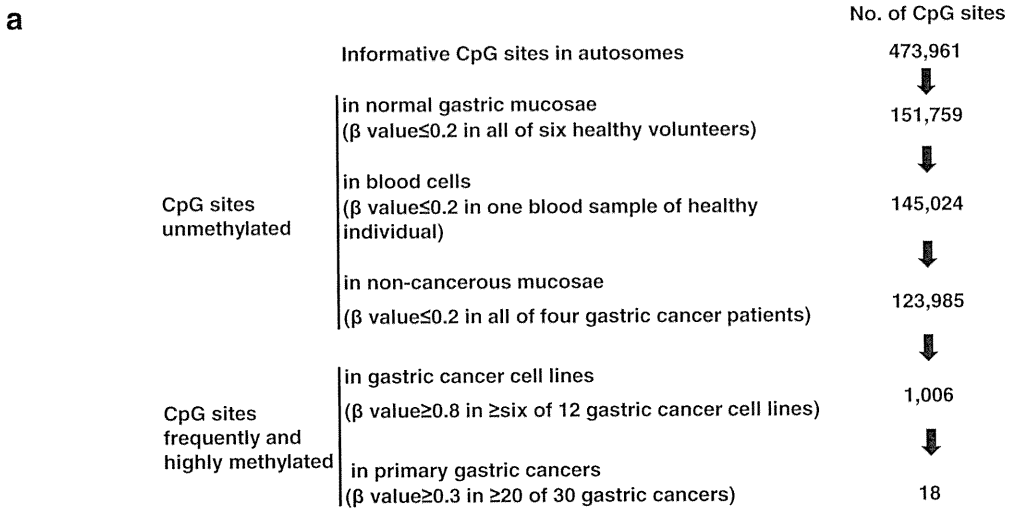

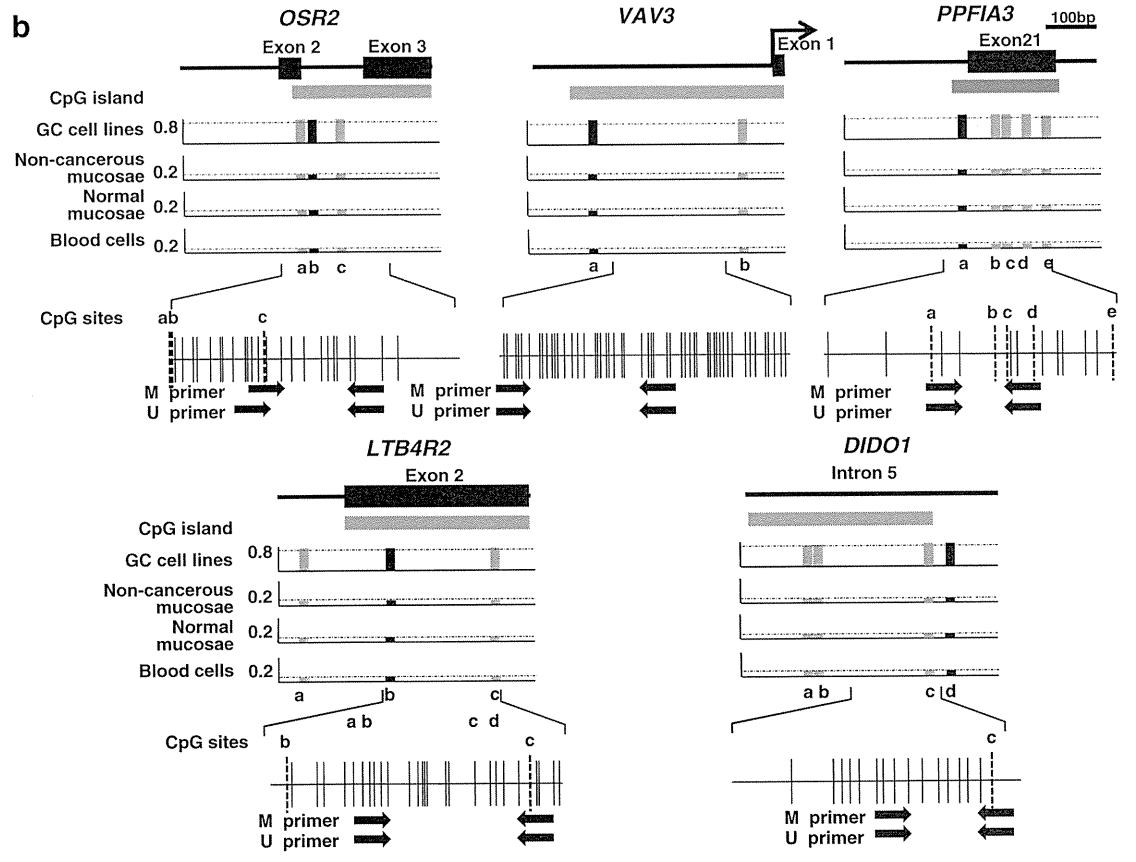

Fig. 1 Selection of specifically methylated regions by a genome-wide screening. **a** Specific genomic regions not methylated in normal cells and fully methylated in cancer cells were selected by a genome-wide screening using an Infinium HumanMethylation450 BeadChip array. Eighteen CpG sites derived from 16 genomic regions were isolated. **b** Five regions of five genes (*OSR2*, *VAV3*, *PPFIA3*, *LTB4R2*, and *DIDO1*) were selected because of their genomic structure and the availability of quantitative methylation-specific PCR (qMSP) primers. The genomic structure, including the location of a CpG island, transcription start site, introns, and exons, is shown at the *top*. The *β* values of the CpG sites analyzed using the bead array are shown in the *middle*, and the *broken lines* show the threshold used in the screening. A CpG map around the CpG site(s) is shown at the *bottom*. *Vertical lines* (*solid* or *broken*) show CpG sites, with *broken lines* showing CpG sites whose *β* values were measured by the bead array. *Arrows* show locations of primers for qMSP. *M* methylated, *U* unmethylated

exclude genes influenced by *H. pylori* infection, the methylation levels of the four genes were analyzed in 23 gastric mucosa samples of *H. pylori*-positive ($n = 14$) and *H. pylori*-negative ($n = 9$) individuals, as well as four samples of peripheral leukocytes different from the one used for the initial screening. The *LTB4R2* methylation level in the *H. pylori*-positive individuals was higher than that in the *H. pylori*-negative individuals and the four samples of peripheral leukocytes, showing that the *LTB4R2* methylation level was affected by *H. pylori* infection. On the other hand, *OSR2*, *VAV3*, and *PPFIA3* were almost unmethylated in the three groups (Fig. 2).

We also analyzed the expression of *OSR2*, *VAV3*, and *PPFIA3* using 17 normal gastric mucosa samples of *H. pylori*-positive ($n = 11$) and *H. pylori*-negative ($n = 6$) individuals. *VAV3* was highly expressed in both *H. pylori*-positive and *H. pylori*-negative gastric mucosae, whereas *OSR2* and *PPFIA3* were only weakly expressed (Fig. S2).

High incidence of methylation of the three genes and their specificity using LCM-purified cells

To examine the incidence of methylation of the three genes in primary GCs, we performed qMSP using 26 independent primary GCs, and observed that at least one of the three genes was methylated in all of the 26 GCs (Fig. 3a). These data showed that if these three genes were used as a panel, they would have a higher coverage (100 %) of primary GCs.

To confirm that the three genes were highly methylated only in GC cells but not in coexisting noncancer cells, four pairs of cancer and noncancer cells were collected by LCM. We found that at least one of the three genes was highly methylated in GC cells (more than 85 %), but that all of them were barely methylated in noncancer cells (less than 5 %) (Fig. 3b). The highest methylation level of the three genes was considered to reflect the fraction of cancer cells, and we defined the panel of the three genes as a DNA methylation marker to estimate the cancer cell fraction in a GC sample.

Because DNA methylation levels of some genes can be influenced by age [24], we also analyzed the correlation between the methylation of the three genes and age. The methylation levels of the three genes were found to be independent of age (Fig. S3).

