RESEARCH ARTICLE

Value of *KRAS*, *BRAF*, and *PIK3CA* Mutations and Survival Benefit from Systemic Chemotherapy in Colorectal Peritoneal Carcinomatosis

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Abstract

Background: It is well known that peritoneal carcinomatosis (PC) from colorectal cancer (CRC) is associated with a poor prognosis. However, data on the prognostic significance of modern chemotherapy containing bevacizumab, cetuximab or panitumumab are not available. Materials and Methods: This retrospective review concerned 526 patients with metastatic CRC who were classified into two groups according to the presence or absence of PC, and were treated with systemic chemotherapy, with or without bevacizumab or anti-EGFR antibodies. The genetic background, in particular *KRAS*, *BRAF*, and *PIK3CA* gene mutations, and overall survival (OS) were compared between the two groups. Results: The median OS values were 23.3 and 29.1 months for PC and non-PC patients, respectively (hazard ratio [HR]=1.20; p=0.17). Among all patients, tumor location, number of metastatic sites and *BRAF* mutation status were significant prognostic factors, whereas the presence of PC was not. In the PC group, chemotherapy with bevacizumab resulted in a significantly longer OS than forchemotherapy without bevacizumab (HR=0.38, p<0.01), but this was not the case in the non-PC group (HR=0.80, p=0.10). Furthermore, the incidence of the *BRAF* V600E mutation was significantly higher in PC than in non-PC patients (27.7% versus 7.3%, p<0.01). *BRAF* mutations displayed a strong correlation with shorter OS in non-PC (HR=2.26), but not PC patients (HR=1.04). Conclusions: Systemic chemotherapy, especially when combined with bevacizumab, improved survival in patients with PC from CRC as well as non-PC patients. While *BRAF* mutation demonstrated a high frequency in PC patients, but it was not associated with prognosis.

Keywords: *BRAF* - colorectal cancer - *KRAS* - peritoneal carcinomatosis - *PIK3CA*

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Introduction

Peritoneal carcinomatosis (PC) from colorectal cancer (CRC) is a form of systemic metastasis that is considered to be prognostic of the terminal stages of disease, and treatment with systemic chemotherapy and/or palliative care is usually recommended. However, it was reported that PC from CRC has a poor prognosis even if treated with chemotherapy, and PC is the second leading cause of death from metastatic CRC (Koppe et al., 2006). Previous studies in patients with PC from CRC, the majority of whom were treated with monotherapy with 5-fluorouracil (5-FU) and leucovorin (LV), demonstrated that the median overall survival (OS) was short, ranging from 5.2 to 12.6 months (Sadeghi et al., 2000; Jayne et al., 2002; Verwaal et al., 2003). Recently, new effective agents such as oxaliplatin and irinotecan have improved the survival of patients with metastatic CRC. Nevertheless, a pooled analysis of two phase III studies revealed that PC from CRC was still associated with significantly shorter OS compared with other manifestations of metastatic CRC (12.7 versus 17.6 months, hazard ratio [HR]=1.3; p<0.01) (Franko et al., 2012). Although the patients enrolled in these studies received either oxaliplatin- or irinotecan-based chemotherapy as first- or second-line treatment, molecularly targeted agents such as bevacizumab and anti-epidermal growth factor receptor (EGFR) drugs were not administered. Some basic and translational research indicates that molecularly targeted agents display specific activity against PC from CRC. Kraft A et al. showed that median VEGF levels were significantly higher in malignant effusions compared with matched

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serum samples in gastrointestinal carcinoma. Thus, the prognostic impact of systemic chemotherapy combined with molecularly targeted agents in patients with PC from CRC has not been clarified (Kraft et al., 1999).

