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ORIGINAL RESEARCH

KRAS Codon 12 Mutation is Associated with More Aggressive Invasiveness in Synchronous Metastatic Colorectal Cancer (mCRC): Retrospective Research

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Objective: To investigate the connection between mutant *KRAS/NRAS/BRAF* and clinicopathological characteristics in therapy-naïve synchronous metastatic colorectal cancer (mCRC) in Chinese populations when compared with all wild type (*KRAS/NRAS/BRAF* wild type).

Patients and Methods: A total of 200 patients with therapy-naïve synchronous mCRC (TNM stage: TanyNanyM1) were retrospectively collected as study objects. Primary tumor tissues from 200 mCRC patients were analyzed through next-generation sequencing panel to assess the mutated regions of *KRAS/NRAS/BRAF*.

Results: The distribution frequency of gene mutation in our study was 41% *KRAS*, 4% *NRAS*, 11.5% *BRAF*, 0.5% both *KRAS* and *BRAF*. Tumors with any gene mutations (any gene mutations in *KRAS/NRAS/BRAF*), *KRAS* and *KRAS codon 12* mutation were more likely to be located in right-sided colon (P=0.007, P=0.008, P=0.026, respectively). For metastasis, tumors with any gene mutations, *KRAS* and *KRAS codon 12* mutation were significantly correlated with peritoneal metastasis (P=0.019, P=0.017, P=0.014, respectively), liver-peritoneum metastases (P=0.004, P=0.003, P=0.002, respectively) and multi-organ metastases (P=0.002, P=0.008, P=0.001, respectively). Tumors with all wild type were significantly correlated with distant lymph node-only metastasis. No statistically significant differences were found between clinicopathological characteristics and *KRAS codon 13* and *NRAS* mutations.

Conclusion: Our study suggests that clinicopathological characteristics (specifically for metastasis) are related to *KRAS/NRAS/BRAF* mutations in therapy-naïve synchronous mCRC population in China. We demonstrated that distant lymph node-only metastasis is visibly linked to all wild-type tumors. We found that patients with any gene mutations, *KRAS* mutation are more likely to carry peritoneal metastasis, liver-peritoneum metastases and multi-organ metastases than those with all wild type. After stratification, *KRAS codon 12* mutation, but not *codon 13* mutation, was remarkably associated with peritoneal metastasis, liver-peritoneum metastases, and multi-organ metastases compared to all wild type. These results may be useful for aiding in the prediction of prognosis and choosing the appropriate regimens for therapy.

Keywords: synchronous metastatic colorectal cancer, *KRAS* mutation, *NRAS* mutation, *BRAF* mutation, *KRAS codon 12* mutation, *KRAS codon 13* mutation

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Background

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the fourth most frequent cause of cancer-related mortality worldwide.[1] In China, with

the rapid development of the economy, people's living standard and food spectrum are gradually westernized, which leads to an increase in the incidence of CRC over time. According to the latest data, China had 521,000 new colorectal cancer cases and 248,000 deaths due to colorectal cancer in 2018 that accounted for approximately 30% of the incidence and mortality in the same periods worldwide. There are no obvious symptoms in early stages of colorectal cancer, so almost 15% to 25% of patients present with synchronous metastasis at diagnosis.[2]

Patients with synchronous metastatic colorectal cancer (mCRC) seem to have much poorer prognosis than those with early and middle stages. With the fast progression of medical technology, we have gradually recognized that CRC is a historically and clinically heterogeneous disease and the accumulation of mutated genes results in CRC tumorigenesis.[3,4] As is well known, the *RAS-RAF-MAPK* pathway, as a signaling pathway downstream of the *epidermal growth factor receptor* (*EGFR*), which can promote carcinogenesis of colorectum,[5,6] can be driven by mutations of oncogenes such as *Kirsten rat sarcoma viral oncogene homolog* (*KRAS*) or *V-raf murine sarcoma viral oncogene homolog B1* (*BRAF*) of the *EGFR*-mediated pathway.[7] In recent decades, the presence of monoclonal antibodies targeted at *EGFR* such as cetuximab and panitumumab, has been shown to be effective for treatment of metastatic colorectal cancer (mCRC). The increasing usage of standard regimens of chemotherapy with or without targeted therapy (including anti-epidermal growth factor receptor antibody and anti-angiogenesis antibody) improves patients' prognosis and relieves pain.[8–10] The combination of chemotherapy and anti-*EGFR* therapy treatment has resulted in a remarkable improvement compared with chemotherapy-only in several clinical trials,[11] the combination therapy as first-line strategy for mCRC patients with all wild type (all of *KRAS*, *NRAS* and *BRAF* genes are wild type) is recommended by the latest American Society of Clinical Oncology (ASCO) guidelines; *KRAS* mutation can be detected in 30–50% of CRC and can be used as a tool to predict resistance to anti-*EGFR* treatment. In addition, almost 90% of *KRAS* mutation present in *codon 12* or 13.[12,13] 3–5% of CRC shows a mutation in *neuroblastoma RAS viral oncogene homolog* (*NRAS*), which has similar clinical and pathological characteristics to *KRAS* mutation.[14,15] Patients with *NRAS* mutation also respond poorly to anti-*EGFR* treatment.[16,17] 5%–10% of CRC can harbor mutant *BRAF* with 90% of its mutation situated in *V600E* which has also shown a negative response to anti-*EGFR* therapy as well as unsatisfying outcomes.[18–20] In a word, it is concluded that patients with any gene mutations (defined as any gene mutations in *KRAS*, *NRAS* or *BRAF*) probably gain resistance to anti-*EGFR* treatment, and may have poorer outcomes and different metastatic patterns than those with all wild type. Therefore, there is an urgent need to comprehensively sequence gene status to select suitable candidates for personalized therapy and regular surveillance. Treatment regimens and prognosis of patients with mutant *KRAS/NRAS/BRAF* have been fully explored. However, the correlation between gene mutations and clinicopathological features (especially for distant organ metastasis) in mCRC has not been fully discussed.

