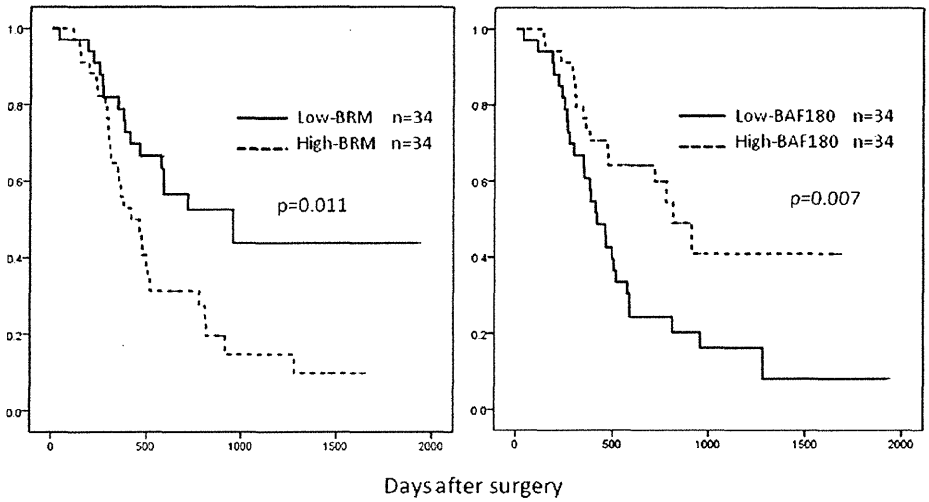

Figure 3. The survival curves were compared by Kaplan-Maier method by the expression level of BRM and BAF180. The statistical significance was evaluated using log-rank test.

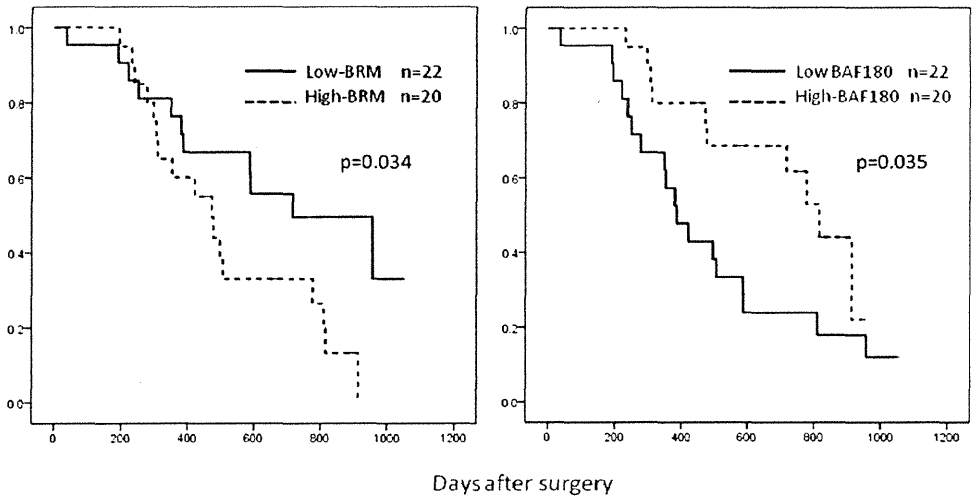

Figure 4. The survival curves of patients with adjuvant gemcitabine were compared by Kaplan-Maier method by the expression level of BRM and BAF180. The statistical significance was evaluated using log-rank test.

organ metastasis, lymphatic invasion, and stage IV disease. Stage IV disease was also correlated to high BRG1, which is reported to have similar biological function as BRM. On the other hand, better clinicopathological features were related to high BAF expression. High BAF180 was related to smaller tumor size, and high BAF47 was associated with negative lymphatic invasion.

In addition, our multivariate analysis revealed both high BRM and low BAF180 were independent prognostic indicators for poor survival, whereas the expression level of BRG1, BAF250a, and BAF47 were not related to overall survival.

As a next step, we investigated the prognostic significance of these factors in the patients with adjuvant gemcitabine. Gemcitabine remains standard therapy in the adjuvant and palliative settings for pancreatic cancer (29,30). However, the response rate of gemcitabine is very low, with only 18% of 1-year survival rate (31). Developing a novel biomarker, which predicts the response for gemcitabine, is urgently needed. In the analysis of the patients with gemcitabine, we reached the same result; both high BRM and low BAF180 were independent prognostic indicators for poor survival.

A previous study showed that BRM or BRG1 is lost in 10-20% of the bladder, colon, breast, esophageal, pancreatic and ovarian cancers by immunohistochemical staining of tissue microarrays (32). Another study reported BRM was lost in approximately 15-20% of primary non-small lung cancers, and silencing of BRM was a prognostic factor for poor outcome (33,34). Although BRM is supposed to be involved in many biological functions, these data showed BRM-containing complexes (BRM/BAF) as tumor suppressor in cancer tissue.

It is also reported that BRM has a role in transcription of CD44 (35), which is important in the process of tumor-endothelium interactions, cell migration, cell adhesion, tumor progression and metastasis (36).

Our result showed that the patient with high BRM had a significantly worse survival than those without (5-year OS:

Table V. Multivariate analysis for overall survival in patients with adjuvant gemcitabine.

Factors	HR (95% CI)	p-value
Age		0.002
<65	1.0	
≥65	0.227 (0.089-0.580)	
Tumor size (cm)		0.280
<4	1.0	
≥4	0.593 (0.230-1.531)	
Histology		0.267
Well/Mod	1.0	
Poor	1.907 (0.610-5.964)	
M		0.923
M0	1.0	
M1	0.947 (0.315-2.847)	
Curability of surgery		0.784
R0	1.0	
R1	1.145 (0.433-3.029)	
BRM		0.017
Low	1.0	
High	3.411 (1.251-9.305)	
BAF180		0.016
Low	1.0	
High	0.336 (0.138-0.819)	

HR, hazard ratio; 95% CI, 95% confidence interval; well, well differentiated adenocarcinoma; mod, moderately differentiated adenocarcinoma; poor, poorly differentiated adenocarcinoma.

9.8 vs. 43.8%, p=0.009), suggesting BRM/BAF in pancreatic cancer may contribute to tumor progression.

We also revealed the significant relationship between high BAF180 expression and smaller-sized tumor, and identified BAF180 as an independent prognostic factor for better survival in pancreatic cancer.

BAF180 maps to the 3p12 region (37) where allele loss is frequent and homozygous deletion have been detected in lung and breast cancer cell lines (38,39). Thus, genes located on this region have been thought as candidates for tumor suppressors. Actually, it is reported that BAF180 mutation is associated with carcinogenesis of breast cancer, and BAF180 suppresses tumorigenesis through its ability to regulate p21 (40), which controls the cell cycle (41). Recent research also clarified BAF180 mutation in clear cell renal cell carcinoma (42). These results suggest the idea that BAF180-containing complexes (PBAF) suppress tumor progression, which does not contradict our present results.

