World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v8.i1.128

World J Gastrointest Oncol 2016 January 15; 8(1): 128-135
ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Impact of *RAS* and *BRAF* mutations on carcinoembryonic antigen production and pattern of colorectal metastases

May Cho, Chie Akiba, Cecilia Lau, David Smith, Milhan Telatar, Michelle Afkhami, Stephen Sentovich, Kurt Melstrom, Marwan Fakih

May Cho, Chie Akiba, Cecilia Lau, Marwan Fakih, Department of Medical Oncology, City of Hope National Medical Center, Duarte, CA 91010, United States

David Smith, Department of Statistics, City of Hope National Medical Center, Duarte, CA 91010, United States

Milhan Telatar, Michelle Afkhami, Department of Pathology, City of Hope National Medical Center, Duarte, CA 91010, United States

Stephen Sentovich, Kurt Melstrom, Department of Surgical Oncology, City of Hope National Medical Center, Duarte, CA 91010, United States

Author contributions: Fakih M designed and supervised the trial; Fakih M and Cho M wrote the paper; Telatar M, Afkhami M, Sentovich S and Melstrom K contributed to the analysis; Cho M, Akiba C and Lau C collected and analysed data; Smith D did the statistical analysis.

Institutional review board statement: This study was reviewed and approved by the City of Hope National Medical Center.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data.

Conflict-of-interest statement: We have no pertinent financial relationships to disclose.

Data sharing statement: No additional data are available.

Correspondence to: Marwan Fakih, MD, Professor, Department of Medical Oncology, City of Hope National Medical Center, Building 51, Room 101, 1500 E Duarte St., Duarte, CA 91010, United States. mfakih@coh.org
Telephone: +1-626-2564673-63087
Fax: +1-626-3018233

Received: July 3, 2015
Peer-review started: July 10, 2015
First decision: September 30, 2015
Revised: October 22, 2015
Accepted: November 23, 2015
Article in press: November 25, 2015
Published online: January 15, 2016

Abstract

AIM: To investigate the impact of *RAS* and *BRAF* mutations on the pattern of metastatic disease and carcinoembryonic antigen (CEA) production.

METHODS: In this retrospective study, we investigated the impact of *RAS* and *BRAF* mutational status on pattern of metastatic disease and CEA production. Only patients presenting with a newly diagnosed metastatic colorectal cancer (CRC) were included. Patients' characteristics, primary tumor location, site of metastatic disease and CEA at presentation were compared between those with and without *RAS* and *BRAF* mutations.

RESULTS: Among 174 patients, mutations in *KRAS*, *NRAS* and *BRAF* were detected in 47%, 3% and 6% respectively. *RAS* mutations (*KRAS* and *NRAS*) were more likely to be found in African American patients (87% *vs* 13%; *P* value = 0.0158). *RAS* mutations were associated with a higher likelihood of a normal CEA (< 5 ng/mL) at presentation. *BRAF* mutations were more likely to occur in females. We were not able to confirm

any association between mutational status and site of metastatic disease at initial diagnosis.

CONCLUSION: No association was found between *RAS* and *BRAF* mutations and sites of metastatic disease at the time of initial diagnosis in our cohort. Patients with *RAS* mutations were more likely to present with CEA levels < 5 ng/mL. These findings may have clinical implications on surveillance strategies for *RAS* mutant patients with earlier stages of CRC.

Key words: *RAS*; *BRAF*; Carcinoembryonic antigen; Pattern of metastatic disease; Surveillance

Core tip: We investigated the impact of *RAS* and *BRAF* mutations on pattern of colorectal cancer (CRC) metastases and carcinoembryonic antigen (CEA) production. Patients with *RAS* mutations were more likely to present with CEA levels < 5 ng/mL. No association was found between *RAS* and *BRAF* mutations and sites of metastatic disease at the time of initial diagnosis in our cohort. Our study is the first study to link low CEA production with a *RAS* mutant status at the time of initial presentation of metastatic CRC. These findings may have clinical implications on surveillance strategies for *RAS* mutant patients with earlier stages of CRC.

Cho M, Akiba C, Lau C, Smith D, Telatar M, Afkhami M, Sentovich S, Melstrom K, Fakih M. Impact of *RAS* and *BRAF* mutations on carcinoembryonic antigen production and pattern of colorectal metastases. *World J Gastrointest Oncol* 2016; 8(1): 128-135 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i1/128.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i1.128

INTRODUCTION

Colorectal cancer (CRC) continues to be the second leading cause of cancer-related death in the United States. It is projected that 136830 individuals will be diagnosed with CRC in 2014 in the United States, 50310 of whom will succumb to this disease[1]. While significant progress has been made in the treatment of metastatic CRC (mCRC) over the last two decades, cure amongst these patients remains rare and is only achievable in approximately 20% of patients who are amenable to metastases resection[2,3].

