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***Retrospective Study***

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

Cho M *et al.* *RAS/BRAF* mutations and CEA and 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.

Cho M, Akiba C, Lau C, Smith D, Telatar M, Afkhami M, Sentovich S, Kurt Melstrom, Fakih M. Impact of *RAS* and *BRAF* mutations on carcinoembryonic antigen production and pattern of colorectal metastases. *World J Gastrointest Oncol* 2015; In press

**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 colorectal cancer (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 (IRB).

***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 AmpliSeqTM Cancer 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 versus clinical and demographic factors with Fisher’s Exact Tests. We also tested for differences in proportions between rate of mutations versus 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).

***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, 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 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[13]. 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 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[24].Similarly, Vauthey et al 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[25]. 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* PCRmutation 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***

To summarize concisely and accurately the relevant background information so that readers may gain some basic knowledge about your study’s relevance and understand its significance for the field as a whole. 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***

To introduce briefly the current hotspots or important areas in the research field as related to your study. *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***

To summarize and emphasize the differences, particularly the advances, achievements, innovations and breakthroughs, as compared to other related or similar studies in the literature, which will allow the readers to assimilate the major points of your article. 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***

To summarize the practical applications of your research findings, so that readers may understand the perspectives by which this study will affect the field and future research. 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***

To describe concisely and accurately any terms that may not be familiar to the majority of the readers, but which are essential for understanding your article. 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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**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.

**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 | 0.30 | 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 | 23 | 38 | 37 | 62 | **0.037** |
| ≥ 5 ng/mL | 114 | 66 | 7 | 6 | 107 | 94 |  | 64 | 56 | 50 | 44 |  |

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

**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*13% | < 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.