Recently, it has been widely recognized that mutations in *KRAS*, *BRAF*, and *PIK3CA* are predictive of the efficacy of EGFR-blocking therapy, and may affect prognosis in CRC. *KRAS* mutations are present in approximately 35% to 40% of CRC cases, with about two-thirds of these mutations in codon 12 and one-third in codon 13 (Neumann et al., 2009). *KRAS* mutational status is predictive of resistance to anti-EGFR monoclonal antibodies in metastatic CRC (Cutsem et al., 2011; Douillard et al., 2013). *BRAF* mutations, specifically V600E, which occur in less than 10% of patients with metastatic CRC, are associated with a poor prognosis (Richman et al., 2009; Van Cutsem et al., 2011). *PIK3CA* mutations are found in about 15% of CRC patients, and occur mainly in exon 9 and exon 20 (Roock et al., 2010; Garrido-Laguna et al., 2012), but their role as a biomarker of resistance to chemotherapy remains controversial (Mao et al., 2012; Eklöf et al., 2013). Thus, mutations in these genes may affect the prognosis of patients with PC from CRC. Moreover, some of these gene mutations are associated with specific clinical features, including histological types, tumor location, and gender. For example, the incidence of PC is higher in poorly differentiated adenocarcinoma and mucinous carcinoma than in other histological types (Garrido-Laguna et al., 2012; Gonsalves et al., 2014). Tran et al. reported that significantly higher rates of PC (46% versus 24%, p=0.001) were observed in *BRAF* mutant tumors compared with *BRAF* wild-type tumors, and the *BRAF* mutation was associated with poorer survival, while PC was not a prognostic factor (Tran et al., 2011).

In this study, the prognostic impact of modern chemotherapy including bevacizumab or anti-EGFR monoclonal antibody was compared between PC from CRC and non-PC patients, taking into account the mutational status of the *KRAS*, *BRAF*, and *PIK3CA* genes.

Materials and Methods

Patient selection

A review was conducted of the medical records of metastatic CRC patients treated with systemic chemotherapy, combined with or without bevacizumab, cetuximab or panitumumab, at the Gastrointestinal Medical Oncology Division of the National Cancer Center Hospital between February 2006 and October 2011. The eligibility criteria were as follows: 1) histologically confirmed metastatic CRC; 2) Eastern Cooperative Oncology Group performance status (PS) ≤2; and 3) no previous chemotherapy for advanced disease. Patients were classified into two groups according to the presence (PC-group) or absence (non-PC group) of PC. PC was diagnosed by a surgical procedure, cytology of ascites, or clear evidence on computed tomography (CT) scans (peritoneal tumor spread, omental thickening, ovarian metastases, and massive ascites).

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the National Cancer Center Hospital.

Treatment

All patients received systemic chemotherapy with fluoropyrimidine (5-FU, capecitabine, and S-1) alone or fluoropyrimidine plus either oxaliplatin or irinotecan as first-line treatment. Some patients also received bevacizumab and anti-EGFR monoclonal antibodies (cetuximab or panitumumab), regardless of the treatment line. Patients receiving concurrent chemoradiotherapy were excluded.

Genomic analysis

DNA was extracted from pre-treatment formalin-fixed paraffin-embedded tumor samples, and the presence or absence of *KRAS* mutations in codons 12 and 13, the *BRAF* V600E mutation and *PIK3CA* mutations in exons 9 and 20 were analyzed. *KRAS* mutations and *PIK3CA* mutations were assessed by direct sequencing using a polymerase chain reaction method (Sanger et al., 1977). Detection of the *BRAF* mutation was conducted with high-resolution melting analysis, as described in detail elsewhere (Pichler et al., 2009). The *BRAF* mutation was only assessed in *KRAS* wild-type tumors because *BRAF* and *KRAS* mutations are mutually exclusive.

Statistical analysis

A chi-squared test was used to evaluate differences in patient and tumor characteristics, including gene mutations between PC and non-PC groups. Survival time was calculated from the date of initiating first-line chemotherapy to the date of death or censored at the last confirmation of survival. Survival curves were plotted using the Kaplan-Meier method, and compared using the log-rank test. Multivariate analysis of OS was carried out using the Cox proportional hazard model, including both clinical and genetic parameters. A p-value of less than 0.05 was considered to be significant.

Results

Patient and tumor characteristics in the PC and non-PC groups

Data from 526 metastatic CRC patients were analyzed, comprising 117 patients with PC (PC group) and 409 patients without PC (non-PC group). Patient and tumor characteristics, including gene mutations according to the presence or absence of PC, are presented in Table 1. There were no remarkable differences in sex, median age, and performance status between the PC group and the non-PC group. However, the PC group had a higher proportion of patients with a primary tumor in the proximal colon (cecum, ascending colon and transverse colon) (51.2% versus 23.5%; p<0.01) and with ≥3 metastatic sites (20.5% versus 5.8%; p<0.01).

The PC group had a significantly higher incidence of the *BRAF* V600E mutation than the non-PC group (27.7% versus 7.3%, p<0.01); in contrast, no differences were observed between the two groups in *KRAS* and *PIK3CA* mutations.