It is estimated that 20% of patients with CRC present with metastatic disease while another 30% develop metastatic disease after an initial presentation with local or regional disease[2,4]. Patients with limited oligometastatic disease are the ones who benefit the most from aggressive surgical strategies[5]. Therefore, early identification of metastatic disease remains key in improving the outcome of patients with metastatic disease. Indeed, intensive surveillance strategies in patients with earlier stages of CRC have been associated with an increased rate of metastectomies in several prospective and retrospective clinical trials[6]. However, these surveillance strategies are not standardized amongst different medical societies and do not take into account the molecular heterogeneity of CRC[7]. It has been recently shown that certain oncogenic alterations have significant impact on disease biology, response to treatment, and overall outcome. For example, *BRAF* mutations, present in 5%-10% of CRCs, are associated with worse prognosis, a worse overall survival after disease recurrence, and a tendency to metastasize to the peritoneum and distant lymph nodes[8,9]. The impact of *KRAS* and *NRAS* mutations, which occur in approximately 50% of CRCs, on the pattern of metastatic disease at initial presentation has been more controversial[10-13].

To better understand the impact of the commonly tested *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations on metastatic disease pattern and on surveillance strategies, we conducted a single institute retrospective study that investigates the impact of *RAS* and *BRAF* mutations on the pattern of metastatic disease and carcinoembryonic antigen (CEA) production.

MATERIALS AND METHODS

Study population

We retrospectively reviewed all cases with metastatic colon cancer patients who presented to City of Hope Comprehensive Cancer Center from 2007 to 2014. Inclusion on study required all the following criteria: (1) confirmed CRC by pathology; (2) availability of imaging studies confirming metastatic disease at the time of presentation; (3) availability of *KRAS* or *BRAF* testing by PCR or by ONCO44 or ONCO48 next generation sequencing; and (4) available CEA level at the time of presentation of metastatic CRC.

Patients' characteristics including age, gender, race, location of the primary tumor, CEA, and sites of metastatic disease at the time of presentation were reviewed and collected from corresponding electronic medical records. Primary tumor location was categorized as right or transverse colon, left colon, and rectum. Metastatic sites were categorized into 3 groups: (1) lung; (2) liver; and (3) mesenteric or distal lymph nodes or peritoneum. The study was approved by the local institutional review board.

RAS and BRAF analysis

To allow for a more powerful sample size, we included *RAS* and *BRAF* analysis performed by either a CLIA certified next generation sequencing or a CLIA certified PCR assay.

Onco 44: Genomic DNA is extracted from micro-dissected cells from formalin-fixed, paraffin-embedded tissue with minimum 30% tumor cellularity. A targeted DNA library is generated using the Ion AmpliSeq™ Cancer

Table 1 *RAS* and *BRAF* status and patient demographics

		All		*BRAF* MT		*BRAF* WT		*P*	*RAS* MT		*RAS* WT		*P*
		n	%	*n*	%	*n*	%		*n*	%	*n*	%	
Age	< 60	87	50	5	6	82	94	1.00	44	51	43	49	1.00
	⩾ 60	87	50	6	7	81	93		43	49	44	51	
Gender	Male	103	59	3	3	100	97	0.052	49	48	54	52	0.54
	Female	71	41	8	11	63	89		38	54	33	46	
Race	White	122	70	7	6	115	94	0.53	57	47	65	53	0.015
	Asian, PI	41	24	4	10	37	90		23	56	18	44	
	Black	8	5	0	--	8	100		7	87	1	13	
	Unknown	3	2	0	--	3	100		0	--	3	100	

n: Number of patients; PI: Pacific Islander; MT: Mutant; WT: Wild type.

Hotspot Panel Kit, and sequenced by semiconductor-based next-generation sequencing technology on an Ion Torrent PGM. The Onco 44 pannel is designed to target 713 mutations in 44 key cancer genes that include *KRAS, NRAS,* and *BRAF.* Tested *KRAS* mutations include codon 12, 13, 61 and 146. Tested NRAS mutations include codon 12, 13, 61.

Onco 48: The Onco48 Panel is designed to target 2800 mutations in 48 key cancer genes. The difference between Onco 44 and 48 is the additional sequencing of 4 target genes: EZH2, GNA11, GNAQ, and IDH2. In addition, the Onco48 panel identifies the rare *KRAS* codon 117 and *NRAS* codon 146 mutations.

***KRAS*-PCR:** DNA was extracted from micro-dissected cells from formalin-fixed, paraffin-embedded tissue with minimum 30% tumor cellularity. A PCR based fragment analysis with 5% sensitivity using "Shift Termination" technology was used to detect mutations in the *KRAS* gene. This assay is CLIA approved and detects 6 mutations on codon 12 and 1 mutation on codon 13 of exon 2.

***BRAF*:** Genomic DNA is extracted from micro-dissected cells from formalin-fixed, paraffin-embedded tissue with minimum 30% tumor cellularity. A real-time PCR assay with 1% sensitivity was performed to detect the c.1799 T > A (V600E) mutation in the *BRAF* gene.

