BRAF Mutation is a Prognostic Biomarker for Colorectal Liver Metastasectomy

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Background and Objectives: In metastatic colorectal cancer, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) is a predictive biomarker for anti-epidermal growth factor receptor (EGFR) treatment and V-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) is a prognostic biomarker. We aimed to determine the impact of *KRAS* and *BRAF* mutation as determined from liver metastases specimens on overall survival (OS) in patients following colorectal liver metastasectomy.

Methods: Liver metastases specimens (n = 292) obtained from patients after liver metastasectomy were used to determine the *KRAS/BRAF* genotype. Associations between clinicopathological parameters and *KRAS*/*BRAF* genotype were identified by univariate and multivariate analyses using the Cox proportional hazards model. The impact of *KRAS*/*BRAF* genotype on survival was analyzed using the Kaplan–Meier method.

Results: The 5-year survival rate of the cohort was 55.8%. The *KRAS* and *BRAF* mutation rates were 38.0 and 2.1%, respectively. *BRAF* genotype, but not *KRAS*, was found to be an independent prognostic biomarker (HR = 5.181, $P = 0.002$) after adjustment for other significant confounding clinicopathological variates: Number of liver metastases (HR = 1.983, $P = 0.009$), concomitant extrahepatic disease (HR = 1.858, $P = 0.014$), and surgical margin (HR = 3.241, $P < 0.001$). *BRAF* genotype was an independent prognostic biomarker in patients with liver metastases only after metastasectomy (HR = 6.245, $P < 0.003$).

Conclusions: *BRAF* mutation is an independent prognostic biomarker for colorectal liver metastasectomy.
J. Surg. Oncol. © 2012 Wiley Periodicals, Inc.

KEY WORDS: biomarker; BRAF; colorectal cancer; liver resection; KRAS

INTRODUCTION

Colorectal cancer is one of the most common malignancies in Taiwan. Liver metastases are present in 15–25% of patients at the time of diagnosis of colorectal cancer, and another 25–50% will develop liver metastases within 3 years following resection of the primary tumor [1]. An abundance of clinical evidence suggests that hepatic resection often leads to a cure [2]. In population-based studies, the 5-year survival averaged 30∼50% among patients who underwent hepatic resection as compared with around 10% among patients who did not undergo hepatic resection [3,4]. Hepatic resection is currently considered the standard treatment and remains the only potentially curative therapy in patients with colorectal liver metastasis.

Central to any discussion of colorectal liver metastasectomy is the linked issue of resectability. Several prognostic scoring systems have been developed [5]. Most of the prognostic factors in the proposed systems include only clinicopathological parameters, such as stage, the extent of liver metastases, and the profiles of tumor markers and biochemistry; however, no biomarkers have been assessed with respect to the prognosis of liver metastasectomy till date.

The v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) is a predictive biomarker of anti-epidermal growth factor receptor (EGFR) treatment in metastatic colorectal cancer [6], but its value as a prognostic biomarker remains controversial [7]. The V-raf murine sarcoma viral oncogene homolog B1 (BRAF) acts as a prognostic biomarker in metastatic colorectal cancer, but its use as a predictive biomarker of anti-EGFR treatment is still under debate. Moreover, in most studies, the *KRAS/BRAF* genotypes have been determined by analysis of primary tumor specimens, not liver

Abbreviations: BRAF, V-raf murine sarcoma viral oncogene homolog B1; CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor; 5-FU, 5-fluorouracil; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; OS, overall survival; PFS, progression-free survival; PET, positron emission tomography.

Grant sponsor: Center of Excellence for Cancer Research at Taipei Veterans General Hospital; Grant number: DOH99-TD-C-111-007; Grant sponsor: Taipei Veterans General Hospital; Grant numbers: 100DHA0100416, 99DHA0100446, 101DHA0100661, 101DHA0100369.

Conflict of interest: None.

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Received 6 October 2011; Accepted 21 January 2012

DOI 10.1002/jso.23063

Published online in Wiley Online Library (wileyonlinelibrary.com).

metastases specimens. Also, no study has focused on the associations between *KRAS*/*BRAF* genotype, clinicopathological parameters, and survival in patients after colorectal liver metastasectomy. Thus, it would be interesting to assess the prevalence and clinicopathological associations of *KRAS*/*BRAF* genotypes obtained from liver metastases specimens in order to provide clues as to the identification of novel biomarkers of serious but potentially curable colorectal liver metastases.

In this study, we first made an extensive survey of *KRAS*/*BRAF* genotypes from liver metastases specimens obtained through liver metastasectomy. We then aimed to evaluate the impact of *KRAF*/*BRAF* genotypes analyzed from liver metastases specimens on clinical outcomes in patients after colorectal liver metastsectomy.

METHODS

Patients and Tissues

From January 2000 to January 2010, a total of 292 consecutive patients with colorectal liver metastases underwent curative-intent hepatic resection at Taipei Veterans General Hospital, Taiwan. Disease stage was assessed based on the American Joint Committee on Cancer staging system, 6th edition. Clinicopathological staging and clinical course were determined by examining a computer database containing detailed information. The study was reviewed and approved by the Institutional Review Board of Taipei Veterans General Hospital.

