3 of the responders to sorafenib. *, CR+PR *vs.* SD+PD. (B) *FGF3*/*FGF4* gene amplification mediates the overexpression of *FGF3*/*FGF4* mRNA. The mRNA expression levels of *FGF3* and *FGF4* were examined in nine HCC samples that were available as frozen samples among forty eight HCC samples that were treated with sorafenib. Rel. mRNA, *target gene*/*GAPD* x10^6.

Fig. 3.

Fluorescence *in situ* hybridization analysis of *FGF3*-amplified HCC. Green, *CEN11P* locus; Red, *FGF3* locus; No., sample numbers; Amp, gene amplification. High-power images are presented for a single cancer cell.

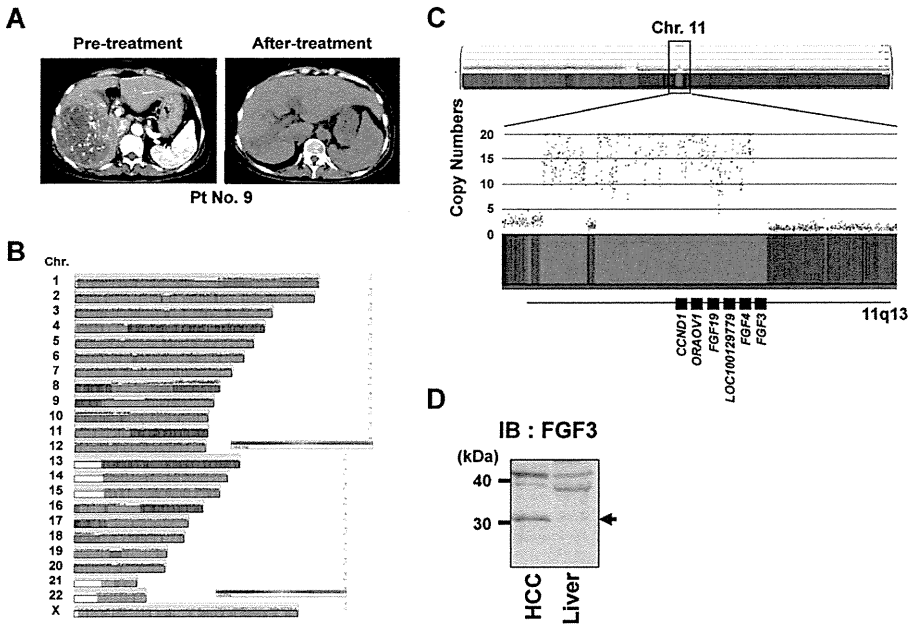
Fig. 4.

FGF3/*FGF4* gene amplification in a series of HCC samples without sorafenib treatment. (A) A TaqMan copy number assay for *FGF3* and *FGF4* was used to examine DNA samples obtained from 82 surgical specimens. Human normal genomic DNA was used as a normal control. Well, well-differentiated HCC; Mod, moderately differentiated HCC; Poor, poorly differentiated HCC.

Fig. 5.

FGF3 and FGF4 overexpression and drug sensitivity to sorafenib *in vitro* and *in vivo*. (A) Growth inhibitory assay examining sorafenib in various cancer cell lines *in vitro*. The growth inhibitory effect of sorafenib was examined using an MTT assay. The IC_{50} values of each cell line are shown in the graph. The black bars show that the IC_{50} values were below 1 μM. Amp, gene amplification. (B) Cancer cell lines stably overexpressing *EGFP*, *FGF3* or *FGF4* were established and designated as A549/EGFP, A549/FGF3 and A549/FGF4. Western blotting confirmed that exogenously expressed FGF3 and FGF4 were secreted into the culture medium. IB, Immunoblotting; Sup., supernatant. (C) The 3T3 cells were exposed to indicated concentrations of sorafenib for 2 hours and were then stimulated with FGF4 conditioned medium for 20 minutes. (D) Mice inoculated with A549/EGFP, A549/FGF3 or A549/FGF4 (n=20 each) were treated with a low dose of sorafenib (n=10, 15 mg/kg/day, p.o.) or without (n=10, vehicle control, p.o.). *p<0.05.

Fig. 1.



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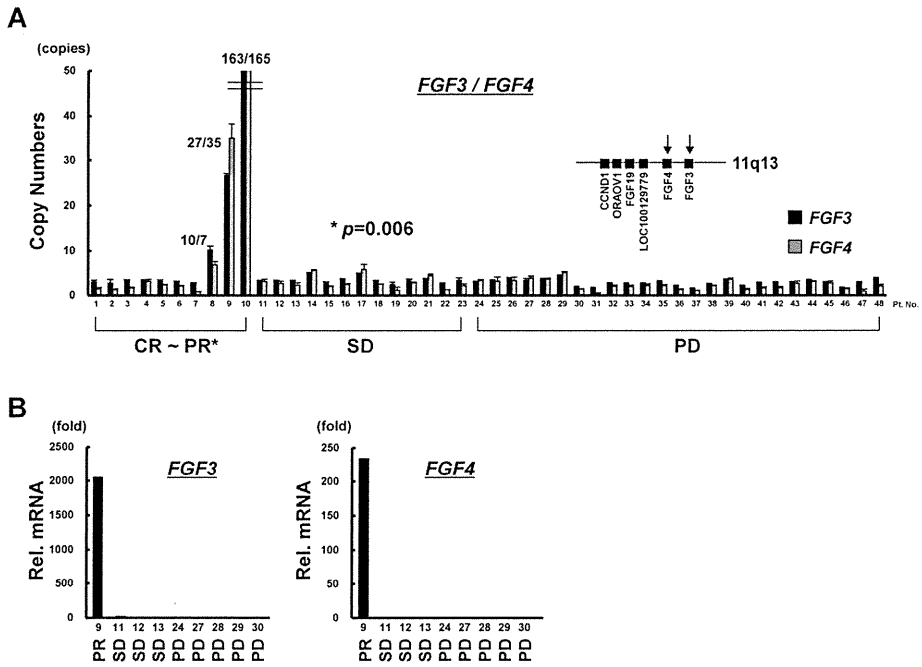

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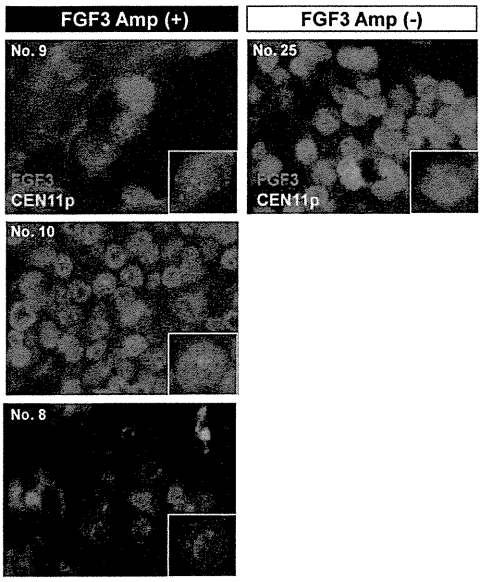

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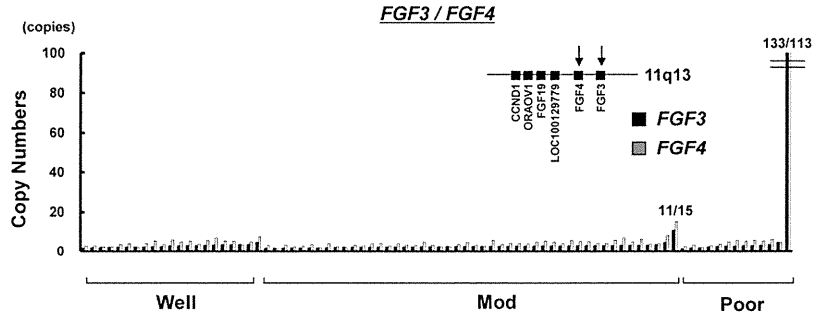

