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Original Article

Clinicopathological and molecular features between synchronous and metachronous metastases in colorectal cancer

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Abstract: The molecular difference between synchronous and metachronous metastases in colorectal cancer (CRC) remains unclear. Between 2000 and 2010, a total of 492 CRC patients were enrolled, including 280 with synchronous metastasis and 212 with metachronous metastasis. Clinicopathological and molecular features were compared between the two groups. Patients with synchronous metastasis were more likely to have right-sided CRC, poorly differentiated tumors, lymphovascular invasion, advanced pathological tumor (T) and node (N) categories, and liver metastases than those with metachronous metastasis. For right-sided CRC, patients with synchronous metastasis had more lymphovascular invasion and liver metastases than those with metachronous metastasis. For left-sided CRC, patients with synchronous metastasis were more likely to have poorly differentiated tumors, lymphovascular invasion, advanced pathological T and N categories, and liver metastases than those with metachronous metastasis. Regarding the genetic mutations, patients with metachronous metastasis had more mutations in *TP53*, *NRAS*, and *HRAS* and fewer mutations in *APC* than those with synchronous metastasis; for right-sided CRC, synchronous metastasis was associated with more *APC* mutations than metachronous metastasis, while for left-sided CRC, metachronous metastasis was associated with more *TP53* and *NRAS* mutations than synchronous metastasis. The 5-year overall survival (OS) rates were significantly higher in metachronous metastasis patients than in synchronous metastasis patients, especially those with left-sided CRC. Multivariate analysis showed that age, sex, lymphovascular invasion, pathological N category, metachronous metastasis, and *BRAF* and *NRAS* mutations were independent prognostic factors affecting OS. CRC patients with synchronous metastasis had a worse OS than those with metachronous metastasis and exhibited distinct genetic mutations.

Keywords: Colorectal cancer, synchronous metastasis, metachronous metastasis, prognostic factor, genetic mutation

Introduction

In Taiwan, colorectal cancer (CRC) is the most common cancer type and the 3rd leading cause of cancer-related death [1]. Approximately 15,000 CRC patients are diagnosed and 5,700 died from cancer every year; hence, CRC is a major public health problem. About half of patients ultimately died either due to progression of metastasis at presentation or recurrent disease after treatment. The most common metastatic site is liver, either synchronous or metachrous [5]. With the improvement of surgical techniques in liver surgery and perioperative care, resection of hepatic metastases has become a more feasible and safe treatment modality and provides an opportunity of long-term survival; however, only approximately 25% of patients could meet the criteria for surgery [2]. A multidisciplinary team is required to discuss the optical treatment strategy for the patients with metastatic CR, and improved survival rate is observed by proper patient selection and aggressive metastasectomy [3].

The timing of occurrence of metastatic disease seems to have an impact on prognosis. The five-year survival rate for CRC patients with synchronous metastasis was 3-11%, which was shorter than 12.8-32.4% for those with metachronous metastasis [4-6]. The discrepancy in the definition of synchronous and metachronous metastasis in previous studies may affect the incidence and survival rate of these patients. To date, there is no consensus regarding the definition of the cutoff time for synchronous and metachronous metastasis of CRC, which varies between 0-12 months after initial diagnosis [2-13]. We selected six months as the cutoff time in this study for the following reasons: (1) a cancer staging procedure is performed in some patients after fully recovered and it may take several months, and a duration of six months will ensure adequate staging for these patients; and (2) metastasis within 6 months after surgery probably has tumor behavior similar to that of metastasis at initial diagnosis [2]. In addition, when the cutoff time was over 4 months after initial diagnosis, the survival was significantly better in the metachronous group than the synchronous group [3].

Previous studies have compared the differences between patients with synchronous and metachronous liver disease and revealed no differences in gender [14], tumor location [15], tumor grading and differentiation [15, 16], extent of vascular invasion [17]. It is our interest whether tumors with different timing of metastasis have different molecular profiles, which may possibly explain the different prognosis between these two groups. For example, *KRAS* mutations have been an important factor in predicting the response to anti-epidermal growth factor receptor (EGFR) therapy [18]. *BRAF* mutations in advanced CRC was associated with a poor prognosis, and *BRAF* inhibitor monotherapy did not show meaningful therapeutic effect for *BRAF*-mutant advanced CRC [19]. The *KRAS/BRAF* mutation status is an important predictor of the treatment response for advanced CRC. There have been few reports investigating the molecular difference between synchronous and metachronous metastasis in CRC patients [12, 13]. In the study by Kim et al [12], mutations in major pathway genes, including *KRAS*, *BRAF, PIK3CA*, *TP53*, *APC*, and *NRAS*, were identified, and similar mutational profiles were observed between patients with synchronous and metachronous metastasis. Fujiyoshi et al [13] reported that the concordance rates of *KRAS* and *BRAF* mutations were high between primary CRC tumor tissues and metastatic tissues; however, the high concordance rates of these genes where not significantly different between patients with synchronous and metachronous metastases. It seems that the genetic alterations were not significantly different between patients with synchronous and metachronous metastases.

To date, there has been a lack of investigation into the correlation among the mutational profiles, metastatic pattern, and prognosis of CRC patients with synchronous and metachronous metastases. The aim of this study was to compare the differences in the clinicopathological and mutational profiles between synchronous and metachronous metastases in CRC patients.