Left colon cancer was defined as malignancy in the splenic flexure, descending colon, sigmoid, and/or rectosigmoid colon, and right colon cancer was defined as that occurring in the cecum, ascending colon, hepatic flexure, and/or transverse colon. The decision as to whether to perform hepatic resection was made by a multidisciplinary specialist committee. The diagnosis of extrahepatic disease was confirmed by reviewing the imaging studies, usually chest and abdomen computed tomography (CT), prior to liver resection. After hepatic resection, decisions regarding adjuvant chemotherapy were made on an individual patient basis at the discretion of the attending physicians. Follow-up was considered to end in March 2011 or at the death of the patient. Patients were followed-up at least every 3 months from the time of hepatic resection for the first 2 years, then every 6 months for 5 years, and subsequently annually until death. Follow-up visits included physical examination, rectodigital examination, carcinoembryonic antigen (CEA) level, chest X-ray, abdominal sonogram, and/or abdominal CT scanning.

DNA Extract, *KRAS*/*BRAF* Genotyping

Liver-metastases tumor regions were marked on standard H&E-stained slides and macrodissected, and were cross-checked to confirm that at least 70% of the cells were cancer cells. DNA extraction was performed using a Nucleon HT DNA extraction kit (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's instructions. Genomic exon 2 of the *KRAS* gene and exon 15 of the *BRAF* gene were separately amplified using previously reported methods [8,9]. Purified PCR products were sequenced using a BigDyeR Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and analyzed using a 3730 ABI capillary electrophoresis system (Applied Biosystems).

Statistical Analysis

The correlations between clinicopathological variates and *KRAS* and *BRAF* genotypes were analyzed by the χ^2 test or Fisher's exact test. The Cox proportional hazards model was applied for univariate and multivariate analyses. Death from any cause was regarded as an event. Overall survival (OS) was defined as the time from hepatic resection to death from any cause. Progression-free survival (PFS) was defined from the date of hepatic resection to the date of confirmation of recurrence. Survival was estimated using the Kaplan–Meier method, and the log-rank test was used for comparison of survival curves. Variates with P-values <0.05 in univariate analyses were entered into multivariate analyses. A two-sided P-value <0.05 was regarded as statistically significant. SPSS software (version 16.00, SPSS, Chicago, IL) was used for all statistical analyses.

RESULTS

Frequencies of *KRAS* and *BRAF* Gene Mutations in Liver Metastases Specimens

A total of 292 patients were included. *KRAS* mutations were present in 38.0% (n = 111) of the patients, including 29.5% with codon 12 mutations, 8.2% with codon 13 mutations, and 0.3% with codon 14 mutations. One sample had both a G12D and a G13D mutation on different alleles. Only 2.1% (n = 6) of the patients tested were positive for *BRAF* mutation. Four patients (1.4%) carried the V600E mutation and two patients (0.7%) carried the V599E mutation. Mutations in *KRAS* and *BRAF* were mutually exclusive in these samples. Table I summarizes the spectrum of *KRAS*/*BRAF* mutations.

Patient Characteristics

The characteristics of the patients are presented in Table II. The median age was 62 years. There were 177 men (60.6%). The majority of the primary colon cancers were adenocarcinoma (96.9%). The percentage of patients with stage I, II, III, and IV disease at initial diagnosis was 2.1, 9.3, 25.3, and 63.4%, respectively. One-hundred and sixty-three patients had synchronous disease. Sixty-one patients (20.9%) had liver metastases with concomitant extrahepatic disease; these included abdominal/peritoneal metastases (n = 19, 6.5%), non-regional lymph node metastases (n = 19, 6.5%), and lung metastases (n = 23, 7.9%). Thirteen of 19 (68.4%) patients with abdominal/peritoneal metastases received aggressive

TABLE I. Distributions of *KRAS* and *BRAF* Mutations (n = 292)

Gene	Mutation site	n (%)
KRAS analysis	n (total)	292 (100)
KRAS mutant		111 (38.0)
	codon 12	86 (29.5)
	G12D	42 (14.4)
	G12V	22 (7.5)
	G12C	14 (4.8)
	G12S	7 (2.4)
	G12R	1 (0.3)
	codon 13	24 (8.2)
	G13D	21 (7.2)
	G13C	1 (0.3)
	G13V	1 (0.3)
	G12D/G13D	1 (0.3)
	codon 14	
	V14I	1 (0.3)
BRAF analysis	n (total)	292 (100)
BRAF mutant		6 (2.1)
	V599E	2 (0.7)
	V600E	4 (1.4)
Co-mutation of *KRAS* and *BRAF*		0 (0)
Wild-type of *KRAS/BRAF*		175 (59.9)

KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *BRAF*, V-raf murine sarcoma viral oncogene homolog B1.