In the present study, we retrospectively enrolled 200 therapy-naïve synchronous mCRC patients at first diagnosis in China. We analyzed the genetic status of *KRAS*, *NRAS* and *BRAF* for each patient with next-generation sequencing to explore whether there is a connection between clinicopathological characteristics and gene mutations in mCRC when compared with all wild type.

Patients and Methods

Ethics Statement

This study was approved by the clinical research ethics committee of the Jiangsu Cancer Hospital and was conducted in accordance with the Declaration of Helsinki. In the context of COVID-19 pandemic and social distancing policy, informed consent from all patients was obtained verbally and confirmed by the clinical research ethics committee of the Jiangsu Cancer Hospital.

Patients and Clinical Data

We retrospectively collected 504 CRC patients in the Affiliated Tumor Hospital of Nanjing Medical University from December 2015 to February 2020. The inclusion criteria were as follows: 1) histologic samples were pathologically demonstrated as colorectal carcinoma; 2) clinical data and genetic test data were completed. The exclusion criteria were: 1) patients with non-metastatic colorectal cancer and non-synchronous metastatic colorectal cancer; 2) preoperative chemotherapy, radiotherapy, targeted therapy and immunotherapy were accepted; 3) primary tumor was located in cecum, appendix and ileocecal junction; 4) samples were histologically confirmed as neuroendocrine carcinoma or containing neuroendocrine components; detailed selection

process is shown in Figure 1. In total, 200 patients with therapy-naïve synchronous mCRC at first diagnosis who underwent primary lesion resection or endoscopic biopsy were included in our study. Clinicopathological data were extracted from medical documents. The pTNM stage system was reviewed according to the 8th edition AJCC cancer staging. Tumor grading and staging were based on the World Health Organization (WHO) criteria. Assessment of distant metastasis was done mainly according to simultaneously confirmed radiological data by two radiologists. Primary lesion of right-sided colon, defined as tumor, was located in ascending colon, hepatic flexure and transverse colon; primary lesion of left-sided colon, defined as tumor, was located in splenic flexure, descending and sigmoid colon; primary lesion of the rectum, defined as large bowel up to the edge of 16 cm from the dentate line.

DNA Extraction and Sequencing

All tissue samples that were extracted from primary tumor through surgical resection or endoscopic biopsy were formalin-fixed, paraffin-embedded, and histologically confirmed.

Five sections (10 μm thick) that were cut from paraffin-embedded tumor tissue blocks were used per analysis. To obtain maximal tumor DNA, we chose tumor-rich paraffin block specimens whose tumor components were greater than at least 30%. DNA in the collected tissue samples was extracted using the QIAamp DNA FFPE Tissue Kit (Cat No. 56404, Qiagen) following the