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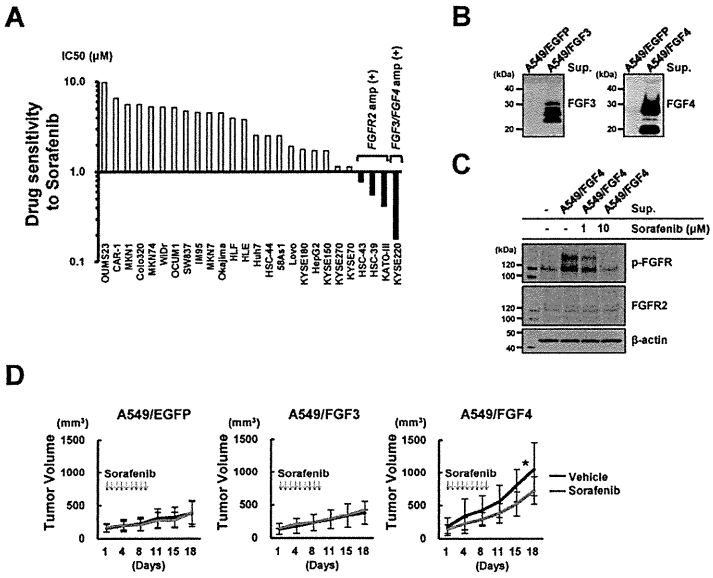

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Original Paper

Intraepithelial Ductal Spread in Colorectal Carcinoma Liver Metastasis

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ABSTRACT

Background/Aims: This study aimed to evaluate the usefulness of immunohistochemical combinations for discrimination between intraepithelial ductal spread of colorectal carcinoma liver metastasis (CRLM) and that of intrahepatic cholangiocarcinoma (ICC).

Methodology: A retrospective analysis of resected specimens from 151 patients with CRLM and 28 patients with ICC was conducted. Intraepithelial ductal spread along the bile ducts was judged positive when tumor cells spreading along the intact basement membranes of intrahepatic bile ducts. We evaluated immunoreactivity of cytokeratin (CK) 7, CK20, CDX2, MUC2, MUC5AC and human gastric mucin (HGM).

Results: Of the 151 patients with CRLM, 21 had intrahepatic bile duct involvement verified histologically. Intraepithelial ductal spread was detected in 17 of 21 (81%) patients with CRLM with bile duct involvement, whereas it was detected in 22 of 28 (79%) patients with ICC. CK20-positive/CK7-negative immunophenotype demonstrated a high accuracy of 95% for evaluation of intraepithelial ductal spread from CRLM. CK7-positive/CK20-negative immunophenotype demonstrated the highest accuracy of 85% for evaluation of intraepithelial ductal spread from ICC.

Conclusion: Intraepithelial ductal spread is a common feature of CRLM with bile duct involvement. Immunohistochemical combination of CK7 and CK20 is useful for discrimination between intraepithelial ductal spread of CRLM and that of ICC.

KEY WORDS: Liver neoplasms; Colorectal carcinoma liver metastasis; Intrahepatic cholangiocarcinoma; Intraepithelial spread; Immunohistochemistry

ABBREVIATIONS: Colorectal Carcinoma Liver Metastasis (CRLM); Intrahepatic Cholangiocarcinoma (ICC); Cytokeratin (CK); Human Gastric Mucin (HGM); Computed Tomography (CT); CT Arterial Portography (CTAP); Endoscopic Retrograde Cholangiopancreatography (ERCP); Percutaneous Transhepatic Cholangiography (PTC)

INTRODUCTION

Since Riopel *et al.* (1) reported eight cases of colonic adenocarcinoma metastatic to the liver that demonstrated prominent spread throughout the biliary tree along intact basement membranes, intraepithelial ductal spread along the bile ducts is recognized behavior of colorectal carcinoma liver metastasis (CRLM), mimicking primary intrahepatic cholangiocarcinoma (ICC). As intraepithelial ductal spread from CRLM closely resembles high-grade dysplasia (*i.e.* carcinoma *in situ*) of the extrahepatic and intrahepatic bile ducts, Riopel *et al.* (1) emphasized that this pattern of intraepithelial ductal spread may make it difficult to discriminate CRLM from primary biliary neoplasms. Although several authors demonstrated that cytokeratin (CK) 7 (specific for biliary epithelium) and CK20 (specific for intestinal epithelium) immunophenotypes are useful for discrimination between CRLM and primary ICC (2-8), a few previous reports which focused on immunohistochemical discrimination of intraepithelial ductal spread from CRLM were case reports (9-12). Therefore, little is known regarding this pattern of intraepithelial ductal spread from CRLM.

CDX2 and MUC2 immunophenotypes are specific markers for intestinal epithelium and adenocarcinoma of the intestinal origin, whereas gastric mucin MUC5AC and human gastric mucin (HGM) immunophenotypes have been proposed as potential markers for ICC (13-16). We evaluated immunoreactivity of CK profile (CK 7 and CK20) and mucin profile (CDX2, MUC2, MUC5AC and HGM) focused on intraepithelial ductal spread of CRLM and that of ICC. This study aimed to evaluate the usefulness of immunohistochemical combinations for discrimination between intraepithelial ductal spread of CRLM and that of ICC.

METHODOLOGY

We examined a total of 179 liver tumors consisting of 151 CRLM and 28 ICC. These tumors came from consecutive Japanese patients who underwent surgical resection as an initial treatment at the Niigata University Medical and Dental Hospital, Niigata, Japan, from January 1990 through December 2009. The patients included 123 men and 56 women with a median age of 66 years (range, 32-82 years). Of 28 patients with ICC, 2 patients had a history of colonic adenocarcinoma. The final

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diagnosis of CRLM and ICC was based on clinical (medical history, follow-up) and radiological data, with consistent histology. The study protocol and the use of human samples were approved by the Institutional Review Board of Niigata University Medical and Dental Hospital, and written informed consent was obtained from all patients involved in the current study.

Radiographical data were available for all patients. These studies included liver ultrasonography, abdominal computed tomography (CT), CT arterial portography (CTAP), endoscopic retrograde cholangiopancreatography (ERCP) and/or percutaneous transhepatic cholangiography (PTC). When bile duct dilatation and/or intrabiliary filling defects were detected on CT images (**Figure 1A**), ERCP was performed for these conditions to investigate the involvement of biliary tree (**Figure 1B**).

Resected specimens were submitted to the Department of Surgical Pathology in our hospital for histological evaluation. In each resected specimen, all available sections (median, 7 sections; range, 2-19 sections) were examined to evaluate intraepithelial ductal spread along the bile ducts histologically. In patients with CRLM, the original tissue sections of primary colorectal carcinoma were available for review. Intraepithelial ductal spread from CRLM was judged positive when tumor cells spreading along the intact basement membranes of intrahepatic bile ducts replaced non-neoplastic biliary epithelium (**Figure 2E**). In the current series, high-grade dysplasia and carcinoma *in situ* were treated as intraepithelial ductal spread of ICC (**Figure 3E**).