Treatment

The majority of patients (80.9%) received oxaliplatin-based regimen as first-line treatment; infusional 5-FU/LV and oxaliplatin (FOLFOX), capecitabine and oxaliplatin (CapeOX), or S-1 and oxaliplatin (SOX). Furthermore, irrespective of treatment line, 63.5% of patients received bevacizumab, and 31.5% received an anti-EGFR monoclonal antibody (cetuximab or panitumumab). Although there were no differences between the PC group and the non-PC group in first-line treatment and bevacizumab administration, a lower proportion of patients in the PC group were treated with anti-EGFR antibodies (22.2% versus 34.2%; p=0.01).

Prognostic impact of PC and gene mutations

There was a trend toward shorter OS in the PC group compared with non-PC group (median OS; 23.3 versus 29.1 months, HR=1.20, 95% confidence interval [CI] 0.92–1.57; p=0.17), but the difference was not statistically significant (Fig 1). In the PC group, patients diagnosed by CT imaging or a surgical procedure had a similar median OS of 22.1 and 23.3 months (HR=1.05, 95% CI 0.63-1.73, p=0.84), respectively. Multivariate analysis, in which gene mutations were included as covariates, revealed that PC was not an independent prognostic factor; in contrast, a primary tumor in the proximal colon, ≥3 metastatic sites, and the *BRAF* V600E mutation were associated with a poor prognosis (Table 2).

Subset analysis

The impact of bevacizumab on survival was greater in the PC group (median 29.0 versus 17.7 months, HR=0.38, 95% CI 0.23-0.63, p<0.01) than the non-PC group (median 30.1 versus 23.4 months, HR=0.80, 95% CI 0.61-1.04, p=0.10) (Fig 2), and, as a consequence, there was no difference in OS between PC and non-PC patients who received bevacizumab-containing chemotherapy (HR=1.07, p=0.70). For patients who were not treated

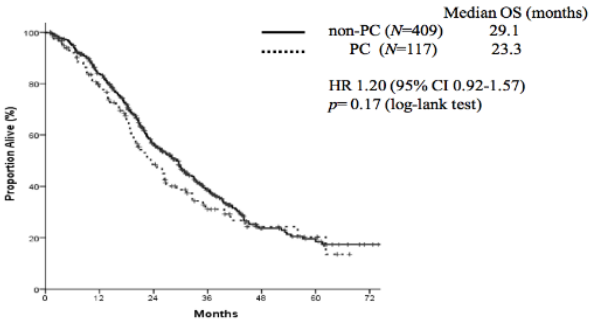

Figure 1. Kaplan-Meier Curves for Overall Survival According to PC Status in the Overall Population. HR, hazard ratio; CI, confidence interval

Table 1. Patient Characteristics According to the Presence or Absence of PC

Characteristics	PC (N=117)	%	non-PC (N=409)	%	p-value
Sex					
Male	61	52.1	251	61.3	0.07
Female	56	47.9	158	38.7	
Age, years					
Median	61		61		0.76
Range	22-78		24-83		
ECOG performance status					
0	70	59.8	221	54	0.55
1	42	35.9	169	41.4	
2	5	4.3	19	4.6	
Tumor location					
Proximal	60	51.2	96	23.5	<0.01
Distal	57	48.8	313	76.5	
Number of metastatic sites					
1	50	42.8	256	62.6	<0.01
2	43	36.7	129	31.6	
≤3	24	20.5	24	5.8	
KRAS					
Wild-type	54	54	163	58.6	0.42
Codon 12, 13 mutation	46	46	115	41.4	
Missing data	17		131		
BRAF (among *KRAS* wild type)					
Wild-type	34	72.3	115	92.7	<0.01
V600E mutation	13	27.7	9	7.3	
Missing data	7		39		
PIK3CA					
Wild-type	53	91.2	181	90.1	0.76
Exon 9, 20 mutation	5	8.8	20	9.9	
Missing data	59		208		