Materials and methods

Between 2000 and 2010, a total of 492 metastatic CRC patients were identified with available tumor samples in the biobank and were included in this study, including 280 patients with synchronous metastasis and 212 patients with metachronous metastasis. Synchronous metastasis was defined as distant metastasis at diagnosis or within 6 months after diagnosis. The molecular and clinicopathological features were collected. Written informed consent for sample collection was signed by all the 492 patients, and the tumor samples were stored at the biobank of Taipei Veterans General Hospital. The Institutional Review Board of Taipei Veterans General Hospital approved the present study.

The exclusion criteria included patients who received preoperative chemoradiotherapy, who did not receive surgical treatment for primary CRC, who did not have available tumor tissue in the biobank, who underwent emergent operations, or died within 30 days after surgery. Right-side CRC was defined as a tumor located from the cecum to the transverse colon, while left-sided CRC was defined as a tumor extending from the splenic flexure to the rectum.

After surgery, patients were followed up every 3 months for the first 2 years and semiannually thereafter. In addition, carcinoembryonic anti-

Table 1. Clinicopathological features between synchronous and metachronous metastasis in CRC

	Synchronous metastasis n=280 n (%)	Metachronous metastasis n=212 n (%)	*P* value
Age (years)			0.547
<70	131 (46.8)	105 (49.5)	
≥70	149 (53.2)	107 (50.5)	
Sex			0.252
Male	179 (63.9)	146 (68.9)	
Female	101 (36.1)	66 (31.1)	
Tumor location			**0.005**
Right-sided	93 (33.2)	46 (21.7)	
Left-sided	187 (66.8)	166 (78.3)	
Tumor differentiation			**0.003**
Well to moderate	247 (88.2)	203 (95.8)	
Poor	33 (11.8)	9 (4.2)	
Lymphovascular invasion			**<0.001**
Absent	156 (55.7)	164 (77.4)	
Present	124 (44.3)	48 (22.6)	
Pathological T category			**<0.001**
T1	1 (0.4)	2 (0.9)	
T2	5 (1.8)	19 (9.0)	
T3	197 (70.4)	164 (77.4)	
T4	77 (27.5)	27 (12.7)	
Pathological N category			**<0.001**
N0	57 (20.4)	79 (37.3)	
N1	79 (28.2)	64 (30.2)	
N2	144 (51.4)	69 (32.5)	
MSI status			0.715
MSS	260 (92.9)	195 (92.0)	
MSI-high	20 (7.1)	17 (8.0)	

MSI: microsatellite instability; MSS: microsatellite stable; TNM: tumor, node, metastasis; bold: statistically significant.

gen analysis, chest radiography, abdominal sonogram, and computerized tomography if needed were arranged. Proton emission tomography or magnetic resonance imaging was arranged when elevation of carcinoembryonic antigen level without determination of tumor recurrence site. Patients with resectable synchronous or metachronous metastasis received surgery and adjuvant chemotherapy with FOLFOX (folinic acid, fluorouracil and oxaliplatin). Patients with unresectable metastasis received palliative chemotherapy with FOLFIRI (folinic acid, fluorouracil and irinotecan) or FOLFOX. Targeted therapies such as bevacizumab, cetuximab, and panitumumab were not reimbursed by the Taiwan National Health Insurance Administration before 2010.

DNA extraction and mutational analysis of the 12-gene panel

The extraction of DNA was performed using the QIAamp DNA Tissue Kit (Qiagen, Valencia, CA, USA) The 12-gene panel with identification of 139 mutations selected from hotspots was investigated according to the COSMIC database and previous studies [20, 21]. As described in a previous report [22], the MassArray method was used to detect the mutations of the 139 hotspots in 12 genes.

Microsatellite instability (MSI) analysis

Five microsatellite markers were used to determine the MSI phenotype according to the international criteria, including D5S345, D2S123, BAT25, BAT26, and D17S250 [23]. MSI-high tumors were defined as samples with 2 or more positive MSI markers, and microsatellite stable (MSS) tumors were defined as samples with 0 or 1 positive MSI marker.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA). The statistical endpoint for overall survival (OS) was measured from the date of diagnosis until the death date. Kaplan-Meier survival curves were compared using the log-rank test. Univariate and multivariate Cox regression analyses were used to assess the impact of the molecular and clinicopathological features on OS. The clinicopathological features were compared using the Chi-squared and two-tailed Fisher's exact tests. Numerical values were compared using Student's t-test. Statistical significance was defined as *p* value less than 0.05.

Results

Clinicopathological features

Among the 492 CRC patients, 280 patients had synchronous metastasis and 212 patients had metachronous metastasis. In **Table 1**, patients with synchronous metastasis had more right-sided tumors, poorly diffferentiated

Table 2. Clinicopathological features of colorectal cancer with synchronous or metachronous metastasis stratified by tumor location