CEA assay

CEA was tested *via* Siemens Advia Centaur chemiluminescent immunoassay and normal range is 0.5 ng/mL to 4.5 ng/mL.

Statistical analysis

We tested for differences in proportions between rate of mutations *vs* clinical and demographic factors with Fisher's Exact Tests. We also tested for differences in proportions between rate of mutations *vs* site of metastatic disease, location of primary disease, and CEA (cut point of 5 ng/mL) with Fisher's Exact Tests. For testing the association between metastases site and CEA as a continuous variable, we transformed CEA using the natural logarithm and used it as the independent variable in a logistic regression. The dependent variable in the logistic regression was presence or absence of a given metastases location.

KRAS and *NRAS* mutations were categorized under *RAS* mutations, irrespective of the testing methodology. Comparative analysis was performed on 4 distinct subgroups: *RAS* mutant, *RAS* wild type, *BRAF* mutant, and *BRAF* wild type populations.

RESULTS

The study population consisted of 174 patients who presented with metastatic colon cancer patients and documented *RAS* and *BRAF* mutational analysis. Genomic evaluation for *KRAS, NRAS*, and *BRAF* was performed by next generation sequencing using ONCO44 or ONCO48 in 122 patients. 52 patients were evaluated for *KRAS* (no *NRAS* evaluation) and *BRAF* mutation by PCR. Eighty-seven (50%) of patients had an identifiable *RAS* mutations (47% *KRAS* and 3% *NRAS*). Only 11 patients (6%) had *BRAF* mutation (Table 1).

RAS and BRAF mutations and patients' demographics

The median age of the study population was 60 years (range 23 to 87 years). There was no difference in *RAS* or *BRAF* mutation status by age, or gender. However, females had a trend towards a higher incidence of *BRAF* mutation. No distinct variations were noted in *KRAS* or *BRAF* mutations by race, with the exception of an increased rate of *RAS* mutations among African Americans. However, African American representation on this study was low (5%), limiting the interpretation of this finding (Table 1).

RAS and BRAF status, primary tumor location and pattern of metastases

There was no difference in primary tumor sites by *KRAS* or *BRAF* status, with the exception of a lower likelihood of *BRAF* mutations among rectal cancers. In addition, no difference in tumor spread pattern at the time of metastatic disease presentation was noted among the 4 molecular subgroups (Table 2).

Table 2 *RAS* and *BRAF* status and primary tumor location and pattern of metastasis

		All		*BRAF* MT		*BRAF* WT		*P*	*RAS* MT		*RAS* WT		*P*
		n	%	*n*	%	*n*	%		*n*	%	*n*	%	
Primary lesion	Rectal	43	25	0	--	43	100	0.022	23	53	20	47	0.23
	Left colon	79	45	5	6	74	94		34	43	45	57	
	Right colon	52	30	6	12	46	88		30	58	22	42	
Site of metastasis	Lung	72	42	3	4	69	96	0.36	41	57	31	42	0.17
	Liver	98	56	5	5	93	95	0.54	46	47	52	53	0.44
	Peritoneal	49	28	5	10	44	90	0.3	23	47	26	53	0.74

n: Number of patients; MT: Mutant; WT: Wild type.

Table 3 *RAS* and *BRAF* status and carcinoembryonic antigen levels

	All		*BRAF* MT		*BRAF* WT		*P*	*RAS* MT		*RAS* WT		*P*
	n	%	*n*	%	*n*	%		*n*	%	*n*	%	
< 5 ng/mL	60	34	4	7	56	93	1.00	37	62	23	38	0.037
⩾ 5 ng/mL	114	66	7	6	107	94		64	56	50	44	

n: Number of patients; MT: Mutant; WT: Wild type.

RAS and BRAF status and CEA production

Thirty-four percent of the total cohort were non-CEA producers (CEA < 5 ng/mL). Patients with liver metastases were more likely to produce CEA (OR = 0.639; $P < 0.0001$) while patients with peritoneal/mesenteric metastases were less likely to produce CEA (OR = 1.315; $P = 0.0010$). Patients with *RAS* mutation were more likely to be low-CEA producers at the time of metastatic disease presentation (Table 3). There was no significant association between *BRAF* mutation status and CEA production.

DISCUSSION

In this study we sought to explore correlations between *RAS* and *BRAF* mutational status, patient demographics, metastatic disease pattern, and CEA production. No distinct demographic characteristics were associated with *RAS* or *BRAF* status, with the exception of *BRAF* mutations which were less likely to occur with a rectal primary. Although not statistically significant, females were more likely to harbor a *BRAF* mutation. These findings are consistent with prior reports[9,12,14,15]. We were not able to confirm an association between *BRAF* mutations and age or a right colon primary, contrary to previous reports[9,12,14,15]. This discordance is likely related to our more limited sample size, especially that the percentages of *RAS*-mutant and *RAS*-wild type patients with right colonic primaries were in line with the above referenced studies. We also investigated the impact of race on *RAS* and *BRAF* mutational status. The only positive association was for *RAS* mutation and African American race. Several studies have previously evaluated the impact of race on *RAS* and *BRAF* mutational status[16,17]. The N0147 adjuvant clinical trial in patients with stage Ⅲ colon cancer reported an increased likelihood of *BRAF* mutation amongst White and an increased *KRAS* mutation frequency in African Americans[18]. In addition, N0147 reported a lower frequency of *KRAS* mutations in Asians, a finding not supported by our study.