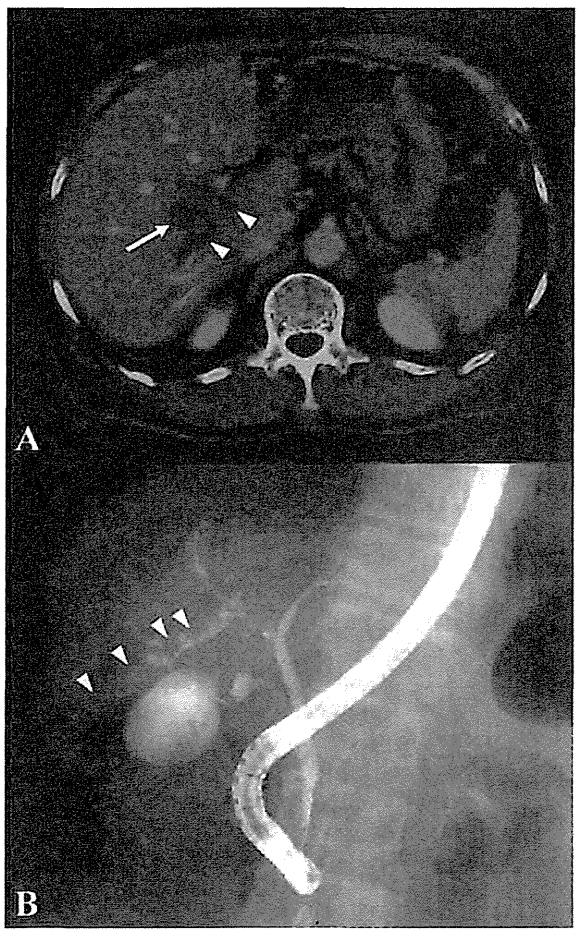

FIGURE 1
Radiological images of patient with colorectal carcinoma liver metastasis (CRLM). (**A**) Abdominal computed tomography (CT) depicts a low density mass (arrow) and dilatation of the posterior intrahepatic bile ducts (arrowheads). (**B**) Endoscopic retrograde cholangiography (ERC) demonstrates intrabiliary filling defects (arrows) within the posterior intrahepatic bile ducts.

One or two representative paraffin-embedded block(s) with the presence of intraepithelial ductal spread along the bile ducts was selected for immunohistochemistry. Nine serial 3-μm sections were re-cut and prepared from each block: one for hematoxylin-eosin staining, one for double staining with hematoxylin-eosin and Victoria blue, six for immunohistochemical staining and one as a negative control. One surgical pathologist (Y.A.) blinded to the clinical details assessed each section.

Paraffin sections were deparaffinized and rehydrated and then microwaved at 500W for 21min or autoclaved at 121°C for 20min in 10mmol/L sodium citrate buffer (pH 6.0) to retrieve antigenic activity. Endogenous peroxidase activity was inhibited by incubation with 0.3% hydrogen peroxide in methanol for 20min. After blocking any non-specific reactions with 10% normal goat serum, sections were incubated overnight at 4°C with the following antibodies: mouse monoclonal antibody against CK7 (Dako, Glostrup, Denmark; dilution at 1:100), mouse monoclonal antibody against CK20 (Dako, Glostrup, Denmark; dilution at 1:100), mouse monoclonal antibody against CDX2 protein (BioGenex Laboratories Inc., San Ramon, CA, USA; dilution at 1:200), mouse monoclonal antibody against Human Muc-2 glycoprotein (Novocastra Laboratories Ltd., Newcastle upon Tyne, UK; dilution at 1:300), mouse monoclonal antibody against Human Muc-5AC glycoprotein (Novocastra Laboratories Ltd., Newcastle upon Tyne, UK; dilution at 1:100) and mouse monoclonal antibody against HGM (Novocastra Laboratories Ltd., Newcastle upon Tyne, UK; dilution at 1:50). The sections were then incubated at room temperature for 30min with goat anti-mouse immunoglobulin conjugated to a peroxidase-labeled amino acid polymer (Simple Stain MAX-PO, MULTI; Nichirei Biosciences, Tokyo, Japan). Diaminobenzidine was used as the chromogen, and the sections were counterstained with hematoxylin. Non-neoplastic epithelium of the intrahepatic bile ducts was used as internal positive control for CK7. Intestinal epithelium was used as the positive control for CK20, MUC2 and CDX2, whereas gastric epithelium was used as the positive control for MUC5AC and HGM. For the negative control, normal mouse immunoglobulin was substituted for each primary antibody.

Immunoreactivity for each antibody was evaluated focused on intraepithelial ductal spread along the bile ducts. CK7, CK20, MUC2 or MUC5AC expression was defined as the presence of cytoplasmic immunoreactivity in the tumor cells, whereas CDX2 expression was defined as the presence of nuclear immunoreactivity according to the criteria of Nagata *et al.* (17). HGM expression was defined as the presence of cytoplasmic and/or apical membranous

immunoreactivity in the tumor cells according to the criteria of Shiroshita *et al.* (18). The expression of CK7, CK20, CDX2, MUC2, MUC5AC or HGM was judged positive when either single tumor cells or cell clusters showed immunoreactivity for each antibody, whereas it was judged negative when no immunoreactivity for each antibody was observed throughout the examined areas of intraepithelial ductal spread.

Medical records were obtained for all 179 patients (151 patients with CRLM and 28 patients with ICC). Categorical variables were compared by the Fisher exact test. The sensitivity, specificity and accuracy of immunophenotypes were calculated for the discrimination between intraepithelial ductal spread from CRLM and that from ICC. All statistical evaluations were performed using the PASW Statistics 17 software package (SPSS Japan, Tokyo, Japan). All tests were 2-tailed and a p value <0.05 was considered statistically significant.

RESULTS

Of the 151 patients with CRLM, 21 had intrahepatic bile duct involvement verified histologically. Intraepithelial ductal spread, characterized by tumor cells spreading along the intact basement membranes of intrahepatic bile ducts replacing non-neoplastic biliary epithelium (**Figure 2E**), was detected in 17 of 21 (81%) patients with CRLM with intrahepatic bile duct involvement. Of the 28 patients with ICC, intraepithelial ductal spread, characterized by high-grade dysplasia or carcinoma *in situ* (**Figure 3E**), was detected in 22 patients with ICC.

Immunophenotypes in the examined areas of intraepithelial ductal spread were comparable with immunophenotypes in main hepatic tumors of CRLM (**Figure 2**) and ICC (**Figure 3**). Immunohistochemical analyses of each monoclonal antibody focused on intraepithelial ductal spread from CRLM (n=17) and ICC (n=22) were summarized in **Table 1**. Positive expression of CK20 (p<0.001) and CDX2 (p<0.001) was significantly associated with intraepithelial ductal spread from CRLM, whereas positive expression of CK7 (p<0.001), MUC5AC (p<0.001) and HGM (p<0.001) was significantly associated with intraepithelial ductal spread from ICC. Among 22 patients with ICC with intraepithelial ductal spread along the bile ducts, 6 (27%), 9 (41%) and 8 (36%) had positive expression of CK20, CDX2 and MUC2, respectively (**Table 1**). Thus, intestinal phenotype, characterized by expression of CK20, CDX2 (**Figure 3H**) and MUC2 was occasionally observed in the areas of intraepithelial ductal spread of ICC.

Immunohistochemical combinations were assessed in the areas of intraepithelial ductal spread from CRLM. The sensitivity, specificity and accuracy of CDX2-positive/CK7-negative immunophenotype were 100%, 95% and 97%, respectively. The sensitivity, specificity and accuracy of CK20-positive/CK7-negative immunophenotype were 94%, 95% and 95%, respectively. CDX2-positive/CK7-negative immunophenotype demonstrated the highest accuracy for evaluation of intraepithelial ductal spread from CRLM.