Variables	Right-sided CRC			Left-sided CRC		
	Synchronous metastasis n=93 n (%)	Metachronous metastasis n=46 n (%)	*P* value	Synchronous metastasis n=187 n (%)	Metachronous metastasis n=166 n (%)	*P* value
Age (years)			0.768			0.782
<70	38 (40.9)	20 (43.5)		93 (49.7)	85 (51.2)	
≥70	55 (59.1)	26 (56.5)		94 (50.3)	81 (48.8)	
Sex			0.768			0.259
Male	53 (57.0)	25 (54.3)		126 (67.4)	121 (72.9)	
Female	40 (43.0)	21 (45.7)		61 (32.6)	45 (27.1)	
Tumor differentiation			0.633			**0.002**
Well to moderate	78 (83.9)	40 (87.0)		169 (90.4)	163 (98.2)	
Poor	15 (16.1)	6 (13.0)		18 (9.6)	3 (1.8)	
Lymphovascular invasion			**0.018**			**<0.001**
Absent	61 (65.6)	39 (84.8)		95 (50.8)	125 (75.3)	
Present	32 (34.4)	7 (15.2)		92 (49.2)	41 (24.7)	
Pathological T category			0.436			**<0.001**
T1	1 (1.1)	0		0	2 (1.2)	
T2	2 (2.2)	3 (6.5)		3 (1.6)	16 (9.6)	
T3	68 (73.1)	35 (76.1)		129 (69.0)	129 (77.7)	
T4	22 (23.7)	8 (17.4)		55 (29.4)	19 (11.4)	
Pathological N category			0.443			**<0.001**
N0	20 (21.5)	12 (26.1)		37 (19.8)	67 (40.4)	
N1	28 (30.1)	17 (37.0)		51 (27.3)	47 (28.3)	
N2	45 (48.4)	17 (37.0)		99 (52.9)	52 (31.3)	
MSI status			0.420			0.250
MSS	83 (89.2)	43 (93.5)		177 (94.7)	152 (91.6)	
MSI-high	10 (10.8)	3 (6.5)		10 (5.3)	14 (8.4)	

MSI: microsatellite instability; MSS: microsatellite stable; T: tumor; N: node; bold: statistically significant.

tumors, lymphovascular invasion, and advanced pathological T and N categories than patients with metachronous metastasis.

In **Table 2**, for right-sided CRC, patients with synchronous metastasis tended to have lymphovascular invasion more often than patients with metachronous metastasis. For left-sided CRC, patients with synchronous metastasis tended to have poorly differentiated tumors, lymphovascular invasion, and advanced T and N categories more often than patients with metachronous metastasis.

Molecular analysis

In **Figure 1**, the most common mutated gene in patients with synchronous metastases was *KRAS*, followed by *APC*, *TP53*, and *PIK3CA*; the most common mutated gene in those with metachronous metastases was *KRAS*, followed by *TP53*, *APC*, and *PIK3CA*. As shown in **Figure 2A**, in right-side colon cancer, the most common mutated gene in those with synchronous metastases was *KRAS*, followed by *APC*, *TP53*, and *PIK3CA*; the most common mutated gene in those with metachronous metastases was *KRAS*, followed by *TP53*, *APC* and *PIK3CA*. As shown in **Figure 2B**, in left-sided colon cancer, the most common mutated gene in those with synchronous metastases was *KRAS*, followed by *APC*, *TP53*, and *PIK3CA*; the most common mutated gene in those with metachronous metastases was *TP53*, followed by *KRAS*, *APC*, and *PIK3CA*. In brief, the most common four mutated genes are the same in these two groups, and *KRAS* was the most common mutated gene. But the order of prevalence of

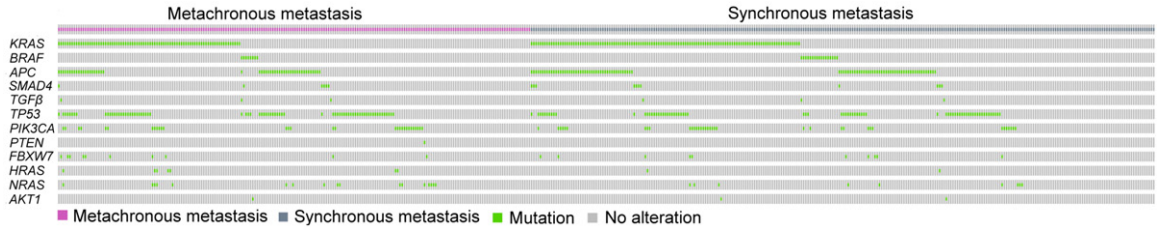

Figure 1. The oncoprint of genetic mutations in CRC with synchronous and metachronous metastases in all CRC patients.

