Association between specific KRAS mutations and the clinicopathological characteristics of colorectal tumors

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Abstract. The aim of this study was to investigate the clinicopathological characteristics and distribution by tumor localization of KRAS point mutations in metastatic colorectal cancer. A total of 189 patients diagnosed with colorectal cancer between 2007 and 2014, who were either metastatic at the time of diagnosis or developed metastasis subsequently, were included in this study. KRAS mutation analysis was performed in the primary tumor tissues and KRAS mutations were identified in 47.6% of the patients. There was a high frequency of the p.G13D point mutation in left-colon tumors (P=0.011), while the p.G12D point mutation was more frequent in right-colon tumors (P=0.004). KRAS wild-type frequency (P=0.02) was higher among patients aged <40 years. A comparison of codon 12 and 13 mutations revealed that codon 12 mutations were more common in the >50-year-old group (P=0.03) and codon 13 mutations were more common in the <70-year-old group (P=0.04). KRAS wild-type tumors were localized in the right colon (P=0.005) and tumors with the p.G13D point mutation (P=0.018) were diagnosed at non-metastatic stages. In conclusion, KRAS point mutations in colorectal cancer exhibited a heterogeneous distribution in terms of tumor localization. In addition, the p.G13D point mutation was found to differ from other mutations in several aspects.

Introduction

The estimated cancer-related mortality rate in Europe for 2014 is >1.3 million, with 7-10% of the cases expected to be due to colorectal cancer (1). The recent developments in colon cancer treatment have increased the need for genetic markers. The RAS oncogene controls numerous cellular functions, including cellular proliferation, apoptosis, migration and differentiation (2). The prevalence of mutant RAS gene is 90% in pancreatic cancer (3), 35% in lung cancer (4), 30-60% in colon cancer (5,6) and 55% in thyroid cancer (4). RAS and Kirsten RAS (KRAS) mutations are present in 30 and 21.6% of all cancers, respectively (7). Recent studies reported that the RAS oncogene may be of predictive and prognostic value (8). KRAS mutation frequency in colon cancer differs by ethnicity and geography (9-11). We analyzed our patients' data to define the association of clinicopathological characteristics and tumor localization with KRAS point mutations in metastatic colorectal cancer.

Materials and methods

Patient characteristics. A total of 189 patients diagnosed with colorectal cancer between 2007 and 2014, who were either metastatic at the time of diagnosis (n=93) or developed metastasis subsequently (n=96), were included in this study. Demographic and clinical characteristics, including age, gender, anatomical location of tumor, histology of the tumor, lymphovascular invasion and number of the involved lymph nodes, were obtained from patient records.

KRAS mutation analysis. DNA was isolated from the primary tumor tissues of all the patients with the QIAamp® DNA FFPE Tissue kit (Qiagen, Hilden, Germany). Therascreen® KRAS Pyro kit (Qiagen) was used for KRAS analysis. The KRAS point mutation status was analyzed with PyroMark Q24 software system (Qiagen).

Statistical analysis. The association between non-parametric variables was assessed with the Chi-square test. Parametric variables were compared with the independent samples t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

Patients. A total of 189 metastatic colorectal cancer patients were included in this study. Of those patients, 122 (64.6%) were male. The median age of the patients was 61 years (27-81 years) and 93 patients (49.2%) were metastatic at the time of diagnosis.

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Table I. Patient characteristics.