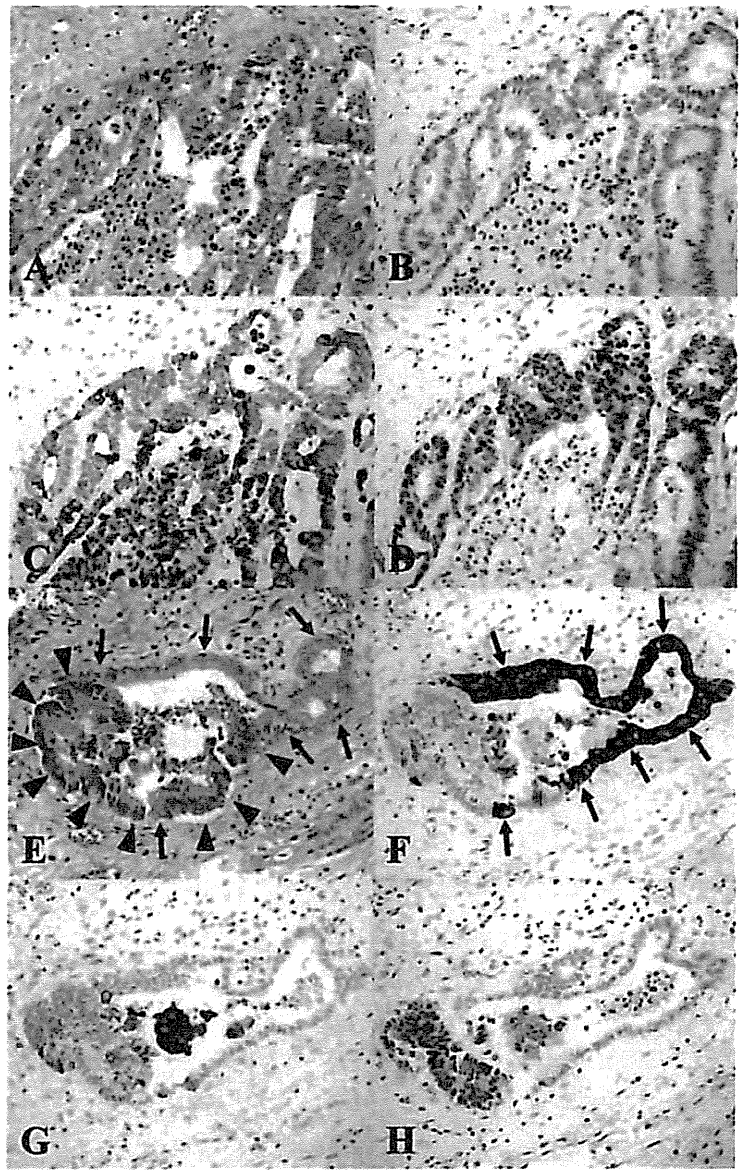

FIGURE 2 Immunophenotypes in colorectal carcinoma liver metastasis (CRLM) with intraepithelial ductal spread along the bile ducts. Tumor cells of main tumor of CRLM (**A–D**) and intraepithelial ductal spread of CRLM (**E–H**) demonstrate positive immunoreactivity for CK20 and CDX2, but negative immunoreactivity for CK7. Intraepithelial ductal spread from CRLM (arrowheads) shows replacement of non–neoplastic biliary epithelium along intact basement membranes (**E**). The remnant non–neoplastic biliary epithelium (arrows) demonstrates positive immunoreactivity for CK7 (**E** and **F**). Double staining with hematoxylin–eosin and Victoria Blue (**A** and **B**). CK7–immunohistochemical staining (**C** and **G**). CK20–immunohistochemical staining (**B** and **F**). CDX2–immunohistochemical staining (**D** and **H**).

Immunohistochemical combinations were assessed in the areas of intraepithelial ductal spread from ICC. The sensitivity, specificity and accuracy of CK7-positive/CK20-negative immunophenotype was 73%, 100% and 85%, respectively. The sensitivity, specificity and accuracy of CK7-positive/CDX2-negative immunophenotype was 59%, 100% and 77%, respectively. CK7-positive/CK20-negative immunophenotype demonstrated the highest accuracy for evaluation of intraepithelial ductal spread from ICC.

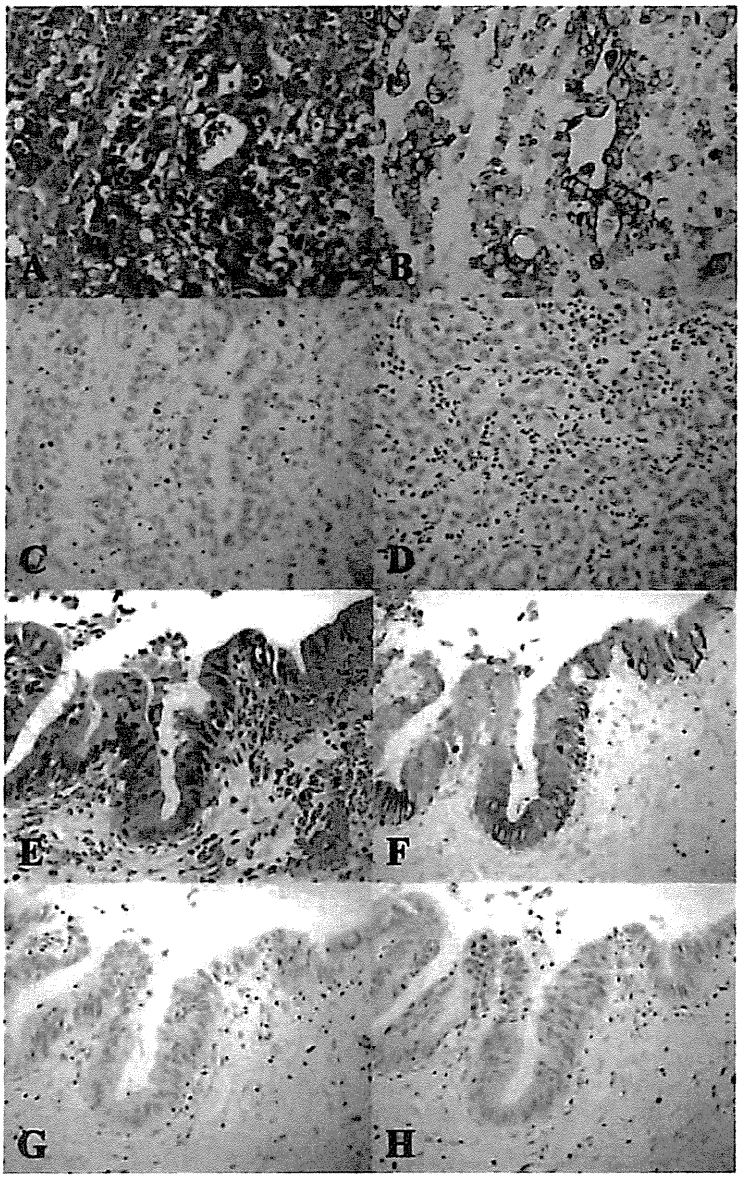

FIGURE 3 Immunophenotypes in primary intrahepatic cholangiocarcinoma (ICC) with intraepithelial ductal spread. Tumor cells of primary ICC (**A–D**) demonstrate positive immunoreactivity for CK7, but negative immunoreactivity for CK20 and CDX2. Intraepithelial ductal spread of ICC (**E–H**) demonstrates positive immunoreactivity for CK7 and CDX2, but negative immunoreactivity for CK20. Double staining with hematoxylin–eosin and Victoria Blue (**A** and **E**). CK7–immunohistochemical staining (**B** and **F**). CK20–immunohistochemical staining (**C** and **G**). CDX2–immunohistochemical staining (**D** and **H**).