CNAs of the three genes

CNAs of a marker gene can affect the methylation level of its region in cancer samples [25]. Therefore, we analyzed CNAs of the three regions in the 20 GCs used for the bead array analysis (Fig. 4). *VAV3* and *PPFIA3* showed no CNAs of more than twofold or less than 0.5-fold. In contrast, *OSR2* showed CNAs at low frequencies (more than

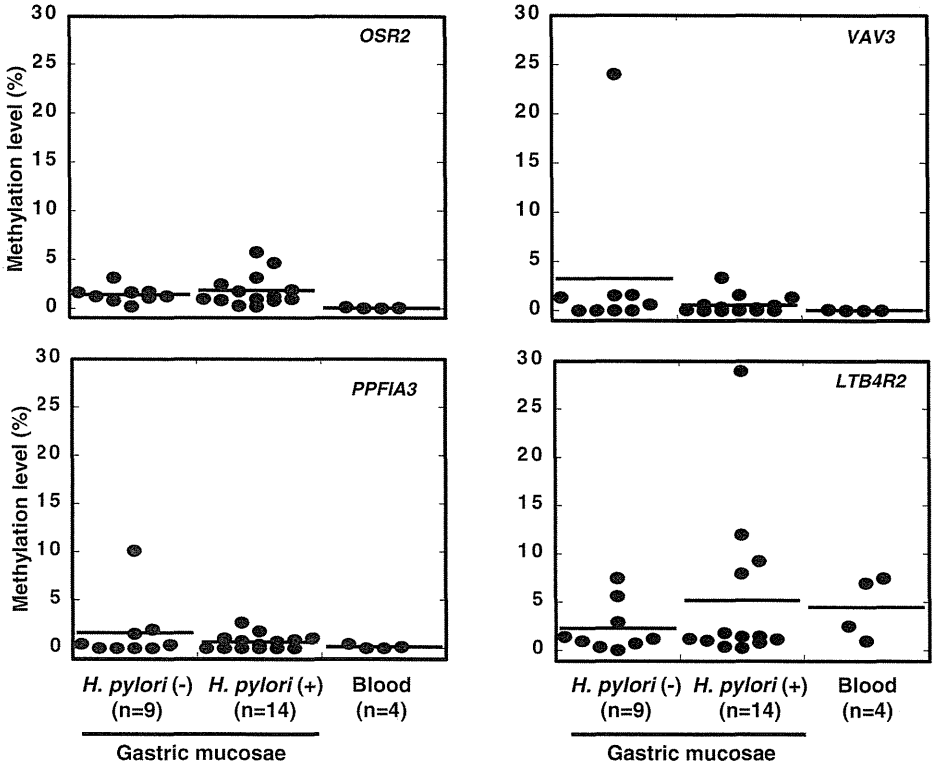

Fig. 2 Isolation of genes not influenced by *Helicobacter pylori* infection. Methylation levels of the four genes were analyzed by quantitative methylation-specific PCR in noncancerous gastric mucosae of *H. pylori*-positive ($n = 14$) and *H. pylori*-negative ($n = 9$) individuals, as well as four samples of peripheral leukocytes. *LTB4R2* was excluded because its methylation level was higher in the *H. pylori*-positive individuals than in the *H. pylori*-negative individuals

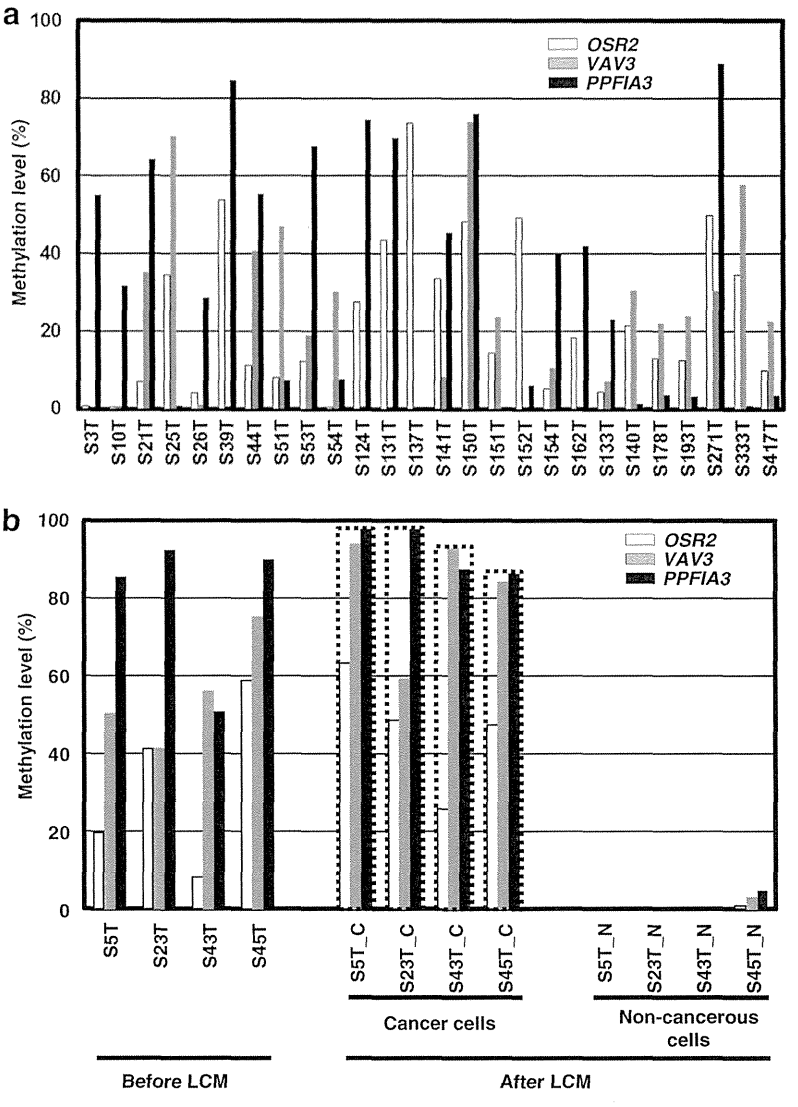

Fig. 3 High incidence of methylation of the three genes and specificity of methylation using cells purified by laser-capture microdissection (LCM). **a** The incidence of hypermethylation of the three genes was analyzed in 26 independent primary gastric cancers (GCs) by quantitative methylation-specific PCR. At least one of the three genes was methylated in all of the 26 GCs. **b** Methylation levels of the three genes were analyzed in four primary GCs before LCM and four pairs of purified cancer and noncancer cells after LCM. At least one of the three genes was highly methylated in GC cells (more than 85 %), but all the three genes were barely methylated in noncancer cells (less than 5 %). *Dotted rectangles* show the panel of the three genes as a DNA methylation marker

twofold in one GC and less than 0.5-fold in two GCs). It was calculated that the deviation of the methylation level from the true cancer cell fraction would be 17.2 % when twofold or 0.5-fold CNA was present in cancer cells [11]. Therefore, the effect of the CNA of *OSR2* was considered to be minimal in the estimation of the cancer cell fraction.

Correlation between the cancer cell fraction estimated by DNA methylation and that estimated by a genetic alteration

To evaluate the accuracy of the DNA methylation marker, 13 GCs with *TP53* mutation were identified among the 30 GCs used for the bead array analysis, and the cancer cell fraction estimated by the marker was compared with the *TP53* mutant frequency. A high correlation between the two methods was observed ($r = 0.77$, $P < 0.001$; Fig. 5). This result showed that the cancer cell fraction estimated by the DNA methylation marker accurately reflected the true fraction of cancer cells in a tumor sample.

Application of the DNA methylation marker to correction of the bead array data

We applied the DNA methylation marker to correct the influence of contamination by normal cells in the data from the epigenomic analysis. For the 30 primary GCs used for the bead array analysis, we measured the fraction of cancer cells using the marker, and corrected the bead array data by division with the evaluated fraction. Unsupervised hierarchical clustering analysis was conducted using 263 genomic blocks selected because their downstream genes

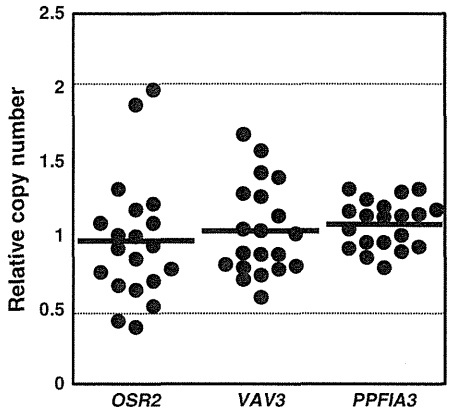

Fig. 4 Copy number alterations (CNAs) of the three genes. CNA of the three genes was analyzed by real-time PCR of the 20 gastric cancers (GCs) used for the bead array analysis. Significant CNA (gain or loss) was defined as a twofold or greater increase or a 0.5-fold or smaller decrease, respectively. Only *OSR2* showed CNAs at low frequencies (twofold or greater in one GC; 0.50-fold or smaller in two GCs)