PC, peritoneal carcinomatosis; ECOG, Eastern Cooperative Oncology Group

Table 2. Multivariate Survival Analysis Using Cox Model for All Patients

Characteristics	HR	95% CI	p-value
PC			
Absent	1		
Present	0.65	0.34-1.23	0.19
Sex			
Male	1		
Female	1.43	0.92-2.22	0.1
Tumor location Proximal			
1			
Distal	0.54	0.31-0.91	0.02
Number of metastatic sites			
≤2	1		
≥3	3.08	1.41-6.72	<0.01
Chemotherapy regimen (first-line)			
Oxaliplatin-based	1		
Irinotecan-based	0.79	0.41-1.53	0.49
Administration of bevacizumab (any line)			
No	1		
Yes	1.05	0.64-1.71	0.83
Administration of cetuximab or panitumumab (any line)			
No	1		
Yes	1.08	0.67-1.74	0.73
KRAS			
Wild-type	1		
Mutant	0.98	0.65-1.47	0.94
BRAF			
Wild-type	1		
Mutant	2.64	1.31-5.35	<0.01
PIK3CA			
Wild-type	1		
Mutant	1.1	0.36-3.36	0.86

PC, peritoneal carcinomatosis; HR, hazard ratio; CI, confidence interval

Table 3. Univariate and Multivariate Analysis with Cox Regression for Factors Associated with Overall Survival in PC Patients

Characteristics	Variable	Univariate analysis			Multivariate analysis		
		HR	95% CI	p-value	HR	95% CI	p-value
Sex	Male	1			1		
	Female	0.71	0.44-1.16	0.18	1.11	0.37-3.35	0.84
Tumor location	Proximal	1			1		
	Distal	0.71	0.44-1.15	0.16	0.82	0.19-3.45	0.79
Chemotherapy regimen (first-line)	Oxaliplatin-based	1			1		
	Irinotecan-based	1.25	0.63-2.47	0.51	1.14	0.16-7.72	0.89
Administration of bevacizumab (any line)	No	1			1		
	Yes	0.39	0.24-0.64	<0.01	0.18	0.04-0.68	0.01
KRAS	Wild-type	1			1		
	Mutant	1.12	0.64-1.95	0.67	1.23	0.51-2.93	0.63
BRAF	Wild-type	1			1		
	Mutant	1.16	0.48-2.81	0.73	2	0.57-7.00	0.27
PIK3CA	Wild-type	1			1		
	Mutant	0.39	0.09-1.64	0.2	0.2	0.77-124.1	0.07

PC, peritoneal carcinomatosis; HR, hazard ratio; CI, confidence interval

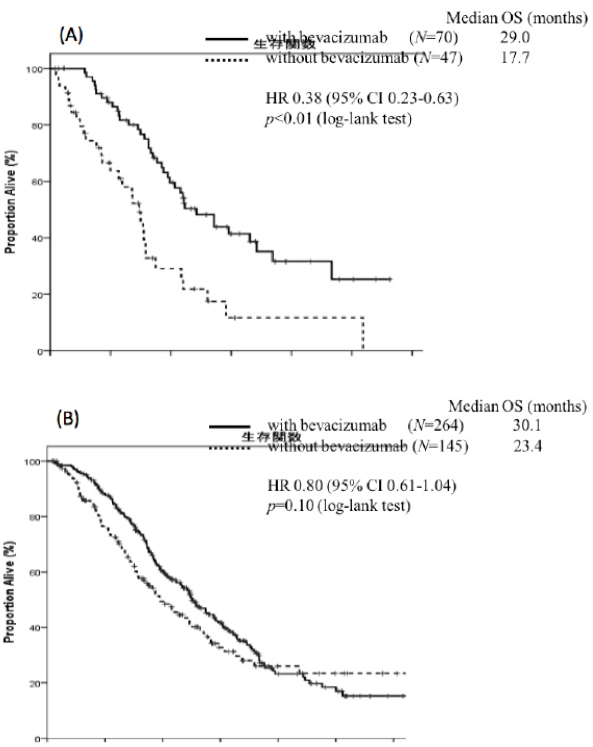

Figure 2. Kaplan-Meier Curves for Overall Survival of (A) PC Patients or (B) non-PC patients Treated with or without Bevacizumab. HR, hazard ratio; CI, confidence interval

