [2]Gastroenterology and Hepatology, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556; [3]Immunotherapy Section, Investigative Treatment Division Research Center for Innovative Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa 277-8577; [4]Center for Animal Resources and Development, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811; [5]Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582; [6]Department of Molecular Pathology, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan

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Heat shock protein (HSP) 105 is overexpressed in various cancers, but is expressed at low levels in many normal tissues, except for the testis. A vaccination with HSP105-pulsed bone marrow-derived dendritic cells (BM-DC) induced antitumor immunity without causing an autoimmune reaction in a mouse model. Because $Apc^{Min/+}$ mice develop multiple adenomas throughout the intestinal tract by 4 months of age, the mice provide a clinically relevant model of human intestinal tumor. In the present study, we investigated the efficacy of the HSP105-pulsed BM-DC vaccine on tumor regression in the $Apc^{Min/+}$ mouse. Western blot and immunohistochemical analyses revealed that the tumors of the $Apc^{Min/+}$ mice endogenously overexpressed HSP105. Immunization of the $Apc^{Min/+}$ mice with a HSP105-pulsed BM-DC vaccine at 6, 8, and 10 weeks of age significantly reduced the number of small-intestinal polyps accompanied by infiltration of both $CD4^+$ and $CD8^+$ T cells in the tumors. Cell depletion experiments proved that both $CD4^+$ and $CD8^+$ T cells play a critical role in the activation of antitumor immunity induced by these vaccinations. These findings indicate that the HSP105-pulsed BM-DC vaccine can provide potent immunotherapy for tumors that appear spontaneously as a result of the inactivation of a tumor suppressor gene, such as in the $Apc^{Min/+}$ mouse model. (*Cancer Sci* 2007; 98: 1930–1935)

Colorectal cancer is the third most common cancer and the fourth most frequent cause of cancer death worldwide. Every year, more than 945 000 people develop colorectal cancer worldwide, and approximately 492 000 patients die.[1] For patients with advanced stages of colorectal cancer, adjuvant systemic chemotherapy is a standard treatment. Major progress has been made by the introduction of regimens containing new cytotoxic drugs such as irinotecan and oxaliplatin; however, the new therapeutic regimens have led to only 8–9 months of progression-free survival.[2] Consequently, the development of new and effective therapeutic approaches, such as immunotherapy, is needed to expand treatment options.

The progression from normal epithelium to colorectal cancer is a multistep process involving the accumulation of multiple genetic alterations.[3] The *APC* gene, a tumor suppressor, is considered to be a gatekeeper in colon tumorigenesis,[4] and one of the earliest molecular events is the loss of function of the *APC* gene product.[5] APC forms a multimeric complex with the axis inhibition protein (AXIN)2 and glycogen synthase kinase 3β, which regulates the nuclear accumulation of β-catenin, a signal transducer of the wnt pathway.[6] When the APC–β-catenin complex is destabilized because of *APC* mutations, β-catenin binds and activates transcription factors that regulate the expression of potent oncogenes such as *c-Myc* and *c-Met*.[7] The importance of the *APC* gene product was confirmed by the demonstration that 80% of all sporadic colorectal cancers are characterized by one or more mutations in the *APC* gene, approximately 60% of which result in the expression of a truncated version of the APC protein.[8]

The $Apc^{Min/+}$ mouse has a nonsense mutation from T to A in the *Apc* gene at codon 850, homologous to the human germline and somatic *APC* mutation.[9] Although homozygous mice die before birth, all heterozygous mice develop multiple adenomas throughout their intestinal tract at an early age.[10] The $Apc^{Min/+}$ mouse model is unique in that tumors appear spontaneously in the intestinal tract, rather than as a result of induction by a carcinogen. This model is particularly advantageous for testing preventive agents targeted against early stage lesions because adenomas grow to a grossly detectable size within a few months on a defined genetic background.[10] Because $Apc^{Min/+}$ mice develop tumors due to the inactivation of the same tumor suppressor gene known to be involved in the pathogenesis of most colon cancers in humans, this model represents a clinically relevant model of human intestinal tumorigenesis.[10] Furthermore, germline mutations in the human *APC* gene cause FAP, whose symptoms resemble those of an $Apc^{Min/+}$ mouse. Therefore, this model provides useful information about not only colon cancer but also FAP.