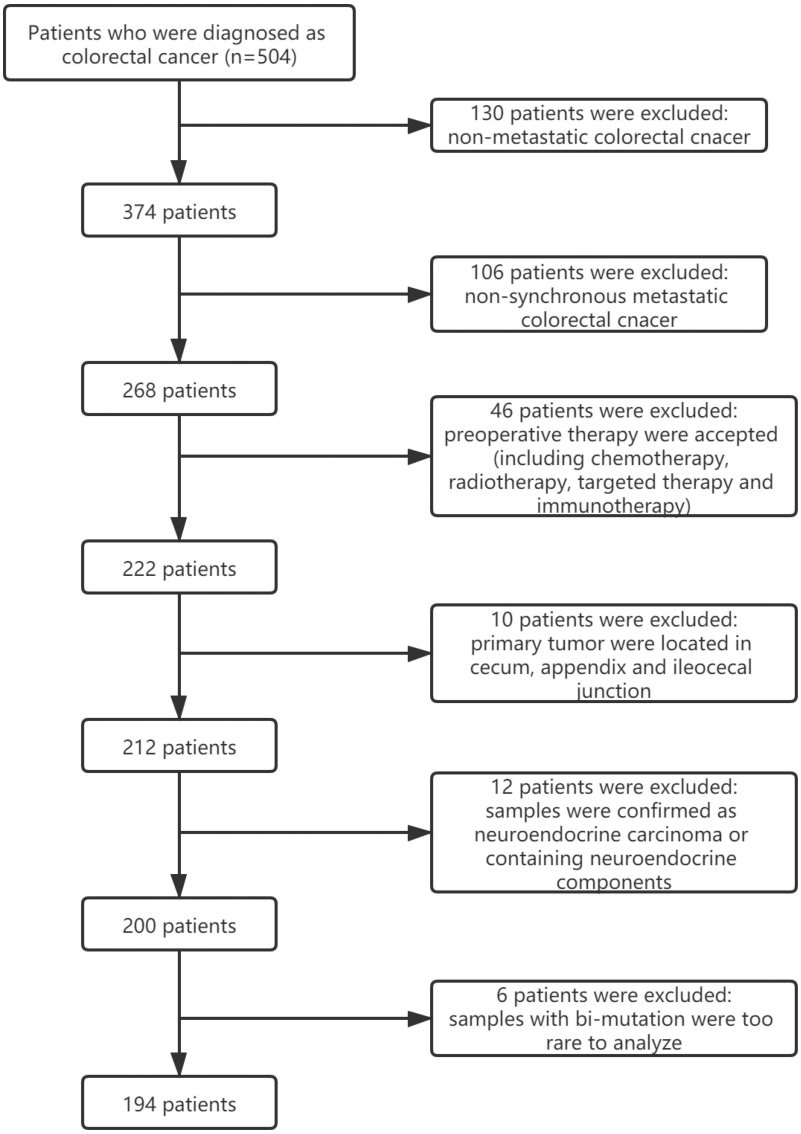

Figure 1 Flow diagram of the patient selection process.

manufacturer's protocol. DNA from each sample was eluted in 50 μL of ATE buffer (included in the kit).

Total DNA was quantified by using the Qubit dsDNA HS Assay kit (Invitrogen), libraries were constructed using the KAPA Hyper library preparation kit (KAPA, KK8504) following Illumina (San Diego, CA) protocols. Sequencing was performed to examine the mutation in *KRAS* (all exons), *NRAS* (all exons), *BRAF* (all exons) through a NovaSeq 6000 sequencing system (Illumina).

Statistical Analysis

Statistical analyses were performed with SPSS software (version 22 of SPSS, Chicago, IL, USA). The relationship between gene mutations and clinical characteristics was compared by Pearson's Chi-squared ($\chi 2$) test or Fischer's exact test. Statistical tests were two-sided, and $P<0.05$ was considered significant.

Results

Frequency of Gene Mutations in Primary Lesions

Within the study period, we retrospectively collected 200 therapy-naïve patients with mCRC at first diagnosis. Primary colorectal samples were analyzed for *KRAS*, *NRAS* and *BRAF* gene mutations. Among these samples, 43% (86/200) of carcinomas were all wild type. *KRAS* mutation occurred in 41% (82/200) of colorectal carcinomas. *NRAS* mutation was observed in 4.0% (8/200) of colorectal carcinomas. *BRAF* mutation was demonstrated in 11.5% (23/200) of colorectal carcinomas. Particularly, there was one sample that harbored both *KRAS* and *BRAF* mutations (*KRAS p.A146T+ BRAF p.D594G*), and in another 5 patients, double *KRAS* mutation existed (*p.G12V+p.D33E*, 2 of *p.G12A+p.G12S*, 2 of *p.G12D +p.G12S*); these cases were excluded from the analysis because they were too rare to analyze. Detailed distribution of mutation subtypes is summed up in Table 1, the percentage of each mutation subtype is shown in Figure 2.