TABLE II. Association of *BRAF* and *KRAS* Mutation Status With Clinicopathological Features in Colorectal Liver Metastases

KRAS/BRAF status	Wild/wild n = 175	*KRAS* mutation n = 111	*BRAF* mutation n = 6		Overall n = 292
Variables				*P*	
Age: median (interquartile range)	62 (54∼73)	61 (51∼74)	54 (41∼65)		62 (53∼74)
Age > 60	97 (55.4%)	59 (53.2%)	3 (50%)	0.909	159 (54.5%)
Gender					
Male	66 (37.7%)	65 (58.6%)	3 (50%)	0.710	177 (60.6%)
Female	109 (62.3%)	46 (41.4%)	3 (50%)		115 (39.4%)
Histology subtype					
Adenocarcinoma	171 (97.7%)	106 (95.5%)	6 (100%)	0.591	283 (96.9%)
Carcinoma	3 (1.7%)	5 (4.5%)	0 (0.0%)		1 (0.3%)
Mucinous adenocarcinoma	1 (0.6%)	0 (0.0%)	0 (0.0%)		8 (2.7%)
Location					
Right-side colon	32 (18.3%)	39 (35.1%)	4 (66.7%)	0.003	75 (25.7%)
Left-side colon	83 (47.4%)	40 (36.0%)	2 (33.3%)		125 (42.8%)
Rectum	60 (34.3%)	32 (28.8%)	0 (0.0%)		92 (31.5%)
Initial stage					
I	6 (3.4%)	0 (0.0%)	0 (0.0%)	0.355	6 (2.1%)
II	19 (10.9%)	8 (7.2%)	0 (0.0%)		27 (9.3%)
III	42 (24.0%)	31 (27.9%)	1 (16.7%)		74 (25.3%)
IV	108 (61.7%)	72 (64.9%)	5 (83.3%)		185 (63.4%)
Synchronous	94 (53.7%)	65 (58.6%)	4 (66.7%)	0.625	163 (55.8%)
No. of liver metastases					
1	57 (32.6%)	44 (39.6%)	2 (33.3%)	0.657	103 (35.3%)
2–3	76 (43.4%)	39 (35.1%)	2 (33.3%)		117 (40.1%)
>3	42 (24.0%)	28 (25.2%)	2 (33.3%)		72 (24.6%)
Maximum size					
<30 mm	95 (54.3%)	50 (45.0%)	4 (66.7%)	0.232	149 (51.0)
>30 mm	80 (45.7%)	61 (55.0%)	2 (33.3%)		143 (49.0)
Distribution					
Unilobar	143 (81.7%)	89 (80.2%)	5 (83.3%)	0.940	247 (81.9%)
Bilobar	32 (18.3%)	22 (19.8%)	1 (16.7%)		55 (18.8%)
Concomitant extrahepatic disease	36 (20.6%)	24 (21.6%)	1 (16.7%)	0.946	61 (20.9%)
Pathologic margin					
Free	155 (88.6%)	98 (88.3%)	5 (83.3%)	0.925	258 (88.4%)
Not free	20 (11.4%)	13 (11.7%)	1 (16.7%)		34 (11.6%)
Neoadjuvant chemotherapy					
With	44 (25.1%)	22 (19.8%)	0 (0.0%)	0.236	66 (22.6%)
Without	131 (74.9%)	89 (80.2%)	6 (100%)		226 (77.4%)
Adjuvant chemotherapy					
With	145 (82.9%)	96 (86.5%)	4 (66.7%)	0.366	245 (83.9%)
Without	30 (17.1%)	15 (13.5%)	2 (33.3%)		47 (16.1%)
Peri-operative anti-EGFR treatment					
With	26(14.9%)	6 (5.4%)	1 (16.7%)	0.044	33 (11.3%)
Without	149(85.1%)	105 (94.6%)	5(83.3%)		259 (88.7%)

KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *BRAF*, V-raf murine sarcoma viral oncogene homolog B1; EGFR, epidermal growth factor receptor.

cytoreductive debulking surgery. One of 19 (5.3%) patients with non-regional lymph node metastases received lymph node dissection, 4 of 19 (21.1%) received radiation therapy, and the others received chemotherapy. Six of 23 (26.0%) patients with lung metastases ultimately received lung metastasectomy. The resection margin-free rate was 88.4%.

There were no significant differences in the baseline characteristics between the *KRAS/BRAF* genotype groups, with the exception of primary tumor location. Compared to *KRAS/BRAF* wild-type tumors (18.3%), *BRAF*-mutated (66.7%) or *KRAS*-mutated tumors (35.1%) had more primary right-side colon locations ($P = 0.003$). There were no significant differences in the use of neoadjuvant treatment or adjuvant treatment between the *KRAS/BRAF* genotypes. Twenty-sixty patients (14.9%) with *KRAS/BRAF* wild-type tumors and 1 patient (16.7%) with a *BRAF*-mutated tumor received peri-operative anti-EGFR therapy, whereas few patients with *KRAS* mutations received anti-EGFR therapy (5.4%).