			KRAS status				Mutant patients							
	Patients		Wild-type		Mutant		Codon 12		Codon 13		Codon 61		Multiple	
Characteristics	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
All	189	100.0	99	52.4	90	47.6	66	73.3	18	20.0	5	5.6	1	1.1
Gender														
Male	122	64.6	66	54.1	56	45.9	41	73.2	13	23.2	2	3.6	0	0.0
Female	67	35.4	33	49.3	34	50.7	25	73.5	5	14.7	3	8.8	1	3.0
Histological type														
Invasive AC	153	81.0	83	54.2	70	45.8	49	70.0	16	22.9	4	5.7	1	1.4
Mucinous AC	34	18.0	15	44.1	19	55.9	16	84.2	2	10.5	1	5.3	0	0.0
Signet ring cell AC	2	1.0	1	50.0	1	50.0	1	100.0	0	0.0	0	0.0	0	0.0
Histological grade														
I	37	19.6	17	45.9	20	54.1	17	85.0	1	5.0	2	10	0	0.0
II	137	72.5	74	54.0	63	46.0	44	69.8	15	23.8	3	4.8	1	1.6
III	15	7.9	8	53.3	7	46.7	5	71.4	2	28.6	0	0.0	0	0.0
Anatomical tumor site														
Rectum	65	34.4	34	52.3	31	47.7	22	71.0	6	19.3	3	9.7	0	0.0
Rectosigmoid junction	12	6.3	8	66.7	4	33.3	4	100.0	0	0.0	0	0.0	0	0.0
Sigmoid	49	26.0	28	57.1	21	42.9	10	47.6	8	38.1	2	9.5	1	4.8
Descending colon	26	13.8	10	38.5	16	61.5	12	75.0	4	25	0	0.0	0	0.0
Transverse colon	12	6.3	6	50.0	6	50.0	6	100.0	0	0.0	0	0.0	0	0.0
Ascending colon	17	9.0	9	52.9	8	47.1	8	100.0	0	0.0	0	0.0	0	0.0
Cecum	8	4.2	4	50.0	4	50.0	4	100.0	0	0.0	0	0.0	0	0.0
AJCC stage														
I	1	0.5	0	0.0	1	100.0	0	0.0	1	100	0	0.0	0	0.0
II	31	16.4	13	41.9	18	58.1	10	55.6	6	33.3	2	11.1	0	0.0
III	64	33.9	36	56.2	28	43.8	21	75.0	7	25	0	0.0	0	0.0
IV	93	49.2	50	53.8	43	46.2	35	81.4	4	9.3	3	7.0	1	2.3

AJCC, American Joint Committee on Cancer; AC, adenocarcinoma.

Mutation distribution. A total of 99 patients (52.4%) were KRAS wild-type and 90 (47.6%) were KRAS mutant. Of the 122 male and 67 female patients, 56 (45.9%) and 34 (50.7%), respectively, were KRAS mutant. The most common mutation site was exon 2 (n=84, 93.3%), followed by exon 3 (n=5, 5.6%) and multiple mutations (n=1, 1.1%). Exon 2 mutations were present in 54 of the male (96.4%) and 30 of the female (88.2%) patients. Codon 12 mutations (n=66, 73.3%) were the most common of exon 2 mutations, whereas codon 13 mutations were less common (n=18, 20%) (Table I). The p.G12D point mutation was the most common of codon 12 mutations (42.4%), followed by p.G12V (34.8%). The p.G12R point mutation was the least frequently observed in codon 12 (Table II). Two (40%) of the male and 3 (60%) of the female patients had exon 3 mutations.

According to the World Health Organization's histological classification, 153 patients (81%) had invasive adenocarcinoma, 34 (18%) (12) had mucinous adenocarcinoma and 2 (1%) had signet ring cell adenocarcinoma. Among the invasive adenocarcinoma patients, 83 (54.2%) were KRAS wild-type and 70 (45.8%) were KRAS mutant. Fifteen (44.1%) patients with mucinous adenocarcinoma were KRAS wild-type. Among the KRAS mutant patients, exon 2 and 3 mutations were present in 65 (92.9%) and 4 (5.7%), of invasive adenocarcinomas, respectively. Exon 2 and 3 mutations were present in 18 (94.7%) and 1 (5.3%), respectively, of patients with mucinous adenocarcinoma. Of the invasive adenocarcinoma cases, 49 had codon 12 (70%) and 16 (22.9%) had codon 13 mutations, while 16 patients (84.2%) with mucinous adenocarcinoma had codon 12 and 2 (10.5%) had codon 13 mutations (Table I).

According to the criteria of the Union for International Cancer Control (http://www.uicc.org/sites/main/files/private/TNM_Classification_of_Malignant_Tumours_Website_15%20MAy2011.pdf), there was grade I differentiation in 37 (19.6%), grade II differentiation in 137 (72.5%) and grade III differentiation in 15 (7.9%) patients. A total of 93 patients were metastatic at the time of diagnosis (Table I).

Statistical analysis. There was no statistically significant difference between KRAS mutant or wild-type patients in

Table II. KRAS point mutation frequency.

Mutations	No.	Frequency in codon (%)	Frequency in mutant tumors (%)	Frequency in all tumors (%)
Codon 12	66	100.0	73.3	34.9
p.G12A	4	6.1	4.4	2.1
p.G12C	4	6.1	4.4	2.1
p.G12D	28	42.4	31.1	14.8
p.G12R	1	1.5	1.1	0.5
p.G12S	6	9.1	6.7	3.1
p.G12V	23	34.8	25.6	12.1
Codon 13	18	100.0	20.0	9.5
p.G13D	18	100.0	20.0	9.5
p.G13R	0	0.0	0.0	0.0
Codon 61	5	100.0	5.6	2.6
61L	3	60.0	3.3	1.5
61H	1	20.0	1.1	0.5
61R	1	20.0	1.1	0.5
Multiple	1	1.5	1.1	0.5

terms of gender (P=0.31), lymphovascular invasion (P=0.30), perineural invasion (P=0.40), tumor histology (P=0.28), histological grade (P=0.62), presence of lymph node involvement (P=0.53), being metastatic at time of diagnosis (P=0.50), number of metastatic lymph nodes (median, 4; P=0.10) and tumor diameter (median, 4.5 cm; P=0.10) (Table III).