Table 1 Mutation Frequency and Subtype Distribution of RAS and BRAF Genes

Genes	Codon	Mutation	Cases (% of 200)
Total cases of KRAS mutation			82(41.0%)
	12	p.G12A	3(1.5%)
	12	p.G12C	2(1.0%)
	12	p.G12D	23(11.5%)
	12	p.G12R	1(0.5%)
	12	p.G12S	5(2.5%)
	12	p.G12V	18(9.0%)
	13	p.G13A	2(1.0%)
	13	p.G13C	1(0.5%)
	13	p.G13D	11(5.5%)
	59	p.A59E	1(0.5%)
	59	p.A59T	1(0.5%)
	61	p.Q61H	1(0.5%)
	61	p.Q61L	2(1.0%)
	117	p.K117N	2(1.0%)
	146	p.A146T	4(2.0%)
	bi-mutation	p.G12A+p.G12S	2(1.0%)
		p.G12D+p.G12S	2(1.0%)
		p.G12V+p.D33E	1(0.5%)
Total cases of NRAS mutation			8(4.0%)
	12	p.G12D	3(1.5%)
	60	p.G60E	1(0.5%)
	61	p.Q61K	1(0.5%)
	61	p.Q61R	3(1.5%)
Total cases of BRAF mutation			23(11.5%)
	466	p.G466V	1(0.5%)
	600	p.V600E	21(10.5%)
	601	p.K601E	1(0.5%)
Total cases of both KRAS and BRAF mutation		p.A146T+p.D594G	1(0.5%)

Patients' Characteristics

Table 2 summarizes the clinicopathological characteristics of study subjects. A total of 194 patients with metastatic colorectal carcinoma who had sufficient clinical data were evaluated. At diagnosis, the median age was 59 years (range 26–83 years); 121 (62.4%) of patients were male, the other 73 (37.6%) were female. Of these patients, 61 (31.4%) had right-sided colon tumors, 80 (41.2%) had left-sided colon tumors, and 53 (27.3%) patients' tumors were located in rectum. For T staging, there were 3 T1, 5 T2, 106 T3, and 55 T4 stage cases, and for N staging, there were 41 N0, 62 N1, and 66 N2 stage cases. For gross type of tumor, 27 (13.9%) were swell type, 131 (67.5%) were ulcer type, and 11 (5.7%) were invasion type. For 25 cases, no surgery was carried out because the patients did not reach the indication of surgery, so their pathologic stage of T and N, tumor gross type was unknown. 105 patients had well/moderate differentiated tumors and 89 patients had poorly differentiated tumors. Regarding the histological type of tumors, 79.4% were classic adenocarcinoma and 20.6% were mucinous/rare histological type.

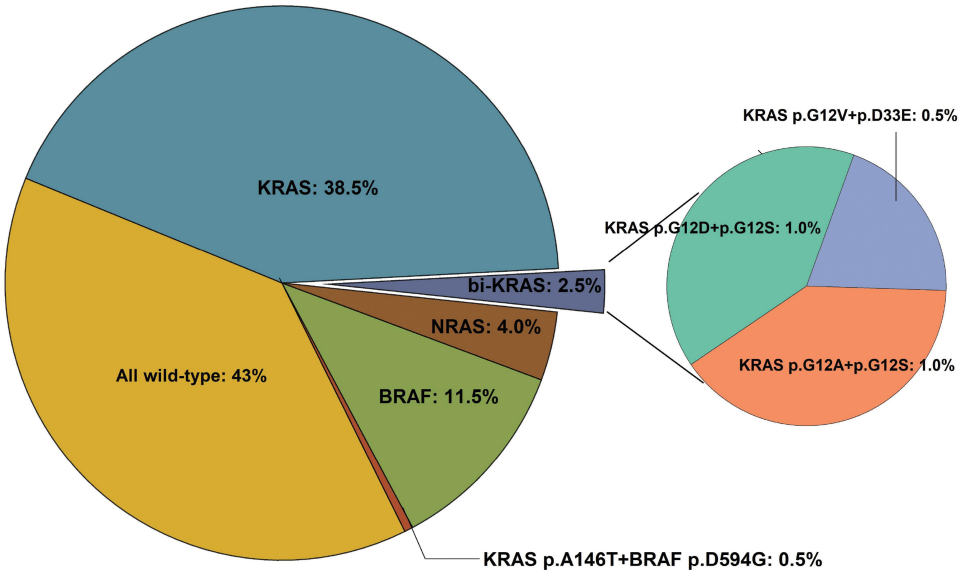

Figure 2 Set diagram illustrates the distribution among KRAS, NRAS and BRAF mutations.

Regarding metastasis, 132 (68%) had liver metastasis, 49 (25.3%) had lung metastasis, 26 (13.4%) had peritoneal metastasis, 34 (17.5%) had abdominal and pelvic implant metastasis, 42 (21.6%) had distant lymph node involvement and 17 (8.8%) had other locations' involvement including spleen (3), bone (4), brain (1), ovary (6) and adrenal gland (3). 116 patients had only one organ involved in metastasis; liver-only metastasis, lung-only metastasis, peritoneum-only metastasis, abdominal and pelvic implant-only metastasis, distant lymph node-only metastasis were respectively observed in 75 (38.7%), 11 (5.7%), 4 (2.1%), 10 (5.2%), 10 (5.2%) of these patients. There were 6 remaining patients, including 3 with bone-only metastasis, 2 with ovary-only metastasis and 1 with spleen-only metastasis, who were excluded due to the low incidence in our study. 78 patients had more than one metastases. Of patients with dual-site metastases, 31 (16%) had liver-lung metastases, 14 (7.2%) had liver-peritoneum metastases and 4 (2.1%) had lung-peritoneum metastases.