BAF250a-containing SWI/SNF complexes (BRG1/BAF) are reported to have different structure and biological properties from PBAF (43,44). A previous study showed that BAF250a was deleted in as many as 30% of renal cell carcinoma and 10% of breast carcinoma (19,45). These results lead to the concept that BRG1/BAF appear to have antagonistic effect on cell cycle progression (46). However, our data did not show the relationship of BAF250a expression to clinicopathological features or overall survival in pancreatic cancer.

Based on this study, we reached the conclusion that high BRM, and low BAF180 are useful biomarker not only for the patients with curative resection, but also for those with adjuvant gemcitabine. Future investigation into biological functions of SWI/SNF components could lead to better management in pancreatic cancer.

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The cellular level of histone H3 lysine 4 dimethylation correlates with response to adjuvant gemcitabine in Japanese pancreatic cancer patients treated with surgery

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Abstract

Background: To search for biomarkers identifying pancreatic cancer patients likely to benefit from adjuvant gemcitabine chemotherapy, we investigated the status of several histone modifications in pancreatic tumors and their relationship to clinicopathological features and outcomes.

Methods: Sixty one pancreatic cancer patients, primarily treated by surgical removal of tumors, were involved in the study. Thirty patients completed postoperative adjuvant gemcitabine, and in 31 it was discontinued. Tumor specimens were examined using immunohistochemistry for di- and tri-methylation of histone H3 lysine 4 (H3K4me2 and H3K4me3), dimethylation and acetylation of histone H3 lysine 9 (H3K9me2 and H3K9ac), and acetylation of histone H3 lysine 18 (H3K18ac). Positive tumor staining for each histone modification was used to classify patients into low- and high-staining groups, which were examined for relationships to clinicopathological features and clinical outcomes.

Results: High expression of H3K4me3 was related to the well and moderately differentiated tumor histological type ($p = 0.012$) and low expression of H3K4me2 was related to the presence of perineural invasion ($p = 0.007$). No cellular histone modifications were associated with overall or disease-free survival of patients as a whole. In the subgroup analyses, a low level of H3K4me2 was significantly associated with worse disease free survival in patients that completed adjuvant gemcitabine ($p = 0.0239$). Univariate and multivariate hazard models also indicated that a low level of H3K4me2 was a significant independent predictor of disease-free survival ($p = 0.007$).

Conclusion: H3K4me2 was found to be a predictor of response to adjuvant gemcitabine in Asian patients with pancreatic cancer.
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Keywords: Histone modification; Pancreatic cancer; Gemcitabine

Introduction

Pancreatic cancer remains an important cause of death in many nations.[1] Surgical removal of tumors is the only curative approach, and gemcitabine chemotherapy is the standard treatment after surgery.[2] Prognosis after resection, even followed by gemcitabine, remains extremely poor. Thus, it is important to identify specific biomarkers of outcomes in order to select patients who could be recommended for more aggressive treatment.

Posttranslational histone modifications of chromatin, including methylation, acetylation, phosphorylation, sumoylation and ubiquitination, play critical roles in creating transcriptional activation and repression patterns, in part through the regulation of chromatin structure.[3] Modifications to histone as a result of methylation, which usually occurs at lysine or arginine residues, are generally associated with gene inactivation[4,5] or silencing.[6–8] On the other hand, acetylation of histone, which mostly occurs at lysine residues in the N-terminal domains, is known to be associated with transcriptional activation.[9–11]

Recent studies have indicated that patterns of certain histone modifications, not at the level of each specific

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gene, but at the level of the individual cell as a whole, are associated with the clinicopathological features and outcomes of several tumor types in humans, including prostate, kidney, lung, gastric, colorectal, ovarian, breast and pancreatic.[6,12–17] Two studies on pancreatic cancers have demonstrated that low cellular levels of methylation of histone H3 at lysine 4 (H3K4), lysine 9 (H3K9) or lysine 27 (H3K27), or in the acetylation of H3 at lysine 18 (H3K18) were independent predictors of poor patient survival among the Caucasian population.[16,17] In particular, low cellular levels of dimethyl-H3K4 (H3K4me2) and dimethyl-H3K9 (H3K9me2) were predictive of survival specifically for those patients receiving adjuvant chemotherapy with fluorouracil, but not with gemcitabine.[17]

In a randomized clinical trial, gemcitabine was found to provide a survival advantage over fluorouracil in addition to symptom-relief in patients with advanced pancreatic cancer.[2] Recent studies have revealed that gemcitabine exhibits ethnic differences in terms of efficacy[18] and adverse reactions, associated in part with cytidine deaminase (CDA) gene polymorphism in the Asian population.[19] The aim of the present study was to determine the patterns of histone modifications in pancreatic cancer among the Japanese population, and to investigate the association between these patterns and clinicopathological features and the benefits of postoperative gemcitabine chemotherapy.

Materials and methods

Patients and samples

This study involved the retrospective analysis of 61 patients with surgically removed pancreatic cancer. All of the patients had undergone curative radical resection for primary pancreatic adenocarcinoma at Kanagawa Cancer Center, Yokohama, Japan, between January 2006 and December 2009. We offered postoperative gemcitabine chemotherapy to all patients. Each patient received adjuvant chemotherapy using one of the following protocols: the gemcitabine standard protocol (gemcitabine 1000 mg/m^2, days 1, 8, and 15, every 4 weeks for 6 months) or the gemcitabine biweekly protocol (gemcitabine 1000 mg/m^2, biweekly for 6 months). Although administration was discontinued in 31 patients, 30 patients completed treatment with gemcitabine at a dose of 12 g, which is considered to be a sufficient dose for adjuvant chemotherapy. Informed consent was obtained from each patient. The Ethics Committees of Kanagawa Cancer Center approved the protocol before initiation of the study. None of the patients had any other malignancies.

Immunohistochemistry

Microarrays consisting of two cores, each 2 mm in diameter, were prepared from formalin-fixed paraffin-embedded tissue blocks of surgically removed primary tumor.

Immunohistochemical staining was performed using commercially available polyclonal rabbit anti-histone antibodies raised against dimethyl histone H3 lysine 9 (H3K9me2), acetyl histone H3 lysine 9 (H3K9ac), dimethyl histone H3 lysine 4 (H3K4me2), trimethyl histone H3 lysine 4 (H3K4me3) and acetyl histone H3 lysine 18 (H3K18ac) (Cell Signaling Technology Inc., Daners, MA, USA).

Tissue microarray sections were deparaffinized with xylene and rehydrated with a graded series of aqueous ethanol. For antigen retrieval, slides were placed in Tris/EDTA pH9.0 buffer and autoclaved at 121 °C for 15 min. Endogenous peroxidases were blocked with 3% hydrogen peroxide solution. Then the sections were incubated with primary rabbit anti-histone polyclonal antibodies for 60 min at room temperature at the following dilutions: anti-H3K9me2, H3K9ac, H3K4me3, H3K18ac at 1:300 and anti-H3K4me2 at 1:600. Thereafter, the sections were treated with HRP polymer kit (Nichirei Biosciences, Tokyo, Japan) for signal amplification. Diaminobenzidine-hydrogen peroxide was used as the chromogen, and counterstained with hematoxylin.