Survival

The surgical mortality rate was only 0.7% (2 patients: 1 patient died from peritonitis, 1 patient from respiratory failure). The 5-year survival rate for the entire cohort was 55.5% (Fig. 1a).The 5-year PFS rate was 23.7% (Fig. 1b). Figure 2a showed the OS according to *KRAS/BRAF* mutation status. The median OS for patients with *BRAF* mutation was only 8.2 months, which was significantly poorer than that of patients with wild-type *KRAS* and *BRAF* (19.7 months) and patients with *KRAS* mutation (19.6 months) ($P = 0.006$). Figure 2b,c shows the OS according to *KRAS* and *BRAF* genotypes. There was no significant difference in OS between *KRAS* codon 12 mutation, *KRAS* codon 13 mutation and wild-type *KRAS/BRAF* patients (Fig. 2b, $P = 0.404$). In addition, *BRAF* V599E/V600E was associated with a short OS as compared with wild-type *KRAS/BRAF* genotypes (Fig. 2c. $P = 0.001$). The clinical courses of these six cases were as follows: Four suffered from recurrence (PFS = 1.7,

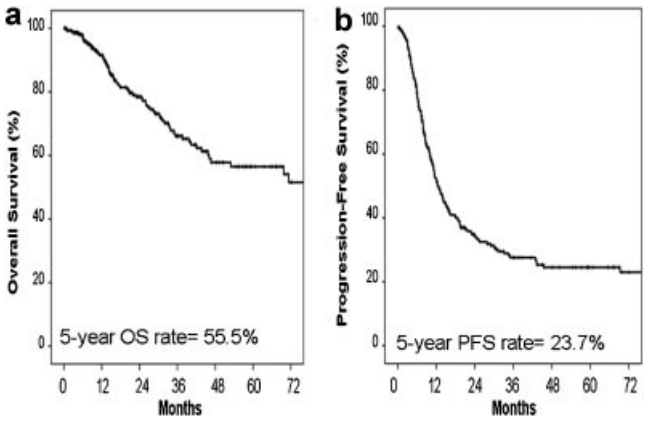

Fig. 1. Panel a: overall survival (OS) for patients with colorectal liver metastases after hepatic resection. Panel b: progression-free survival (PFS) for patients with colorectal liver metastases after hepatic resection.

5.8, 7.5, and 9.8 months), one remains disease free at 66.1 months, and the other was lost to follow-up in 3.1 months. All patients with recurrence died of overwhelming tumor recurrence despite receiving appropriate chemotherapy (three received Oxaliplatin, Irinotecan, and Fluorouracil (5-FU) and one received Oxaliplatin, Irinotecan, 5-FU, Bevacizumab, and Cetuximab).

Prognostic Factors of OS After Liver Metastasectomy

The variables affecting survival were examined (Table III). In the univariate analysis, synchronicity, a number of metastases greater than three, resection margin not free, concomitant extrahepatic disease, and *BRAF* mutation were found to be associated with poor survival. *BRAF* mutation remained an independent prognostic factor in the multivariate analysis (hazard ratio (HR) = 5.181, $P = 0.002$) (Table III).

Prognostic Factors of OS After Liver Metastasectomy in Patients Without Extrahepatic Metastases

After precluding extrahepatic disease, *BRAF* mutation, but not *KRAS* mutation, was still an independent prognostic factor in the multivariate analysis (HR = 6.245, $P = 0.003$) (Table IV and Fig. 3).

DISCUSSION

Till date, our study is the first large-scale assessment of the impact of *KRAS*/*BRAF* genotype (assessed using liver metastases specimens) on survival in patients after colorectal liver metastasectomy. In our results, *BRAF* genotype is an independent prognostic biomarker after adjustment for clinicopathological parameters such as number, lobar distribution, and extrahepatic metastases, but *KRAS* genotype is not a prognostic biomarker. A right-side colon location was found to be significantly associated with the existence of KRAS or *BRAF* mutations.

KRAS genotype, as determined from liver metastases specimens, was not a prognostic biomarker in our patients after liver metastasec-