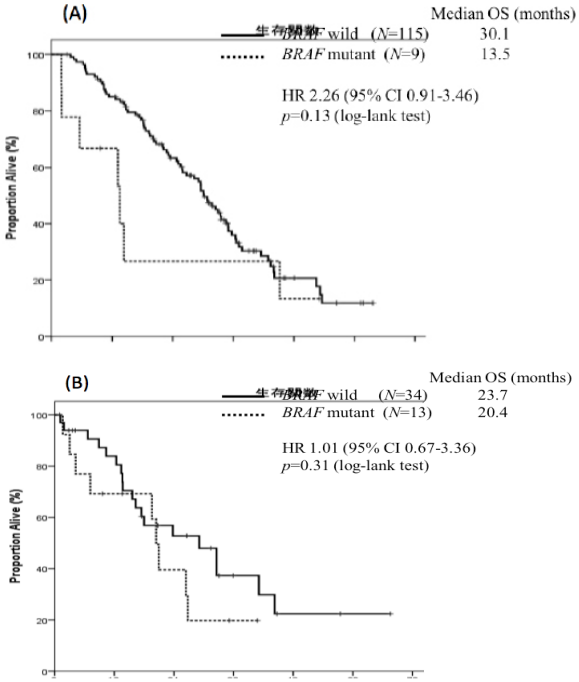

Figure 3. Kaplan-Meier Curves for Overall Survival According to *BRAF* Mutation Status in non-PC Patients (A) and in PC patients (B). HR, hazard ratio; CI, confidence interval

with bevacizumab, OS was significantly shorter in the PC group than the non-PC group (HR=1.82, p<0.01). In addition, the *BRAF* V600E mutation displayed a strong correlation with shorter OS in non-PC patients (HR=2.26, p =0.13), but not in PC patients (HR=1.01, p=0.31) (Fig 3). Both univariate and multivariate analysis in the PC group demonstrated that the addition of bevacizumab to any treatment line was the only prognostic factor (Table 3).

Discussion

In this study, there was no statistically significant difference between the median OS of PC and non-PC patients, despite the PC group having a higher proportion of patients with ≥3 metastatic sites. Median OS in the PC group was more than 23 months, which is considerably longer than in previous studies [2-4]. However, patients in these studies did not receive bevacizumab or anti-EGFR drugs, whereas in the present study, more than 60% of patients received bevacizumab and more than 30% received an anti-EGFR antibody such as cetuximab and panitumumab. Furthermore, the survival of PC patients in the present study was not influenced according to diagnosis by imaging or during surgery. These findings suggest that tumor volume of PC may not have prognostic impact.

This study revealed that chemotherapy with bevacizumab resulted in significantly longer OS than

chemotherapy without bevacizumab in PC patients, whereas there was no difference in OS between patients treated with and without bevacizumab in the non-PC group. Although there was no difference in OS between PC and non-PC patients who received bevacizumab-containing chemotherapy, PC patients had shorter OS than non-PC patients if they were not treated with bevacizumab. These results indicate that the addition of bevacizumab to systemic chemotherapy may confer a survival benefit on patients with PC via its anti-VEGF mode of action. In other words, bevacizumab may recover the poor prognosis of the patients with PC.

On the other hand, the mutational status of *KRAS*, *BRAF*, and *PIK3CA* genes was compared between patients with and without PC from CRC. There was a significant relationship between PC and the *BRAF* V600E mutation, which was at least three-fold more frequent in PC than non-PC patients. In contrast, no significant differences were observed between the two groups in the frequency of *KRAS* and *PIK3CA* mutations. Multivariate analysis, which included gene mutations, demonstrated that the *BRAF* mutation was a poor prognostic factor in all patients, but it was not associated with prognosis in the PC group. It may be speculated that *BRAF* mutation and PC have a confounding impact on OS, and the high co-occurrence of the *BRAF* mutation and PC may reduce their prognostic value. Because the frequency of the *BRAF* mutation is low and it is significantly correlated with PC, a large number of patients are required to confirm the prognostic values of the *BRAF* mutation and PC.

There are several limitations to the present study. First, this was a retrospective investigation at a single institution. Secondly, our data did not include the degree of peritoneal metastasis in the analyses because it is very difficult to evaluate the tumor volume of PC. Severe peritoneal metastasis which is characterized by tumor nodules and ascites sometimes caused bowel obstruction and deterioration of performance status. However, in this study, there was no difference in OS between PC patients diagnosed by imaging and at surgery. These findings suggest that tumor volume of PC may not have prognostic value, and it may be considered that these two types of PC can be classified as a single group.

In conclusion, systemic chemotherapy, especially when combined with bevacizumab, improved survival in patients with PC from CRC as well as non-PC patients. A high frequency of the *BRAF* mutation was observed in PC patients, but it was not associated with prognosis.

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