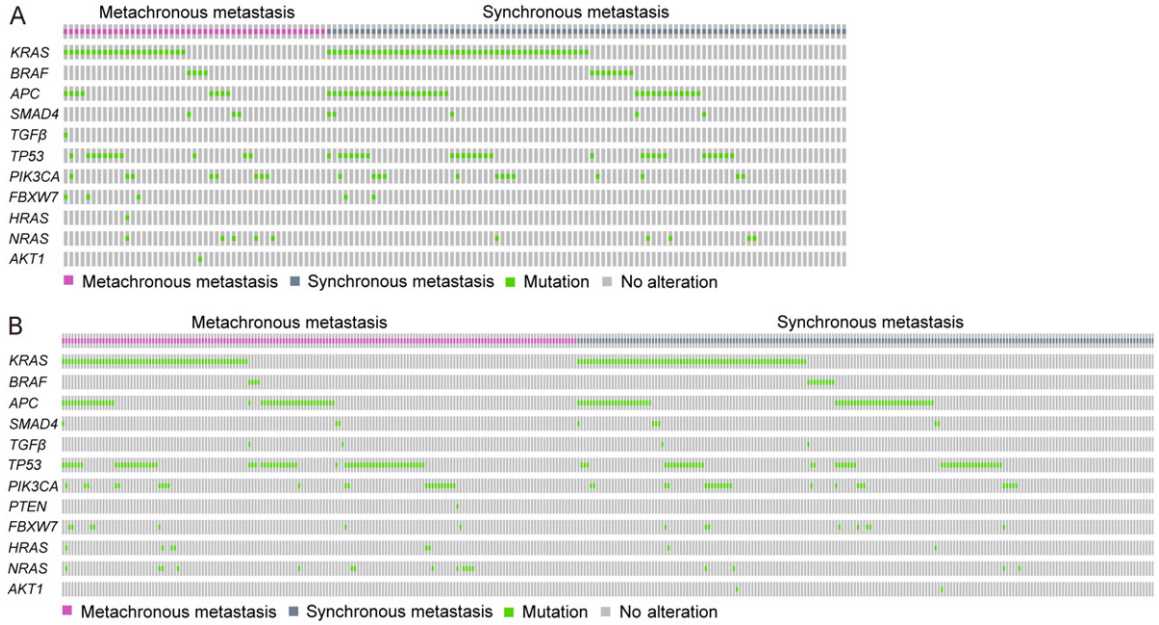

Figure 2. The oncoprint of genetic alteration in CRC with synchronous and metachronous metastases in right-sided and left-sided CRC. The figures of mutation profiles are shown as follows: (A) right-sided CRC patients and (B) left-sided CRC patients.

mutated gene differs in these two groups, with more *APC* and less *TP53* mutation in those with synchronous metastases than in those with metachronous metastasis. The order of prevalence of mutated genes did not differ by sideness in synchronous group but not in metachronous group. For left-sided CRC with metachronous metastases, the most common mutated gene was *TP53* rather than *KRAS* in right-sided CRC with metachronous metastases.

As shown in **Table 3**, patients with synchronous metastasis had more *APC* mutations and fewer mutations in *TP53*, *NRAS*, and *HRAS* than patients with metachronous metastasis. For right-sided CRC, patients with synchronous metastasis had more *APC* mutations than patients with metachronous metastasis, while for left-sided CRC, patients with metachronous metastasis had more *TP53* and *NRAS* mutations than patients with synchronous metastasis.

Metastatic patterns

As shown in **Table 4**, patients with synchronous metastasis had more liver metastases and fewer metastases in the lung and bone than those with metachronous metastasis. For right-sided CRC, patients with synchronous metastasis had more liver metastases than those with metachronous metastasis, while for left-sided CRC, patients with synchronous metastasis had more liver metastases and fewer lung metastases than those with metachronous metastasis.

Table 3. The mutation spectrum of synchronous and metachronous metastasis in CRC stratified by tumor location

Mutation	All CRC			Right-sided CRC			Left-sided CRC		
	Synchronous metastasis n=280 n (%)	Metachronous metastasis n=212 n (%)	*P* value	Synchronous metastasis n=93 n (%)	Metachronous metastasis n=46 n (%)	*P* value	Synchronous metastasis n=187 n (%)	Metachronous metastasis n=166 n (%)	*P* value
TP53	72 (25.7)	74 (34.9)	**0.027**	27 (29.0)	11 (23.9)	0.524	45 (24.1)	63 (38.0)	**0.005**
APC	90 (32.1)	50 (23.6)	**0.037**	34 (36.6)	8 (17.4)	**0.021**	56 (29.9)	42 (25.3)	0.331
PIK3CA	36 (12.9)	30 (14.2)	0.677	13 (14.0)	8 (17.4)	0.597	23 (12.3)	22 (13.3)	0.789
BRAF	17 (6.1)	8 (3.8)	0.250	8 (8.6)	4 (8.7)	0.985	9 (4.8)	4 (2.4)	0.231
KRAS	121 (43.2)	82 (38.7)	0.312	47 (50.5)	22 (47.8)	0.764	74 (39.6)	60 (36.1)	0.508
NRAS	9 (3.2)	17 (8.0)	**0.018**	5 (5.4)	4 (8.4)	0.454	4 (2.1)	13 (7.8)	**0.013**
HRAS	2 (0.7)	7 (3.3)	**0.034**	0	1 (2.2)	0.154	2 (1.1)	6 (3.6)	0.109
FBXW7	10 (3.6)	10 (4.7)	0.524	2 (2.2)	3 (6.5)	0.196	8 (4.3)	7 (4.2)	0.977
PTEN	0	1 (0.5)	0.431	0	0	-	0	1 (0.6)	0.288
SMAD4	11 (3.9)	6 (2.8)	0.509	5 (5.4)	3 (6.5)	0.785	6 (3.2)	3 (1.8)	0.404
*TGF*β	3 (1.1)	3 (1.4)	0.731	0	1 (2.2)	0.154	3 (1.6)	2 (1.2)	0.751
AKT1	2 (0.7)	1 (0.5)	0.732	0	1 (2.2)	0.154	2 (1.1)	0	0.181

Bold: statistically significant.

Table 4. Metastatic pattern of colorectal cancer stratified by tumor location

Metastatic pattern	All CRC			Right-sided CRC			Left-sided CRC		
	Synchronous metastasis n=280 n (%)	Metachronous metastasis n=212 n (%)	*P* value	Synchronous metastasis n=93 n (%)	Metachronous metastasis n=46 n (%)	*P* value	Synchronous metastasis n=187 n (%)	Metachronous metastasis n=166 n (%)	*P* value
Liver	191 (68.2)	83 (39.2)	**<0.001**	55 (59.1)	17 (37.0)	**0.014**	136 (72.7)	66 (39.8)	**<0.001**
Lung	68 (24.3)	88 (41.5)	**<0.001**	21 (22.6)	17 (37.0)	0.074	47 (25.1)	71 (42.8)	**<0.001**
Peritoneum	73 (26.1)	42 (19.8)	0.104	34 (36.6)	18 (39.1)	0.768	39 (20.9)	24 (14.5)	0.117
Bone	6 (2.1)	12 (5.7)	**0.040**	1 (1.1)	3 (6.5)	0.071	5 (2.7)	9 (5.4)	0.187
Others	17 (6.1)	21 (9.9)	0.115	6 (6.5)	5 (10.9)	0.364	11 (5.9)	16 (9.6)	0.185

Bold: statistically significant. Some patients had more than one metastatic pattern.