Heat shock proteins are soluble intracellular proteins that are expressed ubiquitously, and their expression can be induced at much higher levels due to heat shock or other forms of stress. The essential functions of HSP are to bind and protect partially denatured proteins from further denaturation and aggregation.[11] A previous study reported that HSP105 (often called HSP110), identified with serological identification of antigens using the recombinant expression cloning (SEREX) method, is overexpressed in a variety of human cancers, including colorectal, pancreatic, thyroid, esophageal, and breast carcinoma, whereas HSP105 is expressed at lower levels in many normal tissues, except for the testis.[12,13] Immunotherapy targeted at HSP105 in the mouse prophylactic model, such as HSP105-pulsed BM-DC and *HSP105* DNA vaccines, induce antitumor immunity without causing an autoimmune reaction.[14,15] These findings indicate that HSP105 itself could be considered as a valuable tumor-associated antigen for immune-based treatment of various tumors.

[7]To whom correspondence should be addressed.
E-mail: mxnishim@gpo.kumamoto-u.ac.jp
Abbreviations: APC, adenomatous polyposis coli; BM-DC, bone marrow-derived dendritic cell; COX, cyclooxygenase; DC, dendritic cell; ELISPOT, enzyme-linked immunospot; FAP, familial adenomatous polyposis; HSP, heat shock protein; mAb, monoclonal antibody; MBP, myelin basic protein; MHC, major histocompatibility complex; PBS, phosphate-buffered saline.

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Another study reported that HSP105 is involved in tumorigenesis by protecting cancer cells from apoptosis.[16] The constitutive overexpression of HSP105 protein was found to be essential for various cancer cells to survive and, conversely, the apoptosis-inducing effect of HSP105 small interfering RNA (siRNA) is specific for cancer. In contrast, HSP can also stimulate an adaptive immune response against antigens bound to HSP,[17] provided that the vaccine forms a complex of recombinant HSP110 and target tumor-associated antigen.[18,19]

In the present study, $Apc^{Min/+}$ mice were used as a model of a cancer immunotherapy for human colorectal cancer. Because tumors in $Apc^{Min/+}$ mice strongly express HSP105, the efficacy of immunization with HSP105-pulsed BM-DC for preventing the development of tumors in $Apc^{Min/+}$ mice was investigated.

Materials and Methods

Mice and genotyping. Frozen embryos of $Apc^{Min/+}$ mice obtained from the Jackson Laboratory were transferred to C57BL/6J mice (purchased from Charles River Japan, Yokohama, Japan) at the Center for Animal Resources and Development, Kumamoto University. Mice at 4–5 weeks of age were characterized for the *Apc* genotype by polymerase chain reaction analysis of tail DNA with the use of allele-specific primers.[20] The concentrations of these primers were 1.0 μM (5′-TGAGAAAGACAGAAGTTA-3′), 1.0 μM (5′-TTCCACTTTGGCATAAGGC-3′), and 0.2 μM (5′-GCCATCCCTTCACGTTAG-3′). The amplification conditions were 5 min at 94°C before 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 5 min. The mice were maintained by breeding male $Apc^{Min/+}$ mice to female C57BL/6J mice. The mice were kept under specific pathogen-free conditions and these experiments were approved by the Animal Research Committee of Kumamoto University.

Production of recombinant proteins. Highly purified recombinant mouse HSP105 was produced from *Escherichia coli* strain BL21 cells transduced with the mouse *HSP105* gene expression vector, as described previously.[14,21] We also produced highly purified recombinant MBP as a negative control, which was prepared from bacterial lysate in the same way as the preparation of recombinant HSP105. Both recombinant HSP105 and MBP were estimated to be almost endotoxin free using a Limulus amebocyte lysate assay kit (BioWhittaker, Walkersville, MD, USA), and the endotoxin contents in the materials were <10 endotoxin U/mg.