Determination of histone modifications score

Immunohistochemical scoring was undertaken using the modified Histo-score (H-score),[11] which involves semi quantitative assessment of both the intensity of staining (graded as non staining: 0, weak: 1, moderate: 2, strong: 3, adjacent normal pancreatic exocrine cells were graded as the median) and the percentage of positive cells (0–100). The range of possible scores was 0–300, enabling us to explore the rationalization of our patients into biologically relevant groups depending on different levels of detection, which could potentially be missed using simpler scoring methods. Tumor samples with an H-score of <150 for individual chromatin marks were designated as low detection, whereas scores of $\geqq 150$ were designated as high detection.

Statistical analysis

The relationship between histone modification levels and potential explanatory variables, including age, gender, location, tumor size, histological type, depth of invasion, lymph node metastasis, location, lymphatic invasion, venous invasion, perineural invasion, serum CEA and CA19-9 concentrations, was evaluated using the chi-square test. The postoperative survival rate and disease-free survival rate were analyzed using the Kaplan–Meier method, and differences in survival rates were assessed using the log-rank test. A Cox proportional-hazard model was used for univariate and multivariate analyses. Differences were considered as significant when the p value was <0.05. Each statistical analysis was performed using the Dr. SPSS II software program, version 11.0.1J for Windows (SPSS, Inc., Chicago, IL, USA).

Results

Patients characteristics

All patient characteristics are detailed in Table 1 with histone modification levels. Of all 61 patients in the present study, 35 were male and 26 were female, and the median age was 64 (44–84) years. Pancreaticoduodenectomy was performed in 37 patients; 16 patients underwent distal pancreatomy and eight patients underwent total pancreatomy. The median size of the resected tumor was 40 (10–95) mm. The median serum CEA concentrations were 3.7 (0.7–70.2) ng/ml, and the median serum CA19-9 concentrations were 270 (2–14794) ng/ml. TNM stages, based on the UICC 7th edition, were IB:2, IIA:13, IIB:28, III:18.

Within a median follow-up duration of 14.4 (3.8–58.8) months, recurrences were found in 44 patients and deaths occurred in 39 patients.

Immunohistochemistry and H-score distributions

Representative staining for each of the five histone modifications is shown in Fig. 1. Only nuclear staining was regarded as positive, and cases were scored for each mark using a modified H-score as described in the Materials and methods. Histograms showing the distribution of H-scores plotted against the number of cases for each histone modifications are shown in Fig. 2. The median value (range) for each H-score was as follows: H3K9me2, 158 (5–300); H3K9ac, 140 (0–286); H3K4me2, 142 (0–222); H3K4me3, 160 (48–288); H3K18ac, 162 (58–300). H-scores of H3K9me2 and H3K18ac were almost exclusively accumulated in the range 151–200. Although scores in the range 151–200 were also most frequently observed in H3K9ac, H3K4me2 and H3K4me3, scores in the range 51–100 were the second most frequent in these modifications. Based on the finding that the cut off value for the H-score using ROC curve analysis was almost identical to the median value, the expression level of the histone modifications was categorized as being low if they were <150 or high if they were $\geqq 150$, to keep the scores clear and concise.

Relationship between the histone modifications and clinicopathological features

The relationships between the expression levels of histone modifications and the patients' clinicopathological

Table 1
Relationship between the expression of histone modifications and the clinicopathological features.

Variables/ categories	H3K9me2			H3K9ac			H3K4me2			H3K4me3			H3K18ac		
	Low	High	*p* Value	Low	High	*p* Value	Low	High	*p* Value	Low	High	*p* Value	Low	High	*p* Value
	$n = 29$	$n = 32$		$n = 35$	$n = 26$		$n = 36$	$n = 25$		$n = 14$	$n = 47$		$n = 15$	$n = 46$	
Location															
Head	20	24	0.600	24	20	0.472	27	17	0.549	10	34	0.947	9	35	0.228
Body/tail	9	8		11	6		9	8		4	13		6	11	
Tumor size															
≦2 cm	1	5	0.111	3	3	0.700	1	5	**0.026***	0	6	0.159	1	5	0.635
>2 cm	28	27		32	23		35	20		14	41		14	41	
Histological type															
Well, mod	21	31	**0.007***	28	24	0.180	31	21	0.819	9	43	**0.012***	12	40	0.509
Por, others	8	1		7	2		5	4		5	4		3	6	
Depth of invasion															
T1, T2	2	1	0.496	1	2	0.388	1	2	0.354	0	3	0.332	1	2	0.718
T3, T4	27	31		34	24		35	23		14	44		14	44	
Lymph node metastasis															
Absent	7	8	0.938	12	3	**0.041***	7	8	0.263	3	12	0.754	2	13	0.244
Present	22	24		23	23		29	17		11	35		13	33	
Venous invasion															
Absent	9	11	0.933	11	9	0.770	11	9	0.628	4	16	0.630	4	16	0.630
Present	18	21		23	16		24	15		10	29		10	29	
Perineural invasion															
Absent	9	7	0.409	8	8	0.47	5	11	**0.007***	5	11	0.297	6	10	0.194
Present	19	24		26	17		30	13		8	35		9	34	
CA19-9															
Normal	4	7	0.42	3	8	0.056	4	7	**0.047***	2	9	0.813	1	10	0.284
Abnormal	21	21		25	17		29	13		9	33		10	32	

Well: well differentiated, Mod: moderately differentiated, Por: poorly differentiated.
Bold values represent less than 0.05.

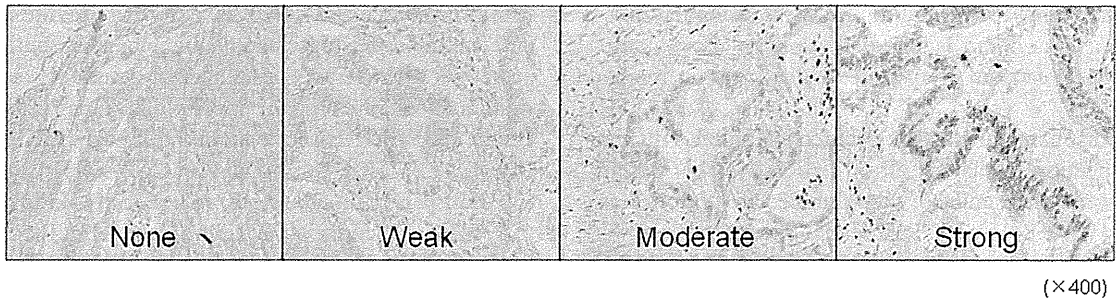

Figure 1. Representative examples of H3K4me2 immunohistochemical staining in pancreatic cancer tissues. Demonstrative images for each criterion are shown. Scale-bars: 100 μm.

features were then examined (Table 1). The low H3K9me2 expression group was significantly associated with the group of poorly differentiated adenocarcinomas or histological types other than adenocarcinoma ($p = 0.007$). In contrast, the high H3K4me3 expression group was significantly associated with the group of well and moderately differentiated adenocarcinomas ($p = 0.012$). Other modifications were not associated with tumor histological type. The low H3K4me2 expression group was significantly associated with the presence of perineural invasion ($p = 0.007$) and elevated serum CA19-9 concentrations ($p = 0.047$). The high H3K4me2 expression group was associated with smaller tumor size ($p = 0.026$). The low H3K9ac expression group was related to the absence of lymph node metastasis ($p = 0.041$). Histone modifications were unrelated to age, gender, tumor location, lymphatic invasion, venous invasion, depth and serum CEA concentrations.