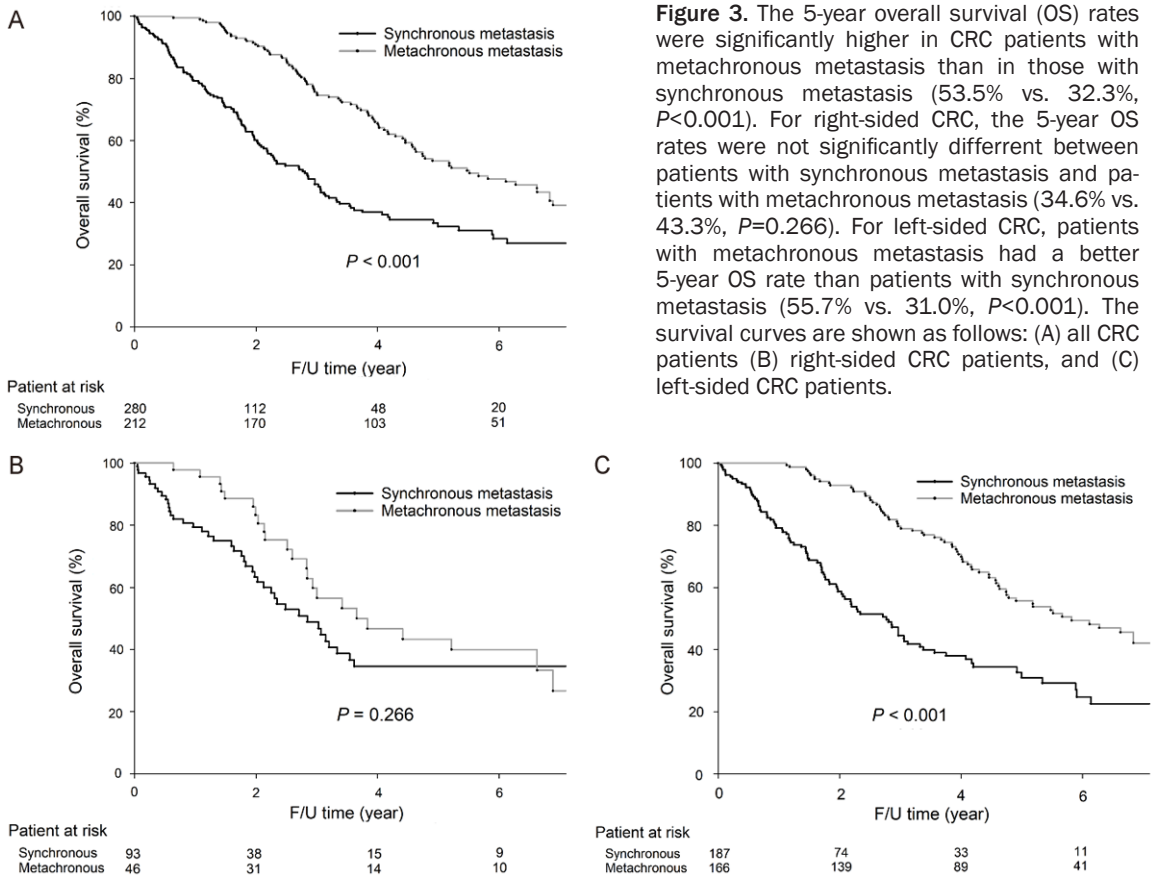

Figure 3. The 5-year overall survival (OS) rates were significantly higher in CRC patients with metachronous metastasis than in those with synchronous metastasis (53.5% vs. 32.3%, *P*<0.001). For right-sided CRC, the 5-year OS rates were not significantly different between patients with synchronous metastasis and patients with metachronous metastasis (34.6% vs. 43.3%, *P*=0.266). For left-sided CRC, patients with metachronous metastasis had a better 5-year OS rate than patients with synchronous metastasis (55.7% vs. 31.0%, *P*<0.001). The survival curves are shown as follows: (A) all CRC patients (B) right-sided CRC patients, and (C) left-sided CRC patients.

Survival analysis

In **Figure 3A**, patients with metachronous metastasis had better 5-year OS than patients with synchronous metastasis (53.5% vs. 32.3%, *P*<0.001). For right-sided CRC, there was no significant difference in 5-year OS between patients with synchronous metastasis and patients with metachronous metastasis (34.6% vs. 43.3%, *P*=0.266, **Figure 3B**). For left-sided CRC, patients with metachronous metastasis had a better 5-year OS rate than patients with synchronous metastasis (55.7% vs. 31.0%, *P*<0.001, **Figure 3C**).

In **Table 5**, the univariate analysis showed that eight covariates were significantly correlated with OS: age, sex, lymphovascular invasion, pathological N category, MSI status, synchronous metastasis, and *BRAF* and *NRAF* mutations. The eight covariates were included in the multivariate analysis, which showed that age, sex, lymphovascular invasion, pathological N category, synchronous metastasis, and *BRAF* and *NRAS* mutations were independent prognostic factors affecting OS.