In the current series, 2 patients with ICC had a past history of surgical resection for colorectal adenocarcinoma. One patient had intraepithelial ductal spread along the bile ducts (**Figure 3E-H**). Tumor cells of primary ICC (**Figure 3A**) showed positive expression of CK7 (**Figure 3B**), whereas the expression of CK20 (**Figure 3C**) and CDX2 (**Figure 3D**) was negative. The area of intraepithelial ductal spread from ICC (**Figure 3E**) showed positive expression of CK7 (**Figure 3F**) and CDX2 (**Figure 3H**), whereas the expression of CK20 (**Figure 3G**) was negative. Another patient had ICC with no evidence of intraepithelial ductal spread along the bile ducts. CT depicted a mass without bile duct dilatation in the anterior section of the liver. The tumor cells of primary ICC showed positive expression of CK7, whereas the expression of CK20, CDX2 and MUC5AC was negative.

DISCUSSION

Intraepithelial ductal spread along the bile ducts may make it difficult to discriminate CRLM from primary biliary neoplasms without knowledge of the clinical details because intraepithelial ductal spread from CRLM closely resembles high-grade dysplasia or carcinoma *in situ* of the bile ducts, as described by Riopel *et al.* (1). Past history of colorectal adenocarcinoma is informative for pathologists to discriminate between CRLM and ICC. Two recent patients with primary ICC who had a past history of surgical resection for colorectal adenocarcinoma prompted the current study based on immunohistochemical analysis, which demonstrated that among the tested immunophenotypes, immunohistochemical combination of CK7 and CK20 for the histological evaluation of intraepithelial ductal spread is useful for discrimination between CRLM and ICC.

Although studies of bile duct involvement in CRLM vary widely with respect to cited incidence from 6.1%-42% (19-24), the incidence of intraepithelial ductal spread from CRLM is unknown (1, 9-12). We found a high incidence of intraepithelial ductal spread along the bile ducts in patients with CRLM with bile duct involvement in the current study, which relied on histological examination of one section only from each tissue block. Our evaluation therefore represented the minimum incidence of intraepithelial ductal spread from CRLM, suggesting that intraepithelial ductal spread along the bile ducts is a common feature of CRLM with bile duct involvement.

CDX2 is a new, highly specific and sensitive marker of the intestinal origin of adenocarcinoma (13). Tot (8) reported that CK20-positive/CK7-negative immunophenotype is more specific in predicting the colorectal origin of liver metastasis than CDX2 expression. In the current series, CDX2-positive/CK7-negative immunophenotype showed higher accuracy (accuracy, 97%) than CK20-positive/CK7-negative immunophenotype (accuracy, 95%) for evaluation of intraepithelial ductal spread from CRLM. However, positive expression of CDX2 was occasionally observed in the areas of intraepithelial ductal spread from ICC (**Table 1** and **Figure 3H**). Furthermore, CK7-positive/CDX2-negative immunophenotype showed lower accuracy (accuracy, 77%) than CK7-positive/CK20-negative immunophenotype (accuracy, 85%) for evaluation of intraepithelial ductal spread from ICC. Taken together, these findings suggest that immunohistochemical combination of CK7 and CK20 for the histological evaluation of intraepithelial ductal spread along the bile ducts allows discrimination between CRLM and ICC.

New effective chemotherapy regimens, such as irinotecan (25, 26), FOLFOX (5-fluorouracil, leucovorin, oxaliplatin) (27), bevacizumab (28) and cetuximab (29) provide survival benefits for patients

with CRLM, whereas cisplatin plus gemcitabine is an appropriate option for the treatment of patients with advanced biliary cancer (30). As the majority of CRLM with bile duct involvement showed intraepithelial ductal spread along the bile ducts, mimicking primary ICC, firm histological diagnosis with knowledge of the clinical details is of importance for making a decision regarding chemotherapy regimen (31). When intraepithelial ductal spread along the bile ducts is found to be positive in the resected specimens, discrimination between intraepithelial ductal spread of CRLM and that of ICC is important. To make a firm histological evaluation of intraepithelial ductal spread along the bile ducts, immunohistochemical combination of CK7 and CK20 appears to be useful for discriminating between CRLM and ICC.

There are two main limitations to the current study. First, it was a retrospective analysis of a small number of patients with intraepithelial ductal spread along the bile ducts. Second, the amount of tested immunophenotypes was limited. To our knowledge, however, this is one of the largest series dealing with intraepithelial ductal spread along the bile ducts of CRLM and that of ICC.

TABLE 1 Immunohistochemical Analysis in Tumor Specimens of Intraepithelial Ductal Spread for Discrimination between CRLM and ICC

Variable	Modality	No. of patients		*p* value
		CRLM (n=17)	ICC (n=22)	
CK7	Negative	17 (100%)	1 (5%)	<0.001
	Positive	0 (0%)	21 (95%)	
CK20	Negative	1 (6%)	16 (73%)	<0.001
	Positive	16 (94%)	6 (27%)	
CDX2	Negative	0 (0%)	13 (59%)	<0.001
	Positive	17 (100%)	9 (41%)	
MUC2	Negative	8 (47%)	14 (64%)	0.345
	Positive	9 (53%)	8 (36%)	
MUC5AC	Negative	15 (88%)	4 (18%)	<0.001
	Positive	2 (12%)	18 (82%)	
HGM	Negative	14 (82%)	4 (18%)	<0.001
	Positive	3 (18%)	18 (82%)	

CRLM: colorectal carcinoma liver metastasis; ICC: intrahepatic cholangiocarcinoma; HGM: human gastric mucin

CONCLUSIONS

Intraepithelial ductal spread along the bile ducts is a common feature of CRLM with bile duct involvement. Among the tested immunophenotypes, immunohistochemical combination of CK7 and CK20 for the histological evaluation of intraepithelial ductal spread is useful for discrimination between CRLM and ICC.

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PHASE II STUDIES

A randomized phase II trial of intra-arterial chemotherapy using SM-11355 (Miriplatin) for hepatocellular carcinoma

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Abstract *Background* SM-11355 is a platinum complex developed to treat hepatocellular carcinoma (HCC) via administration into the hepatic artery as a sustained-release suspension in iodized oil. We conducted a multicenter phase II trial in patients with HCC to evaluate the efficacy and safety of SM-11355, using a Zinostatin stimalamer suspension in iodized oil as a reference. *Methods* Patients with unresectable HCC were randomized 2:1 to receive administration of the SM-11355 or Zinostatin stimalamer suspension into the hepatic artery. A second injection was given 4–12 weeks later. Efficacy was evaluated by CT 3 months after treatment and categorized as therapeutic

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effect (TE) V to I, where TE V was defined as disappearance or 100% necrosis of all treated tumors. *Results* A total of 122 patients were evaluated for efficacy and toxicity (SM-11355, n=83; Zinostatin stimalamer, n=39). Baseline characteristics were similar in the two groups. The TE V rates were 26.5% (22/83) and 17.9% (7/39) in the SM-11355 and Zinostatin stimalamer groups, respectively. In the SM-11355 group,the most frequent drug-related adverse events (AEs) of≥grade 3 were elevated AST, elevated ALT, thrombocytopenia, and hyperbilirubinemia. The AEs with the largest difference between the two groups (SM-11355 vs. Zinostatin stimalamer) were hepatic vascular injury (0 vs. 48.4%) and eosinophilia (84.3 vs. 41.0%). The 2-year and 3-year survival rates were 75.9% vs. 70.3% and 58.4% vs. 48.7%, respectively. *Conclusions* The results suggest that SM-11355 in iodized oil has similar efficacy to Zinostatin stimalamer and that repeated dosing of SM-11355 is possible without hepatic vascular injury in cases of relapse.