When the patients were separated into age groups, out of the 10 patients aged <40 years, 9 were KRAS wild-type and 1 was KRAS mutant. This difference was statistically significant (P=0.02), but disappeared with increasing age (50 years, P=0.057; and 70 years, P=0.08) (Table III).

The only difference observed when comparing exon 2 and 3 mutation patients was that those with exon 3 mutations had less perineural invasion (P=0.04). Although all the exon 3 mutations were localized in the left colon, the difference did not reach statistical significance, most likely due to the limited number of patients (P=0.3) (data not shown).

The comparison of codon 12 and 13 mutations revealed that codon 13 mutations were more frequent in patients at non-metastatic stages (P=0.032) and those aged <70 years (P=0.04), whereas codon 12 mutations were more frequent in patients aged >50 years (P=0.03). Codon 12 and 13 patients did not exhibit statistically significant differences in terms of gender, perineural/lymphovascular involvement, histological subtype, disease grade, presence of lymph node metastasis, mean number of involved lymph nodes and median tumor diameter (Table III).

When the tumor localizations were grouped as right and left colon (splenic flexure), it was observed that the p.G12D point mutation was more frequent in right-colon tumors (P=0.004) and the p.G13D point mutation was more frequent in left-colon tumors (P=0.011); (Table IV); in addition, when the localization groups were right, left and transverse colon (hepatic flexure, splenic flexure), the p.G12D point mutation was more frequent in right-colon tumors (P=0.001) and the p.G13D point mutation was more frequent in left-colon tumors (P=0.033) (data not shown). All 8 patients who were diagnosed with ascending colon tumors had p.G12D point mutations significantly more frequently compared to other localizations (P=0.001) (Table V). When the patients were grouped based on colon localization regarding embryogenesis development, no association with KRAS status was detected (P=0.9). p.G12D point mutations were higher in frequency in colon segments developing from the midgut (P=0.001) and all the p.G13D point mutations were localized in colon segments developing from the hindgut (P=0.019) (data not shown).

Right- and left-colon tumors were not associated with the presence or absence of metastasis at the time of diagnosis (P=0.17). There was no association between localization in the left or the right colon and the presence of metastasis at the time of diagnosis among KRAS mutant patients (P=0.8). KRAS wild-type tumors located in the right colon, compared to KRAS wild-type tumors located in the left colon, were more frequently diagnosed during non-metastatic stages (P=0.005) (data not shown).

Discussion

KRAS mutations are significantly involved in the carcinogenesis of colorectal cancer. KRAS mutations play a definitive role in the anti-epidermal growth factor receptor (EGFR)-directed therapy of colorectal cancer (8,13). However, tumors harboring KRAS mutations constitute a heterogeneous group of colorectal cancers. KRAS mutation frequency has been reported to be 31.9-56% by different studies (10,14-17). The present study was performed to determine the association of KRAS mutation status with demographic and clinicopathological characteristics and anatomical location of the tumor.

A Brazilian cohort study reported an association between KRAS mutations and gender (16). However, ours and a number of other studies found no association between KRAS mutations and gender (P=0.31). KRAS mutations were reported to

Table III. Comparison of clinicopathological characteristics of tumors according to KRAS mutation status and location.