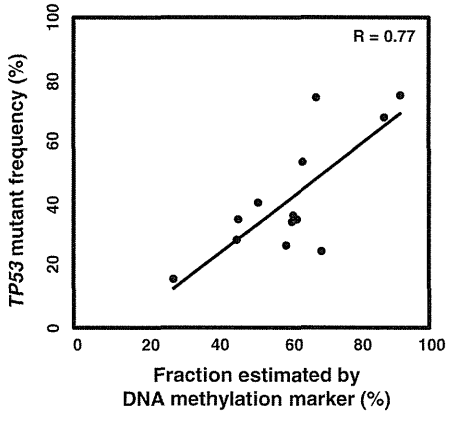

Fig. 5 Correlation between the cancer cell fraction estimated by DNA methylation and that estimated by a genetic alteration. The cancer cell fraction estimated by the DNA methylation marker was compared with the *TP53* mutant frequency. A high correlation between the two methods was observed ($r = 0.77$, $P < 0.001$)

were silenced by aberrant methylation [1] (Fig. 6b). Compared with the heatmap before the correction (Fig. 6a), two samples, S20T and S22T, moved from the CpG island methylator phenotype (CIMP)-negative group to the CIMP-high group. The cancer cell fraction in these two samples was less than 20 % (Fig. 3a). After exclusion of these two samples and correction of the methylation levels, the clustering of the CIMP-high, CIMP-moderate, CIMP-low, and CIMP-negative GCs became much clearer (Fig. 6c). From these data, we concluded that the DNA methylation marker could be used to identify and exclude samples with an extremely low fraction of cancer cells, and to correct the molecular data.

Discussion

We successfully established a panel of three genes (*OSR2*, *VAV3*, and *PPFIA3*) as a marker to estimate the fraction of cancer cells in primary GCs. Using the DNA methylation marker, we were also able to identify and exclude samples with a low fraction of cancer cells, and to correct the methylation levels by the fraction of cancer cells. After this, the genome-wide DNA methylation profiles yielded clearer clustering of CIMP by unsupervised hierarchical clustering analysis. This is the first molecular marker for the cancer cell fraction in GC.

The DNA methylation marker has the advantages of simplicity without the need for experienced pathologists or paired normal samples, compared with microscopic examination and genomic alterations. Also, the DNA methylation marker is likely to have a broad coverage in primary GCs because the DNA methylation marker was methylated in 100 % of the 26 primary GCs used for validation. Further, we were easily able to use the DNA methylation marker to assess the cancer cell fraction, even in diffuse-type GCs, for which even an expert pathologist has difficulty in estimating the cancer cell fraction. Finally, since the methylation levels of the three genes were independent of age, this marker was regarded to be useful to estimate the cancer cell fraction irrespective of age.

The correlation of the cancer cell fraction estimated by the DNA methylation marker with *TP53* mutant frequency was high ($r = 0.77$, $P < 0.001$). However, in two samples, the cancer cell fraction estimated by the marker was twice as large as that estimated by the *TP53* mutant frequency. Since loss of heterozygosity can coexist with a mutation of *TP53* in GCs, we speculated that the discrepancy between the two methods in the two GC samples might have been caused by the loss of heterozygosity of *TP53*.

Gastric mucosae, especially when infected with *H. pylori*, can have very high levels of DNA methylation, so we paid special attention to isolation of marker genes in this study. The panel of the three genes was not affected by *H. pylori* infection because the genes were barely methylated in *H. pylori*-positive mucosae. Only two samples in *H. pylori*-negative individuals had a high methylation of *VAV3* or *PPFIA3*, respectively. One possible reason for detection of such high methylation levels in *H. pylori*-negative samples is that these two samples were contaminated with cancer cells because they were resected from samples from GC patients. Another possible reason is that they were methylated in noncancer cells during past *H. pylori* infection.

A CNA can affect the methylation level of a marker gene. Therefore, we analyzed the CNAs of the three genes in 20 primary GCs used for the bead array analysis, and found CNAs of the three genes had little influence on the

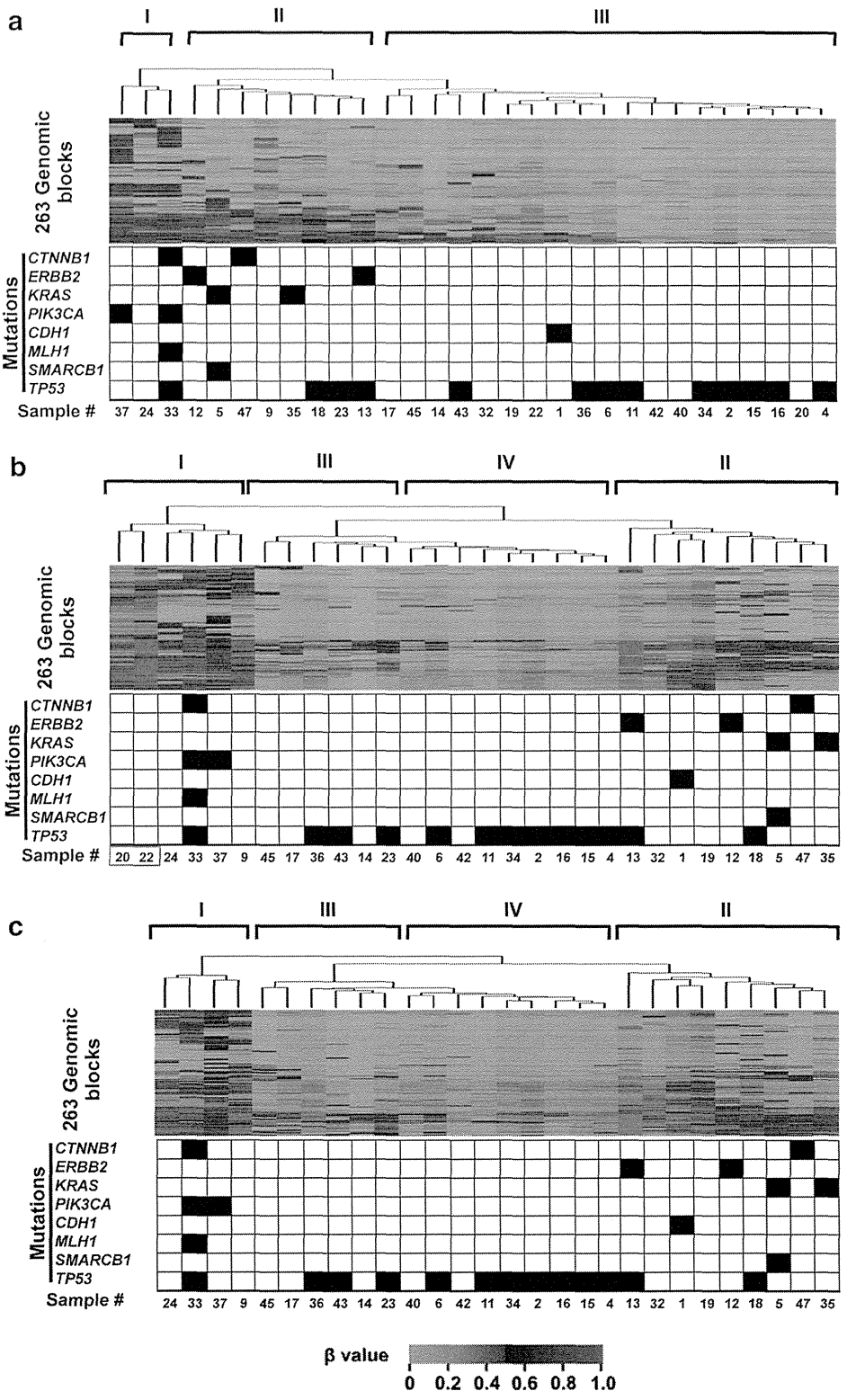

Fig. 6 Application of the DNA methylation marker to the correction of the bead array data. **a** Unsupervised hierarchical clustering analysis of the 30 primary gastric cancers using DNA methylation profiles of 263 genomic blocks. **b** Two samples surrounded by a *red square* (S20T and S22T) moved from the CpG island methylator phenotype (CIMP)-negative group to the CIMP-high group after the Infinium HumanMethylation450 BeadChip array data had been corrected by the DNA methylation marker. **c** After exclusion of two samples with a low fraction of cancer cells, a heatmap using the corrected bead array data showed a much clearer clustering of CIMP-high, CIMP-moderate, CIMP-low, and CIMP-negative gastric cancers

estimation of the cancer cell fraction. Regarding the expression of the three marker genes, only *VAV3* was highly expressed in normal gastric mucosae. The region of *VAV3*, for which DNA methylation was analyzed, was outside the nucleosome-free region, suggesting that its transcription is not necessarily suppressed by the methylation.

