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Relationships between KRAS mutation status and baseline radiographic distribution of disease in patients with stage IV colorectal cancer

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Abstract

Purpose: KRAS oncogene testing is recommended in all patients with metastatic colorectal cancer due to its impact on treatment selection, but we do not know if KRAS genotype affects extent or pattern of metastases. We investigated whether the initial radiographic distribution of disease varies by KRAS genotype in stage IV colorectal cancer.

Materials and methods: This retrospective study of 65 patients with stage IV colorectal cancer was derived from an institutional clinical trials database. Inclusion criteria required KRAS testing and pretreatment CT studies to be available. Disease burden was characterized by two radiologists.

Results: Univariate analysis showed that there was no significant difference in the initial distribution of disease between KRAS mutant and wild type tumors ($P > 0.05$). Exploratory analyses showed that patients with poorly differentiated histology had a statistically significant increase in hepatic metastases in the presence of KRAS mutations vs. KRAS wild type genotype (median 5.0 vs. 0.5, $P = 0.02$).

Conclusions: No overall difference was found in the initial radiographic distribution of disease between KRAS mutant and wild type colorectal cancers. Patients with both poorly differentiated histology and KRAS mutations had more liver metastases in subgroup analyses.

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Colorectal cancer is the second most common cause of cancer death in the United States [1]. Up to 50% of patients with colorectal cancers develop metastases, and the burden of disease at some sites of metastasis is a known prognostic factor in colorectal cancer [2]. The treatment of metastatic colorectal cancer entered a new era with development of effective chemotherapy regimens as well as molecular-targeted therapeutic agents [3–6]. In the era of these targeted agents, colorectal cancer has evolved from being regarded as a single disease entity to a heterogeneous group of diseases according to clinically relevant molecular markers or pathways. It is reasonable to hypothesize that the characteristics of tumors, including the initial volume and distribution of metastasis, might be influenced in part by unique genetic alterations occurring during primary tumor development [7, 8].

Tumor genotype has been found to carry treatment and prognostic significance in colorectal cancer. For example, agents that target the epidermal growth factor receptor (EGFR) have been found to offer a significant survival benefit in a subset of patients with colorectal cancer [4–6, 9, 10]. These anti-EGFR agents have diminished efficacy in the presence of mutations in the downstream components of the EGFR signaling pathway, including the Ras, Raf, and MAPK pathway [11].

The KRAS gene, which belongs to the RAS family of oncogenes in this signaling pathway, is mutated in approximately 40% of colorectal cancers [3]. 90% of such mutations occur in codon 12 or 13 [12]. Due to the frequency and therapeutic implications of this mutation, KRAS testing is the only genotype testing that is recommended by the National Comprehensive Cancer Network for all patients with metastatic colorectal cancer [13].

KRAS mutations and TP53 inactivation are known to promote the emergence of aggressive subclones in the evolution of colorectal cancer [14, 15], and some studies have suggested an association with advanced tumor staging [16, 17] or increased occurrence of liver metastasis [18, 19]. There have also been conflicting reports of the prognostic significance of KRAS mutation status beyond its effect on anti-EGFR therapies, with some studies suggesting that KRAS mutations conferred worse outcomes while others showed no significant differences [6, 20–22]. No report has been made of the relationship between KRAS mutations and the global radiographic volume and distribution of metastases in stage IV colorectal cancers.

We hypothesize that the initial distribution and volume of disease in patients presenting with stage IV colorectal cancer will vary by tumor KRAS genotype. The following sections detail a retrospective cohort study that we performed to test this hypothesis.

Materials and methods

Study population

This study was approved by our institutional review board and the requirement for informed consent was waived. A cohort of subjects with stage IV colorectal cancer (Tx Nx M1, AJCC 7th edition) was identified using a gastrointestinal oncology clinical trials database at our institution [23]. This database included all the subjects who enrolled in one of the indexed gastrointestinal oncology clinical trials from 2004 to 2010, and all 65 subjects from the database that matched our criteria were included in our study. Inclusion criteria included biopsy-proven colorectal adenocarcinoma, stage IV disease at initial diagnosis, no prior chemotherapy at the time of baseline imaging, the presence of pre-chemotherapy CT imaging of the abdomen and pelvis in our radiology system, and tumor KRAS mutation testing documented in our medical record. Subjects were initially diagnosed between March 2003 and August 2009, and median follow-up was 39 months (range 8–115 months). No subjects were lost to follow-up.

