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

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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

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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.

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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 metastrectomies 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
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).

| Race     | All | BRAF | BRAF | n | % | n | % | n | % | n | % | n | % | n | % |
|----------|-----|------|------|---|---|---|---|---|---|---|---|---|---|---|---|
| Male     | 103 | 59   | 6    | 3 | 3 | 100| 97| 0.052| 49| 48| 54| 52| 0.54|
| Female   | 71  | 41   | 8    | 11| 63| 89| 38| 54| 33| 46|
| 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.
and African American race. Several studies have previously evaluated the impact of race on mutational status. The only positive association was for patients with right colonic primaries were in line with the percentages of related to our more limited sample size, especially that to previous reports findings are consistent with prior reports mutations and age or a right colon primary, contrary to previous reports. We were not able to confirm an association between BRAF mutations and age or a right colon primary, contrary to previous reports. 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. 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. 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 metastases 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. Yaeger et al. 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. Tran et al. 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. reported a higher likelihood of BRAF mutations in patients with distant lymph node metastases at the site of first recurrence. Finally, Kawazoe et al. 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. 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 2 RAS and BRAF status and primary tumor location and pattern of metastasis

| Site of metastasis | RAS WT | RAS MT | BRAF WT | BRAF MT | \( P \) | \( n \) | \% |
|-------------------|--------|--------|---------|---------|------|------|-----|
| Primary lesion    |        |        |         |         |      |      |     |
| Rectal            | 43     | 25     | 43      | 100     | 0.022| 23   | 53  |
| Left colon        | 79     | 45     | 74      | 94      | 0.34 | 34   | 43  |
| Right colon       | 52     | 30     | 46      | 88      | 0.30 | 30   | 58  |
| Site of metastasis|        |        |         |         |      |      |     |
| Liver             | 58     | 96     | 93      | 95      | 0.54 | 46   | 47  |
| Peritoneal        | 49     | 28     | 44      | 90      | 0.3  | 23   | 47  |

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

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

| CEAs above 5 ng/mL | RAS | BRAF | \( P \) |
|--------------------|-----|------|-------|
| \( < 5 \text{ ng/mL} \) |     |      |      |
| 60                 | 54  | 4    | 0.001|
| \( \geq 5 \text{ ng/mL} \) | 114 | 66   | 0.037|

\( n \): 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. We were not able to confirm an association between BRAF mutations and age or a right colon primary, contrary to previous reports. 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. 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. In addition, N0147 reported a lower frequency of KRAS mutations in Asians, a finding not supported by our study.
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. reported no predictive role for KRAS mutations on the site(s) of metastatic disease at the time of presentation. Pereira et al. 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. reported on the initial metastatic disease patterns in South Korean patients with mCRC. Lung metastases were more frequent
as the initial metastatic site in KRAS mutant patients while liver and distant lymph node metastases were less likely\cite{23}. Yaeger et al\cite{11} reported on the impact of KRAS mutations on the pattern of metastastic 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\cite{11}. KRAS mutations have also been associated with a higher risk of lung relapse while KRAS mutations were associated with increased local recurrence after curative resection of primary CRC or after curative intent hepatectomy\cite{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\cite{10}. Kemeny et al\cite{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\cite{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\cite{26} which reported a higher percentage of patients with CEA > 5 ng/mL among the KRAS mutant cohort. Selcukbiricik 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\cite{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\cite{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 carcinomembrionic 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 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.

**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.

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