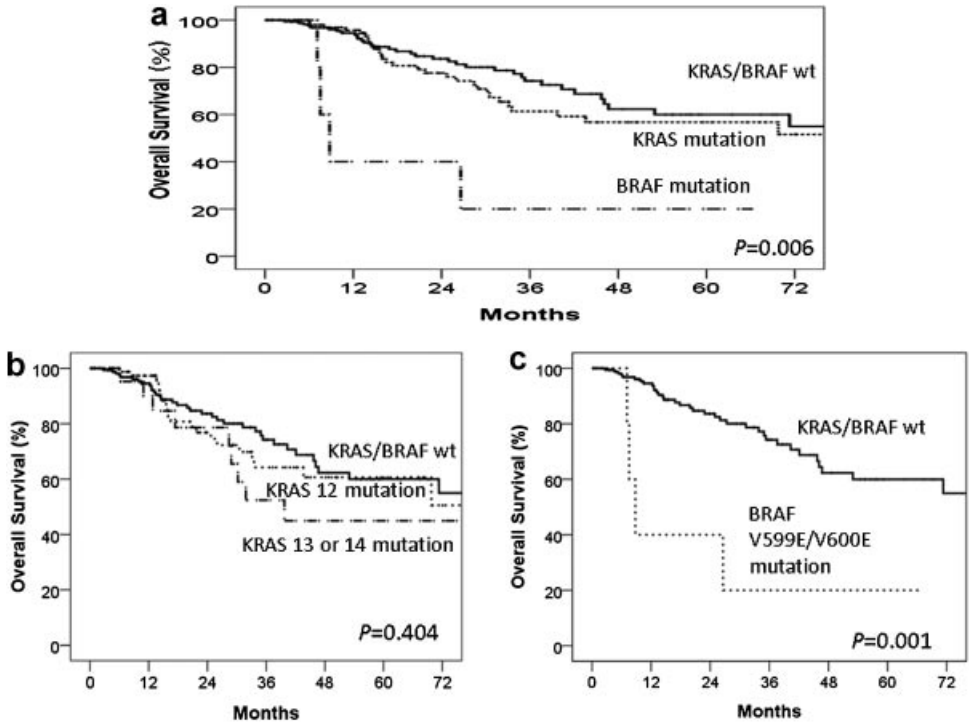

Fig. 2. Panel a: Overall survival (OS) for patients with colorectal liver metastases after hepatic resection according to *KRAS/BRAF* mutation status. Panel b: OS for patients with colorectal liver metastases after hepatic resection according to *KRAS* genotype. Panel c: OS for patients with colorectal liver metastases after hepatic resection according to *BRAF* genotype. *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *BRAF*, V-raf murine sarcoma viral oncogene homolog B1; wt, wild type.

TABLE III. Prognostic Factors for Overall Survival According to Univariate and Multivariate Analyses (n = 292)

Variable	Hazard ratios (95% CI)	*P*	Hazard ratios (95% CI)	*P*
Male	0.956 (0.604∼1.513)	0.849	—	—
Age > 60	0.838 (0.534∼1.315)	0.442	—	—
Initial Stage 3 or 4	2.275 (0.918∼5.639)	0.076		
Bilobar liver metastases	1.758 (0.892∼3.463)	0.103	—	—
Synchronous	1.644 (1.028∼2.629)	0.038	1.518 (0.930∼2.479)	0.095
Size > 30 mm	0.994 (0.632∼1.561)	0.978		
Number > 3	1.884 (1.136∼3.126)	0.014	1.983 (1.188∼3.311)	0.009
Positive margin	3.418 (1.848∼6.323)	<0.001	3.241 (1.728∼6.077)	<0.001
Concomitant extrahepatic disease	1.940 (1.193∼3.154)	0.008	1.858 (1.131∼3.053)	0.014
Neoadjvuant treatment	1.128 (0.593∼2.146)	0.714	—	—
Adjuvant chemotherapy	0.761 (0.411∼1.409)	0.385	—	—
Peri-operative anti-EGFR treatment	0.815 (0.328∼2.027)	0.660	—	—
KRAS mutation	1.240 (0.791∼1.945)	0.349	—	—
BRAF mutation	4.080 (1.485∼11.207)	0.006	5.181 (1.859∼14.437)	0.002

KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *BRAF*, V-raf murine sarcoma viral oncogene homolog B1; EGFR, epidermal growth factor receptor.

tomy. This observation is in agreement with most reports indicating that the use of *KRAS* genotype as a prognostic biomarker in metastatic colorectal cancer is controversial [7,10–14]. In previous studies, the *KRAS* genotype was analyzed from primary tumor specimens. The *KRAS* mutation rate in our liver metastases specimens was 38%, which is similar to the previously reported frequencies (range of 30–42%) in primary tumor specimens. Survival analysis did not clearly demonstrate that the OS for patients with *KRAS* codon 12, codon 13, or codon 14 mutations was poorer than that of *KRAS* wild-type patients. This is not consistent with previous reports using primary tumor specimens, which indicated that metastatic colorectal cancer patients with a *KRAS* codon 13 mutation, but not a *KRAS* codon 12 mutation, had a poorer prognosis than *KRAS* wild-type patients [14,15].

In our study, only one patient had a *KRAS* codon 14 mutation. This was also rare (0.1%) in the report of Vaughn et al. [16] and has previously been described in a limited number of colorectal tumors [17–19]. It may be pathogenic, based on studies showing the recombinant protein containing this mutation to have reduced GTPase activity [20] and to increase colony growth [20,21]. The rare incidence made the results difficult to interpret.