Assessment of metastatic disease

All subjects had baseline pre-chemotherapy CT imaging of the abdomen and pelvis ($n = 65$), and almost all had baseline CT imaging of the chest ($n = 63$). Among the 65 subjects, 13 had baseline CT scans performed at outside hospitals that were imported into our PACS, and 52 patients had baseline CT performed in our institution according to the protocol described as below.

All baseline staging CT scans were evaluated in consensus by two radiologists (M.R. and K.W.K., with 7 and 8 years of postgraduate radiology experience) for the location and volume of metastatic disease using counts of individual lesions. Soft tissue, lung, and bone windows were reviewed. To estimate the whole-organ burden of disease, we counted the number of discrete lesions that measured 1 cm or greater in longest axial dimension in the liver and lung. Discrete peritoneal implants greater than 1 cm in longest axial dimension were also counted. The 1-cm axial threshold was chosen for consistency with the accepted RECIST thresholds [24]. We assessed for osseous metastases, but none were present in this cohort. For retroperitoneal and thoracic lymph nodes, we counted lesions with an axial short-axis measurement greater than 1 cm to remain consistent with clinical practice [24].

If any lesion was felt to be indeterminate for metastasis on the baseline CT scan, then two radiologists reviewed all available follow-up imaging and clinical information and made an assignment decision in consensus. Disagreements in scoring and lesion confirmation were resolved by consensus with a third reviewer (N.R.).

KRAS testing

All subjects included in this study had KRAS testing in a CLIA approved laboratory. 52 subjects had testing at our institution and 13 had testing at an outside institution with a formal report reviewed by our oncologists. For the subjects who were tested at our institution, standard testing included sequencing of codons 12 and 13 in exon 2. Genomic DNA was extracted from paraffin-embedded tumor tissue and KRAS sequence analysis was performed on a PCR product using primers spanning exon 2. The sequence was analyzed by pyrosequencing using a Biotage Pyromark MD instrument.

Medical record review

Information was retrieved for each subject using the previously described oncology database, the radiology patient archiving and communication system, and the electronic medical record. Among 65 subjects, 13 had baseline imaging that was performed at an outside hospital but imported into our system, and 52 had baseline imaging at our institution. All data abstraction was performed by two licensed radiologists (K.W.K. and M.R.).

The collected data included subject age at diagnosis, sex, history of cancer diagnosis and treatment, location of the primary colorectal tumor, clinical and

pathological cancer staging, tumor histologic grade, the presence or absence of mucinous histologic features, and the results of tumor KRAS genotype tests. In addition, the primary tumor was categorized as proximal or distal (divided at the middle of the transverse colon). The tumor differentiation was categorized as poorly differentiated vs. not poorly differentiated based on the clinical pathology report.

Statistical analysis

Statistical analysis was performed using SAS 9.2 (SAS Institute Inc., Cary, NC). All tests of significance were performed using an α of 0.05. Continuous variables were assessed for normality using the Shapiro–Wilk test. The associations between the KRAS status of cancers, the distribution and volume of metastatic disease, and related clinical features were analyzed with the Fisher's exact or Chi square tests for categorical variables, with an unpaired Student's *t* tests for age, and with Mann–Whitney tests for the remaining continuous variables. Missing data were excluded from the associated analyses.

Results

Baseline characteristics of the cohort

The baseline characteristics of the cohort are summarized in Table 1 by KRAS mutation status. Among 65 subjects, there were 30 subjects with KRAS wild type genotypes and 35 subjects with KRAS mutations. Among those with KRAS mutations, 25 were in codon 12, 8 were in codon 13, and 2 were without specific codon mutation data. Between subjects with KRAS mutation and KRAS wild type, there was no significant difference in age, sex, histologic differentiation, and histologic presence of mucin, Subjects with KRAS mutations had proximal colonic tumors more frequently than those with KRAS wild type genotypes (51% vs. 23%, $P = 0.024$).

Initial distribution of disease

Table 2 shows the baseline distribution of disease by KRAS mutation status. The liver was the most common metastatic site and was involved in 77% of subjects. Distant lymph nodes in the chest or retroperitoneum (42%), peritoneal implants (39%), and lung metastases (31%) comprised the remainder of the common sites of disease. Less common sites of metastasis included the ovaries, brain, and adrenal glands; these were not included in the analysis due to their low numbers. No bone metastasis was observed in this cohort.