In summary, a DNA methylation marker—namely, the panel of the three genes—was isolated, and was shown to be qualified to estimate the cancer cell fraction in GCs. Application of the marker to correction of the bead array data showed promising results for improving the accuracy of molecular analysis. The DNA methylation marker is expected to be useful in many aspects of GC research.

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

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Comparative Outcomes Between Initially Unresectable and Recurrent Cases of Advanced Pancreatic Cancer Following Palliative Chemotherapy

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Objectives: The objective of this study was to compare the clinical outcomes between initially unresectable and recurrent advanced pancreatic cancer (APC) patients after palliative chemotherapy.

Methods: Data of a total of consecutive 269 patients with pathologically confirmed APC patients who received palliative chemotherapy between January 2006 and April 2012 were reviewed. Patients were classified into initially unresectable and recurrent group, and overall survival (OS) was compared between the 2 groups.

Results: The median OS was significantly longer in the recurrent group compared with the initially unresectable group (383 vs 308 days; hazard ratio [HR], 0.59; 95% confidence interval, 0.44–0.80; $P < 0.01$). After adjustment for distant metastasis, performance status, and levels of carbohydrate antigen 19-9, carcinoembryonic antigen, C-reactive protein, and lactate dehydrogenase, the status of recurrent or unresectable disease remained as an independent prognostic factor with a clinically relevant HR value (HR, 0.66; 95% confidence interval, 0.48–0.90; $P = 0.01$). In addition, the 2-year OS rate of the recurrent group was significantly higher than that of the unresectable group (24.2% vs 9.6%, $P = 0.01$).

Conclusions: Our results suggested that the status of recurrent or initially unresectable disease was an independent prognostic factor in APC patients receiving palliative chemotherapy.

Key Words: pancreatic cancer, gemcitabine, palliative chemotherapy, prognostic factor

Abbreviations: APC — advanced pancreatic cancer, CA-19-9 — carbohydrate antigen 19-9, CEA — carcinoembryonic antigen, CRP — C-reactive protein, LDH — lactate dehydrogenase, AST — aspartate transaminase, ECOG PS — Eastern Cooperative Oncology Group Performance Status, BSLD — baseline sum of longest diameter, HR — hazard ratios, CI — confidence interval

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Pancreatic cancer is one of the most lethal malignancies worldwide,[1] and surgery remains the only modality to potentially cure this disease; however, most patients are diagnosed too late for curative resection.[2] Even after curative resection, relapse of disease within 2 years occurs in more than 80% of patients.[3] Although gemcitabine-based chemotherapy has been established as the standard treatment for patients with advanced pancreatic cancer (APC) for more than a decade, the long-term treatment efficacy and disease prognosis remain dismal.[4]

A combination chemotherapy regimen, consisting of oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX), was recently developed as an alternative treatment option for metastatic pancreatic cancer patients with good performance status and other stringent inclusion criteria.[5] Philip et al[6] reported that APC patients are a heterogeneous group with a variety of clinical characteristics. Although the treatment progress of APC as a whole is slow, there still exist subgroups of patients who achieve favorable outcomes after palliative chemotherapy. Thus, it is important to identify prognostic factors to aid in the selection of an appropriate treatment regimen and prediction of life expectancy in daily clinical practice.[6] Previous studies have identified various pathological, clinical, and laboratory prognostic factors for APC in patients after palliative chemotherapy.[7–9] However, the survival difference between unresectable and recurrent disease after palliative chemotherapy remains to be clarified.

In the present study, we retrieved clinical data of 269 consecutive APC patients who received palliative chemotherapy and analyzed prognostic factors by mainly focusing on the status of unresectable and recurrent disease.

MATERIALS AND METHODS

Patients and Treatment

We retrieved the clinical data of 269 consecutive patients with pathologically confirmed pancreatic ductal adenocarcinoma who received palliative first-line chemotherapy at Kyoto University Hospital (Kyoto, Japan) between January 2006 and April 2012 using a prospective cohort database system (CyberOncology; Cyber Laboratory Inc, Tokyo, Japan)[10] and electronic medical charts. The baseline patient characteristics including laboratory data before the first cycle of palliative chemotherapy were retrieved and analyzed. Patients who had once undergone radical resection (R0 or R1) for primary tumors and developed recurrent disease was categorized into the recurrent group (n = 83) and those who had an initial diagnosis of unresectable disease into the initially unresectable group (n = 186). Among the patients with measurable target lesions by RECIST (Response Evaluation Criteria in Solid Tumors) version 1.1,[11] the tumor volume was estimated using baseline sum of longest diameter (BSLD).[12] All patients provided written informed consent for the use of their clinical data in the medical records system for the purposes of research. This study was approved by the Ethics Committee of Kyoto University Graduate School of Medicine (E1606).

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The authors declare no conflict of interest.

TABLE 1. Patient Characteristics

	All (n = 269)	Initially Unresectable (n = 186)	Recurrent (n = 83)	*P*
Age				
≥65 y	155 (57.6%)	106 (57%)	49 (59%)	0.79*
<65 y	114 (42.4%)	80 (43%)	34 (41%)	
Sex				
Male	139 (51.7%)	100 (53.8%)	39 (47%)	0.36*
Female	130 (48.3%)	86 (46.2%)	44 (53%)	
PS score				
0-1	256 (95.2%)	175 (94.1%)	81 (97.6%)	0.36*
2	13 (4.8%)	11 (5.9%)	1 (2.4%)	
Distant metastasis				
Yes	202 (75.1%)	143 (76.9%)	59 (71.1%)	0.36*
No	67 (24.9%)	43 (23.1%)	24 (28.9%)	
No. metastases				
Single	7 (3.5%)	4 (2.8%)	3 (5.1%)	0.42*
Multiple	195 (96.5%)	139 (97.2%)	56 (94.9%)	
Metastatic site				
Liver	132 (49.6%)	96 (49.5%)	36 (50%)	0.24*
Peritoneum	103 (38.7%)	78 (40.2%)	25 (34.7%)	0.08*
Lung	23 (8.7%)	14 (7.2%)	9 (12.5%)	0.36*
Bone	8 (3%)	6 (3.1%)	2 (2.8)	1.00*
Primary tumor location				
Head	161 (59.9%)	100 (53.8%)	61 (73.5%)	<0.01*
Body and tail	108 (40.1%)	86 (46.2%)	22 (26.5%)	
Palliative first line				
Gemcitabine monotherapy	171 (63.6%)	123 (66.1%)	48 (57.8%)	0.01†
Gemcitabine and S-1	87 (32.3%)	59 (31.7%)	28 (33.7%)	
S-1 monotherapy	9 (3.3%)	2 (1.1%)	7 (8.4%)	
Gemcitabine and erlotinib	2 (1.1%)	2 (1.1%)	0	
CA-19-9, U/mL				
Median	184	333	93	<0.01‡
Range	1–72,663	1–72,663	1–16,213	
CEA, ng/mL				
Median	4.2	4.0	4.3	0.50‡
Range	0.2–1563.0	0.2–1563.0	0.7–322.8	
CRP, mg/dL				
Median	0.2	0.2	0.1	<0.01‡
Range	0–21.8	0–21.8	0–7.7	
LDH, IU/L				
Median	184	178	192	<0.01‡
Range	111–735	111–735	121–471	
Total bilirubin, mg/dL				
Median	0.7	0.7	0.7	0.05‡
Range	0.2–15.9	0.3–10.2	0.2–15.9	
AST, IU/L				
Median	25	24	26	0.02‡
Range	11–466	11–422	15–466	
Alanine transaminase, IU/L				
Median	26	27	24	0.73‡
Range	7–564	7–564	10–501	
Creatinine, mg/dL				
Median	0.7	0.7	0.6	0.09‡
Range	0.3–3.2	0.3–3.2	0.3–1.3	

(Continued on next page)

TABLE 1. *(Continued)*

	All (n = 269)	Initially Unresectable (n = 186)	Recurrent (n = 83)	*P*
Hemoglobin, g/dL				
Median	11.7	11.9	11.4	0.01‡
Range	7.2–15.8	7.2–15.8	8.7–14.4	

Gemcitabine monotherapy and other chemotherapeutic regimens were compared.
*Fisher exact test.
†χ^2 Test.
‡Mann-Whitney *U* test.