Relationship of histone modifications to patient overall and disease-free survival

We compared the overall and disease-free survival rates among the cases with different levels of histone modification using the log-rank test. The overall and disease-free survival (DFS) rates did not appear to differ according to H3K9me2, H3K9ac, H3K4me2, H3K4me3 or H3K18ac status (data not shown).

Histone modification levels and adjuvant gemcitabine chemotherapy

We next examined whether histone levels were able to predict patient response to gemcitabine chemotherapy. We stratified patients on the basis of postoperative therapy; the patients in group A received gemcitabine chemotherapy

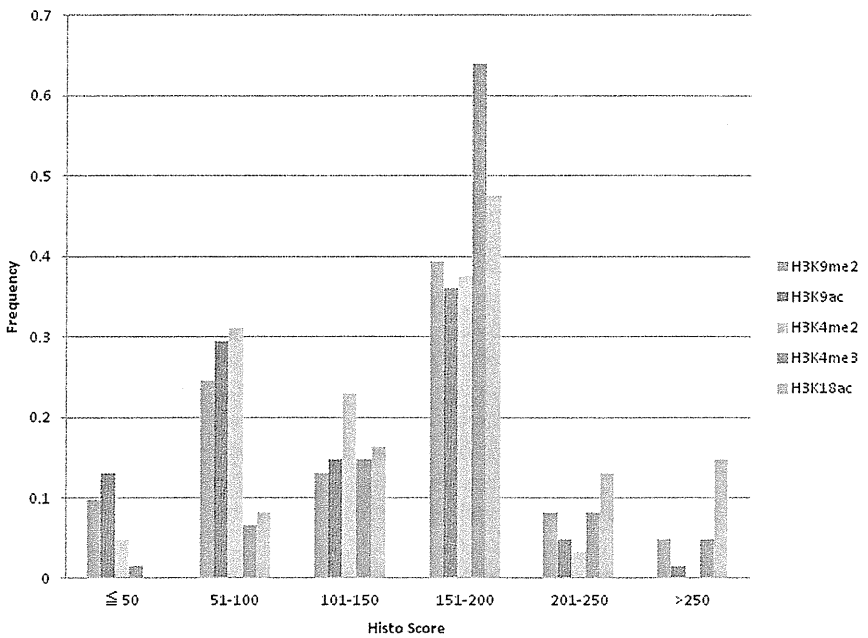

Figure 2. Histograms showing the distribution of H-scores plotted against the number of cases exhibiting the histone modifications.

at a dose of 12 g and those group B did not received chemotherapy or did not achieved a dose of 12 g. In group B, 10 patients did not start gemcitabine because of their unwillingness to undergo treatment, and the remaining 21 patients commenced gemcitabine but abandoned it during the course of treatment, generally due to the adverse effects. Evaluation of clinicopathological factor groups A and B using the chi-square test, revealed that only the presence of lymph node metastasis was significantly associated with Group B ($p = 0.031$). Using Kaplan–Meier survival analysis it was found that the low H3K4me2 expression group was significantly associated with the worse DFS in group A that received the full-dose of gemcitabine (Fig. 3). Both univariate and multivariate hazard models also indicated that the low H3K4me2 expression group was a significant independent predictor of DFS ($p = 0.007$) (Table 2).

Discussion

We used immunohistochemistry to evaluate the modification patterns of five different histone residues at the cellular level in 61 surgically removed pancreatic tumors and examined the relationship between histone modifications and patient clinicopathological features and outcomes.

Relationship between the histone modifications and clinicopathological features

A low level of cellular methylation of H3K4 was associated with perineural invasion and elevated serum CA19-9 concentrations, and a low level of cellular methylation of H3K9 was associated with the histology of the group, including poorly differentiated adenocarcinoma and tumors other than adenocarcinoma. In contrast, a high cellular level of methylation of H3K4 was associated with smaller tumor size and a well or moderately differentiated adenocarcinoma histology. Although different effects of methylation on gene transcription, namely activation or repression, have been reported for H3K4 (activation),[17] H3K9 (activation/repression)[5,7,8] or H3K27 (repression),[3] cellular methylation levels of histone H3 were generally considered to be associated with unfavorable clinicopathological characteristics in our study. This feature was consistent with the preceding two reported studies on pancreatic cancer.[16,17] A similar association has been reported in ovarian and breast cancers.[16] In stage I non-small-cell lung cancer (NSCLC) patients, a high level of H3K4me2 has been reported to be associated with the best survival rates,[13] which can be considered as a similar trend to that found in pancreatic cancer. In contrast, gastric adenocarcinomas with a high level of H3K9me3 were associated with unfavorable characteristics such as higher T stage, nodal metastasis and recurrence.[14] Cellular histone methylation levels may have different impacts on different tumor types, and also on the location of methylated lysine residues.

Impact of histone modification levels on disease free survival

In the present study, we did not find any association between a low cellular level of H3K4me2, H3K4me3 or H3K9me2 and the overall and disease-free survival rates of patients, in spite of a positive correlation with unfavorable characteristics. Because previous papers have revealed a correlation with poorer survival,[16,17] we may need a larger

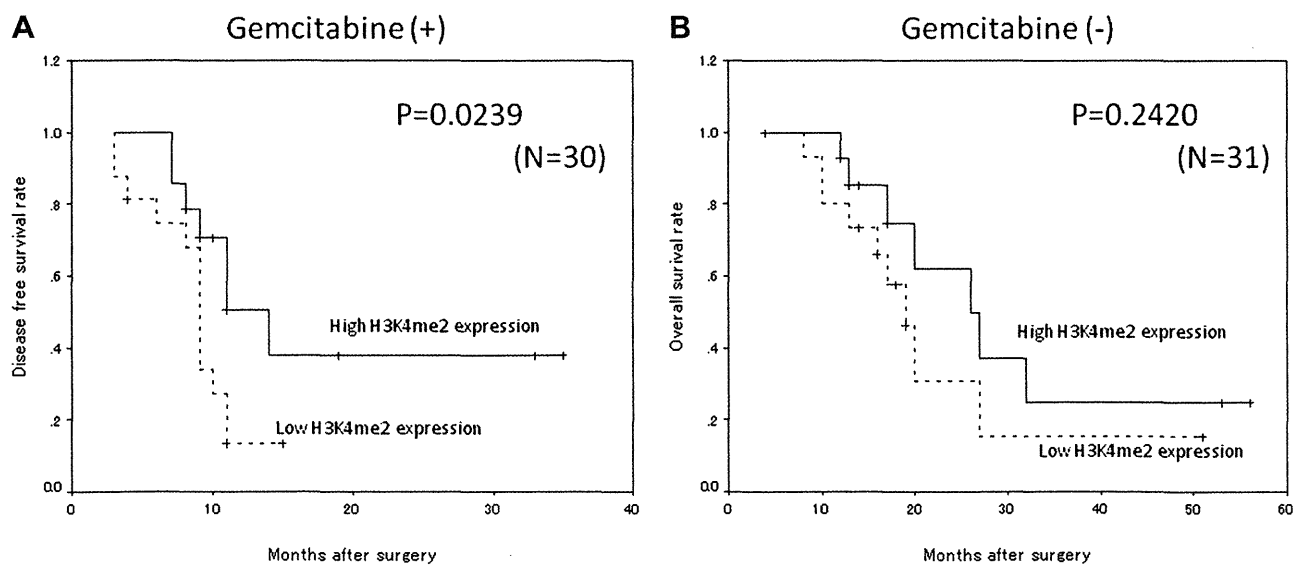

Figure 3. Disease-free survival according to postoperative chemotherapy. The low H3K4me2 expression group was significantly associated with the worse DFS in group A.