In **Figure 4A**, the 5-year post-metastasis survival rates were not significantly different between CRC patients with synchronous metastasis and those with metachronous metastasis (33.0% vs. 32.3%, *P*=0.822). For right-sided CRC, the 5-year post-metastasis rates were not significantly different between patients with synchronous metastasis and patients with metachronous metastasis (34.6% vs. 25.0%, *P*=0.127, **Figure 4B**). For left-sided CRC, the 5-year post-metastasis rates were not significantly different between patients with synchronous metastasis and patients with metachronous metastasis (31.0% vs. 34.6%, *P*=0.637, **Figure 4C**).

As shown in **Figure 5A**, for patients with synchronous metastasis, the 3-year OS rates (18% vs. 47.5%, *P*=0.006) were significantly lower in patients with *BRAF* mutations than in those without *BRAF* mutations; the 3-year OS rates were not significantly different between pa-

Table 5. Univariate and multivariate analysis of overall survival in colorectal cancer

	Univariate analysis			Multivariate analysis		
	Odds ratio	Confidence interval	*P* value	Odds ratio	Confidence interval	*P* value
Age (year)			**<0.001**			**<0.001**
<70	1.00			1.00		
≥70	1.65	1.270-2.130		1.79	1.361-2.347	
Sex			**0.034**			**0.018**
Male	1.00			1.00		
Female	0.74	0.559-0.978		0.70	0.524-0.942	
Tumor location			0.157			
Right-sided	1.00					
Left-sided	0.82	0.614-1.082				
Lymphovascular invasion			**<0.001**			**0.008**
Absent	1.00			1.00		
Present	1.75	1.3389-2.279		1.38	1.031-1.859	
Pathological T category			0.078			
T1	1.00					
T2	1.36	0.175-10.533				
T3	1.86	0.260-13.293				
T4	2.67	0.368-19.392				
Pathological N category			**<0.001**			**<0.001**
N0	1.00			1.00		
N1	1.33	0.951-1.865		1.06	0.751-1.491	
N2	2.04	1.487-2.791		1.86	1.336-2.575	
MSI status			**0.044**			
MSS	1.00					
MSI-high	1.56	1.012-2.401				
Metastasis			**<0.001**			**<0.001**
Synchronous metastasis	1.00			1.00		
Metachronous metastasis	0.48	0.366-0.617		0.47	0.356-0.613	
BRAF mutation			**<0.001**			**<0.001**
No	1.00			1.00		
Yes	2.50	1.545-4.057		3.32	2.024-5.450	
NRAS mutation			**0.031**			**0.001**
No	1.00			1.00		
Yes	1.76	1.054-2.934		2.30	1.434-3.696	
HRAS mutation			0.242			
No	1.00					
Yes	0.51	0.162-1.583				
TP53 mutation			0.987			
No	1.00					
Yes	1.00	0.760-1.322				
APC mutation			0.754			
No	1.00					
Yes	0.96	0.721-1.267				

T: tumor; N: node; MSI: microsatellite instability; MSS: microsatellite stable; bold: statistically significant.

tients with *NRAS* mutations and those without *NRAS* mutation (0% vs. 47.2%, *P*=0.068, **Figure 5B**). For patients with metachronous metastasis, the 3-year OS rates were signifi-