Chemotherapy regimens consisted of gemcitabine monotherapy (n = 171),[13] gemcitabine and S-1 combination therapy (n = 87),[14] S-1 monotherapy (n = 9),[15] and gemcitabine and erlotinib combination therapy (n = 2).[16] The standard doses and regimen schedules were adjusted at the discretion of the treating physicians according to incidence of adverse events or the general condition of each patient.

Statistical Method

Baseline patient characteristics were compared using the χ^2 test or Fisher exact test for dichotomous variables or the Mann-Whitney *U* test for continuous variables. The follow-up period was measured from the date of palliative chemotherapy initiation and terminated on January 2013 or on the date of death. Overall survival (OS) was defined as the period between the date of palliative chemotherapy initiation and the date of death for any reason or the last follow-up visit. The OS was estimated using the Kaplan-Meier method, and differences were compared using the log-rank test. The following putative prognostic factors were evaluated: the status of initially unresectable or recurrent disease, age (<65 or ≥65 years), sex (male or female), Eastern Cooperative Oncology Group Performance Status (ECOG PS) score (0–1 or 2), primary tumor location (pancreatic head or body and tail), disease extensions (locally advanced or metastatic), and first-line chemotherapy regimen (gemcitabine alone or other regimens). The continuous parameters were categorized for the convenience of prognostic analysis as follows [7,17,18]: carbohydrate antigen 19-9 (CA-19-9) (<1000 or ≥1000 U/mL), carcinoembryonic antigen (CEA) (<5 or ≥5 ng/mL), C-reactive protein (CRP) (<0.5 or ≥0.5 mg/dL), and lactate dehydrogenase (LDH) (<250 or ≥250 IU/L). The hazard ratio (HR) and 95% confidence interval (CI) were calculated using the Cox regression model. Prognostic factors shown to be significant in the univariate analysis were tested via multivariate analysis. A 2-tailed $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS statistical software (version 17.0; SPSS Inc, Chicago, IL).

RESULTS

Patient Characteristics

A total of 269 consecutive patients with pathologically confirmed pancreatic cancer who received palliative first-line chemotherapy was investigated. Patient characteristics were

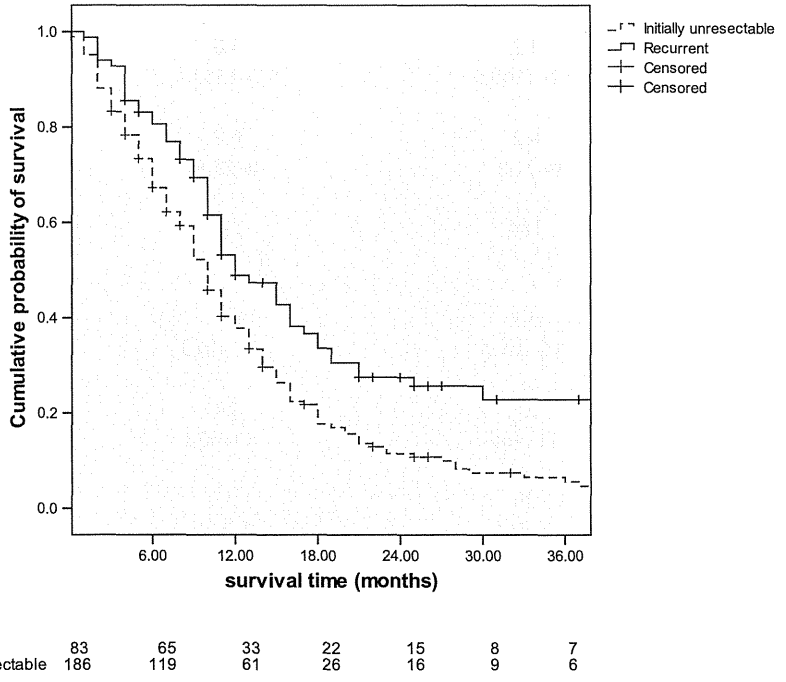

FIGURE 1. Overall survival for patients with recurrent and unresectable APC.

TABLE 2. Univariate Analysis of Prognostic Factors

	n	Median OS, d	HR	95% CI	*P*
The status of initially unresectable/recurrent					
Initially unresectable	186	308	1	0.44–0.80	<0.01
Recurrent	83	383	0.59		
Age					
≥65 y	155	339	1	0.75–1.28	0.89
<65 y	114	323	0.98		
Sex					
Female	130	337	1	0.69–1.18	0.45
Male	139	334	0.9		
ECOG PS					
2	13	131	1	0.26–0.83	<0.01
0-1	256	338	0.46		
Distant metastasis					
Yes	202	317	1	0.35–0.69	<0.01
No	67	444	0.5		
Primary tumor location					
Head	161	331	1	0.89–1.53	0.26
Body and tail	108	342	1.17		
Palliative first-line chemotherapy regimen					
Gemcitabine monotherapy	171	335	1	0.84–1.47	0.46
Gemcitabine and S-1	87	337	1.11		
CA-19-9, U/mL					
≥1000	62	179	1	0.33–0.61	<0.01
<1000	207	366	0.45		
CEA, ng/mL					
≥5	114	290	1	0.43–0.74	<0.01
<5	155	403	0.56		
CRP, mg/dL					
≥0.5	85	212	1	0.39–0.70	<0.01
<0.5	184	369	0.52		
LDH, IU/L					
≥250	38	124	1	0.39–0.81	<0.01
<250	231	356	0.56		
Hemoglobin, g/dL					
≥10	236	337	1	0.70–1.67	0.73
<10	33	278	1.08		

stratified by the status of unresectable or recurrent disease and summarized in Table 1.

The incidence of pancreatic head cancer was more common among the recurrent group (73.5% vs 53.8%, $P < 0.01$), and the difference in first-line palliative chemotherapy was significant between the 2 groups ($P < 0.01$). Carbohydrate antigen 19-9, CRP, and hemoglobin levels were significantly higher in the initially unresectable group, whereas LDH and aspartate transaminase (AST) levels were significantly higher in the recurrent group. Proportion of patients with distant metastasis was comparable between the 2 groups (76.9% and 71.1%, $P = 0.36$).

Overall Survival

With the median follow-up period of 333 days (range, 17–2358 days), the OS period was 383 days (95% CI, 263–502 days) and 308 days (95% CI, 277–339 days) in the recurrent and initially unresectable groups, respectively (HR, 0.59; 95% CI, 0.44–0.80; $P < 0.01$; Fig. 1). The 2-year survival rate was 24.2% (95% CI, 14.2%–34.3%) and 9.6% (95% CI, 5.1%–14.1%) in the recurrent and initially unresectable groups, respectively, which was statistically significant ($P = 0.01$). Next, we investigated whether putative prognostic factors affected the OS rate in our cohort. The log-rank test demonstrated that the factors of recurrent disease, no metastatic disease, good performance status, and lower levels of CA-19-9, CEA, CRP, and LDH were significantly associated with an improved OS rate (Table 2).

Multivariate Analysis of Prognostic Factors

As mentioned above, univariate analysis demonstrated that the status of recurrent disease, no distant metastasis, good performance status, and lower levels of CA-19-9, CEA, CRP, and LDH were significantly associated with improved prognosis. After adjustment for distant metastasis, performance status, and CA-19-9, CEA, CRP, and LDH via multivariate analysis, the status of recurrent disease remained a favorable independent prognostic factor (HR, 0.66; 95% CI, 0.48–0.90; $P = 0.01$). Other favorable prognostic factors were no distant metastasis (HR, 0.57; 95% CI, 0.40–0.80; $P < 0.01$) and lower levels of CA-19-9 (HR, 0.66;

TABLE 3. Multivariate Analysis of Prognostic Factors

	n	Median OS, d	HR	95% CI	*P*
The status of initially unresectable/recurrent					
Initially unresectable	186	308	1	0.48–0.90	0.01
Recurrent	83	383	0.66		
Distant metastasis					
Yes	202	317	1	0.40–0.80	<0.01
No	67	444	0.57		
ECOG PS					
2	13	131	1	0.33–1.11	0.1
0-1	256	338	0.6		
CA-19-9, U/mL					
≥1000	62	179	1	0.47–0.93	0.02
<1000	207	366	0.66		
CEA, ng/mL					
≥5	114	290	1	0.48–0.86	<0.01
<5	155	403	0.65		
CRP, mg/dL					
≥0.5	85	212	1	0.47–0.85	<0.01
<0.5	184	369	0.63		
LDH, IU/L					
≥250	38	124	1	0.41–0.89	0.01
<250	231	356	0.6		

95% CI, 0.47–0.93; $P = 0.02$), CEA (HR, 0.65; 95% CI, 0.48–0.86; $P < 0.01$); CRP (HR, 0.63; 95% CI, 0.47–0.85; $P < 0.01$), and LDH (HR, 0.60, 95% CI, 0.41–0.89; $P = 0.01$) (Table 3).