Gene Mutation and Clinical Characteristics

Table 3 summarizes the connection between clinicopathological characteristics and gene mutations. Tumor with any gene mutations was significantly correlated with mucinous/rare histological subtypes (P=0.016), peritoneal metastasis (P=0.019) and multi-organ metastases (P=0.002), and was more likely located in right-sided colon (P=0.007) than all wild-type tumors. There was no statistically significant association between any gene mutations and other clinicopathological features like gender, age, differentiation degree, etc. When compared with all wild-type tumor, mutant *KRAS* tumor had a higher rate in right-sided colon (P=0.008) and had a significant relevance with peritoneum metastasis (P=0.017) and multi-organ metastases (P=0.008). When compared with all wild type, differences between *NRAS* mutation and clinical features did not reach statistical significance, *BRAF* mutation showed significant association with mucinous/rare histological subtypes, distant lymph node metastasis and multi-organ metastases in comparison with all wild type (P=0.026 and P=0.029, P=0.003, respectively). Moreover, mutant *BRAF* carcinomas also tended to be located in right-sided colon (P=0.052) and had peritoneal metastasis (P=0.052) compared to all wild-type carcinomas, although it did not reach statistical significance.

Differences Among Specific KRAS Mutations in mCRC

For specific *KRAS* mutations, detailed information is shown in Table 4. Tumor with *KRAS codon 12* mutation was more likely to present in right-sided colon (P=0.026) and present with peritoneal metastasis (P=0.014) and multi-organ metastases (P=0.001) than all wild-type tumors. After stratification, patients with peritoneal

Table 2 Patients' Characteristics

Characteristics	Cases (% of 194)
Gender	
Male	121(62.4%)
Female	73(37.6%)
Age	
<60	98(50.5%)
≥60	96(49.5%)
Primary location	
Right-sided colon	61(31.4%)
Left-sided colon	80(41.2%)
Rectum	53(27.3%)
Tumor infiltration	
T1	3(1.5%)
T2	5(2.6%)
T3	106(54.6%)
T4	55(28.4%)
Unknown#	25(12.9%)
Nodal status	
N0	41(21.1%)
N1	62(32.0%)
N2	66(34.0%)
Unknown#	25(12.9%)
Gross type	
Swell type	27(13.9%)
Ulcer type	131(67.5%)
Invasion type	11(5.7%)
Unknown#	25(12.9%)
Pathological type	
Adenocarcinoma	154(79.4%)
Mucinous/rare adenocarcinoma	40(20.6%)
Differentiation	
Well-moderate	105(54.1%)
Poor	89(45.9%)
Location of metastasis	
Liver	132(68.0%)
Lung	49(25.3%)
Peritoneum	26(13.4%)
Distant lymph node	42(21.6%)
Abdominal and pelvic implant	34(17.5%)
Others	17(8.8%)
Single-site metastasis	
Liver-only metastasis	75(38.7%)
Lung-only metastasis	11(5.7%)
Peritoneum-only metastasis	4(2.1%)
Distant lymph node-only metastasis	10(5.2%)
Abdominal and pelvic implant-only metastasis	10(5.2%)
Dual-site metastases	
Liver-lung metastases	31(16.0%)
Liver-peritoneum metastases	14(7.2%)
Lung-peritoneum metastases	4(2.1%)
Number of metastases	
1	116(59.8%)
≥1	78(40.2%)

Note: #25 patients without surgery were excluded here.

metastasis had a tendency to carry mutant *KRAS G12D* (P=0.052). *KRAS G12V* was more frequent in right-sided colon (P=0.022) than all wild type. Both *KRAS G12D* and *G12V* mutated carcinomas were significantly linked to multi-organ metastases compared to all wild-type carcinomas (P=0.01 and P=0.002, respectively). There were no significant differences found in patients with mutant *KRAS codon 13* and *G13D*.

Single-Site and Dual-Site Metastases

For single-site metastasis, all wild type tumors were significantly correlated with distant lymph node metastasis compared to any gene mutations, *KRAS* mutation and *KRAS codon 12* mutation cases (P=0.006, P=0.003 and P=0.014, respectively). For dual-site metastases, patients with any mutations, *KRAS* mutation, *BRAF* mutation, *KRAS codon 12* and *KRAS G12D* mutations were statistically significantly more likely to carry liver-peritoneum metastases than those with all wild type (P=0.004, P=0.003, P=0.029, P=0.002, and P=0.007, respectively).