Based on our clinical experience regarding patterns of disease, we also performed a stratified analysis of sites of metastasis. 34% of subjects had metastases only in the liver, 8% had metastases only in the peritoneum, 42% had metastases in the liver or peritoneum and at least one additional site, and 17% had no metastases in the liver and peritoneum. The stratified distribution of metastasis did not vary significantly between the KRAS wild type and mutant groups ($P = 0.202$).

Univariate analysis showed no statistically significant difference in the counts of lesions larger than 1 cm in the liver, retroperitoneum, lungs, and thoracic and retroperitoneal lymph nodes between the KRAS mutant and wild type groups ($P > 0.05$ for all comparisons). There was a weak trend toward significance in the count of liver metastases ($P = 0.16$), so exploratory subgroup analysis was performed using histologic features and primary site as potential covariates. Among subjects with poorly differentiated histology, there was a significant difference in the count of liver metastases between the KRAS mutant and wild type groups (median 5.0 vs. 0.5, interquartile range 14 vs. 2, $P = 0.02$).

The initial distribution of disease was also compared between the KRAS codon 12 and codon 13 mutation groups, and no significant difference was found ($P > 0.05$ for all comparisons).

Table 1. Baseline characteristics by KRAS mutation status

Characteristics	Overall ($n = 65$)	KRAS wild type ($n = 30$)	KRAS mutation ($n = 35$)	P[b]
Age at diagnosis (years)[a]	55.7 ± 11.7	54.9 ± 12.8	56.4 ± 10.8	0.609
Sex				
Male	37 (56.9)	17 (56.7)	20 (57.1)	1.000
Female	28 (43.1)	13 (43.3)	15 (42.9)	
Differentiation				
Well or moderate	46 (70.8)	20 (66.7)	26 (74.3)	0.589
Poor	19 (29.2)	10 (33.3)	9 (25.7)	
Presence of mucin				
Non-mucinous	48 (73.8)	24 (80.0)	24 (68.6)	0.398
Mucinous	17 (26.2)	6 (20.0)	11 (31.4)	
Primary tumor location				
Proximal	25 (38.5)	7 (23.3)	18 (51.4)	0.024
Distal	40 (61.5)	23 (76.7)	17 (48.6)	

Unless otherwise specified, data are reported as count (percentage)
[a]Age data are reported as mean ± standard deviations
[b]Fisher's exact test

Table 2. Distribution of disease by KRAS mutation status

Disease assessment	Overall (n = 65)	KRAS wild type (n = 30)	KRAS mutation (n = 35)	P
Sites of metastasis[a]				
Distant nodes	27 (41.5)	13 (43.3)	14 (40.0)	0.806[c]
Liver	50 (76.9)	21 (70.0)	29 (82.9)	0.251[c]
Lung	20 (30.8)	8 (26.7)	12 (34.3)	0.595[c]
Peritoneum	25 (38.5)	12 (40.0)	13 (37.1)	1.000[c]
Stratified site(s) of metastasis[a]				
Liver only	22 (33.9)	8 (26.7)	14 (40.0)	0.202[c]
Peritoneum only	5 (7.7)	3 (10.0)	2 (5.7)	
Liver or peritoneum plus another site	27 (41.5)	11 (36.7)	16 (45.7)	
Liver and peritoneum not involved	11 (16.9)	8 (26.7)	3 (8.6)	
Count of metastatic lesions >1 cm[b]				
Retroperitoneal lymph nodes	0 (1, 0–25)	0 (1, 0–8)	0 (1, 0–8)	0.310[d]
Thoracic lymph nodes	0 (0, 0–15)	0 (0, 0–15)	0 (0, 0–10)	0.566[d]
Liver	3 (7, 0–213)	2 (6, 0–25)	3 (8, 0–213)	0.163[d]
Lung	0 (0, 0–43)	0 (0, 0–43)	0 (0, 0–17)	0.993[d]
Peritoneum	0 (1, 0–13)	0 (1, 0–10)	0 (4, 0–13)	0.584[d]
Liver lesion count by histology[b]				
Well or moderately differentiated (n = 46)	3.5 (7, 0–60)	5 (8, 0–25) [n = 20]	3 (7, 0–60) [n = 26]	0.98[d]
Poorly differentiated (n = 19)	2 (5, 0–213)	0.5 (2, 0–3) [n = 10]	5 (14, 0–213) [n = 9]	0.02[d]