Keywords Iodized oil · MIRIPLA · Liver cancer · Suspension · Parallel study

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Introduction

International cancer statistics from 2002 indicate that hepatocellular carcinoma (HCC) ranks third behind lung and gastric cancer in the number of deaths [1]. The impact of current standard treatments for advanced HCC, including conventional transcatheter arterial chemoembolization (TACE) using doxorubicin or cisplatin is limited and the prognosis is unsatisfactory [2]. Therefore, there is a clear need for new treatments in management of this disease.

SM-11355, (SP-4-2)-[(1*R*,2*R*)-cyclohexane-1,2-diamine-*N*,*N*']bis (tetradecanoato-*O*) platinum monohydrate (Fig. 1) is a highly lipophilic platinum derivative that can be delivered suspended in iodized oil, an oily lymphographic agent, via injection into the hepatic artery [3]. Following injection into an HCC-feeding artery, iodized oil selectively accumulates in the tumor. Similarly, an iodized oil suspension of SM-11355 accumulates selectively within HCC nodules, allowing continuous release of active platinum compounds into tumor tissues. A phase I dose-finding study using different injection levels indicated a recommended dose of 20 mg/mL and an upper limit of the injection volume of 6 mL [4]. In an early phase II trial , SM-11355 showed a promising anticancer effect with a mild toxicity profile in patients with advanced HCC. Responses were evaluated by computed tomography (CT) three months after treatment, with complete response (CR) defined as disappearance or 100% necrosis of all tumors. Iodized oil accumulation in tumors was taken to indicate necrosis. Of 16 eligible patients, 9 (56%) showed CR [5]. This CR rate was superior to our expectation, because the CR rate in conventional TACE is 15–20% based on the same evaluation criteria [6, 7]. Therefore, the results of the early phase II study

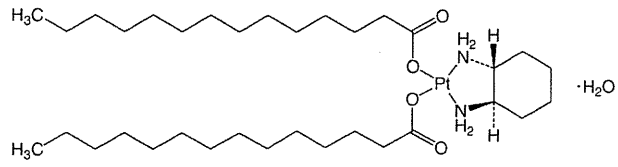

Fig. 1 Structural formula of SM-11355

indicated that SM-11355 has potential as an alternative to TACE in treatment of advanced HCC.

Based on these findings, we conducted a late phase II open-label trial of SM-11355. The aims of the study were to re-evaluate the efficacy, safety and pharmacokinetics of SM-11355 in a larger population, since only 16 eligible patients were included in the previous phase II study, and to confirm the candidacy of SM-11355 as an experimental treatment in a forthcoming clinical study in comparison with conventional TACE. To achieve regulatory approval of SM-11355 in Japan, it was necessary to undertake a parallel study. Therefore, we conducted a randomized phase II trial using Zinostatin stimalamer as a reference, because this agent is the only commercially available lipophilic drug for HCC in Japan and chemolipiodolization of Zinostatin stimalamer has been approved for treatment of advanced HCC in Japan [8, 9]. However, statistical comparisons between the two treatment groups were not planned since the goal of the study was re-evaluation of outcomes for SM-11355, and because the sample size required to conduct a statistical analysis was larger than expected.

Patients and methods

Inclusion criteria

Consecutive patients with HCC were eligible for the study if they had no indication for resection or local ablation therapy. The diagnosis was confirmed histologically and/or clinically using angiography and enhanced CT. Each patient was required to meet the following criteria: at least one measurable intrahepatic lesion that showed tumor staining by CT; tumor stage II or III in the staging system of the Liver Cancer Study Group of Japan [6, 7]; Child-Pugh classification A or B; adequate hematological function (WBC≥3000 /μL, blood platelets≥50000 /μL, hemoglobin≥9.5 g/dL), adequate hepatic function (AST and ALT≤ 5-fold the upper limit of normal, serum bilirubin <3 mg/dL, serum albumin≥3 g/dL), adequate renal function (serum creatinine≤the upper limit of normal); an Eastern Cooperative Oncology Group performance status of 0–2; age 20 to 74 years old; minimum life expectancy≥3 months, and provision of written informed consent. Patients who had undergone hepatic resection, local ablation therapy, and/or TACE were eligible if they showed no evidence of local tumor recurrence in the treated lesions. Patients who had undergone chemolipiodolization with anti-cancer agents other than Zinostatin stimalamer or a platinum-containing agent were also eligible if the treated lesions were resected. The previous anticancer treatment had to have been discontinued for at least 4 weeks before enrollment in this study.

Exclusion criteria

Patients were excluded if they met any of the following criteria: history of allergy to iodine-containing agents and/or contrast material; history of systemic chemotherapy; serious complication such as a cardiac disease or a thyroid disease ; concomitant malignancy; bile duct invasion; pregnant or lactating women and fertile patients who were not using effective contraception; and participation in another trial within 6 months before giving informed consent.

Study treatment

Patients who met the entry criteria were provisionally registered and randomly assigned to the SM-11355 or Zinostatin stimalamer group before undergoing angiography. Each investigator then confirmed registration after establishing that the patient met the following additional requirements based on angiographic findings: intrahepatic lesions that showed tumor staining and were fed by an artery with an appropriate structure for catheter insertion; no evidence of tumor thrombus in the portal or hepatic vein; no evidence of intrahepatic arteriovenous shunting; and no evidence of local tumor recurrence in previously treated lesions. The central random assignment by dynamic allocation to either a SM-11355 group or Zinostatin stimalamer group was stratified according to center and maximun tumor diameter.

A suspension of SM-11355 (MIRIPLA; Dainippon Sumitomo Pharma Co., Japan) or Zinostatin stimalamer (SMANCS; Astellas Pharma Inc., Japan) in iodized oil was injected into the hepatic artery using Seldinger's technique. Patients in the SM-11355 group received SM-11355 suspended in iodized oil (20 mg/mL) in a volume of up to 6 mL according to tumor size. Patients in the Zinostatin stimalamer group received Zinostatin stimalamer suspended in iodized oil (1 mg titer/mL) in a volume of up to 6 mL. When iodized oil accumulation in the treated tumor was insufficient and tumor staining was found in diagnostic imaging 5 weeks (±10 days) after the first injection, a second injection was given within 12 weeks after the first injection.

Efficacy and safety assessment

The antitumor effect was evaluated by CT or MRI 3 months after the last injection according to the response criteria proposed by the Liver Cancer Study Group of Japan [10], which are similar to the criteria proposed by the European Association for the Study of the Liver (EASL) Panel of Experts on HCC [11]. Tumor size was measured using the sum of the products of the perpendicular longest diameters of all measurable lesions. In the response evaluation criteria, iodized oil accumulation in a tumor is regarded as an indication of necrosis because significant positive correlations

have been reported between iodized oil accumulation observed on CT images and necrotic regions in resected tumors examined pathologically after TACE and after intra-arterial chemotherapy with iodized oil [5, 8, 12, 13]. Therapeutic effect (TE) was defined as follows: TE V, disappearance or 100% necrosis of all treated tumors; TE IV, more than 50% reduction in tumor size and/or more than 50% necrosis; TE III, more than 25% reduction in tumor size and/or more than 25% necrosis; and TE I, more than 25% increase in tumor size regardless of the necrotic effect. TE II was defined as a response not qualifying for classification as TE V, IV, III, or I. When a patient assigned to the SM-11355 group and judged to be TE V developed a tumor in a different region and requested SM-11355, the drug was given continuously after the study, provided that this was felt to be necessary by the investigator. The primary endpoint was the TE V rate. The secondary endpoints were the response rate based on the Response Evaluation Criteria in Solid Tumors (RECIST) and on the Japan Society for Cancer Therapy Criteria [14], which are similar to the World Health Organization (WHO) Criteria. The serum α-fetoprotein (AFP) level of each patient was measured before and 5 weeks after each treatment. Survival was evaluated using the Kaplan-Meier method. Toxicity was assessed according to the criteria of the Japan Society for Cancer Therapy [15], which are also fundamentally similar to WHO criteria.