Contrary to the current literature, we did not find an association between *BRAF* mutation and peritoneal mestastases at the time of presentation, likely due to our small *BRAF* mutant sample size. Several studies have reported an increased likelihood of peritoneal dissemination in *BRAF* mutant mCRC patients[8,9,19]. Yaeger *et al*[9] reported that patients with *BRAF* mutations were more likely to present with peritoneal metastases at initial diagnosis and less likely to have liver-limited metastases. Moreover, the 2-year cumulative incidence of peritoneal metastases was higher with *BRAF* mutated tumors[9]. Tran *et al*[8] reported a higher rate of peritoneal and distant lymph node metastases and a lower rate of lung metastases in *BRAF* mutated tumors. Similarly, Russo *et al*[19] reported a higher likelihood of *BRAF* mutations in patients with distant lymph node metastases at the site of first recurrence. Finally, Kawazoe *et al*[12] retrospectively studied the clinical-pathological features of *BRAF* mutations in Japanese patients with metastatic CRC and found that peritoneal metastases are more frequently observed in *BRAF* mutated patients. Since the presence of peritoneal metastases has been identified as a poor prognostic factor, a higher incidence of peritoneal metastases in *BRAF* tumors may partly explain the poor prognosis associated with this subgroup[12,20,21]. These studies are summarized in Table 4.

Our study did not confirm an association between *RAS* mutations and lung metastases at initial mCRC presentation. There is discordance among studies on the impact of *RAS* mutational status on lung metastases at the time of initial mCRC presentation. However,

Table 4 *BRAF* status and pattern of colon cancer metastases

Ref.	*n* (%*BRAF*)	End point	%*BRAF* MT *vs* %*BRAF* WT	*P*
Tran *et al*[8]	524 (11%)	Rate of peritoneal metastases	46% *vs* 24%	0.001
		Rate of distant lymph node metastases	53% *vs* 38%	0.008
		Rate of lung metastases	35% *vs* 49%	0.049
Yaeger *et al*[9]	515 (18%)	Peritoneal involvement at presentation	26% *vs* 14%	< 0.01
Kawazoe *et al*[12]	264 (5%)	Peritoneal metastasis	50% *vs* 18%	0.009

n: Total number of patients; %*BRAF*: %patients with *BRAF* mutation; MT: Mutant; WT: Wild type.

Table 5 *RAS* status and pattern of colon cancer metastases

Ref.	*n* (%MT)	Results	*P*
Cejas *et al*[29]	110 (34% *KRAS* MT)	Frequency of *KRAS* mutation in primary tumor of patients with lung *vs* liver metastases 59% *vs* 32%	0.054
Tie *et al*[10]	Cohort A 161 (48.4% *KRAS* MT)	Mutation frequencies in lung in *KRAS* MT *vs* WT 62% *vs* 38%	0.003
		Mutation frequencies in brain in *KRAS* MT *vs* WT 56.5% *vs* 43.5%	0.003
	Cohort C 859 (33.8% *KRAS* MT)	Relapse in lung in *KRAS* MT HR 2.1, 95%CI: 1.2-3.5	0.007
Kim *et al*[23]	143 (43.4% *KRAS* MT)	Lung as initial metastatic site in *KRAS* MT *vs* WT 45.3% *vs* 22.1%	0.003
		Liver as initial metastatic site in *KRAS* MT *vs* WT 37.3% *vs* 70.6%	< 0.001
		Distant lymph node as initial metastatic site in *KRAS* MT *vs* WT 6.7% *vs* 19.1%	0.025
Vauthey *et al*[25]	193 (18% All *RAS* MT)	3-yr lung RFS rate in patients undergoing curative resection of liver metastases in *RAS* MT *vs* WT 34.6% *vs* 59.3%	< 0.001
Yaeger *et al*[11]	918 (48% All *RAS* MT)	Lung as site of first metastasis in *RAS* MT *vs* WT 22% *vs*%	< 0.01
		Cumulative incidence of lung as subsequent metastasis at 2 yr after diagnosis in RAS MT *vs* WT 32.5% *vs* 19%	< 0.001
Kemeny *et al*[24]	169 (30% *KRAS* MT)	3-yr cumulative recurrence rate to lung after hepatic resection and HAI in *KRAS* MT *vs* WT 58% *vs* 32%	< 0.01
		3-yr cumulative recurrence rate to brain after hepatic resection and HAI in *KRAS* MT *vs* WT 14.5% *vs* 2%	0.05
		3-yr cumulative recurrence rate to bone after hepatic resection and HAI in *KRAS* MT *vs* WT 13.4% *vs* 2%	< 0.01
Pereira *et al*[13]	494 (41% *KRAS* MT)	Time to lung metastasis (median months) in *KRAS* MT *vs* WT 15.2 *vs* 22.4 (HR 1.4)	0.002

n: Total number of patients; %MT: %patients with mutation; MT: Mutant; WT: Wild type; RFS: Recurrence free survival; HAI: Hepatic arterial infusion.