Table 2
Univariate and multivariate Cox regression analyses of factors affecting disease-free survival.

Variables/categories	*n*	Univariate			Multivariate		
		HR	95% CI	*p* Value	HR	95% CI	*p* Value
Location							
Head	21	1	0.874–7.061	0.088			
Body/tail	9	0.248					
Tumor size							
≦2 cm	3	1	0.300–17.02	0.428			
>2 cm	27	2.261					
Lymph node metastasis							
Absent	11	1	0.678–4.665	0.242			
Present	19	1.778					
H3K4me2 expression							
Low	16	1	0.132–0.904	**0.030***	1	0.038–0.600	**0.007***
High	14	0.346			0.151		
Histological type							
Well, mod	24	1	0.370–4.357	0.703			
Por, others	6	1.271					
Vascular invasion							
Absent	12	1	0.446–2.915	0.783			
Present	17	1.141					
Lymphatic invasion							
Absent	9	1	1.039–19.74	**0.044***	1	0.568–11.739	0.220
Present	21	4.498			2.582		
Perineural invasion							
Absent	9	1	0.613–5.525	0.277			
Present	21	1.841					

CI: confidence interval, Well: well differentiated, Mod: moderately differentiated, Por: poorly differentiated.
Bold values represent less than 0.05.

cohort to clarify this issue. However, in a subgroup analysis, we found that a low level of H3K4me2 was associated with worse disease free survival in patients receiving adjuvant gemcitabine. This result was different from that reported in a preceding study by Manuyakorn et al.[17] that indicated a positive association between a low level of H3K4me2 and disease free survival only for those patients that had received adjuvant fluorouracil, but not for those that had received gemcitabine. Differences in drug efficacy and toxicity have been reported between Asians and Caucasians.[20] Polymorphic variations in genes involved in gemcitabine pharmacology could be a cause of these differences.[21] Actually, Ross et al.[18] found significant differences in the distribution of genotypes between healthy Asians and Caucasians in 13/19 loci in the genes involved in gemcitabine pharmacology. It has been further reported that the variant of the CDA gene, involved in gemcitabine detoxification,[22,23] was associated with response rate and time to progression, and that the variation of the SLC28A1 gene, a gemcitabine transporter,[24,25] was associated with hematologic toxicity in patients with NSCLC receiving gemcitabine-based treatment.[18] Ethnic genetic background could be responsible for the difference in response to adjuvant gemcitabine between previous studies involving the Caucasian population and the present study involving the Japanese population.

Conclusion

We indicated that H3K4me2 at the cellular level might be useful in identifying pancreatic cancer patients who would be likely to derive benefit from adjuvant gemcitabine. Although our study had several limitations including the small sample size and its retrospective nature, we believe that the results obtained are meaningful, and should be strengthened by adequately powered future studies.

Conflict of interest statement

The authors declare that they have no potential conflict of interest.

Acknowledgment

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Objective: Gemcitabine has been widely used, and cisplatin plus gemcitabine is considered as standard first-line chemotherapy for patients with advanced biliary tract cancer. However, no standard therapy was established following the progression to gemcitabine-containing first-line therapy. As S-1 monotherapy as second-line chemotherapy is still not well known in a practical setting this study aimed to clarify its efficacy and safety.
Methods: We retrospectively reviewed 55 consecutive patients who received S-1 monotherapy as second-line chemotherapy after failure of a gemcitabine-containing regimen at our institution from September 2007 to March 2011. The inclusion criteria were preserved organ function and an Eastern Cooperative Oncology Group performance status of 0–2 and without massive ascites or pleural effusion. S-1 was administered orally twice a day at a dose of 40 mg/m^2 for 28 days, followed by 14 days of rest.
Results: Fifty-one patients were selected for this analysis. The overall response rate was 4.0% and the disease control rate was 38.0%. The median survival time was 6.0 months and the median progression-free survival was 2.3 months. Adverse events were generally mild, and treatment-related death did not occur. In the subgroup analysis, overall survival was significantly shorter in the patients with peritoneal dissemination and those who had shown no response to the first-line chemotherapy ($P = 0.033$ and 0.023, respectively).
Conclusions: S-1 monotherapy as the second-line chemotherapy for patients with gemcitabine-refractory advanced biliary tract cancer is also feasible in a practical setting and its efficacy is almost the same as in the previous prospective study.

Key words: S-1 – biliary tract cancer – second-line – gemcitabine refractory

INTRODUCTION

Biliary tract refers to all routes that bile juice passes through from hepatocytes to the duodenum, including intrahepatic bile duct, extrahepatic bile duct, gall bladder and ampulla of Vater. Therefore, biliary tract cancer (BTC) includes intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder carcinoma and ampullary carcinoma. Sometimes, intrahepatic cholangiocarcinoma is classified as primary liver cancer by UICC (1) and Japanese classification (2), but it is more often classified as BTC because of its development, as well as pathological and clinical features.

BTC is not a common disease throughout the world; however, it is more commonly encountered in East Asia and Latin America than any other countries (3). Furthermore, it is the sixth leading cause of cancer-related death in Japan.

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They are usually found in unresectable stage; however, resection surgery is the only way to cure BTC. Moreover, recurrence after curative surgery is common because BTC has high malignant potential and propensity to metastasize. Therefore, systemic chemotherapy is important for the treatment of BTC. Gemcitabine (GEM) has shown efficacy and safety for advanced BTC in many reports (4–6). GEM is considered the key drug for the treatment of advanced BTC, and GEM monotherapy was recognized as a community standard in Japan until 2010. In 2010, the results of the Phase III study of cisplatin (CDDP) plus GEM versus GEM for advanced BTC were reported (7) and GEM and CDDP combination therapy showed superiority to GEM monotherapy. Similar results were also reported in Japanese Phase II study (8). CDDP and GEM combination therapy is now considered as a standard first-line regimen for advanced BTC. In 2011, CDDP received approval from social insurance in Japan for advanced BTC.