Pharmacokinetics

Pharmacokinetic data were determined in patients in the SM-11355 group who gave written informed consent and were treated at institutions where a pharmacokinetic study could be conducted. Peripheral blood samples (5 ml) were collected 3 weeks after each treatment for determination of the total plasma platinum concentration and the platinum concentration in methanol extracts (SM-11355 metabolite concentration). The total platinum concentration in resected tissue was also determined in a patient who underwent surgery after evaluation of efficacy.

Statistical analysis

We anticipated enrollment of 120 patients at 17 participating hospitals over the study period of 3 years. A 2:1 ratio for SM-11355 to Zinostatin stimalamer randomization was chosen as a balance between the goals of the study, which were to re-evaluate the efficacy, safety and pharmacokinetics of SM-11355 in a larger population than that in the previous phase II study, and the current limited use of Zinostatin stimalamer. The number of subjects was determined based on the feasibility of the study because the sample size required to conduct a statistical analysis was larger than expected. Assuming a baseline 15% TE V rate for conventional TACE [6], the SM-11355 arm would be considered 'favorable' if there was a 10% improvement in this endpoint (to 25%) with an acceptable toxicity profile. A total of 80 patients in the SM-11355 arm is needed to estimate the TE-V rate with an accuracy of ±10%.

This study was not powered to permit formal statistical comparison between the two treatment arms. However, it does allow an initial assessment of SM-11355 in terms of TE-V, response rate, overall survival and toxicity with a view to performance of a follow-on phase III study.

Results

Patient

From April 2002 to October 2004, 131 patients were enrolled in the study, and 126 were assigned randomly at a 2:1 ratio to receive SM-11355 (85 patients) or Zinostatin stimalamer (41 patients) (Fig. 2). Five patients were excluded from the randomization because tumor staining was not observed in angiography and/or an appropriate hepatic artery for selective catheter insertion was not found (n=3), multiple tumors were observed in angiography that required reconsideration of the treatment strategy (n=1), and withdrawal of consent (n=1). After administration, 4 patients were identified as ineligible due to a platelet count <50,000/μL (n=1), esophageal cancer (n=1) in the SM-11355 group, and deviation from correct use of the investigational products (n=2) in the Zinostatin stimalamer group. Therefore, 122 patients (SM-11355 group, n=83; Zinostatin stimalamer group, n=39) were analyzed for efficacy and safety. The baseline demographic and disease characteristics of the patients are listed in Table 1.

Of the 85 original patients in the SM-11355 group, 18 were withdrawn from the study before the planned evaluation of efficacy 3 months after the first injection because of marked progression of the primary disease (n=5), serious adverse events (n=4), use of prohibited concomitant therapeutic agents or a requirement for combination therapy (n=3), and other reasons (duplicated count). Treatment was terminated in 11 patients after evaluation of the the first injection because complete necrosis of tumors (TE V) was obtained. The remaining 56 patients received a second injection.

Of the 41 patients in the Zinostatin stimalamer group, 9 were withdrawn before the planned evaluation of efficacy 3 months after the first injection, due to marked progression of the primary disease (n=2), serious adverse events (n=1), contravention of the protocol (n=1), appearance of hepatic injury (n=1), and other reasons (duplicated count). Treatment was terminated in 7 patients after evaluation of the first injection because complete necrosis of tumors (TE V) was obtained. The remaining 25 patients received a second injection.

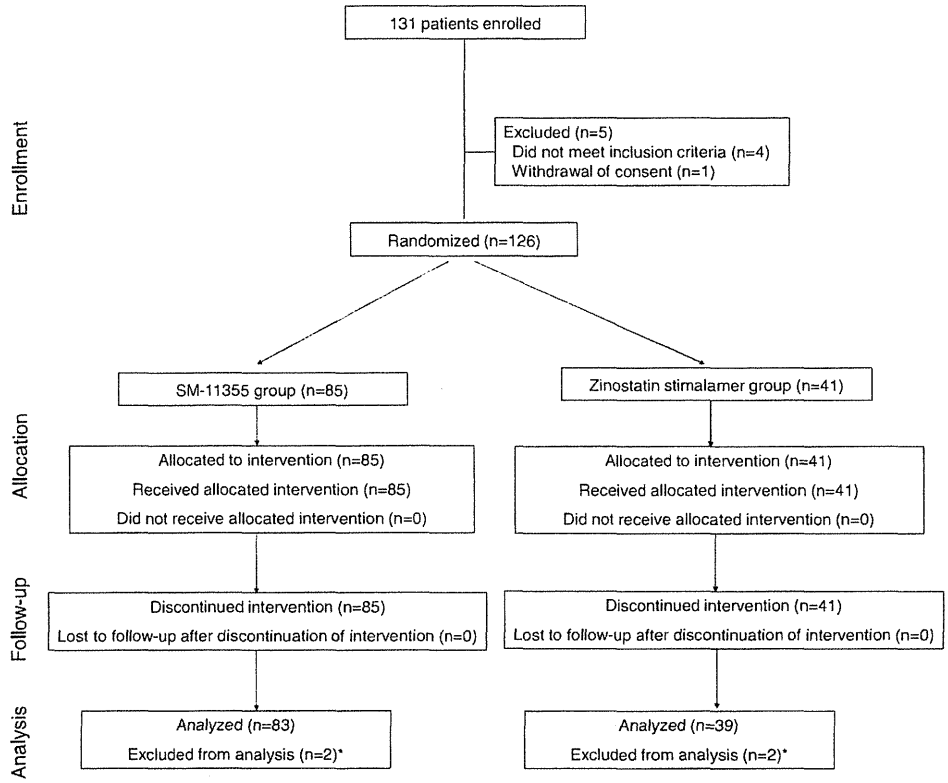

Fig. 2 Study flow diagram

Table 1 Patient background

	SM-11355	Zinostatin stimalamer
Number of patients	83	39
Sex (male:female)	70:13 (84.3%:15.7%)	30:9 (76.9%:23.1%)
Age (median)	67.0 (48–74)	68.0 (52–74)
PS (0:1:2:3:4)	80:3:0:0:0	35:4:0:0:0
HBs antigen positive	9 (13.6%)	1 (3.2%)
HCV antibody positive	55 (83.3%)	30 (96.8%)
HBs antigen · HCV antibody positive	2 (3.0%)	0 (0%)
Tumor stage (I:II:III:IV-A:IV-B)	0:43:40:0:0	0:19:20:0:0
Child-Pugh Classification (A:B:C)	61:22:0	32:7:0
Previously treated	25 (30.1%)	13 (33.3%)
Number of tumors 1	24 (28.9%)	9 (23.1%)
2	19 (22.9%)	11 (28.2%)
3	16 (19.3%)	7 (17.9%)
≥4	24 (28.9%)	12 (30.8%)
Maximum tumor diameter (mm) (Min-Max)	29.0 (10.0–80.0)	29.0 (10.0–94.0)