BRAF genotype as determined from liver metastases specimens was an independent prognostic biomarker in our patients who underwent liver metastasectomy after adjustment for significant clinicopathological parameters. This observation was consistent with reports that the *BRAF* genotype obtained from primary tumor specimens is a prognostic biomarker in metastatic colorectal cancer [7,10–14]. However, the *BRAF* mutation rate was only 2.1% in our study, which is lower than previously reported frequencies obtained from primary tumor specimens in Caucasian populations, which were found to be in the range of 7.9–13.3% [22–26]. Indeed, geographical variations have been reported, and the *BRAF* mutation rate has been found to be around 5–7% in Asian populations [14,27]. However, the *BRAF* mutation rate in our liver metastases specimens was far lower. Interestingly, our results were similar to those obtained in the CELIM trial (Caucasian population), which enrolled patients with potentially convertible colorectal liver metastases without extra-hepatic metastases. In that trial, it was found that the BRAF mutation rate was 3.2% (obtained from primary tumor specimens) [28]. One possible explanation for this discrepancy is selection bias. Patients with metastatic colorectal cancer with a *BRAF* mutation have a poor survival, which implies a relative resistance to

TABLE IV. Prognostic Factors for Overall Survival According to Univariate and Multivariate Analyses in Patients With Liver Metastases Only (n = 231)

Variable	Hazard ratios (95% CI)	*P*	Hazard ratios (95% CI)	*P*
Male	1.077 (0.609∼1.905)	0.799	—	—
Age >60	0.939 (0.543∼1.623)	0.821	—	—
Initial stage 3 or 4	2.260 (0.893∼5.723)	0.085		
Bilobar liver metastases	1.260 (0.494∼3.215)	0.629	—	—
Synchronous	3.088 (1.740∼5.479)	<0.001	3.258 (1.768∼6.002)	<0.001
Size >30 mm	0.853 (0.496∼1.469)	0.567		
Number >3	2.175 (1.189∼3.977)	0.012	1.543 (0.812∼2.933)	0.186
Positive margin	3.505 (1.625∼7.557)	0.001	4.464 (1.977∼10.080)	<0.001
Neoadjvuant treatment	0.716 (0.322∼1.592)	0.413	—	—
Adjuvant chemotherapy	0.863 (0.444∼1.678)	0.385	—	—
Peri-operative anti-EGFR treatment	0.616 (0.149∼2.539)	0.503	—	—
KRAS mutation	1.483 (0.860∼2.558)	0.156	—	—
BRAF mutation	4.735 (1.466∼15.295)	0.009	6.245 (1.894∼20.591)	0.003

KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *BRAF*, V-raf murine sarcoma viral oncogene homolog B1; EGFR, epidermal growth factor receptor.

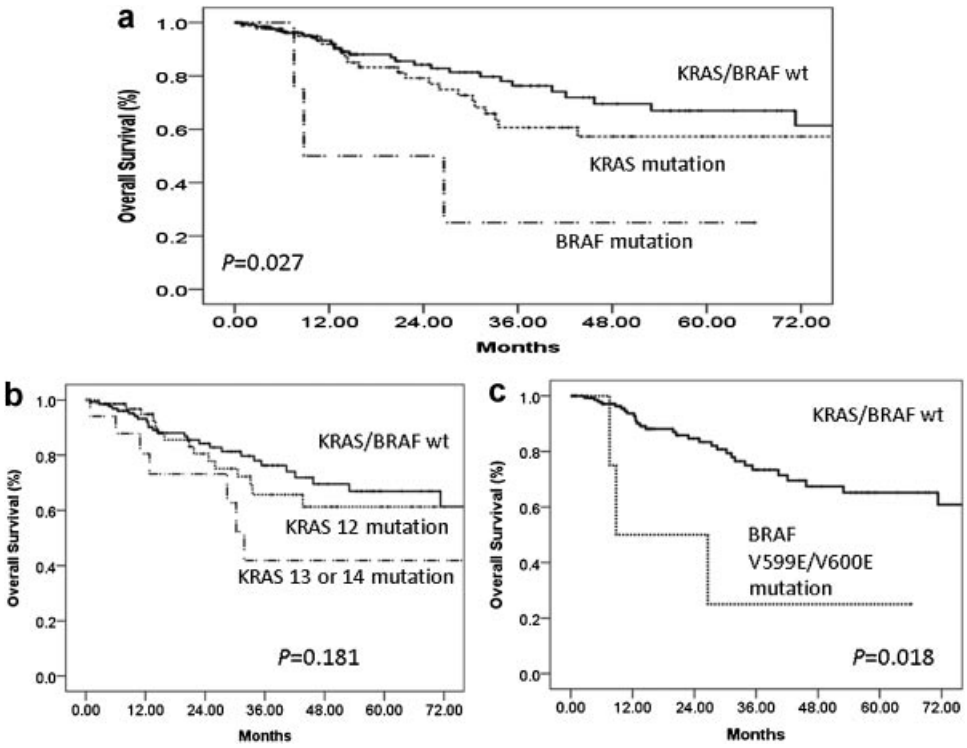

Fig. 3. Panel a: Overall survival (OS) for patients with colorectal liver metastases only (no extrahepatic metastases) after hepatic resection according to *KRAS/BRAF* mutation status. Panel b: OS for patients with colorectal liver metastases only after hepatic resection according to *KRAS* genotype. Panel c: OS for patients with colorectal liver metastases only after hepatic resection according to *BRAF* genotype. *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *BRAF*, V-raf murine sarcoma viral oncogene homolog B1; wt, wild type.