	KRAS status			Codon 12 vs. codon 13		
Characteristics	Wild-type, n (%)	Mutant, n (%)	P-value	Codon 12, n (%)	Codon 13, n (%)	P-value
LV invasion						0.70
Yes	42 (74.0)	41 (77.0)	0.30	27 (63.0)	10 (71.0)	
No	15 (26.0)	22 (23.0)		16 (27.0)	4 (29.0)	
Perineural invasion			0.40			0.46
Yes	30 (55.0)	25 (46.0)		19 (53.0)	6 (46.0)	
No	25 (450)	29 (54.0)		17 (47.0)	7 (54.0)	
Histological type			0.28			0.21
Invasive	83 (84.0)	70 (79.0)		49 (75.0)	16 (88.0)	
Mucinous	15 (15.0)	19 (21.0)		16 (25.0)	2 (12.0)	
Histological grade			0.62			0.06
I	17 (17.0)	20 (22.0)		17 (26.0)	1 (6.0)	
II	74 (75.0)	63 (70.0)		44 (67.0)	15 (83.0)	
III	8 (8.0)	7 (8.0)		5 (7.0)	2 (11.0)	
Tumor localization			0.80			0.009
Right	19 (19.0)	18 (20.0)		18 (27.0)	0 (0.0)	
Left	80 (81.0)	72 (80.0)		48 (73.0)	18 (100.0)	
Tumor localization (colon)			0.90			0.04
Right	13 (13.0)	12 (13.0)		12 (18.0)	0 (0.0)	
Left	80 (81.0)	72 (80.0)		48 (73.0)	18 (100.0)	
Transverse	6 (6.0)	6 (7.0)		6 (9.0)	0 (0.0)	
Gender			0.31			0.32
Female	33 (33.0)	34 (37.0)		25 (39.0)	5 (28.0)	
Male	66 (67.0)	56 (73.0)		41 (61.0)	13 (72.0)	
Age (years)						
≤40	9 (10.0)	1 (1.0)	0.02	-	-	
>40	90 (90.0)	89 (99.0)				
Age (years)						0.03
≤50	19 (21.0)	9 (10.0)	0.057	4 (6.0)	4 (22.0)	
>50	80 (79.0)	81 (90.0)		62 (94.0)	14 (78.0)	
Age (years)						0.04
≤70	81 (82.0)	64 (71.0)	0.08	43 (65.0)	16 (88.0)	
>70	18 (18.0)	26 (29.0)		23 (35.0)	2 (12.0)	
Metastasis at diagnosis						0.032
Metastatic	51 (52.0)	43 (47.0)	0.50	35 (53.0)	4 (22.0)	
Non-metastatic	48 (48.0)	47 (53.0)		31 (47.0)	14 (78.0)	
LN involvement						0.56
Yes	34 (58.0)	34 (59.0)	0.53	26 (59.0)	8 (57.0)	
No	25 (42.0)	24 (41.0)		18 (41.0)	6 (43.0)	
Median no. of involved LNs						0.30
≥4	48 (81.0)	41 (70.0)	0.10	11 (25.0)	6 (43.0)	
<4	11 (19.0)	17 (30.0)		33 (75.0)	8 (57.0)	
Median tumor diameter (cm)						0.80
≥4.5	28 (51.0)	37 (65.0)	0.10	27 (64.0)	9 (64.0)	
<4.5	26 (49.0)	20 (35.0)		15 (36.0)	5 (36.0)	

LV, lymphovascular; LN, lymph node.

Table IV. Comparison of KRAS point mutations according to tumor location and metastatic status.

Variables	All patients (n=90)	p.G12D		p.G13D		p.G12V		Codon 61	
		No. (%)	P-value	No. (%)	P-value	No. (%)	P-value	No. (%)	P-value
Location of tumor									
Right colon	18	11 (61.0)	0.004	0 (0.0)	0.011	4 (22.0)	0.3	0 (0.0)	0.3
Left colon	72	17 (23.0)		18 (25.0)		19 (26.0)		5 (7.0)	
Metastasis at diagnosis									
Metastatic	43	14 (32.0)	0.8	4 (9.0)	0.018	12 (28.0)	0.6	3 (7.0)	0.6
Non-metastatic	47	14 (30.0)		14 (30.0)		11 (23.0)		2 (40)	

Table V. Frequency of KRAS point mutations according to anatomical location of the tumor.

Mutations	Rectum		Rectosigmoid junction		Sigmoid		Descending colon		Transverse colon		Ascending colon		Cecum	
	No.	[a]/[b] (%)	No.	[a]/[b] (%)	No.	[a]/[b] (%)	No.	[a]/[b] (%)	No.	[a]/[b] (%)	No.	[a]/[b] (%)	No.	[a]/[b] (%)
KRAS mutant	31	34.4/16.4	4	4.4/2.1	21	23.1/11.1	16	17.6/8.4	6	6.6/3.1	8	8.8/4.2	4	4.4/2.1
Codon 12	22	24.4/11.6	1	1.1/0.5	10	11.1/5.2	12	13.2/6.3	6	6.6/3.1	8	8.8/4.2	0	0
p.G12A	2	2.2/1.05	0	0/0	1	1.1/0.5	0	0	1	1.1/0.5	0	0	0	0
p.G12C	1	1.1/0.5	1	1.1/0.5	0	0	1	1.1/0.5	1	1.1/0.5	0	0	0	0
p.G12D	8	8.9/4.2	0	0	3	3.3/1.6	6	6.6/3.1	1	1.1/0.5	8	8.8/4.2	2	2.2/1.05
p.G12R	0	0	0	0	0	0	0	0/0	1	1.1/0.5	0	0	0	0
p.G12S	4	4.5/2.1	0	0	0	0	2	2.2/1.05	0	0/0	0	0	0	0
p.G12V	7	7.8/3.7	0	0	6	6.6/3.1	3	3.3/1.6	2	2.2/1.05	0	0	2	2.2/1.05
Codon 13	6	6.6/3.1	3	3.3/1.6	8	8.8/4.2	4	4.5/2.1	0	0	0	0	0	0
p.G13D	6	6.6/3.1	3	3.3/1.6	8	8.8/4.2	4	4.5/2.1	0	0	0	0	0	0
p.G13R	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Codon 61	3	3.3/1.6	0	0	2	2.2/1.05	0	0	0	0	0	0	0	
Multiple	0	0	0	0	1	1.1/0.5	0	0	0	0	0	0	0	0