Immunizations and scoring of tumors. HSP105-pulsed BM-DC were prepared as described previously.[14,22] The mice were inoculated intraperitoneally with HSP105-pulsed BM-DC (5×10^5) suspended in 200 μL PBS at 6, 8, and 10 weeks of age. The mice were treated with BM-DC alone, MBP-pulsed BM-DC, or PBS as controls. At 12 weeks of age the mice were killed and their small intestines were removed and fixed with formaldehyde. The intestines were then opened and stained with methylene blue and the number of tumors was counted.

Western blot and immunohistochemical analysis. Western blotting and the immunohistochemical detection of HSP105 were carried out as described previously.[12,16] Rabbit polyclonal antihuman HSP105 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as the primary antibody in this study. The immunohistochemical staining of $CD4^+$ and $CD8^+$ T cells was carried out as described previously.[14] mAb specific to CD4 (L3T4; BD PharMingen, San Diego, CA, USA) and CD8 (Ly-2; BD PharMingen) were used for staining.

Depletion of $CD4^+$ or $CD8^+$ T cells in mice. Rat mAb GK1.5 specific to mouse CD4 and 2.43 specific to mouse CD8 were used to deplete $CD4^+$ and $CD8^+$ T cells, respectively, *in vivo*. The 6-week-old $Apc^{Min/+}$ mice were injected with ascites (500 μg/mouse) from hybridoma-bearing nude mice six times intraperitoneally with an interval of 3–4 days between injection. Normal rat IgG (Chemicon, Temecula, CA, USA) was used as a control. The depletion of T cell subsets was monitored by a flow cytometric analysis, which showed a more than 90% specific depletion in the number of splenocytes.

ELISPOT assay. The $Apc^{Min/+}$ mice were immunized with HSP105-pulsed BM-DC or BM-DC alone at 6 and 8 weeks of age. At 10 weeks of age, spleen cells were harvested and depleted of $CD4^+$ or $CD8^+$ T cells using a magnetic cell-sorting system with antimouse CD4 mAb and antimouse CD8a (Mittenyi Biotec GmbH, Bergisch Gladbach, Germany) mAb, respectively. The purity of these T-cell subsets exceeded 95% based on a flow cytometric analysis. $CD4^-$ T cells were used as a source of $CD8^+$ T cells and antigen-presenting cells, and $CD8^-$ T cells were used as a source of $CD4^+$ T cells and antigen-presenting cells. Five hundred thousand $CD4^-$ or $CD8^-$ T cells were added to each well in triplicate cultures of RPMI-1640 medium containing 10% fetal calf serum (FCS) together with 2 μg/mL HSP105, MBP, and one with medium only at 37°C for 24 h. Then ELISPOT assays were carried out as described previously.[12]

Statistical analysis. The statistical significance of differences between the experimental groups was determined using Student's *t*-test. The overall survival rate was calculated using the Kaplan–Meier method, and statistical significance was evaluated using Wilcoxon's test. A value of $P < 0.05$ was considered to be statistically significant.

Results

Overexpression of HSP105 in intestinal adenomas of the $Apc^{Min/+}$ mice. A previous study reported that mouse HSP105 is overexpressed in liver metastasis of a murine colorectal adenocarcinoma cell line (Colon26), and in lung metastasis of a murine melanoma cell line (B16-F10).[15] The expression of HSP105 in tumors of $Apc^{Min/+}$ mice were thereby analyzed. The small intestines of $Apc^{Min/+}$ mice were excised, and the expression level of HSP105 was evaluated by both western blot and immunohistochemical analyses. The $Apc^{Min/+}$ mice developed adenomatous polyps spontaneously, predominantly in and throughout the small intestine at 4 months of age (Fig. 1a). Both western blot and immunohistochemical analyses confirmed the strong expression of HSP105 in the tumors of $Apc^{Min/+}$ mice (Fig. 1b,c). Based on these observations, the $Apc^{Min/+}$ mouse was chosen as a murine model of cancer immunotherapy targeted at HSP105.

Immunization with HSP105-pulsed BM-DC vaccine reduced the number of small intestinal polyps in $Apc^{Min/+}$ mice. The preventive effects of HSP105-pulsed BM-DC vaccination on the development of adenomatous polyps in the $Apc^{Min/+}$ mice were investigated. The mice were divided into four groups consisting of 10 mice each, inoculated intraperitoneally with PBS (group 1), BM-DC (group 2), MBP-pulsed BM-DC (group 3), or HSP105-pulsed BM-DC (group 4) at 6, 8, and 10 weeks of age. Two weeks after the last immunization, the number of tumors in the small intestine was counted.