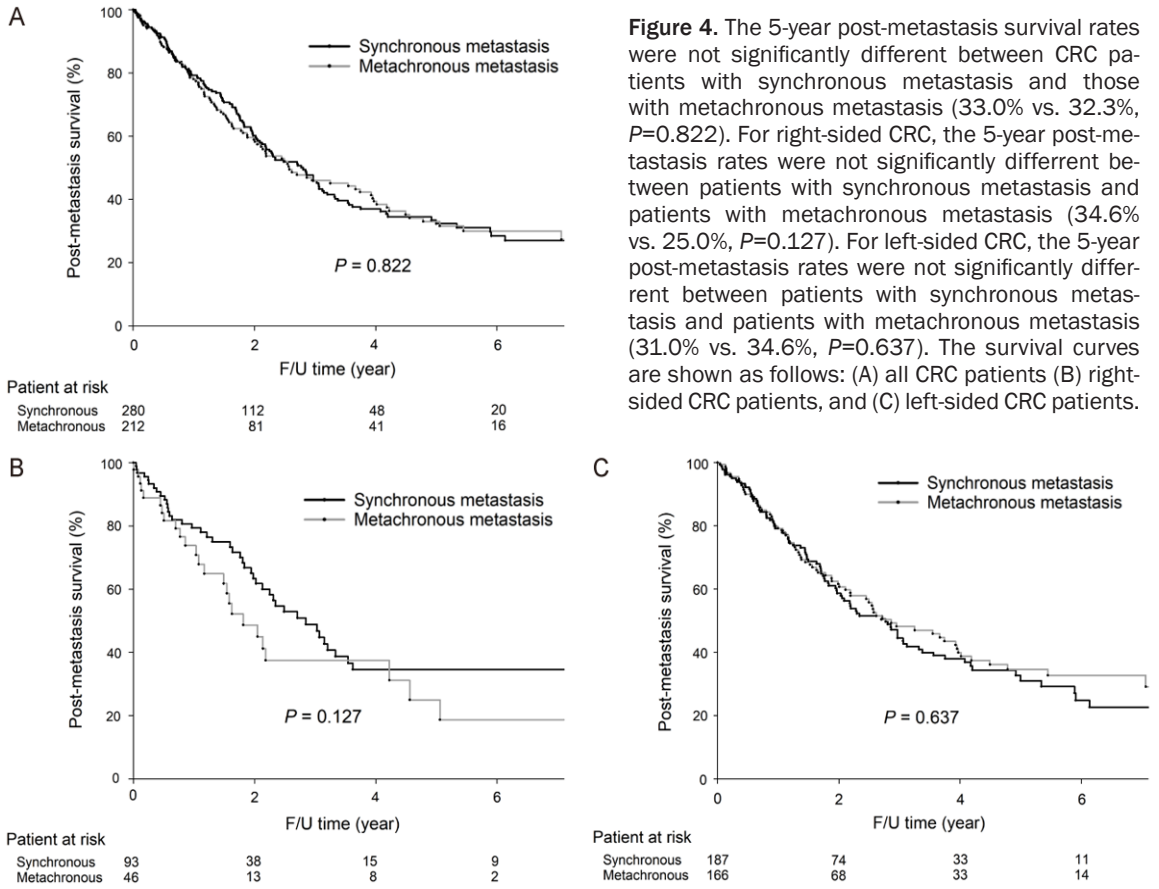

Figure 4. The 5-year post-metastasis survival rates were not significantly different between CRC patients with synchronous metastasis and those with metachronous metastasis (33.0% vs. 32.3%, *P*=0.822). For right-sided CRC, the 5-year post-metastasis rates were not significantly differrent between patients with synchronous metastasis and patients with metachronous metastasis (34.6% vs. 25.0%, *P*=0.127). For left-sided CRC, the 5-year post-metastasis rates were not significantly differrent between patients with synchronous metastasis and patients with metachronous metastasis (31.0% vs. 34.6%, *P*=0.637). The survival curves are shown as follows: (A) all CRC patients (B) right-sided CRC patients, and (C) left-sided CRC patients.

cantly lower in patients with *BRAF* mutations than in those without *BRAF* mutations (42.9% vs. 77.1%, *P*=0.030, **Figure 5C**); the 3-year OS rates (56.5% vs. 77.6%, *P*=0.005, **Figure 5D**) were significantly lower in patients with *NRAS* mutations than in those without *NRAS* mutations.

Discussion

To the best of our knowledge, the present study includes the largest population investigating the genetic alterations between synchronous and metachronous metastasis in CRC patients. The novel finding of the present study is that different genetic mutations exist between synchronous and metachronous metastasis in CRC. In addition, the differences in mutational profiles between synchronous and metachronous metastases are also distinct in right-sided and left-sided CRC.

The major difference between the present study is that patients with metachronous metastasis had more mutations in *TP53*, *NRAS*, and *HRAS* and fewer mutations in *APC* than those with synchronous metastasis, while no difference in genetic mutations was noted between synchronous and metachronous metastases in other studies [12, 13]. In addition, our results demonstrated that synchronous metastasis was associated with more *APC* mutations in right-sided colon cancer and metachronous metastasis was associated with more *TP53* and *NRAS* mutations in left-sided colon cancer. The reason for the discrepancy between the present study and others might be due to sample size, racial difference, the definition of synchronous and metachronous metastases, and environmental factors.

In the present study, patients with synchronous metastasis had a worse OS rate than patients with metachronous metastasis, especially among those with left-sided CRC. Our result is interesting, and the possible reason is that patients with synchronous metastasis had more advanced pathological T and N catego-