clinical studies have consistently shown an association between *KRAS* mutation and lifetime likelihood of lung metastases in patients with mCRC, but not at initial presentation (Table 5). In our previous study, conducted on a different patient data set, Sharma *et al*[22] reported no predictive role for *KRAS* mutations on the site(s) of metastatic disease at the time of presentation. Pereira *et al*[13] retrospectively evaluated patients with mCRC who were tested for *KRAS* mutation at MD Anderson Cancer Center. They did not report an increase rate of lung metastases in *KRAS* mutated patients at the time of diagnosis of mCRC. However, *KRAS* mutation was found to have a shorter time to lung metastases and a two-fold greater odd of developing lifetime lung metastases in a cohort of a liver-limited CRC. However, several other studies reported that *KRAS* mutant patients were more likely to present with lung metastases than *KRAS* wild type patients. Kim *et al*[23] reported on the initial metastatic disease patterns in South Korean patients with mCRC. Lung metastases were more frequent

as the initial metastasic site in *KRAS* mutant patients while liver and distant lymph node metastases were less likely[23]. Yaeger *et al*[11] reported on the impact of *KRAS* mutations on the pattern of metastatic spread in CRC. In this retrospective study, *KRAS* mutant patients had a higher incidence of lung metastases at initial presentation compared to *KRAS* wild type patients. In addition, *KRAS* mutated patients had higher cumulative incidence of lung, bone and brain metastases at two years from initial mCRC presentation. Fewer patients had liver-limited disease at the initial presentation in *KRAS* mutated patients than *KRAS* wild type patients[11]. *KRAS* mutations have also been associated with a higher risk of lung relapse while *NRAS* mutations were associated with increased local recurrence after curative resection of primary CRC or after curative intent hepatectomy[10,24,25]. Review of patients with stage Ⅱ and Ⅲ primary CRC who participated in VICTOR clinical trial showed an association between *KRAS* mutations and an increased relapse rate in the lung. Relapse in the liver was similar between *KRAS* mutant and wild type patients[10]. Kemeny *et al*[24] reported on the pattern of metastatic disease recurrence in patients who underwent hepatic resection and adjuvant HAI plus systemic chemotherapy. The three-year cumulative incidence of lung metastases was higher in the *KRAS* mutant patients. The cumulative incidence of bone and brain metastases was also increased in the *KRAS* mutant patients. Similarly, Vauthey *et al*[25] reported that patients with *KRAS* mutant tumors who underwent curative intent liver resection at MD Anderson cancer center had a lower three-year lung RFS in comparison to patients with *KRAS* wild type tumors. Based on the above studies (summarized in Table 5), *KRAS* mutant mCRC patients have an increased lifetime risk of developing lung metastases. However, the impact of *KRAS* mutational status on the incidence of lung metastases at the initial time of diagnosis of metastatic disease remains controversial. Whether the lack of association between lung metastases at presentation and *KRAS* mutations is related to a limited sample size on those studies *vs* being the result of tumor biology remains unclear.

We have studied the impact of *RAS* and *BRAF* mutational status on CEA levels at the time of initial diagnosis of metastatic disease. We did not find any difference in CEA levels between *BRAF* mutant and *BRAF* wild type mCRC at initial presentation. In contrast, *RAS* mutant mCRC patients were more likely to be non-CEA producers (62% *RAS*-MT *vs* 38% *RAS*-WT) (Table 3). Our findings are in contrast to a study by Selcukbiricik *et al*[26] which reported a higher percentage of patients with CEA > 5 ng/mL among the *KRAS* mutant cohort. Selcurkbiricik study was limited by stage heterogeneity (stages Ⅰ-Ⅳ) and did not include an analysis of the impact of *RAS* mutation within the stage Ⅳ disease cohort. Our study also showed an association between CEA levels and site of metastatic disease. CEA was more likely to be elevated in patients with liver metastases and lower in patients with peritoneal or mesenteric recurrence, which is consistent with prior reports[27].