Table 2 Antitumor efficacy

Group	N	Antitumor efficacy						
"Criteria for Evaluation of Direct Effects on Hepatocellular Carcinoma" of the Liver Cancer Study Group of Japan								
		V	IV	III	II	I	NE	Percentage of TE V (%) [95% CI]
SM-11355	83	22	21	12	7	17	4	26.5 [17.4–37.3]
Zinostatin stimalamer	39	7	14	4	10	1	3	17.9 [7.5–33.5]
Response Evaluation Criteria in Solid Tumors (RECIST)								
		CR	PR	SD		PD	NE	Percentage of CR + PR
SM-11355	83	0	20	52		10	1	24.1 [15.4–34.7]
Zinostatin stimalamer	39	0	10	23		6	0	25.6 [13.0–42.1]
"Clinical Response Evaluation Criteria for Solid Tumor Chemotherapy" of the Japan Society for Cancer Therapy								
		CR	PR	MR	NC	PD	NE	Percentage of CR + PR
SM-11355	83	0	17	10	36	19	1	20.5 [12.4–30.8]
Zinostatin stimalamer	39	0	9	5	19	6	0	23.1 [11.1–39.3]

Efficacy

The antitumor efficacy is shown in Table 2. The percentages of TE V patients were 26.5% (22/83) [95% confidence interval (CI): 17.4–37.3%] in the SM-11355 group and 17.9% (7/39) [95% CI: 7.5–33.5%] in the Zinostatin stimalamer group. In a RECIST assessment, response rates were 24.1% (20/83) [95% CI: 15.4–34.7%] and 25.6% (10/39) [95% CI: 13.0–42.1%] in the respective groups. Based on the Japan Society for Cancer Therapy Criteria, the tumor responses were 20.5% (17/83) [95% CI: 12.4–30.8%] and 23.1% (9/39) [95% CI: 11.1–39.3%] in the respective groups (Table 2).

Of 61 patients with a pre-treatment AFP level above the upper limit of normal in the SM-11355 group, 6 / 60 (10%) had an AFP level within the normal range 5 weeks after the first injection. No data for the AFP level were available for 1 patient in the SM-11355 group at 5 weeks after the first injection. Among the 61 patients, 37 received a second injection and 6 (16%) had a normal AFP level 5 weeks after the second injection. Of the 26 patients in the Zinostatin stimalamer group with a pre-treatment AFP level above the upper limit of normal, none had an AFP level within the normal range 5 weeks after the first injection. Among the 26 patients, 18 received a second injection, but none had a normal AFP level 5 weeks after the second injection.

Cumulative survival rates are shown in Fig. 3. The follow-up period was approximately 3 years after the treatment period. The longest follow-up periods in the SM-11355 and Zinostatin stimalamer groups were both 5.6 years, and the median periods were 3.0 years and 2.8 years, respectively. The one-year survival rates in the SM-11355 and

Fig. 3 Cumulative survival rate

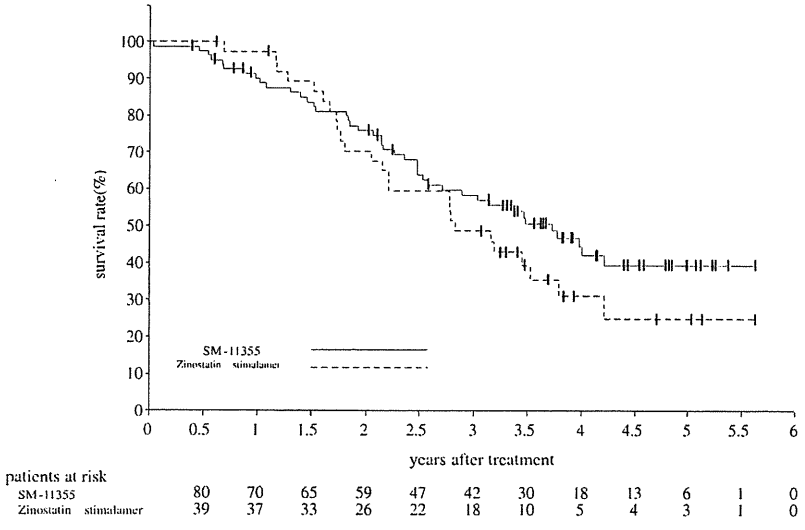

Table 3 Hematological and non-hematological adverse events

	SM-11355			Zinostatin stimalamer		
	No. of patients	All (%)	≥ Grade 3 (%)	No. of patients	All (%)	≥ Grade 3 (%)
Decrease in leukocytes	83	41.0	1.2	39	66.7	0
Decrease in lymphocytes	83	79.5	0	39	79.5	0
Decrease in neutrophils	83	53.0	8.4	39	43.6	2.6
Decrease in platelets	83	50.6	12.0	39	74.4	10.3
Decrease in hemoglobin	83	15.7	0	39	10.3	0
Increase in eosinophils	83	84.3	0	39	41.0	0
Increase in monocytes	83	57.8	0	39	76.9	0
Fatigue	83	39.8	0	39	46.2	0
Fever	83	96.4	3.6	39	97.4	0
Chills	83	39.8	0	39	51.3	0
Vomiting	83	55.4	1.2	39	51.3	0
Pain at injection site	83	43.4	0	39	41.0	2.6
Decrease in albumin	83	50.6	0	39	28.2	0
Increase in ALP	83	30.1	1.2	39	51.3	0
Increase in ALT	83	59.0	24.1	39	66.7	20.5
Increase in AST	83	62.7	26.5	39	79.5	38.5
Increase in bilirubin	83	57.8	12.0	39	71.8	5.1
Decrease in calcium	83	38.6	0	39	51.3	0
Increase in γ-GTP	83	49.4	0	39	61.5	0
Increase in glycemia	83	56.6	12.0	39	56.4	5.1
Increase in LDH	83	60.2	0	39	69.2	0
Increase in CRP	83	95.2	0	39	79.5	0
Prolonged PT time	83	42.2	1.2	39	28.2	0
Decrease in urinary creatinine	83	54.2	0	39	56.4	0
Increase in urinary creatinine	83	49.4	0	39	38.5	0
Increase in urinary NAG	83	89.2	0	39	87.2	0

Adverse events that occurred at a rate of >40% are shown

Zinostatin stimalamer groups were 90.1% and 97.4%, the 2-year survival rates were 75.9% and 70.3%, respectively, and the 3-year survival rates were 58.4% and 48.7%, respectively. The median survival time (MST) was 3.7 years in the SM-11355 group and 2.8 years in the Zinostatin stimalamer group.

Safety

Hematological adverse events were relatively mild and transient in both groups (Table 3). The incidences of neutropenia and decreased hemoglobin were similar in the two groups, but the incidence of eosinophilia was higher in the SM-11355 group, and the incidences of leukopenia and thrombocytopenia were higher in the Zinostatin stimalamer group. Most non-hematological adverse events (Table 3) were also mild and transient in both groups. Major events of grade 3 or higher involved liver dysfunction (including elevations in AST, ALT and hyperbilirubinemia) and hyperglycemia, but these had similar incidences in both groups and most were reversible.

One patient in the SM-11355 group died of esophageal variceal rupture, which occurred 12 days after the first injection, and one patient in the Zinostatin stimalamer group died of hepatic failure 168 days after the second injection. Esophageal variceal rupture was considered not to be related to the treatment because the condition was recognized before initiation of treatment and the event was not classified as a toxicity. Other serious adverse events occurred in 8 patients in the SM-11355 group (increase in AST in 2 patients; and increase in ALT, sepsis, systemic inflammatory response syndrome (SIRS: a syndrome characterized by systemic inflammation and extensive tissue damage associated with serious infection), decrease in neutrophils, acute myocardial infarction (AMI), and hypotension in 1 case each) and in 2 patients in the Zinostatin stimalamer group (respiratory distress and arrhythmia, and abdominal pain in 1 case each). All the patients recovered with appropriate treatment. Most of these events