Tumors had already developed in the small intestine of $Apc^{Min/+}$ mice at the time of the first vaccination (6 weeks of age). Each mouse had a mean of 6.3 ± 3.4 tumors at that time. The mean number of tumors at 12 weeks of age was 20.9 ± 9.6 in group 4, which was significantly less ($P = 0.006$) than the numbers in group 1 (37.8 ± 11.0), group 2 (40.8 ± 11.0), and group 3 (34.8 ± 9.5) (Fig. 2a). It was therefore concluded that the HSP105-pulsed BM-DC vaccine has the potential to prevent the growth of tumors expressing HSP105. The survival time in group 4 (175.3 ± 32.6 days) tended to be longer than that in group 1 (146.7 ± 13.0 days) and in group 2 (152.7 ± 25.5 days); however, the difference between group 4 and group 2 was not statistically significant ($P = 0.081$; Fig. 2b). No apparent abnormalities, such as weight loss, hair abnormality, or paralysis, were observed in

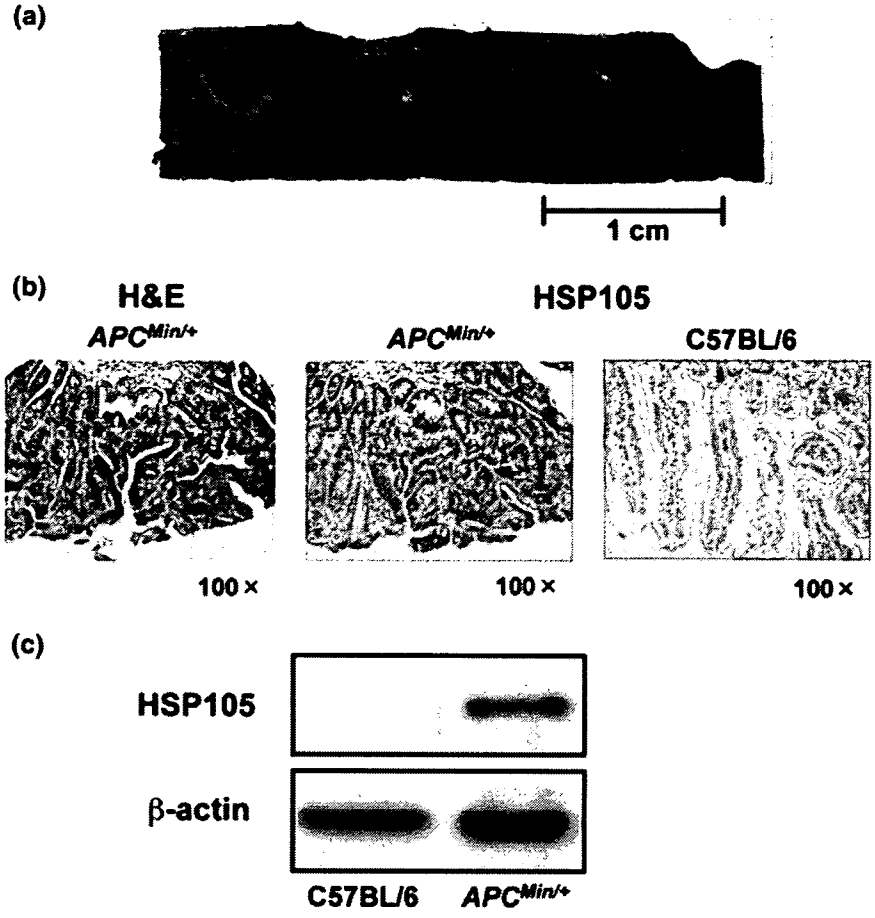

Fig. 1. Overexpression of heat shock protein (HSP) 105 in adenomatous polyps of $Apc^{Min/+}$ mice. (a) Macroscopic polyps in the small intestine of 4-month-old $Apc^{Min/+}$ mice. (b) A microscopic analysis of polyps in the small intestine of 12-week-old $Apc^{Min/+}$ mice stained with hematoxylin–eosin (left) and anti-HSP105 monoclonal antibody (middle). A normal small intestine was stained with anti-HSP105 monoclonal antibody as a negative control (right). Objective magnification was ×100. (c) Western blot analysis of HSP105 in the small intestine of 4-month-old $Apc^{Min/+}$ mice. The samples were small intestines of $Apc^{Min/+}$ and C57BL/6J mice homogenized in lysis buffer. The small intestines of three mice per group were pooled.