Our study has several limitations. This is a single institution study with a relatively small size. Modest associations between *RAS* and *BRAF* status and other clinical variables may have therefore been missed due to the lack of adequate power. In addition, the diagnosis of metastatic disease on this study could have been made during surveillance for disease recurrence or during the work-up of symptomatic disease. Therefore, the conclusions derived from this study may not be clearly generalizable to the surveillance population or to the population presenting with symptomatic stage Ⅳ disease. Other limitations include the inclusion of patients with *KRAS* PCR mutation assay (no ONCO48 analysis). This implies that some patients may have been assigned to the *RAS* wild type subgroup without ruling out the possibility of *NRAS* or non-exon 2 *KRAS* mutations. The likelihood of this event impacting our overall results is low as only 52 patients (30%) of our study population was analyzed by *KRAS*-PCR only. Given that less than 10% of the general population carries a non-exon 2 *KRAS* mutation or *NRAS* mutations, we expect that less than 10 patients may have been inappropriately labeled.

In summary, our study is the first study to link low CEA production with a *RAS* mutant status at the time of initial presentation of metastatic CRC. If validated in larger studies, especially in surveillance settings, our findings would have major clinical significance. It has been recently confirmed that *RAS* mutations increase the risk of systemic disease recurrence after a curative resection in patients with stage Ⅲ colon cancer[28]. Reliable screening strategies are especially important in this high risk population in order to diagnosis early recurrence and increase the likelihood of curative-intent metastectomies. If CEA is confirmed as a less reliable screening strategy, intense radiographic screening will be especially important as a complement to CEA screening in this population.

COMMENTS

Background

It is estimated that 20% of patients with colorectal cancer (CRC) present with metastatic disease while another 30% develop metastatic disease after an initial presentation with local or regional disease. Patients with limited oligometastatic disease are the ones who benefit the most from aggressive surgical strategies. Therefore, early identification of metastatic disease remains key in improving outcome. It has been recently shown that certain oncogenic alterations have significant impact on disease biology, response to treatment, and overall outcome. To better understand the impact of the commonly presented *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations on metastatic disease pattern and on surveillance strategies, the authors conducted a single institute retrospective study that investigates the impact of *RAS* and *BRAF* mutations on the pattern of metastatic disease and carcinoembryonic antigen (CEA) production.

Research frontiers

BRAF mutations, present in 5%-10% of CRCs, are associated with worse prognosis, a worse overall survival after disease recurrence, and a tendency to metastasize to the peritoneum and distant lymph nodes. The impact of *KRAS* and *NRAS* mutations, which occur in approximately 50% of CRCs, on the

pattern of metastatic disease at initial presentation has been more controversial. No studies have reported on the impact of either *RAS* or *BRAF* mutations on CEA production.

Innovations and breakthroughs

The authors did not find any difference in CEA levels between *BRAF* mutant and *BRAF* wild type mCRC at initial presentation. In contrast, *RAS* mutant mCRC patients were more likely to be non-CEA producers (62% *RAS*-MT *vs* 38% *RAS*-WT).

Applications

The study is the first study to link low CEA production with a *RAS* mutant status at the time of initial presentation of metastatic CRC. If validated in larger studies, especially in surveillance settings, the authors' findings may indicate that CEA surveillance is less reliable in curatively resected *RAS* mutant CRC patients. Alternative surveillance strategies may be required in this patients population.

Terminology

The ONCO 44/48 is the next-generation sequencing technology at the City of Hope which is designed to target 713 mutations in 44 and 48 key cancer genes.

Peer-review

It is a well-written paper.

REFERENCES

1 **Siegel R**, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 9-29 [PMID: 24399786 DOI: 10.3322/caac.21208]

2 **Fong Y**, Blumgart LH. Hepatic colorectal metastasis: current status of surgical therapy. *Oncology* (Williston Park) 1998; **12**: 1489-1498; discussion 1489-1500; 1503 [PMID: 9798202]

3 **Park JH**, Kim TY, Lee KH, Han SW, Oh DY, Im SA, Kang GH, Chie EK, Ha SW, Jeong SY, Park KJ, Park JG, Kim TY. The beneficial effect of palliative resection in metastatic colorectal cancer. *Br J Cancer* 2013; **108**: 1425-1431 [PMID: 23481187 DOI: 10.1038/bjc.2013.94]

4 **Fong Y**, Cohen AM, Fortner JG, Enker WE, Turnbull AD, Coit DG, Marrero AM, Prasad M, Blumgart LH, Brennan MF. Liver resection for colorectal metastases. *J Clin Oncol* 1997; **15**: 938-946 [PMID: 9060531]

5 **Van Cutsem E**, Nordlinger B, Adam R, Köhne CH, Pozzo C, Poston G, Ychou M, Rougier P. Towards a pan-European consensus on the treatment of patients with colorectal liver metastases. *Eur J Cancer* 2006; **42**: 2212-2221 [PMID: 16904315]

6 **Pita-Fernández S**, Alhayek-Aí M, González-Martín C, López-Calviño B, Seoane-Pillado T, Pértega-Díaz S. Intensive follow-up strategies improve outcomes in nonmetastatic colorectal cancer patients after curative surgery: a systematic review and meta-analysis. *Ann Oncol* 2015; **26**: 644-656 [PMID: 25411419]