DISCUSSION

The prognosis of patients with pancreatic cancer remains dismal,[19] and radical resection is the only hope for long survival. [20] Even after potentially curative resection, this disease relapses in more than 80% of patients,[3] and palliative chemotherapy has been the standard treatment for patients with recurrent diseases as well as for initially unresectable disease.[4] Previous studies have reported several prognostic factors of APC patients treated with chemotherapy,[7,9] and performance status or disease extension (locally advanced or metastatic) has been commonly used as a stratification factor in randomized clinical trials[21,22]; however, little is known regarding the prognostic differences between recurrent and initially unresectable diseases after palliative chemotherapy. To the best of our knowledge, this is the first study to demonstrate a significantly better prognosis of recurrent disease compared with initially unresectable disease.

Baseline CA-19-9, CRP, and hemoglobin levels were significantly higher in the initially unresectable group, whereas LDH and AST levels were significantly higher in the recurrent group. Elevation of baseline CA-19-9 or CRP levels has been reported to be associated with poorer prognosis of APC patients treated with chemotherapy.[7,23] Therefore, higher baseline levels of CA-19-9 and CRP in the initially unresectable group may have reflected the poorer status of patients with this disease. Multiple testing may have affected differences in other baseline characteristics (hemoglobin, LDH, and AST). The ECOG PS score was shown to be a prognostic factor by univariate analysis, but not by multivariate analysis, probably because the majority of patients had a good performance status (0 or 1) in this study. The log-rank test demonstrated a significantly longer median OS period in the recurrent group compared with the initially unresectable group (383 vs 308 days), and the HR was clinically relevant (HR, 0.59; 95% CI, 0.44–0.80; $P < 0.01$). After adjustment for CA-19-9 and CRP levels and other putative prognostic factors, including distant metastasis, performance status, and CEA and LDH levels, the status of recurrent disease remained favorable independent of prognostic factors with a clinically relevant HR value (HR, 0.66; 95% CI, 0.48–0.90; $P = 0.01$). This suggests that the favorable prognosis of recurrent disease was not merely attributable to differences in baseline characteristics between the 2 groups. It was also unlikely that the differences of chemotherapy regimens affected the current results because almost 99% of patients received gemcitabine, S-1, or gemcitabine/S-1 combination therapy, and the efficacies of these 3 regimens have demonstrated no statistical differences in a large randomized phase III study.[24] Furthermore, we investigated OS in 171 patients who received gemcitabine monotherapy. The median OS period of 48 patients with recurrent disease was significantly greater than that of 123 patients with initially unresectable disease (344 vs 305 days, $P = 0.02$).

The issue of prognostic differences stratified by the history of surgery in APC patients has been previously discussed. Hashimoto et al[25] reported better OS rates in patients with recurrent disease than in those with primary metastasis (270 vs 185 days, $P < 0.01$) in their study of 326 APC patients receiving gemcitabine monotherapy. As in the current study, recurrent status was one of the significant favorable prognostic factors after univariate analysis (HR, 0.53; 95% CI, 0.38–0.74; $P < 0.01$) in their study; however, this difference was not statistically significant after multivariate analysis (HR, 0.76; 95% CI, 0.53–1.09; $P = 0.14$). van Cutsem et al[26] also reported a trend toward better OS in APC patients who had previously undergone Whipple resection compared with that in patients who had not. This result also supported our current results.

There are several possible explanations for the better prognosis of recurrent disease. First, the intensive follow-up after surgery allowed for the detection of disease recurrence,

whereas tumor volume remained relatively small. The lower tumor burden could have potentially contributed to favorable chemotherapy response.[12] Therefore, we estimated tumor volume using the BSLD. Tumors from 176 patients (94%) were evaluable for BSLD in the initially unresectable group, whereas tumors from 41 patients (50%) were evaluable in the recurrent group. Baseline sum of longest diameter was significantly larger for the initially unresectable group (mean, 40.3 [SD, 18.9] vs 29.8 [SD, 12.8] mm; $P < 0.01$). However, among patients with measurable lesions (n = 217), a trend toward better OS was observed for those in the recurrent group after adjustment for BSLD (HR, 0.66; 95% CI, 0.43–1.00; $P = 0.05$). Furthermore, this difference in survival was unlikely to be caused by lead-time bias because the difference in survival curves between the 2 groups increased at later time points, suggesting that the HR was constant over time. Second, biological differences may exist between recurrent and unresectable disease. Generally, the tumor burden in pancreatic cancer patients eligible for surgical resection at the initial presentation was comparably less than that in unresectable disease, which may reflect the slow growth rate associated with initially resectable disease. Thus, potential biological differences may have contributed to the better prognosis of recurrent disease. Further studies are needed to clarify the underlying mechanisms.

This study was limited by its nonrandomized, retrospective design, although we expect future prospective trials to confirm the current results.

In conclusion, the status of recurrent or initially unresectable disease was identified as an independent prognostic factor for patients with APC who received palliative chemotherapy. Because patients with different prognostic characteristics are preferred for separate clinical trials,[6] our results should contribute to the design of future clinical trials to predict the prognosis of patients with pancreatic cancer treated with palliative chemotherapy.

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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (14): Pancreatic cancer

Therapeutic applications of curcumin for patients with pancreatic cancer

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Abstract

A number of preclinical studies have demonstrated anticancer effects for curcumin in various types of tumors, including pancreatic cancer. Curcumin has anticancer effects both alone and in combination with other anticancer drugs (*e.g.*, gemcitabine, 5-fluorouracil, and oxaliplatin), and it has been shown to modulate a variety of molecular targets in preclinical models, with more than 30 molecular targets identified to date. Of these various molecules, NF-κB is thought to be one of the primary targets of curcumin activity. Based on these promising preclinical results, several research groups, including our own, have progressed to testing the anticancer effects of curcumin in clinical trials; however, the poor bioavailability of this agent has been the major challenge for its clinical application. Despite the ingestion of gram-level doses of curcumin, plasma curcumin levels remain at low (ng/mL) levels in patients, which is insufficient to yield the anticancer benefits of curcumin. This problem has been solved by the development of highly bioavailable forms of curcumin (THERACURMIN®), and higher plasma curcumin levels can now be achieved without increased toxicity in patients with pancreatic cancer. In this article, we review possible therapeutic applications of curcumin in patients with pancreatic cancer.

Key words: Curcumin; Pancreatic cancer; Nuclear factor-kappa B; Bioavailability; THERACURMIN

Core tip: A growing body of evidence supports the idea that curcumin is a promising anticancer drug. Curcumin has anticancer effects, both alone and in combination with other anticancer drugs, through the modulation of a variety of molecular targets in preclinical models. However, the poor bioavailability of curcumin has been the major challenge to its clinical application. This problem has been overcome by the development of highly bioavailable forms of curcumin (THERACURMIN®), and higher plasma curcumin levels can now be achieved without increased toxicity. Further clinical trials will be necessary to test the therapeutic applications of this promising agent in patients with pancreatic cancer.

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INTRODUCTION

Pancreatic cancer is one of the most lethal malignancies worldwide[1], and the majority of patients are diagnosed too late for curative resection. Even in patients who have undergone curative resection, the disease relapse rate within 2 years is greater than 80%[2]. Systemic gemcitabine-based chemotherapy has been a standard therapy for patients with advanced pancreatic cancer since 1997, when a randomized phase III study demonstrated that gemcitabine monotherapy significantly improved cancer-

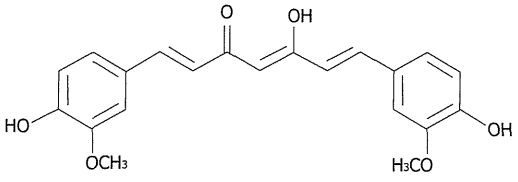

Figure 1 Chemical structure of curcumin.

related symptoms compared with 5-fluorouracil[3]. Over the past decade, many efforts have been made to improve the overall survival of patients with this disease by combining gemcitabine with a second cytotoxic agent. However, most of these gemcitabine combination therapies have failed to show significant survival advantages over gemcitabine monotherapy[4-11]. Therefore, novel approaches - other than simply adding additional cytotoxic agents to gemcitabine - are warranted. In addition, it is important to consider the balance between efficacy and quality of life when choosing a palliative chemotherapy, as patients with pancreatic cancer often suffer from cancer-related symptoms, such as fatigue, appetite loss, and pain.