Discussion

As one of the most devastating diseases in the world, colorectal carcinoma is a pathologically and clinically heterogeneous malignancy. With the changing global lifestyle, the number of people suffering from CRC continues to rise. China is also facing a similar situation, and even worse. Early detection of CRC is often missed because of its unclear symptoms, while chemotherapy at advanced stages is generally unsatisfying. The appearance of monoclonal antibodies (MoAbs), like cetuximab and panitumumab, has significantly improved the outcome of mCRC patients. Since the efficacy of anti-*EGFR* therapy is bound up with *KRAS* status in CRC, many studies have estimated the role of

Table 3 Associations Between Gene Mutations and Clinicopathological Characteristics of Patients

	All Wild Type	Any Gene Mutations	P-value	KRAS Mutation	P-value	NRAS Mutation	P-value	BRAF Mutation	P-value
Gender									
Male	56/86 (65.1%)	65/108 (60.2%)	0.481	48/77 (62.3%)	0.712	6/8 (75.0%)	0.712*	11/23 (47.8%)	0.130
Female	30/86 (34.9%)	43/108 (39.8%)		29/77 (37.7%)		2/8 (25.0%)		12/23 (52.2%)	
Age									
<60	46/86 (53.5%)	52/108 (48.1%)	0.460	37/77 (48.1%)	0.488	4/8 (50.0%)	1*	11/23 (47.8%)	0.629
≥60	40/86 (46.5%)	56/108 (51.9%)		40/77 (51.9%)		4/8 (50.0%)		12/23 (52.2%)	
Primary Location									
Right-sided colon	20/86 (23.3%)	41/108 (38.0%)	0.007	30/77 (39.0%)	0.008	1/8 (12.5%)	0.780	10/23 (43.5%)	0.052
Left-sided colon	46/86 (53.5%)	34/108 (31.5%)		23/77 (29.9%)		5/8 (62.5%)		6/23 (26.1%)	
Rectum	20/86 (23.3%)	33/108 (30.6%)		24/77 (31.2%)		2/8 (25.0%)		7/23 (30.4%)	
Tumor infiltration#									
T1	2/80 (2.5%)	1/89 (1.1%)	0.664	1/63 (1.6%)	0.583	0/8 (0.0%)	0.460	0/18 (0.0%)	0.756
T2	2/80 (2.5%)	3/89 (3.4%)		3/63 (4.8%)		0/8 (0.0%)		0/18 (0.0%)	
T3	47/80 (58.8%)	59/89 (66.3%)		42/63 (66.7%)		7/8 (87.5%)		10/18 (55.6%)	
T4	29/80 (36.3%)	26/89 (29.2%)		17/63 (27.0%)		1/8 (12.5%)		8/18 (44.4%)	
Nodal Status#									
N0	21/80 (26.3%)	20/89 (22.5%)	0.407	15/63 (23.8%)	0.765	3/8 (37.5%)	0.309	2/18 (11.1%)	0.178
N1	32/80 (40.0%)	30/89 (33.7%)		23/63 (36.5%)		1/8 (12.5%)		6/18 (33.3%)	
N2	27/80 (33.8%)	39/89 (43.8%)		25/63 (39.7%)		4/8 (50.0%)		10/18 (55.6%)	
Gross type#									
Swell type	13/80 (16.3%)	14/89 (15.7%)	0.753	13/63 (20.6%)	0.271	0/8 (0.0%)	0.349	1/18 (5.6%)	0.284
Ulcer type	63/80 (78.8%)	68/89 (76.4%)		43/63 (68.3%)		8/8 (100.0%)		17/18 (94.4%)	
Invasion type	4/80 (5.0%)	7/89 (7.9%)		7/63 (11.1%)		0/8 (0.0%)		0/18 (0.0%)	
Pathological type									
Adenocarcinoma	75/86 (87.2%)	79/108 (73.1%)	0.016	60/77 (77.9%)	0.117	5/8 (62.5%)	0.094*	15/23 (65.2%)	0.026*
Mucinous/rare adenocarcinoma	11/86 (12.8%)	29/108 (26.9%)		17/77 (22.1%)		3/8 (37.5%)		8/23 (34.8%)	
Differentiation									
Well-moderate	45/86 (52.3%)	60/108 (55.6%)	0.654	47/77 (61.0%)	0.263	4/8 (50.0%)	1*	9/23 (39.1%)	0.261
Poor	41/86 (47.7%)	48/108 (44.4%)		30/77 (39.0%)		4/8 (50.0%)		14/23 (60.9%)	
Location of metastasis									
Liver	55/86 (64.0%)	77/108 (71.3%)	0.276	55/77 (71.4%)	0.309	6/8 (75.0%)	0.708*	16/23 (69.6%)	0.616
Lung	22/86 (25.6%)	27/108 (25.0%)	0.926	20/77 (26.0%)	0.954	3/8 (37.5%)	0.435*	4/23 (17.4%)	0.413
Peritoneum	6/86 (7.0%)	20/108 (18.5%)	0.019	15/77 (19.5%)	0.017	0/8 (0.0%)	1*	5/23 (21.7%)	0.052*
Distant lymph node	21/86 (24.4%)	21/108 (19.4%)	0.403	10/77 (13.0%)	0.063	0/8 (0.0%)	0.192*	11/23 (47.8%)	0.029
Abdominal and pelvic implant	11/86 (12.8%)	23/108 (21.3%)	0.122	17/77 (22.1%)	0.117	1/8 (12.5%)	1*	5/23 (21.7%)	0.322*
Single-site metastasis									
Liver-only metastasis	39/86 (45.3%)	36/108 (33.3%)	0.088	27/77 (35.1%)	0.182	3/8 (37.5%)	0.728*	6/23 (26.1%)	0.096
Lung-only metastasis	4/86 (4.7%)	7/108 (6.5%)	0.758*	5/77 (6.5%)	0.736*	1/8 (12.5%)	0.366*	1/23 (4.3%)	1*
Peritoneum-only metastasis	2/86 (2.3%)	2/108 (1.9%)	1*	1/77 (1.3%)	1*	0/8 (0.0%)	1*	1/23 (4.3%)	0.513*
Distant lymph node-only metastasis	9/86 (10.5%)	1/108 (0.9%)	0.006*	0/77 (0.0%)	0.003*	0/8 (0.0%)	1*	1/23 (4.3%)	0.685*
Abdominal and pelvic implant-only metastasis	5/86 (5.8%)	5/108 (4.6%)	0.753*	5/77 (6.5%)	1*	0/8 (0.0%)	1*	0/23 (0.0%)	0.582*