existing chemotherapy and tumor aggressiveness. Thus, these patients have fewer opportunities to receive hepatic resection due to advanced disease, a poor response to induction chemotherapy, and multiple metastasis [6,7,13,14,22,23,25]. Based on the above, *BRAF* mutation might be shown to be a potential biomarker for resectability of colorectal liver metastases in the future. More studies are warranted to confirm our observation.

The approach to the treatment of metastatic or recurrent colorectal cancer is evolving. Our study showed the results of actual clinical practice. According to the NCCN guidelines (2012. v2), resectable lung and extrapulmonary lesions do not preclude hepatic resection. However, patients with extrahepatic disease differ biologically to the liver-only population (more disseminated disease and a significantly poorer prognosis (Table III)). We performed the analysis after precluding extrahepatic disease, and *BRAF*, but not *KRAS*, was still an independent prognostic factor (Table IV and Fig. 3).

None of our patients carried both a *KRAS* and a *BRAF* mutation, which is similar to previous reports of metastatic colorectal cancer [17,29,30].

We also investigated the *KRAS/BRAF* genotype with respect to clinicopathological parameters, and found that both *KRAS* and *BRAF* mutations were dominant in right-side colon cancers, which was similar to previous reports using primary tumor specimens [14,31–33].

Our study had some limitations. First, the *KRAS/BRAF* genotype was obtained from liver metastases specimens. We did not obtain counterpart primary tumor specimens, and thus we were unable to directly prove our speculation that the lower *BRAF* mutation rate was due to selection bias. However, Knijn et al. [34] demonstrated a high concordance of *KRAS* mutation status between primary colorectal tumors and their corresponding liver metastases. The authors suggested that both primary tumors and liver metastases can be used for *KRAS* mutation analysis. In addition, another research group found a high concordance in *BRAF* status between primary and metastatic tumors [27][35]. These findings could partially validate our hypothesis. Second, although our sample size was large, only 33 (11.3%) patients received anti-EGFR treatment, and thus we were not able to assess whether *KRAS* or *BRAF* genotype was a predictive biomarker for anti-EGFR treatment. Third, we identified mutations at two *BRAF* codons. We were unable to perform further analysis of the two *BRAF* codon mutations due to the small population (n = 6).

In conclusion, the *BRAF* mutation rate determined from liver metastases specimens was lower than the rates stated in previous reports using primary tumor specimens. *BRAF* mutation might act as an independent prognostic biomarker in patients with colorectal liver metastases after hepatic resection.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Po-Min Chen for his discussions. This work was supported in part by the Division of Experimental Surgery of the Department of Surgery, Taipei Veterans General Hospital, and also by Taipei Veterans General Hospital, Taiwan Clinical Oncology Research Foundation, Department of Health, Taiwan (Center of Excellence for Cancer Research at Taipei Veterans General Hospital: DOH99-TD-C-111-007 and DOH99-TD-C-111-007); Taipei Veterans General Hospital (100DHA0100416, 99DHA0100446, 101DHA0100661, 101DHA0100369).