the mice immunized with HSP105-pulsed BM-DC, suggesting that serious autoimmunity was not observed in the mice. A histological analysis of the major organs (brain, lung, heart, liver, small intestine, kidney, and testis) of the immunized mice revealed no pathological inflammation (data not shown).

Both CD4⁺ and CD8⁺ T cells are required for antitumor immunity. To determine the role of CD4⁺ and CD8⁺ T cells in the reduction of tumor development in $Apc^{Min/+}$ mice immunized with HSP105-pulsed BM-DC, mice were depleted of CD4⁺ or CD8⁺ T cells by treatment with anti-CD4 or anti-CD8 mAb, respectively, *in vivo*. During the depletion procedure, the mice were immunized with PBS or HSP105-pulsed BM-DC vaccine (Fig. 3a). In the group of mice immunized with HSP105-pulsed BM-DC, together with inoculation of anti-CD4 mAb (35.5 ± 10.8) or anti-CD8 mAb (30.2 ± 9.6), the tumor numbers were significantly larger than those in the mice given rat IgG (18.8 ± 5.9) or left untreated (19.9 ± 7.7). The differences in the tumor numbers between the anti-CD4 mAb-treated group and the rat IgG-treated group ($P = 0.002$), and between the anti-CD8 mAb-treated group and the rat IgG-treated group ($P = 0.013$) were statistically significant. In the group of mice inoculated with PBS, the numbers of tumors in the mice given either anti-CD4 mAb (38.1 ± 5.7) or anti-CD8 mAb (38.1 ± 5.6) did not differ significantly from those in the mice given rat IgG (37.8 ± 4.8) or in the untreated mice (40.8 ± 6.1) (Fig. 3b). These results suggest that both CD4⁺ and CD8⁺ T cells play a crucial role in the protective antitumor immunity induced by the HSP105-pulsed BM-DC vaccine, because the HSP105-pulsed BM-DC vaccine was not effective in the mice showing a depletion of either CD4⁺ or CD8⁺ T cells.

Detection of HSP105-specific T cells in mice immunized with the HSP105-pulsed BM-DC vaccine. The $Apc^{Min/+}$ mice were immunized with HSP105-pulsed BM-DC or BM-DC at 6 and 8 weeks of age. At 10 weeks of age, spleen cells were harvested and depleted of CD4⁺ or CD8⁺ T cells using magnetic cell-sorting system, and the ELISPOT assay was carried out. The ELISPOT assay showed that the CD8⁻ cells (CD4⁺ T cells and antigen-presenting cells) derived from the mice immunized with HSP105-pulsed BM-DC produced a significantly larger amount of interferon-γ in response to HSP105 than did CD8⁻ cells derived from mice immunized with BM-DC. Similar results were observed for the CD4⁻ cells (CD8⁺ T cells and antigen-presenting cells) (Fig. 4a). These observations clearly indicate that both HSP105-specific CD4⁺ and CD8⁺ T cells were induced in the mice immunized with HSP105-pulsed BM-DC vaccine.

To investigate the antitumor effect of the HSP105-pulsed BM-DC vaccination, the tumor was evaluated histopathologically. The small intestines derived from the mice used for the ELISPOT assay were stained with anti-CD4 or anti-CD8 mAb. Both CD4⁺ and CD8⁺ T cells infiltrated into the tumors of mice immunized with HSP105-pulsed BM-DC; however, this was not the case in tumors derived from the mice immunized with BM-DC (Fig. 4b). These results suggest that HSP105-pulsed BM-DC have the potential to sensitize many HSP105-specific CD4⁺ and CD8⁺ T cells to kill tumor cells.