[a]Data are reported as count (percentage)
[b]Data are reported as median (interquartile range, full range)
[c]Fisher's exact test
[d]Mann–Whitney U test

Discussion

The main objective of this study was to assess whether KRAS mutation status affects the initial volume and distribution of metastatic disease of subjects with stage IV colorectal cancers. KRAS mutations confer resistance to treatments that target the EGFR pathway, so KRAS mutation testing is currently the only genotype test that is recommended for patients with stage 4 colorectal cancer in the NCCN guidelines [5, 13]. If there were associations between genotype and the distribution of disease, then these results could be used to determine if genotype-specific imaging strategies would offer potential benefit in such patients. In this cohort, few differences were observed in the baseline distribution and volumes of disease between subjects with KRAS mutant and wild type tumors. These differences included a bias toward distal primary sites for KRAS wild type tumors (P = 0.024), as has been previously described [11], and an increased count of liver lesions in KRAS mutant tumors with poorly differentiated histology (P = 0.02), which does not have a known biological basis at this time. These limited findings require further investigation, but the bulk of the analysis suggests that there is no significant difference in the volume and distribution of metastatic disease by KRAS mutation status at the time of initial presentation of stage IV colorectal cancer.

The lack of association between KRAS mutation status and the initial burden of disease in this cohort suggests that, if there are factors that drive poorer outcomes in some KRAS-mutated tumors, they were either not reflected in the baseline anatomic imaging or were confounded by an unmeasured factor. One possibility is that colorectal cancer patients become symptomatic at a relatively similar disease burden regardless of the underlying tumor aggressiveness, which would mean that a patient with slowly growing, indolent disease may initially present with the same absolute burden of disease as a patient with rapidly growing, aggressive disease. In this scenario, a single anatomic assessment would be unable to distinguish between the indolent and aggressive forms of the disease. Assessments of growth velocity at two or more time points, or functional assessments of aggressiveness, including glucose metabolism (via FDG-PET), angiogenesis (via perfusion imaging), or cellular proliferation (via 18-F fluorothymidine PET), could potentially add complementary information in this scenario [25].

As with all observational studies, it is possible that an unmeasured confounder has masked the effects of KRAS mutations in this cohort. Potential considerations include selection bias and burdens of disease that are occult on anatomic imaging [26]. This cohort was derived from a database of clinical trials, which means that the subjects all qualified to participate in clinical trials. This results in a selection bias against subjects with poor performance status, and the magnitude of that effect is not known in our cohort.

Our study has several limitations. First, the statistical power of some of the analyses is modest due to the relatively small number of subjects, especially in subgroup analysis of patients with KRAS mutations. Analysis of a larger cohort will be necessary for confirmation of our findings. Second, we used semiquantitative counts of lesions to approximate disease burden rather than quantitative 3D volumetry. While theoretically superior to

lesion counts, the validation of 3D volumetry is ongoing, and it is not widely implemented in practice at this time. We plan to validate our methodology against the volumetric methods that arise from the ongoing work of the Quantitative Imaging Biomarkers Alliance of the Radiological Society of North America. Third, our counts of lesions only included lesions measuring at least 1 cm in size to maintain consistency with RECIST standards. It is possible that the burden of subcentimeter tumor foci could also impact patient outcomes, but this possibility was not assessed in our study. Fourth, our analysis of KRAS mutational status was limited to codons 12 and 13 and did not include KRAS mutations in codon 61 or 146 or NRAS mutations. Nonetheless, since codon 12/13 mutations in KRAS represent the vast majority of RAS mutations in colorectal cancer, our analysis provides a reasonable representation of RAS activated tumors [22].

While this initial study found no significant relationship between the radiographic distribution of disease and KRAS tumor genotype in this cohort, additional work is needed to determine if such relationships exist in other stages of disease, genotype groups, or types of cancers. In particular, mutations in BRAF, NRAS, and PIK3CA have been shown to hold additional prognostic significance in patients with colorectal cancer and may be adopted into global treatment guidelines [4]. If such differences do exist, then it possible that genotype-specific imaging strategies may be necessary to maximize the cost-effectiveness of imaging for cancer staging, treatment response assessment, and subsequent surveillance.

In conclusion, our study has shown that KRAS mutation status confers no significant overall difference in the amount and distribution of metastatic disease at the baseline assessment of stage IV colorectal cancer. The exploratory finding that patients with both poorly differentiated histology and KRAS mutations had more liver metastases will require confirmation in subsequent studies.

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