REFERENCES

1. Khatri VP, Petrelli NJ, Belghiti J: Extending the frontiers of surgical therapy for hepatic colorectal metastases: Is there a limit? J Clin Oncol 2005;23:8490–8499.
2. Primrose JN: Surgery for colorectal liver metastases. Br J Cancer 2010;102:1313–1318.
3. Morris EJ, Forman D, Thomas JD, et al.: Surgical management and outcomes of colorectal cancer liver metastases. Br J Surg 2010;97:1110–1118.
4. Cummings LC, Payes JD, Cooper GS: Survival after hepatic resection in metastatic colorectal cancer: A population-based study. Cancer 2007;109:718–726.
5. Schindl M, Wigmore SJ, Currie EJ, et al.: Prognostic scoring in colorectal cancer liver metastases: Development and validation. Arch Surg 2005;140:183–189.
6. Van Cutsem E, Kohne CH, Hitre E, et al.: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360:1408–1417.
7. Roth AD, Tejpar S, Delorenzi M, et al.: Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: Results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol 2010;28:466–474.
8. Samowitz WS, Sweeney C, Herrick J, et al.: Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res 2005;65:6063–6069.
9. Baldus SE, Schaefer KL, Engers R, et al.: Prevalence and heterogeneity of KRAS, BRAF, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases. Clin Cancer Res 2010;16:790–799.
10. Andreyev HJ, Norman AR, Cunningham D, et al.: Kirsten ras mutations in patients with colorectal cancer: The multicenter ''RASCAL'' study. J Natl Cancer Inst 1998;90:675–684.
11. French AJ, Sargent DJ, Burgart LJ, et al.: Prognostic significance of defective mismatch repair and BRAF V600E in patients with colon cancer. Clin Cancer Res 2008;14:3408–3415.
12. Kakar S, Deng G, Sahai V, et al.: Clinicopathologic characteristics, CpG island methylator phenotype, and BRAF mutations in microsatellite-stable colorectal cancers without chromosomal instability. Arch Pathol Lab Med 2008;132:958–964.
13. Ogino S, Nosho K, Kirkner GJ, et al.: CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut 2009;58:90–96.
14. Yokota T, Ura T, Shibata N, et al.: BRAF mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer. Br J Cancer 2011;104:856–862.
15. Farina-Sarasqueta A, van Lijnschoten G, Moerland E, et al.: The BRAF V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. Ann Oncol 2010;21:2396–2402.
16. Vaughn CP, Zobell SD, Furtado LV, et al.: Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. Genes Chromosomes Cancer 2011;50:307–312.
17. Ahlquist T, Bottillo I, Danielsen SA, et al.: RAS signaling in colorectal carcinomas through alteration of RAS, RAF, NF1, and/or RASSF1A. Neoplasia 2008;10:680–686 682 p following 686.
18. Ferraz JM, Zinzindohoue F, Lecomte T, et al.: Impact of GSTT1, GSTM1, GSTP1 and NAT2 genotypes on KRAS2 and TP53 gene mutations in colorectal cancer. Int J Cancer 2004;110:183–187.
19. He Y, Van't Veer LJ, Mikolajewska-Hanclich I, et al.: PIK3CA mutations predict local recurrences in rectal cancer patients. Clin Cancer Res 2009;15:6956–6962.
20. Schubbert S, Zenker M, Rowe SL, et al.: Germline KRAS mutations cause Noonan syndrome. Nat Genet 2006;38:331–336.
21. Tyner JW, Erickson H, Deininger MW, et al.: High-throughput sequencing screen reveals novel, transforming RAS mutations in myeloid leukemia patients. Blood 2009;113:1749–1755.
22. Barault L, Veyrie N, Jooste V, et al.: Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. Int J Cancer 2008;122:2255–2259.
23. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al.: Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res 2007;67:2643–2648.
24. Richman SD, Seymour MT, Chambers P, et al.: KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: Results from the MRC FOCUS trial. J Clin Oncol 2009;27:5931–5937.
25. Tol J, Nagtegaal ID, Punt CJ: BRAF mutation in metastatic colorectal cancer. N Engl J Med 2009;361:98–99.
26. Price TJ, Hardingham JE, Lee CK, et al.: Impact of KRAS and BRAF gene mutation status on outcomes from the phase III AGITG MAX trial of capecitabine alone or in combination with bevacizumab and mitomycin in advanced colorectal cancer. J Clin Oncol 2011.
27. Park JH, Han SW, Oh DY, et al.: Analysis of KRAS, BRAF, PTEN, IGF1R, EGFR intron 1 CA status in both primary tumors and paired metastases in determining benefit from cetuximab therapy in colon cancer. Cancer Chemother Pharmacol 2011;68:1045–1055.
28. Folprecht G, Gruenberger T, Bechstein WO, et al.: Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: The CELIM randomised phase 2 trial. Lancet Oncol 2010;11:38–47.
29. Frattini M, Balestra D, Suardi S, et al.: Different genetic features associated with colon and rectal carcinogenesis. Clin Cancer Res 2004;10:4015–4021.
30. Rajagopalan H, Bardelli A, Lengauer C, et al.: Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature 2002;418:934.
31. Deng G, Kakar S, Tanaka H, et al.: Proximal and distal colorectal cancers show distinct gene-specific methylation profiles and clinical and molecular characteristics. Eur J Cancer 2008;44:1290–1301.
32. Kim IJ, Kang HC, Jang SG, et al.: Oligonucleotide microarray analysis of distinct gene expression patterns in colorectal cancer tissues harboring BRAF and K-ras mutations. Carcinogenesis 2006;27:392–404.
33. Zlobec I, Bihl MP, Schwarb H, et al.: Clinicopathological and protein characterization of BRAF- and K-RAS-mutated colorectal cancer and implications for prognosis. Int J Cancer 2010;127:367–380.
34. Knijn N, Mekenkamp LJ, Klomp M, et al.: KRAS mutation analysis: A comparison between primary tumours and matched liver metastases in 305 colorectal cancer patients. Br J Cancer 2011;104:1020–1026.
35. Santini D, Spoto C, Loupakis F, et al.: High concordance of BRAF status between primary colorectal tumours and related metastatic sites: Implications for clinical practice. Ann Oncol 2010;21:1565.
36. *BRAF*, but not *KRAS*, mutation was found to be a poor prognostic biomarker of liver metastasectomy and might be a potential biomarker for resectability of colorectal liver metastases.