No standard therapy was established following the progression to GEM-containing first-line therapy. S-1 is an oral agent consisting of a mixture of tegafur, 5-chloro-2, 4-dihydroxypyrimidine and potassium oxonate at a molar ratio of 1:0.4:1 (9), which has mainly been investigated in Asian countries. In a Phase II study of S-1 as a drug for first-line chemotherapy for advanced BTC, it was reported that the objective response rate was 32.5%, and the median survival time (MST) was 9.4 months with median time to progression (TTP) 3.7 months (10,11). Because of the good anti-tumor activity, two prospective studies of S-1 monotherapy as second-line therapy after the progression to GEM (12,13) were conducted. In these studies, the objective response rates were 22.7 and 7.5% and the values of MST were 13.5 and 7.5 months. S-1 is practically used as a drug for second-line chemotherapy in Japan to treat advanced BTC.

However, these results were quite different from one another. Consequently, the efficacy and safety of S-1 monotherapy as second-line therapy for advanced BTC is still not established in a practical setting, which is why we performed this retrospective analysis.

PATIENTS AND METHODS

Patients

The subjects were 55 consecutive patients who received S-1 monotherapy as second-line chemotherapy after the failure to GEM-containing regimen at Kanagawa Cancer Center between September 2007 and March 2011. We retrospectively reviewed their medical records. All the patients received a pathological and graphical diagnosis of BTC (intrahepatic or extrahepatic cholangiocarcinoma, gallbladder cancer or ampullary carcinoma). Advanced BTC was defined as (i) metastasis to other organs or to a distant lymph node, (ii) metastasis to form a bulky lymph node of hepatoduodenal ligament, (iii) invasion to common hepatic artery or proper hepatic artery or celiac artery or superior mesenteric artery, (iv) invasion to the bilateral branches of hepatic artery, (v) invasion to the trunk of portal vein which leads to the growth of collateral vessels, or invasion to the bilateral branches of portal vein, (vi) invasion to the bilateral secondary branch of the bile duct, (vii) invasion to one side of the hepatic artery/portal vein and invasion to another side of the secondary branches of the bile duct and (viii) recurrence after curative surgery. In addition to these criteria, intrahepatic cholangiocarcinoma with intrahepatic metastasis in the bilateral lobe is also defined as advanced BTC. Additional criteria for this retrospective analysis included an Eastern Cooperative Oncology Group performance status (PS) of 0–2, good bone marrow function, white blood cell count $\geq 3000/mm^3$, neutrophil count $\geq 1500/mm^3$, hemoglobin ≥ 8.5 g/dl, platelet count $\geq 100\,000/mm^3$, good renal function (serum creatinine ≤ 1.5 mg/dl) and good liver function (total bilirubin ≤ 2.0 mg/dl and transaminase levels ≤ 2.5 times the upper limit of the normal ranges). Patients with obstructive jaundice were eligible after receiving adequate biliary drainage and decreasing transaminase levels (less than five times the upper limit of the normal range). Patients were excluded if they had not received GEM in the first-line regimen or had already received S-1, or if they had massive ascites, pleural effusion, active concomitant malignancy, brain metastasis, interstitial pneumonia, uncontrolled diabetes mellitus and regular use of warfarin, phenytoin or frucitocin.

Treatment

S-1 was administered orally twice a day at a dose of 40 mg/m^2. The initial doses were determined according to the body surface area (BSA) calculated by body weight and height as follows: BSA $< 1.25\ m^2$, 80 mg/day; $1.25\ m^2 \leq$ BSA $< 1.5\ m^2$, 100 mg/day; $1.5\ m^2 \leq$ BSA, 120 mg/day. S-1 was given for 28 days followed by 14 days of rest. Dose reduction and interruption were considered in the case of severe toxicities (graded as 3–4) according to the Common Terminology Criteria of Adverse Event version 4.0 (CTCAE v4.0). No dose re-escalation was conducted following the dose reduction. This treatment course was repeated until disease progression, unacceptable toxicities or patients' refusal.

Evaluation

Tumor response was assessed approximately every 2 months in contrast-enhanced computed tomography according to the Response Evaluation Criteria In Solid Tumor (RECIST, version 1.1). Toxicities were evaluated according to the CTCAE v4.0. Overall survival was defined as the duration from the date of treatment initiation to the date of death of any cause or the last follow-up. Progression-free survival (PFS) was defined as the duration from the date of S-1 treatment initiation to the date of documented disease progression

or death. The overall survival and PFS were calculated using the Kaplan–Meier method. Subgroup analyses were evaluated with the log-rank test and the Cox proportional hazard model. This study was approved by Kanagawa Cancer Center institutional review board.

RESULTS

Subjects

One hundred and thirteen patients with advanced BTC received GEM monotherapy or GEM plus CDDP combination therapy as the first-line treatment and 83 patients discontinued. Among these 83 patients, 55 patients received S-1 monotherapy as the second-line treatment and 51 patients were selected for this study according to the eligibility criteria. The reason for exclusion was anemia due to the first-line treatment in one patient, massive ascites in one patient, PS 3 in one patient and patient's refusal for surgical treatment in one patient. The patient characteristics are shown in Table 1. Among the 51 patients, the median age was 69 years (range 39–81), 29 (57%) were male and all the patients except only one had an Eastern Cooperative Oncology Group PS of 0–1. The number of patients with gallbladder carcinoma was 26 (51%), and that with recurrent disease after the curative surgery was 8 (16%). Regarding the first-line treatment, the number of patients who had received GEM monotherapy was 47 (92%), while the number of patients who received GEM plus CDDP combination therapy was 4 (8%). In GEM monotherapy and GEM plus CDDP combination therapy, PFS was 4.0 and 3.4 months, 5 patients (10.6%) and 1 (25%) patient showed a partial response and 26 (55.3%) and 2 (50%) showed stable disease, respectively.

Treatment

A total of 176 courses were administered, with a median of two courses per patient (range 1–18). Dose reduction due to the adverse events was conducted in 17 (33%) patients, and treatment was interrupted during the course in 15 (29%) patients. The median dose intensity of S-1 was 87.3% (range 38.4–100%) compared with the planned dosage. S-1 monotherapy was discontinued in 43 (84%) patients because of the disease progression and in 4 (8%) patients because of the adverse events (Grade 2 nausea in two patients, Grade 2 gastrointestinal bleeding in one and Grade 2 anorexia in one). Four patients (8%) had been receiving S-1 monotherapy at the time of this analysis.

Efficacy

Excluding 1 patient who could not be evaluated, 2 (4.0%) patients showed partial responses and 19 (38%) showed stable disease, resulting in an overall objective response rate of 4% and a disease control rate of 42%. The overall MST was 6.0 months and the PFS was 2.3 months (Fig. 1). In subgroup analysis according to the presence of ascites, indicating the presence of peritoneal dissemination, the MSTs of patients with and without ascites were 2.2 and 6.8 months ($P = 0.033$), respectively. And there was a significant difference in overall survival between patients who had progressive disease against the first-line chemotherapy and who had any response (3.5 and 7.2 months, respectively, $P = 0.023$). These two factors were also significant in multivariate analysis; the hazard ratios were 3.2 and 2.3, respectively. However, there was no significant difference between gallbladder carcinoma and non-gallbladder carcinoma (Table 2).