[a]Frequency in mutant tumors. [b]Frequency in all tumors.

be more frequent in right-colon tumors by Bleeker *et al* (17), but in left-colon tumors by Zulhabri *et al* (18). In this study, similar to the prior studies (14), there was no significant difference in KRAS mutation frequency according to tumor localization (P=0.8). Zulhabri *et al* (18) reported an association between the presence of KRAS mutations and tumor size; by contrast, Minamoto *et al* (19) reported that there was no association between the two. Our results were consistent with those of Minamoto *et al* (18). Bazan *et al* (20) reported that mutations were associated with mucinous histology and that mutations were associated with lymph node metastasis and advanced Dukes' stage. In our study, there was no significant difference in histological subtype based on codon (P=0.21) and exon mutations (P=0.9). By contrast, codon 13 mutations were found at high frequency in non-metastatic stages (P=0.018). Zahrani *et al* (14) reported that the p.G12V point mutation was frequent in tumors of the sigmoid colon. However, the frequency of the p.G12V point mutation in the sigmoid tumors was not significantly higher in this study (P=0.1).

When the patients were grouped as aged under and over 40, 50 and 70 years, KRAS wild-type frequency was significantly higher among those aged <40 years (P=0.02), but this significance disappeared as the cut-off age increased (50 years, P=0.057; and 70 years, P=0.08). A comparison of codon 12 and 13 mutations revealed that codon 12 mutations were more common in patients aged >50 years (P=0.03) and that codon 12 mutations were more common in those aged <70 years (P=0.04). We did not identify any study in the medical literature comparing KRAS mutation frequency by age groups.

Yamauchi *et al* (21) reported that KRAS mutations were more common in cecum tumors. In our study, cecum tumors had equal numbers of KRAS wild- and mutant-types. The RASCAL II study included clinical findings suggesting that p.G12V point mutation had a more aggressive clinical course (11). Lymph node metastasis and distant organ metastases are indicators of tumor aggressiveness. Distant organ (P=0.6) and lymph node metastases (P=0.9) were not

significantly higher in patients harboring the p.G12V point mutation; thus, p.G12V point mutation may not necessarily reflect a more aggressive clinical course.

All codon 13 mutations were p.G13D point mutations. Although p.G13D point mutations were more frequent in grade II tumors, this high frequency was not statistically significant (P=0.062). Brink *et al* (14) reported that the G>T transversion was significantly higher among females with rectal tumors, while we did not observe such a significant difference (P=0.7). Similar to the findings of Brink *et al* (14), the codon 13 mutation frequency was higher in tumors of the distal colon (P=0.011).

An *in vitro* study by Guerrero *et al* (22) demonstrated that tumors with codon 13 mutations tended to exhibit increased apoptosis. Patients with p.G13D point mutations were diagnosed more commonly as non-metastatic (P=0.018) and tumors with p.G13D point mutations appeared to have latent metastasis due to apoptosis. The higher rates of apoptosis, response to anti-EGFR treatment (22,23), left-colon localization and diagnosis at non-metastatic stage distinguish p.G13D from the remaining mutations investigated.

The main limitation of the present study was its retrospective nature. In addition, we only analyzed cases diagnosed at our Oncology Center to uniform KRAS testing; therefore the patient number was limited.

In conclusion, despite conflicting results between the existing literature and the present study, certain KRAS mutations were found to be associated with the clinicopathological characteristics and anatomical location of the tumor. Our data indicate that localization of KRAS mutations in a specific codon or exon may not be coincidental. The definition of the colon cancer subgroups according to KRAS mutational status may be helpful in developing new preventive and treatment strategies.

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