7 **Charnsangavej C**, Clary B, Fong Y, Grothey A, Pawlik TM, Choti MA. Selection of patients for resection of hepatic colorectal metastases: expert consensus statement. *Ann Surg Oncol* 2006; **13**: 1261-1268 [PMID: 16947009 DOI: 10.1245/s10434-006-9023-y]

8 **Tran B**, Kopetz S, Tie J, Gibbs P, Jiang ZQ, Lieu CH, Agarwal A, Maru DM, Sieber O, Desai J. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *Cancer* 2011; **117**: 4623-4632 [PMID: 21456008 DOI: 10.1002/cncr.26086]

9 **Yaeger R**, Cercek A, Chou JF, Sylvester BE, Kemeny NE, Hechtman JF, Ladanyi M, Rosen N, Weiser MR, Capanu M, Solit DB, D'Angelica MI, Vakiani E, Saltz LB. BRAF mutation predicts for poor outcomes after metastasectomy in patients with metastatic colorectal cancer. *Cancer* 2014; **120**: 2316-2324 [PMID: 24737664 DOI: 10.1002/cncr.28729]

10 **Tie J**, Lipton L, Desai J, Gibbs P, Jorissen RN, Christie M, Drummond KJ, Thomson BN, Usatoff V, Evans PM, Pick AW, Knight S, Carne PW, Berry R, Polglase A, McMurrick P, Zhao Q, Busam D, Strausberg RL, Domingo E, Tomlinson IP, Midgley R, Kerr D, Sieber OM. KRAS mutation is associated with lung metastasis in patients with curatively resected colorectal cancer. *Clin Cancer Res* 2011; **17**: 1122-1130 [PMID: 21239505]

11 **Yaeger R**, Cowell E, Chou JF, Gewirtz AN, Borsu L, Vakiani E, Solit DB, Rosen N, Capanu M, Ladanyi M, Kemeny N. RAS mutations affect pattern of metastatic spread and increase propensity for brain metastasis in colorectal cancer. *Cancer* 2015; **121**: 1195-1203 [PMID: 25491172 DOI: 10.1002/cncr.29196]

12 **Kawazoe A**, Shitara K, Fukuoka S, Kuboki Y, Bando H, Okamoto W, Kojima T, Fuse N, Yamanaka T, Doi T, Ohtsu A, Yoshino T. A retrospective observational study of clinicopathological features of KRAS, NRAS, BRAF and PIK3CA mutations in Japanese patients with metastatic colorectal cancer. *BMC Cancer* 2015; **15**: 258 [PMID: 25886136 DOI: 10.1186/s12885-015-1276-z]

13 **Pereira AA**, Rego JF, Morris V, Overman MJ, Eng C, Garrett CR, Boutin AT, Ferrarotto R, Lee M, Jiang ZQ, Hoff PM, Vauthey JN, Vilar E, Maru D, Kopetz S. Association between KRAS mutation and lung metastasis in advanced colorectal cancer. *Br J Cancer* 2015; **112**: 424-428 [PMID: 25535726 DOI: 10.1038/bjc.2014.619]

14 **Kalady MF**, Dejulius KL, Sanchez JA, Jarrar A, Liu X, Manilich E, Skacel M, Church JM. BRAF mutations in colorectal cancer are associated with distinct clinical characteristics and worse prognosis. *Dis Colon Rectum* 2012; **55**: 128-133 [PMID: 22228154 DOI: 10.1097/DCR.0b013e31823c08b3]

15 **Roth AD**, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, Dietrich D, Biesmans B, Bodoky G, Barone C, Aranda E, Nordlinger B, Cisar L, Labianca R, Cunningham D, Van Cutsem E, Bosman F. 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 [PMID: 20008640 DOI: 10.1200/JCO.2009.23.3452]

16 **Gonsalves WI**, Mahoney MR, Sargent DJ, Nelson GD, Alberts SR, Sinicrope FA, Goldberg RM, Limburg PJ, Thibodeau SN, Grothey A, Hubbard JM, Chan E, Nair S, Berenberg JL, McWilliams RR. Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147. *J Natl Cancer Inst* 2014; **106**: pii: dju106 [PMID: 24925349 DOI: 10.1093/jnci/dju106]

17 **Sylvester BE**, Huo D, Khramtsov A, Zhang J, Smalling RV, Olugbile S, Polite BN, Olopade OI. Molecular analysis of colorectal tumors within a diverse patient cohort at a single institution. *Clin Cancer Res* 2012; **18**: 350-359 [PMID: 22114137 DOI: 10.1158/1078-0432.CCR-11-1397]

18 **Yoon HH**, Shi Q, Alberts SR, Goldberg RM, Thibodeau SN, Sargent DJ, Sinicrope FA, Alliance for Clinical Trials in Oncology. Racial differences in KRAS/BRAF mutation rates and survival in colon cancer (NCCTG N0147 [Alliance]). *J Clin Oncol* 2014; **32**: 5s, (suppl; abstr 3536)