Toxicities

Table 3 presents the adverse events that occurred during the S-1 monotherapy as the second-line treatment. No treatment

Table 1. Patient characteristics

	Patients ($n = 51$)	Percent
Median age (range)	69 (39–81)	
Gender		
Male	29	56.9
Female	22	43.1
ECOG PS		
0	40	78.4
1	10	19.6
2	1	2.0
Location of primary tumor		
Intrahepatic bile duct	15	29.4
Extrahepatic bile duct	9	17.6
Gallbladder	26	51.0
Ampulla of Vater	1	2.0
Extent of disease		
Local advanced	16	31.4
Metastatic (prior curative surgery)	35 (8)	68.6 (15.7)
With ascites	5	9.8
CEA before treatment (ng/ml)		
≤5.0	22	43.1
>5.0	29	56.9
CA19-9 before treatment (mU/ml)		
≤37	13	25.5
>37	38	74.5
Prior treatment regimen		
Gemcitabine alone	47	92.2
Gemcitabine + cisplatin	4	7.8

ECOG PS, Eastern Cooperative Oncology Group performance status; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

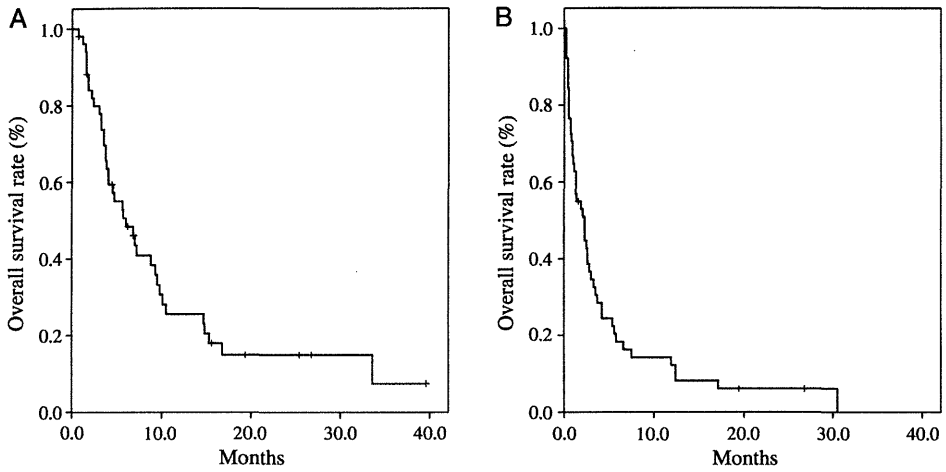

Figure 1. (A) The Kaplan–Meier curves for overall survival. The median survival time was 6.0 months [95% confidence interval (CI): 3.4–8.6]. (B) The Kaplan–Meier curves for progression-free survival (PFS). The median PFS was 2.3 months (95% CI: 1.2–3.4).

death occurred, and generally, toxicities were mild: Grade 3/4 hematologic adverse events, which include anemia, leukopenia, neutropenia and thrombocytopenia, were observed in only one patient for each, and non-hematologic adverse events with Grade 3/4 were diarrhea (three patients, 6%), anorexia (one patient, 2%), nausea (one patient, 2%), mucositis oral (one patient, 2%) and rash (one patient, 2%).

DISCUSSION

In this analysis, ~64% of the patients received second-line chemotherapy after being refractory to GEM-containing regimen similar to 70% of the patients who received the second-line chemotherapy in the past study (8). These findings indicate that the development of effective second-line chemotherapy is critical to the treatment of advanced BTC. However, there is no standard regimen after the refractory condition to the GEM plus CDDP regimen, as National Comprehensive Cancer Network guideline shows no recommendation about it.

5-Fluorouracil (5-FU) was expected to have an anti-tumor effect for advanced BTC, and some studies of 5-FU monotherapy or 5-FU combination regimen as first-line treatment were reported previously (14–19). According to these studies, it is considered that 5-FU was ineffective as an agent for first-line treatment. S-1, which is a 5-FU derivative, is a promising agent for first-line treatment (10,11). However, the agent effective in the first-line treatment is not always effective in the second-line treatment, and it is necessary to evaluate the efficacy and safety of the agent in the second-line treatment. The results of the current study were similar to the report published by Suzuki et al. (13) at the 2010 annual meeting of the American Society of Clinical Oncology. On the other hand, the results reported by Sasaki et al. (12) were largely better than those of the current study. One of the reasons for the difference may be the patient's characteristics, especially the primary site of tumor and peritoneal dissemination. It was reported that gallbladder cancer has a poor prognosis (6,20). Gallbladder cancer was included 51% in the current study, while only 27% in the Phase II study reported by Sasaki et al. (12). As for the peritoneal dissemination, it was not mentioned in the report so it cannot be compared. Instead, they insisted on the tumor volume rather than on the primary site. From this point of view, patients with recurrent disease show better prognosis than those with non-resectable disease because careful observation results in small tumor volume when the recurrence is pointed out (8). However, no survival difference was observed between the patients with recurrent disease and non-resectable disease in our study, and since the outcome that recurrent case had better prognosis may mean lead time bias, further studies are needed to address this issue.

Subgroup analysis of our study indicates that patients who had shown progressive disease for the first-line chemotherapy administering GEM tended to have worse prognosis despite the second-line chemotherapy of S-1 than those who had shown disease control. It means that S-1 monotherapy as second-line treatment may not salvage patients who did not show any response to the GEM-containing regimen. Neither GEM nor CDDP cross-reacts with S-1 in pharmacokinetics (21–25), and patients who showed disease progression against both first-line and second-line chemotherapy may have other complex factors. Nonetheless, it is important to exercise caution while interpreting the results of this retrospective study, as the patients' backgrounds are different from one another.

Concerning the toxicities, Grade 3–4 adverse events were not frequent and no treatment-related death was observed. Moreover, treatment discontinuation was needed for only four (8%) patients. Therefore, two prospective studies and the current study showed similar results, indicating that S-1 monotherapy is tolerable in the second-line treatment after the GEM failure.

Table 2. Prognostic factors for overall survival

Factor	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Gender				
Male	1			
Female	0.82 (0.43–1.57)	NS		
Age ≤65 years old				
Yes	1			
No	1.46 (0.76–2.80)	NS		
Performance status				
0	1			
1	1.60 (0.76–3.33)	NS		
CEA ≤5.0 ng/ml				
Yes	1			
No	1.70 (0.88–3.31)	NS		
CA19-9 ≤37 IU/ml				
Yes	1			
No	1.19 (0.56–2.51)	NS		
Recurrent disease				
Yes	1			
No	1.30 (0.59–2.86)	NS		
Metastatic disease				
Yes	1			
No	0.71 (0.37–1.37)	NS		
Gallbladder carcinoma				
Yes	1			
No	1.62 (0.84–3.11)	NS		
Without ascites				
Yes	1		1	
No	2.77 (1.04–7.17)	0.033	3.21 (1.20–8.61)	0.020
Any response to first-line chemotherapy				
Yes	1		1	
No	2.10 (1.09–4.05)	0.023	2.29 (1.17–4.47)	0.015

CI, confidence interval; NS, not significant.