Discussion

In the present study, the HSP105-pulsed BM-DC vaccine could sensitize HSP105-specific T cells *in vivo* and inhibited the spontaneous development of intestinal tumors overexpressing HSP105 in $Apc^{Min/+}$ mice. For diseases of germline mutations that cause malignancy throughout the body, such as FAP, novel strategies for the prevention of cancer are needed urgently because there is no satisfactory treatment for FAP. Therefore,

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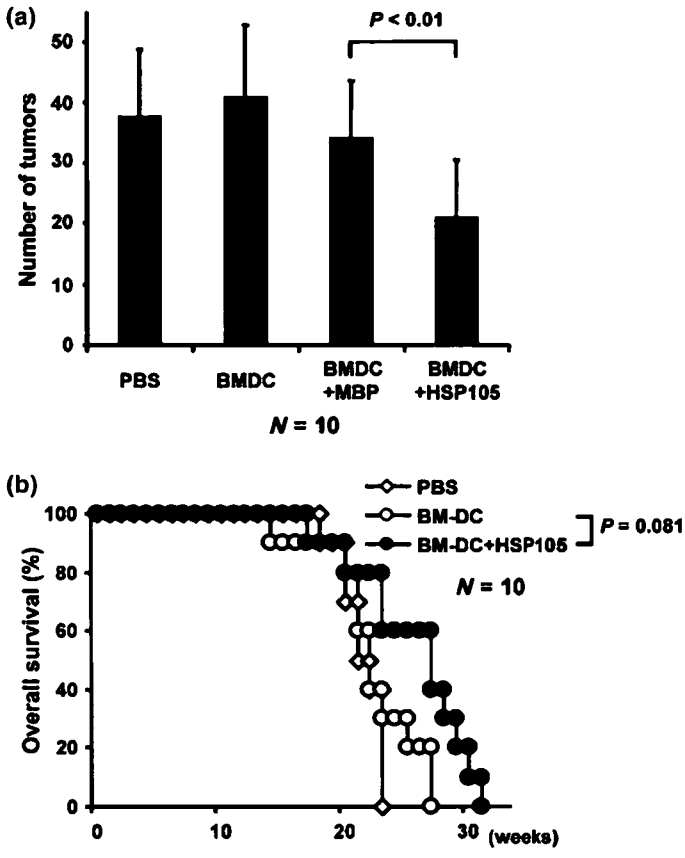

Fig. 2. Vaccination with heat shock protein (HSP) 105-pulsed bone marrow-derived dendritic cells (BM-DC) decreased the number of polyps in the small intestine of the $Apc^{Min/+}$ mice. (a) The $Apc^{Min/+}$ mice were inoculated intraperitoneally with HSP105-pulsed BM-DC (5×10^5), BM-DC alone, or myelin basic protein-pulsed BM-DC or phosphate-buffered saline (PBS) at 6, 8, and 10 weeks of age. At 12 weeks of age, the small intestines of the $Apc^{Min/+}$ mice were excised, stained with methylene blue, and the number of tumors was counted by the naked eye. Each group consisted of 10 $Apc^{Min/+}$ mice. The statistical significance of the differences in results was determined using an unpaired *t*-test. (b) The survival rate of $Apc^{Min/+}$ mice immunized with HSP105-pulsed BM-DC, BM-DC alone, or PBS as a control. The immunization protocol was the same as that of (a). The overall survival rate was calculated using the Kaplan–Meier method, and statistical significance was evaluated using Wilcoxon's test.