Curcumin is a natural polyphenol compound derived from turmeric (*Curcuma longa*). Constituting 1%-5% of turmeric preparations, curcumin has a molecular weight of 368.37 and the molecular formula $C_{21}H_{20}O_6$ (Figure 1). Curcumin has long been used as a food (*e.g.*, in the popular Indian curry), a coloring agent and in traditional medicine[12,13]. A number of preclinical studies have demonstrated that curcumin has anticancer effects against a variety of tumors, including pancreatic cancer, both *in vitro* and *in vivo*[14-32]. These promising results have attracted the interest of many researchers hoping to develop this agent as a chemopreventive as well as a chemotherapeutic drug[33,34]. In contrast with conventional cytotoxic drugs - which often have side effects such as nausea, vomiting or fatigue - curcumin has minimal toxicity. This is a great advantage when treating patients with pancreatic cancer, who generally show poor tolerance to intensive therapy due to their poor clinical conditions. Safety is another advantage of this agent. The safety of curcumin has been approved by the Food and Drug Administration and World Health Organization; In addition, its safety is strongly supported by the fact that this agent has been used in traditional Hindu and Chinese medicine for thousands of years.

In this article, we review possible therapeutic applications of curcumin for the treatment of patients with pancreatic cancer.

ANTICANCER EFFECTS OF CURCUMIN AGAINST PANCREATIC CANCER *IN VITRO* AND *IN VIVO*

A PubMed search using the key words "curcumin" and "cancer" reveals that over 2000 articles have been published on this topic since 1983, with that number increasing rapidly year after year. Numerous preclinical studies have demonstrated anticancer effects for curcumin against not only pancreatic cancer[14,17,22,24,26-28,32,35] but also a variety of other malignancies, including breast[21], colon[23,29], gastric[30], head and neck[25], hepatic[15], ovarian[20], lung[31]and prostate cancers[19], as well as lymphoma and leukemia[16,18].

Li *et al*[14] were the first to report the anticancer effects of curcumin against pancreatic cancer cells. They demonstrated that curcumin can suppress tumor growth in pancreatic cancer cell lines in a time- and dose-dependent manner by inhibiting nuclear transcription factor-kappa B (NF-κB). The efficacy of curcumin has also been demonstrated using an orthotopic mouse model of pancreatic cancer[36]. Although treatment with either curcumin (1 g/kg orally) or gemcitabine (25 mg/kg *via* intraperitoneal injection) had modest antitumor effects, the combination of curcumin and gemcitabine suppressed tumor growth more effectively than either agent alone. In addition to gemcitabine, curcumin has also been shown to potentiate the effects of other cytotoxic agents, including cisplatin, oxaliplatin, and 5-fluorouracil, in preclinical models[25,29,37].

Curcumin can modulate the activity of a variety of molecules that play important roles in cancer progression, with more than 30 molecular targets identified to date[38]. Of these molecules, NF-κB appears to be one of the primary targets of curcumin[14,27,36]. Interestingly, recent studies have demonstrated that changes in microRNA (miRNA) expression levels following treatment with curcumin or a curcumin analog are involved in the anticancer effects of these agents[28,39]. For example, curcumin can upregulate the expression of miR-200[28], which plays important roles in regulating the epithelial-to-mesenchymal transition (EMT) and cancer progression[40]. Conversely, curcumin can downregulate the expression of miR-21[28], which is overexpressed in a variety of tumors, including pancreatic cancer, and is considered to be an oncogenic miRNA[41]. Representative preclinical studies of the anticancer effects of curcumin against pancreatic cancer are summarized in Table 1.

Based on these promising preclinical results, several researcher groups, including our own, have progressed to testing the anticancer effects of curcumin in clinical trials.

CLINICAL TRIALS INVOLVING CURCUMIN IN PATIENTS WITH PANCREATIC CANCER

Despite numerous published preclinical studies, relatively few clinical trials have been reported so far. Several phase I and pharmacokinetic studies have been conducted using curcumin, and they found no dose-limiting toxicity (DLT) up to at least 12 g/d when administered orally to both healthy volunteers[42,43] and cancer patients[44-46]. The minor toxicities of Grade 1-2 diarrhea and nausea have been reported, although these were likely due to the

Table 1 A summary of representative preclinical studies on the anticancer effects of curcumin against pancreatic cancer

Reported molecular targets	Curcumin dose required for the reported effects	
	in vitro (μmol/L)	*in vivo*
NF-κB↓ (Ref. 14)	≥ 5.4	NA
NF-κB↓, cyclin-D1↓ c-myc↓, Bcl-2↓ Bcl-xL↓, cIAP-1↓ MMP↓, COX2↓ VEGF↓ (Ref. 36)	≥ 25	1 g/kg per day, *po*
NF-κB↓, Sp-1, Sp-3, Sp4↓ cyclin-D1↓, survivin↓ VEGF↓ (Ref. 27)	≥ 25	100 mg/kg per day, intraperitoneal injection
NF-κB↓, PGE2↓ VEGF↓, miR-21↓ miR-200↑ (Ref. 28)	≥ 4	NA

cIAP1: Cellular inhibitor of apoptosis portein-1; MMP: Matrix metalloproteinase; COX2: Cyclooxygenase-2; VEGF: Vascular endothelial growth factor; PGE2: Prostaglandin E2; NA: Not available.

ingestion of large volumes of curcumin at one time. Due to poor bioavailability, curcumin doses greater than 8 g/d do not lead to further increases in plasma curcumin levels; therefore, daily oral doses of 8 g or less have been most commonly used in clinical trials.

Dhillon *et al*[47] were the first to report a phase II clinical trial of the effects of curcumin against pancreatic cancer. Twenty-five patients, including 3 chemo-naive patients, were enrolled in this study. Of the 22 patients that could be evaluated for responses, one patient showed a stable disease course for over 18 mo and another patient showed a partial response in a liver metastasis (73% decrease in size), although this effects lasted for only 1 month. Furthermore, curcumin treatment was found to be safe in patients with pancreatic cancer, and no toxicity was associated with curcumin intake.

Our group conducted a phase I / II clinical trial of curcumin in patients with pancreatic cancer who had become resistant to gemcitabine-based chemotherapy[48]. In contrast with the study by Dhillon *et al*[47], which tested the safety and efficacy of curcumin monotherapy, our study evaluated the efficacy of combined gemcitabine-based chemotherapy and curcumin treatment, which we tested based on the preclinical results showing that curcumin could potentiate the anticancer effects of gemcitabine[36]. As no previous studies had demonstrated the safety and feasibility of this drug combination in cancer patients, we began with a phase I study involving an 8-g daily oral dose of curcumin in combination with gemcitabine-based chemotherapy. The first 3 patients that could be assessed completed their first treatment cycle without a predefined DLT. Therefore, we selected this dose for the following phase II study. In total, 21 patients who showed disease progression during previous gemcitabine-based chemotherapy were enrolled in the study. The addition of an 8-g daily oral curcumin dose did not increase the risk of clinically relevant toxicity, and the toxicity profile of the combined drugs was comparable with that observed in pancreatic cancer patients treated with gemcitabine-based chemotherapy alone. Cumulative toxicity from curcumin was not observed, and 4 patients were able to continue this intake regimen for over 6 mo, indicating that this agent is safe for long-term use. Even though the preliminary results were from a small sample, the observed median survival time (MST) of 5.4 (95%CI 3.6-7.4) mo and a 1-year survival rate of 19% (95%CI 4.4%-41.4%) are promising results, particularly considering the poor prognosis of patients with pancreatic cancer with resistance to gemcitabine-based chemotherapy.

Epelbaum *et al*[49] reported the results from another clinical trial testing the efficacy and feasibility of curcumin in combination with gemcitabine monotherapy in chemo-naive patients with advanced pancreatic cancer. Seventeen patients were enrolled in the study, and they received the standard dose and schedule of gemcitabine in combination with an 8-g daily oral dose of curcumin. In contrast to the previous 2 studies that showed low toxicity for 8-g daily oral doses of curcumin[47,48], this study reported that 5 patients (29%) discontinued the curcumin regimen after a period of several days to 2 wk due to intractable abdominal fullness and/or pain. Indeed, the dose of curcumin was eventually reduced to 4 g/d due abdominal complaints in 2 other patients. The researchers discussed the possibility that increased gastrointestinal toxicity could be caused by the combination of curcumin and gemcitabine, and they concluded that 8 g oral curcumin is not a viable treatment dose when combined with gemcitabine in patients with pancreatic cancer. One possible explanation for the discrepancy between our results and those of Epelbaum *et al*[49] is that the baseline clinical condition of the patients was poorer in the Epelbaum *et al*[49] study than in ours, and therefore, the abdominal fullness or pain experienced by these patients may have been primarily attributable to cancer-related symptoms.