Table 3. Adverse events that occurred during S-1 monotherapy as the second-line treatment, according to CTCAE version 4.0

	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic				
Anemia	23	17	1	0
Leucopenia	8	7	1	0
Neutropenia	8	5	1	0
Thrombocytopenia	15	4	1	0
Non-hematologic				
Anorexia	18	5	1	0
Nausea	6	7	1	0
Diarrhea	5	2	3	0
Mucositis oral	5	2	1	0
Fatigue	4	2	0	0
Dysgeusia	6	0	0	0
Skin hyperpigmentation	4	0	0	0
Vomit	2	1	0	0
Constipation	3	0	0	0
Rash	0	1	1	0
Watering eyes	2	0	0	0

Grade 3–4 adverse events were not frequent and no treatment-related death did occur.

Other treatment regimens were reported for the patients with BTC refractory to GEM (Table 4). Lee et al. (26) reported that the Conti-FAM regimen showed a response rate of 12% and an MST of 6.7 months with a TTP of 2.3 months. Pino et al. (27) reported that the CapCel regimen showed a response rate of 9% and an MST of 4.4 months with a PFS of 4.0 months. These studies suggest modest efficacy and safety; however, it is a problem that these studies included more patients with pancreatic cancer rather than with BTC. Recently, many molecular-targeting drugs are developed, and some of these are expected to be efficacious for advanced BTC. Paule et al. suggested the efficacy of the cetuximab plus GEM-oxaliplatin (GEMOX) regimen for patients who are refractory to GEMOX (28). The study enrolled a few patients and was limited to intrahepatic cholangiocarcinoma. However, cetuximab plus GEMOX was expected to be useful for the first-line treatment in the single-arm Phase II study (29), and cetuximab plus GEMOX will be one of the candidates for the standard care of second-line treatment after the GEM plus platinum. Lastly, sunitinib is also expected in the second-line treatment (30).

Brandi (31) analyzed EM plus platinum compound, capecitabine or irinotecan as a drug for second-line treatment for patients refractory to GEM in the first-line treatment. It asks the clinical questions whether or not GEM should be used in the second-line treatment for patients refractory to GEM in the first-line treatment. Indeed, 5-FU is the key drug in metastatic colorectal cancer, which should be used after failure to first-line regimen including itself (32,33). In advanced BTC, some clinical trials that investigate the usefulness of GEM-containing second-line treatment after the failure to GEM are ongoing in Japan, such as GEMOX (UMIN000003650) and fix-dose rate GEM plus S-1(UMIN000005918).

The efficacy of second-line chemotherapy by S-1 monotherapy and these reported regimens should be evaluated by placebo control studies because the result will change

Table 4. Other regimens reported about the second-line treatment of advanced biliary tract cancer

Author	Regimen	Patients (*n*)	GBC	Response rate (%)	Median TTP or PFS (months)	Median survival time (months)
Lee et al.	Conti-FAM	16	31.3%	12	2.3	6.7
Pino et al.	CapCel	35	14%	9	4.0	4.4
Paule et al.	GEMOX + Cet	9	0	22	4.0	7.0
Yi et al.	Sunitinib	56	26.8%	8.9	1.7	4.8
Brandi et al.	GEM + platinum or GEM + capecitabine or GEM + CPT-11	49	12.2%	—	3.5	8.1

Conti-FAM; continuous 5-fluorouracil, doxorubicin and mitomycin-C; CapCel, capecitabine and celecoxib; GEMOX, gemcitabine and oxaliplatin; Cet, cetuximab; GEM, gemcitabine; CPT-11, irinotecan; GBC, gallbladder carcinoma; TTP, time to progression PFS; progression free survival.

because of the patient's background. Nevertheless, it is difficult to carry out a randomly controlled study, which compares S-1 monotherapy with placebo, since S-1 is approved for advanced biliary tract cancer by social insurance in Japan. Therefore, S-1 monotherapy can be the control arm in the clinical trials that test new promising regimens in the future.

In conclusion, S-1 monotherapy in a practical setting is well tolerated, and its efficacy is almost the same as the prospective clinical trials for patients with advanced BTC refractory to a GEM-containing regimen. Further development and randomized controlled studies of the second-line treatment are warranted.

Conflict of interest statement

None declared.

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Comparison of the Chemosensitivity of the Primary Lesion and a Pancreatic Metastasis of Colon Cancer: A Case Report

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Abstract. *Pancreatic metastasis from colorectal cancer is rare, and accounts for less than 2% of all pancreatic metastases. There have been no studies that have reported the differences in the sensitivity to chemotherapy between the primary lesion and the pancreatic metastasis in colorectal cancer. We experienced a rare example of pancreatic metastasis from colorectal cancer, and report here the difference in the sensitivity to the antitumor drug. A 68-year-old female underwent colectomy for rectal carcinoma with a mass in the pancreatic tail and the liver. The patient also underwent a distal pancreatectomy and a segmental liver resection at the same time. v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) and tumor protein 53 (TP53) gene mutation analyses, in addition to the histopathological examinations, revealed tumors of the liver and the pancreatic tail as being metastases from the primary carcinoma. We employed a collagen gel droplet-embedded culture drug sensitivity test for both the primary lesion and the pancreatic metastasis. The sensitivity to oxaliplatin and FOLFOX (5-flurouracil, folinic acid and oxaliplatin) were lower in the pancreatic metastasis compared to the primary lesion. In conclusion, pancreatic metastasis from colorectal malignancy is rare, and the present results suggest that there are potential differences in the sensitivity to chemotherapy between the primary colorectal tumor and its pancreatic metastasis.*

Colorectal cancer (CRC) is the third most common type of cancer and the fourth leading cause of death due to cancer worldwide (1). In spite of progress made in chemotherapy for CRC, the outcomes of CRC with distant metastasis still remain poor. The pancreas is an uncommon location for solitary metastasis from other primary carcinomas (2). But in many autopsy series, the prevalence of pancreatic metastasis has been described as being as high as 1.6% to 11% (3, 4). The metastases usually derive from a primary tumor of the kidney, lung, breast, gastrointestinal tract (stomach, small bowel or colorectum) or from melanoma (5). There have only been 29 reported cases of a solitary resectable pancreatic metastasis from colorectal cancer (6). Although hepatic resection is a potentially curative therapy for liver metastases from CRC, the benefits of resection of pancreatic metastases are unclear.

The collagen gel droplet-embedded culture drug sensitivity test (CD-DST), using various types of malignant neoplasms, has been safely and widely applied in Japan (7-9). However, to date, CD-DST data for a pancreatic metastasis from CRC have not been reported. This case study was performed in order to evaluate the differences in the CD-DST results between the primary lesion and its pancreatic metastasis. An accumulation of this type of information may be helpful in the future in order to establish treatment modalities for unresectable metastatic pancreatic tumors, or may allow for resectable tumors to be treated with chemotherapy instead of surgical removal.

Case Report

A 68-year-old female in good general condition presented to our department in May 2011 complaining of constipation and tested positive for occult fecal bleeding. There was an adenocarcinoma of the rectum detected by colorectal endoscopy, and computed tomography also revealed an inhomogeneous mass in the pancreatic body, measuring 35 mm in the largest diameter, and in segment 6 of the liver, measuring 30 mm in the largest diameter (Figure 1).

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Key Words: Colorectal cancer, pancreatic metastasis, CD-DST, *p53* mutation, FOLFOX, KRAS.