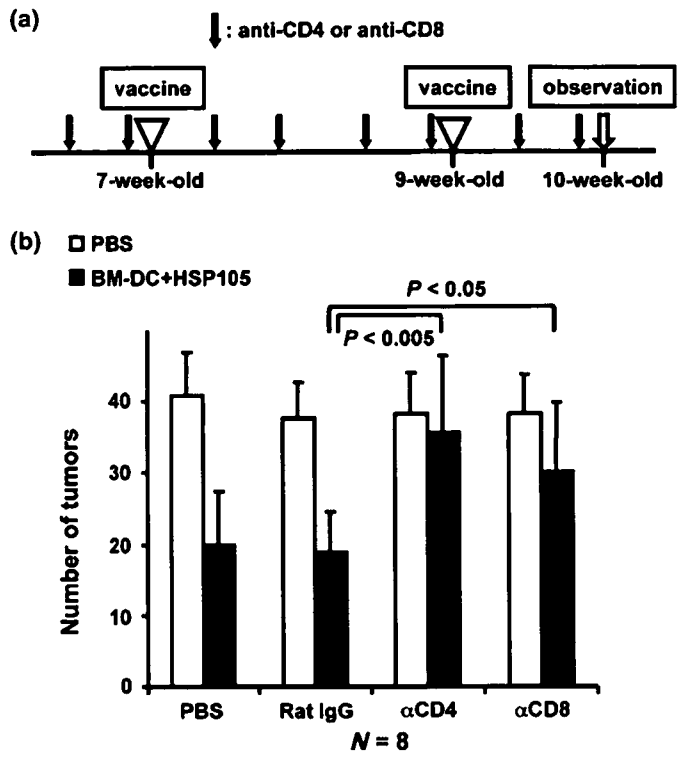

Fig. 3. Both CD4⁺ and CD8⁺ T cells are involved in the antitumor immunity elicited by the heat shock protein (HSP) 105-pulsed dendritic cell vaccine. (a) The protocol for the vaccination and the depletion of T cell subsets. (b) The number of polyps in the small intestine of $Apc^{Min/+}$ mice with various treatments. The number of tumors was counted as described in the legend for Fig. 2. Each group consisted of eight $Apc^{Min/+}$ mice. The statistical significance of the difference between the results was determined using the unpaired *t*-test.

the specific objective of the present study was to find out whether HSP105-pulsed DC-based immunotherapy can be used as a potent new strategy for the prevention of spontaneously arising tumors in FAP patients.

The ELISPOT assay shown in Figure 4a shows that both CD4⁺ and CD8⁺ HSP105-reactive T cells were primed in the mice immunized with HSP105-pulsed BM-DC. In this assay, we cannot completely rule out the possibility that responses were directed against contaminated bacteria-derived molecules in the HSP105 recombinant protein preparation. However, we consider this unlikely because practically no response was observed against BM-DC loaded with recombinant MBP protein, which was prepared from bacterial lysate in the same way as the preparation of recombinant HSP105. These recombinant proteins were purified extensively as described in a previous paper,[14] and contamination of lipopolysaccharide (LPS) or other DC-stimulants was ruled out.

Previous studies have reported that HSP105 is overexpressed specifically in a variety of human cancers and mouse tumor cells.[13,14] The present study demonstrated that HSP105 was also strongly expressed in the adenomatous polyps of $Apc^{Min/+}$ mice. In human tissue, the overexpression of HSP105 is a late event in the adenoma–carcinoma sequence, because immunohistochemical analysis revealed that HSP105 is strongly expressed in adenocarcinoma but not in adenoma.[13] Although the $Apc^{Min/+}$ mouse model has provided useful information about the pathogenesis of colorectal cancer, it is limited because it does not completely mimic the disease in humans. In humans, patients with FAP develop hundreds to thousands of adenomatous polyps, predominantly in the distal colon, and have a high risk of malignancies before the age of 40 years.[23] In contrast, $Apc^{Min/+}$ mice develop dozens to hundreds of adenomas and have a shortened life span. However, these adenomas are located mainly in the small intestine and they generally do not become malignant.[10] Furthermore, mice carrying different *Apc* mutations have been established. Tumors arising in these mice are histologically similar, but vary with respect to age of onset, number of tumors, and location.[24] Given this variation, the pattern of HSP105 expression in intestinal tumors may be different between human and $Apc^{Min/+}$ mice. Regardless of these differences, the $Apc^{Min/+}$ mice provide an appropriate model for analysis of the efficacy of the HSP105-pulsed BM-DC vaccine for inhibition of the development of human colorectal cancer, because the loss of *APC* function is the initiating event in not only FAP but also in the vast majority of sporadic colon cancers.

Recent findings regarding the cellular and molecular pathogenesis of colorectal cancer have led to the development of new targeted therapeutic options. Overexpression of COX-2 is one of the most significant observations in this respect.[25] The use of COX-2 inhibitor suppresses the development of colon cancer in