19 **Russo AL**, Borger DR, Szymonifka J, Ryan DP, Wo JY, Blaszkowsky LS, Kwak EL, Allen JN, Wadlow RC, Zhu AX, Murphy JE, Faris JE, Dias-Santagata D, Haigis KM, Ellisen LW, Iafrate AJ, Hong TS. Mutational analysis and clinical correlation of metastatic colorectal cancer. *Cancer* 2014; **120**: 1482-1490 [PMID: 24500602 DOI: 10.1002/cncr.28599]

20 **Assersohn L**, Norman A, Cunningham D, Benepal T, Ross PJ, Oates J. Influence of metastatic site as an additional predictor for response and outcome in advanced colorectal carcinoma. *Br J Cancer* 1999; **79**: 1800-1805 [PMID: 10206296 DOI: 10.1038/sj.bjc.6990287]

21 **Catalano V**, Loupakis F, Graziano F, Torresi U, Bisonni R, Mari D, Fornaro L, Baldelli AM, Giordani P, Rossi D, Alessandroni P, Giustini L, Silva RR, Falcone A, D'Emidio S, Fedeli SL. Mucinous histology predicts for poor response rate and overall survival of patients with colorectal cancer and treated with first-line oxaliplatin- and/or irinotecan-based chemotherapy. *Br J Cancer* 2009; **100**: 881-887 [PMID: 19259089 DOI: 10.1038/sj.bjc.6604955]

22 **Sharma N**, Saifo M, Tamaskar IR, Bhuvaneswari R, Mashtare T, Fakih M. KRAS status and clinical outcome in metastatic colorectal cancer patients treated with first-line FOLFOX chemotherapy. *J Gastrointest Oncol* 2010; **1**: 90-96 [PMID: 22811812]

23 **Kim MJ**, Lee HS, Kim JH, Kim YJ, Kwon JH, Lee JO, Bang SM, Park KU, Kim DW, Kang SB, Kim JS, Lee JS, Lee KW. Different metastatic pattern according to the KRAS mutational status and site-specific discordance of KRAS status in patients with colorectal cancer. *BMC Cancer* 2012; **12**: 347 [PMID: 22876814 DOI: 10.1186/1471-2407-12-347]

24 **Kemeny NE**, Chou JF, Capanu M, Gewirtz AN, Cercek A, Kingham TP, Jarnagin WR, Fong YC, DeMatteo RP, Allen PJ, Shia J, Ang C, Vakiani E, D'Angelica MI. KRAS mutation influences recurrence patterns in patients undergoing hepatic resection of colorectal metastases. *Cancer* 2014; **120**: 3965-3971 [PMID: 25155157 DOI: 10.1002/cncr.28954]

25 **Vauthey JN**, Zimmitti G, Kopetz SE, Shindoh J, Chen SS, Andreou A, Curley SA, Aloia TA, Maru DM. RAS mutation status predicts survival and patterns of recurrence in patients undergoing hepatectomy for colorectal liver metastases. *Ann Surg* 2013; **258**: 619-626; discussion 626-627 [PMID: 24018645 DOI: 10.1097/SLA.0b013e3182a5025a]

26 **Selcukbiricik F**, Bilici A, Tural D, Erdamar S, Soyluk O, Buyukunal E, Demirelli F, Serdengecti S. Are high initial CEA and CA 19-9 levels associated with the presence of K-ras mutation in patients with metastatic colorectal cancer? *Tumour Biol* 2013; **34**: 2233-2239 [PMID: 23625655]

27 **Fakih MG**, Padmanabhan A. CEA monitoring in colorectal cancer. What you should know. *Oncology* (Williston Park) 2006; **20**: 579-587; discussion 588, 594, 596 passim [PMID: 16773844]

28 **Blons H**, Emile JF, Le Malicot K, Julié C, Zaanan A, Tabernero J, Mini E, Folprecht G, Van Laethem JL, Thaler J, Bridgewater J, Nørgård-Petersen L, Van Cutsem E, Lepage C, Zawadi MA, Salazar R, Laurent-Puig P, Taieb J. Prognostic value of KRAS mutations in stage III colon cancer: post hoc analysis of the PETACC8 phase III trial dataset. *Ann Oncol* 2014; **25**: 2378-2385 [PMID: 25294886]

29 **Cejas P**, López-Gómez M, Aguayo C, Madero R, de Castro Carpeño J, Belda-Iniesta C, Barriuso J, Moreno García V, Larrauri J, López R, Casado E, Gonzalez-Barón M, Feliu J. KRAS mutations in primary colorectal cancer tumors and related metastases: a potential role in prediction of lung metastasis. *PLoS One* 2009; **4**: e8199 [PMID: 20020061 DOI: 10.1371/journal.pone.0008199]

P- Reviewer: Lyakhovich A
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Jiao XK

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com