(*Continued*)

Table 3 (Continued).

	All Wild Type	Any Gene Mutations	P-value	KRAS Mutation	P-value	NRAS Mutation	P-value	BRAF Mutation	P-value
Dual-site metastases									
Liver-lung metastases	14/86 (16.3%)	17/108 (15.7%)	0.919	13/77 (16.9%)	0.918	2/8 (25%)	0.620*	2/23 (8.7%)	0.515*
Liver-peritoneum metastases	1/86 (1.2%)	13/108 (12.0%)	0.004	10/77 (13.0%)	0.003	0/8 (0.0%)	1*	3/23 (13.0%)	0.029*
Lung-peritoneum metastases	1/86 (1.2%)	3/108 (2.8%)	0.631*	2/77 (2.6%)	0.603*	0/8 (0.0%)	1*	1/23 (4.3%)	0.379*
Number of metastases									
1	62/86 (72.1%)	54/108 (50.0%)	0.002	40/77 (51.9%)	0.008	5/8 (62.5%)	0.685*	9/23 (39.1%)	0.003
≥1	24/86 (27.9%)	54/108 (50.0%)		37/77 (48.1%)		3/8 (37.5%)		14/23 (60.9%)	

Notes: #25 patients without surgery were excluded here. *Two-sided Fischer's exact test, others are two-sided $\chi 2$ test.

KRAS status in CRC. Some studies indicated that anti-*EGFR* therapy shows a response to mCRC people with wild-type *KRAS*.[21,22] However, even in *KRAS* wild-type cohorts, more than 65% of patients were still resistant to anti-*EGFR* MoAbs.[23] In further studies, Yuan et al concluded that patients with *BRAF* mutation are unlikely to gain benefit from anti-*EGFR* therapy.[24,25] For mutant *NRAS*, a similar conclusion was also reported by De Roock et al.[16] Furthermore, the latest ASCO guidelines also suggest that response to anti-*EGFR* treatment is confined to patients with all wild type. In addition, Foltran et al illustrated that patients with any mutation of the oncogenes have poorer survival compared to those with all wild type.[26] Despite the fact that the relation between gene mutation and survival of mCRC patients has been fully examined, there is ambiguous understanding of the linkbetween mutant genes and clinicopathological features in mCRC patients in China, especially for distant metastasis.