Table 2 summarizes the published clinical trials that have tested the effects of curcumin in patients with pancreatic cancer.

APPLICATION OF A HIGHLY BIOAVAILABLE FORM OF CURCUMIN (THERACURMIN®) IN CLINICAL TRIALS

Several investigators, including ourselves, have tested plasma curcumin levels in clinical trials, and most studies have reported that plasma curcumin levels remained at low (ng/mL) levels, despite multi-gram doses of curcumin[42,45,46,48]. As described in the previous section, the intake of oral doses of curcumin greater than 8 g did not lead to further increases in plasma curcumin levels in human subjects[42-44]. Therefore, the poor bioavailability of curcumin has been the primary challenge to its clinical application. As a result, many efforts have been made to improve the bioavailability of this agent using a variety of approaches, including innovative drug delivery systems (nanoparticles, liposomes and phospholipids)[50-65] and the development of new curcumin analogs[66,67]. For

Table 2 A summary of published clinical trials testing curcumin in patients with pancreatic cancer

	Dhillon *et al*[47]	Kanai *et al*[48]	Epelbaum *et al*[49]	Kanai *et al*[69]
Sample size	25	21	17	14
Study design	Phase II	Phase I / II	Phase II	Phase I
Study period	2008[1]	2008-2009	2004-2006	2011-2012
Dose of curcumin	8 g/d	8 g/d	8 g/d	200 mg/d[2] (n = 9) 400 mg/d[2] (n = 5)
Prior history of chemotherapy	Yes (n = 22)	Yes (n = 21)	None	yes (n = 14)
Concomitant use of anticancer drug	No	Yes	Yes	Yes
Major toxicity associated with curcumin	None	None	Abdominal discomfort (n = 5)	Abdominal pain (n = 2)
Median survival time (mo)	NA	5.4	5	4.4

[1]Publication year; [2]THERACURMIN® was used in this study. NA: Not available.

Table 3 A comparison of representative studies reporting plasma curcumin levels in human subjects

	Lao *et al*[42]	Sharma *et al*[45]	Garcea *et al*[46]	Kanai *et al*[68]
Sample size	3 (1)[1]	3	3	6
Dose of curcumin (g/d)	12	3.6	3.6	0.21[1]
Plasma curcumin levels (ng/mL, mean ± SE)	57	4 ± 0.2	< 1	275 ± 67

[1]Plasma curcumin was detected in only one subject.

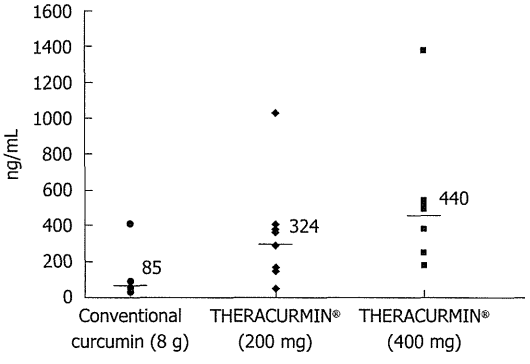

Figure 2 Plasma curcumin levels following administration of conventional curcumin and THERACURUMIN®. Each point corresponds to an individual patient. Bars denote the median value. Adapted from Kanai *et al*[69].

example, a nanoparticle-based drug delivery system has been shown to improve the water solubility of hydrophobic agents such as curcumin, and several different types of nanoparticle-based curcumin have been published[52,56-59,61,62,64,65].

Of these new varieties of nanoparticle-based curcumin, we chose THERACURMIN® for further study, as it showed a greater than 30-fold increase in bioavailability compared with conventional curcumin in rat models[64]. THERACURMIN® was prepared as follows[64,68]. First, gum ghatti - which primarily consists of polysaccharides obtained from ghatti tree exudates - was dissolved in water to make a gum ghatti solution. Curcumin powder was mixed into this solution, and water and glycerin were added to adjust the final weight. This mixture was ground using a wet grinding mill (DYNO-MILL®KDL, Willy A Bachofen AG) and then dispersed with a high-pressure homogenizer (Homogenizer 15MR-8TA, APV Gaulin). Stable THERACURMIN® is obtained from this procedure.

To verify the improved bioavailability of THERACURMIN® in human subjects, we conducted a dose-escalation and pharmacokinetic study[68]. Six healthy human volunteers were recruited and given THERACURMIN® *via* a single oral dose of 150 mg. Following an interval of 2 wk, the same subjects were then given THERACURMIN® *via* a single oral dose of 210 mg. The C_{max} values for THERACURMIN® at the 150 and 210 mg doses were 189 ± 48 and 275 ± 67 ng/mL (mean ± SEM), respectively. No toxicity associated with THERACURMIN® intake was observed in this study.

These results indicate that the ingestion of THERACURMIN® can lead to higher plasma curcumin levels than those achieved with conventional curcumin (Table 3). Therefore, we considered this new form of curcumin to be a promising tool for testing the potential anticancer effects of curcumin in clinical trials, and we conducted a phase I study testing the safety of THERACURMIN® in patients with pancreatic cancer[69].

A total of 16 patients (14 patients with pancreatic cancer and 2 patients with biliary tract cancer) who failed standard gemcitabine-based chemotherapy were enrolled in the study. Based on our previous pharmacokinetic study, we chose to use THERACURMIN® containing 200 mg curcumin (Level 1) as the starting dose. THERACURMIN® was administered orally every day in combination with standard gemcitabine-based chemotherapy.

Ten patients were assigned to the Level 1 group and six to the Level 2 group (THERACURMIN® containing 400 mg curcumin). Peak plasma curcumin levels (median) following THERACURMIN® administration were 324 ng/mL (range = 47-1029 ng/mL) for Level 1 and 440 ng/mL (range = 179-1380 ng/mL) for Level 2. Importantly, these values were significantly higher than the median value (85 ng/mL) observed in our previous study using 8-g doses of conventional curcumin (Figure 2). With respect to safety, two patients reported increased abdominal pain following THERACURMIN® administration. Computed tomography scans performed prior to THERACURMIN® administration in these patients revealed dilated colons, which could have been due to in-

testinal obstructions caused by peritonitis carcinomatosa. As described in the previous section, Epelbaum *et al*[49] reported abdominal fullness or pain following curcumin administration in patients with pancreatic cancer. We speculate that curcumin may irritate the intestine, potentially increasing abdominal pain in patients with intestinal obstructions due to peritonitis carcinomatosa or other complications. In future clinical trials, we advise caution when administering curcumin to these types of patients.

Other observed toxicities were comparable to those for gemcitabine-based chemotherapy alone, and repetitive exposure to high concentrations of curcumin did not cause any unexpected serious adverse events, nor did they increase the incidence of adverse events in patients with pancreatic cancer receiving gemcitabine-based chemotherapy. In fact, three patients safely continued THERACURMIN® treatment for > 9 mo. With respect to efficacy, no responses were observed in this study based on RECIST; however, the MST was 4.4 mo (95% confidence interval: 1.8-7.0 mo) for the 14 patients with pancreatic cancer, and three patients (21%) survived for > 12 mo following initiation of THERACURMIN®.

Interestingly, fatigue- and functioning-associated quality of life (QOL) scores scaled by EORTC QLQ-C30 significantly improved following THERACURMIN® administration. In five patients, the fatigue score improved by > 20, which was interpreted as a significant and clinically relevant change[70]. Preclinical and clinical studies demonstrating the benefits of curcumin on heart disease, depression, and fatigue, also support these findings[71-73]. As improved QOL has been demonstrated to contribute to better outcomes in cancer patients[74], it is tempting to speculate that THERACURMIN® may prolong the overall survival of patients with pancreatic cancer through QOL improvements. A randomized placebo-controlled clinical trial is now underway to verify this hypothesis (UMIN000010326).

CONCLUSION

A growing body of evidence supports the idea that curcumin is a promising anticancer drug. In preclinical models, curcumin has been shown to have anticancer effects, both alone and in combination with other anticancer drugs, through the modulation of a variety of molecular targets. However, the poor bioavailability of curcumin has been the major challenge to its clinical application. This problem has now been solved by the development of highly bioavailable forms of curcumin (THERACURMIN®), which can induce higher plasma curcumin levels without increased toxicity. Further clinical trials will be necessary to test the therapeutic applications of this promising agent in patients with pancreatic cancer.

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