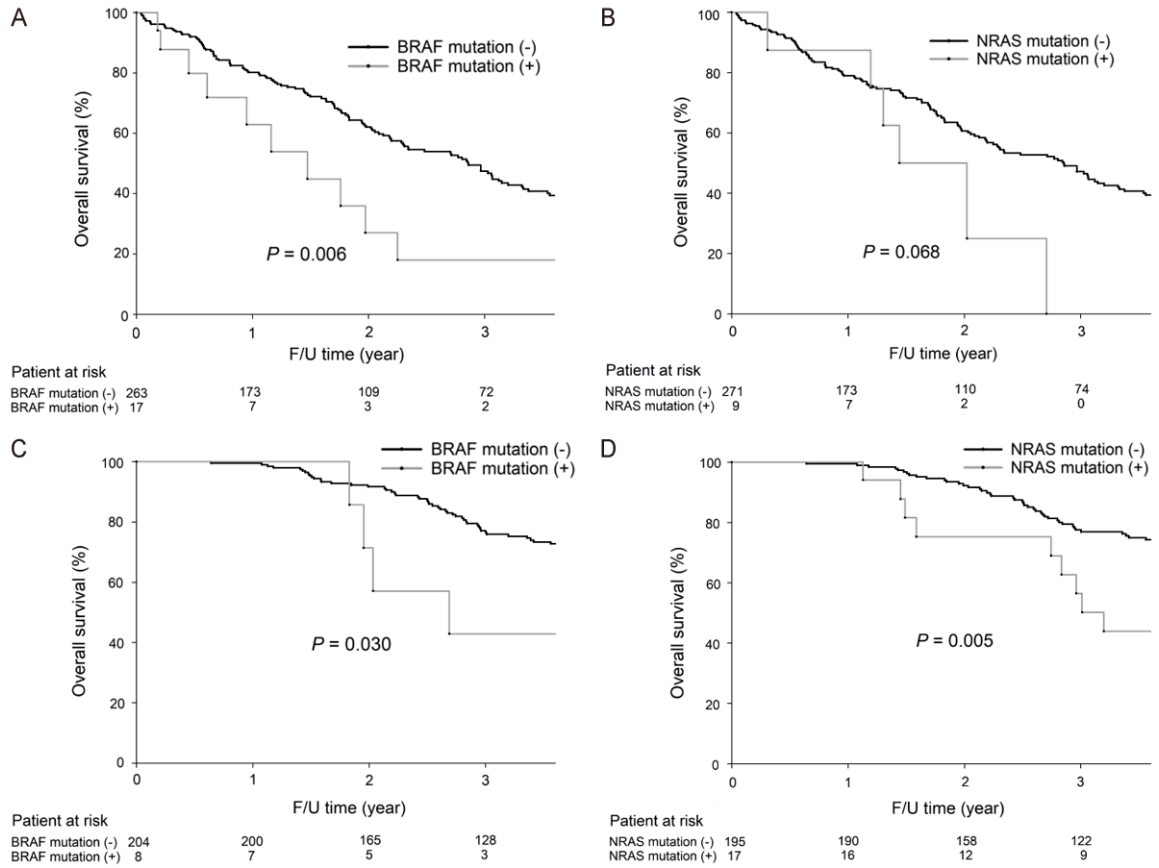

Figure 5. A. For CRC patients with synchronous metastasis, the 3-year OS rates were significantly lower in patients with *BRAF* mutations than in patients without *BRAF* mutations (18.0% vs. 47.5%, *P*=0.006). B. For CRC patients with synchronous metastasis, the 3-year OS rates were not significantly different between patients with *NRAS* mutations and those without *NRAS* mutation (0% vs. 47.2%, *P*=0.068). C. For CRC with metachronous metastasis, the 3-year OS rates were significantly lower in patients with *BRAF* mutations than in those without *BRAF* mutations (42.9% vs. 77.1%, *P*=0.030). D. For metachronous metastatic CRC patients, the 3-year OS rates were significantly lower in patients with *NRAS* mutations than in patients without *NRAS* mutations (56.5% vs. 77.6%, *P*=0.005).

ries than patients with metachronous metastasis, especially among those with the left-sided CRC.

Mutations in *APC*, *KRAS*, and *TP53* were frequently found in primary CRC and were correlated with a higher frequency of liver metastasis [24]. In addition, mutations in *APC*, *TP53*, and *KRAS* were also frequently detected in both primary CRC and lung metastasis tissues [25]. In the present study, for right-sided CRC, synchronous metastasis was associated with more *APC* mutations and liver metastases than metachronous metastasis; for left-sided CRC, metachronous metastasis was associated with more *TP53* and *NRAS* mutations and more lung metastases than synchronous metastasis. For right-sided CRC, patients with *APC* mutations had more liver metastases than patients without *APC* mutations (69.0% vs. 44.3%, *P*= 0.007). For left-sided CRC, compared with patients with none or either *TP53* or *NRAS* mutations, patients with both *TP53* and *NRAS* mutations had a higher frequency of lung metastases (37.5% vs. 10.6%, *P*=0.015). It was reported that concurrent mutations of *TP53* and *RAS/BRAF* are associated with extrahepatic metastases in CRC [26]. According to our results, it seems that *APC* mutation may play an important role in liver metastases in right-sided CRC with synchronous metastasis. In addition, concurrent *TP53* and *NRAS* mutations may be associated with lung metastases in left-sided CRC with metachronous metastasis.

Among our 212 patients with metachronous metastasis, 17 patients (8%) had *NRAS* muta-

tions. Our results showed that for patients with metachronous metastasis, *NRAS*-mutated CRC was associated with a worse 3-year OS rate than *NRAS*-nonmutant CRC. It was reported that *BRAF* and *NRAS* mutations were correlated with distant metastasis, and *BRAF* mutation rather than *NRAS* mutation was correlated with a poorer OS rate in stage I-III CRC [27]. In their study, the number of patients with *NRAS* mutation (n=7) was too small to investigate the prognostic role of *NRAS* mutation. The discrepancy between the present study and the study of Guo et al [27] is the patient population and the number of patients with genetic mutations. Since the present study enrolled patients with synchronous and metachronous metastasis and most of them had advanced CRC, the frequency of genetic mutations was higher than that in the study by Guo et al [27], which enrolled patients with stage I-III CRC. Consequently, according to our results, both *BRAF* and *NRAS* mutations are prognostic indicators for CRC patients with synchronous or metachronous metastasis. Meta-analysis demonstrated that mutations in *BRAF*, *PIK3CA*, *NRAS* and exons 3 and 4 of *KRAS* predict resistance to ant-EGFR therapy [28]. Our results might be helpful for evaluation of clinical benefit of anti-EGFR therapy in metastatic CRC.

There are limitations to the present study. This is a retrospective study, and the patients who had metastatic CRC without surgical treatment and available tumor tissue in the biobank were excluded and so selection bias exists. Although a significant difference was noted in some genetic mutations, the patient number is small and the difference in some genetic mutations could not be detected due to low prevalence of mutation. More patients enrolled from different countries and different races are required to validate our findings.

Conclusions

The genetic mutations are distinct between CRC patients with synchronous and metachronous metastasis, and these differences are also observed in patients with different tumor locations. Our results may have clinical impact and remind physicians to be aware of the genetic mutations and the metastatic patterns in the management of CRC patients.

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Disclosure of conflict of interest

None.

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