To our best knowledge, this is the first study to discuss the association between gene mutations and clinicopathological features, especially for metastatic patterns, in first diagnosed mCRC of Chinese population compared with all wild type. We are the first to demonstrate that among patients with any gene mutations, those with *KRAS* mutation and *BRAF* mutation are more likely to carry peritoneal metastasis, liver-peritoneum metastases and multi-organ metastases compared to all wild-type patients. After stratification, *KRAS codon 12* mutation, but not *codon 13* mutation, was remarkably associated with peritoneal metastasis, liver-peritoneum metastases and multi-organ metastases. We are also the first to demonstrate that distant lymph node-only metastasis is visibly linked to all wild-type tumors. In addition, we also found that tumors with any mutations are statistically located in right-sided colon, having mucinous or signet-ring cell component compared to all wild type, which is consistent with a previous study.[27]

It is well known that there is a strong association between survival outcome and site of metastasis. Previous studies found that the survival of patients with peritoneal metastasis was poorer than those with metastasesin other sites,[28,29] which could partly be blamed on local complications like ascites formation. However, the molecular mechanism is still controversial and not well-established. In addition, patients with any gene mutations had a shorter survival time than those with all wild type. Further, mutations in *KRAS* or *BRAF*, which also suffer inferior prognosis compared with all wild-type counterparts,[30] were reported by Liu et al. In our study, we observed that among tumors with any gene mutations, *KRAS* or *BRAF* mutation more frequently metastasized to peritoneum and liver-peritoneum compared to those with all wild type, which might be an explanation of previous studies.

The frequency of mutant *KRAS* in our study was 41%, which was similar to other studies.[12,13] Previous studies have examined the distribution of *KRAS* mutation from western populations, which reported that the most frequent subtype in *codon 12* was *G12D*, followed by *G12V*, *G12C*, *G12S* and *G12A* and *G12R*. In *codon 13* mutations, the majority was *KRAS G13D*, followed by *G13C*, *G13R*.[31,32] However, in the present study, the corresponding order was *G12D*, *G12V*, *G12S*, *G12A*, *G12C* and *G12R* in *codon 12*; in *codon 13*, the corresponding order was *KRAS G13D* and *G13A*. Moreover, we also found some rare mutations, including *A59E*, *A59T* in *KRAS codon 59* and *Q61H*, *Q61L* in *codon 61*, but they were too rare in our study to analyze. Surprisingly, we found five cases

Table 4 Associations Between Different Mutations of KRAS and Clinicopathological Characteristics of Patients

	All Wild Type	KRAS Codon 12 Mutation	P-value	KRAS Codon 13 Mutation	P-value	KRAS G12D Mutation	P-value	KRAS G12V Mutation	P-value	KRAS G13D Mutation	P-value
Gender											
Male	56/86 (65.1%)	34/52 (65.4%)	0.974	8/14 (57.1%)	0.564	13/23 (56.5%)	0.447	13/18 (72.2%)	0.562	6/11 (54.5%)	0.519*
Female	30/86 (34.9%)	18/52 (34.6%)		6/14 (42.9%)		10/23 (43.5%)		5/18 (27.8%)		5/11 (45.5%)	
Age											
<60	46/86 (53.5%)	26/52 (50.0%)	0.691	6/14 (42.9%)	0.460	14/23 (60.9%)	0.527	6/18 (33.3%)	0.120	6/11 (54.5%)	0.947
≥60	40/86 (46.5%)	26/52 (50.0%)		8/14 (57.1%)		9/23 (39.1%)		12/18 (66.7%)		5/11 (45.5%)	
Primary location											
Right-sided colon	20/86 (23.3%)	21/52 (40.4%)	0.026	4/14 (28.6%)	0.181	9/23 (39.1%)	0.063	10/18 (55.6%)	0.022	3/11 (27.3%)	0.196
Left-sided colon	46/86 (53.5%)	16/52 (30.8%)		4/14 (28.6%)		6/23 (26.1%)		6/18 (33.3%)		3/11 (27.3%)	
Rectum	20/86 (23.3%)	15/52 (28.8%)		6/14 (42.9%)		8/23 (34.8%)		2/18 (11.1%)		5/11 (45.5%)	
Tumor infiltration#											
T1	2/80 (2.5%)	0/44 (0.0%)	0.628	1/11 (9.1%)	0.319	0/21 (0.0%)	0.622	0/15 (0.0%)	0.237	1/8 (12.5%)	0.201
T2	2/80 (2.5%)	2/44 (4.5%)		1/11 (9.1%)		0/21 (0.0%)		2/15 (13.3%)		1/8 (12.5%)	
T3	47/80 (58.8%)	28/44 (63.6%)		7/11 (63.6%)		15/21 (71.4%)		9/15 (60.0%)		4/8 (50.0%)	
T4	29/80 (36.3%)	14/44 (31.8%)		2/11 (18.2%)		6/21 (28.6%)		4/15 (26.7%)		2/8 (25.0%)	
Nodal status#											
N0	21/80 (26.3%)	10/44 (22.7%)	0.432	3/11 (27.3%)	0.906	3/21 (14.3%)	0.389	5/15 (33.3%)	0.617	2/8 (25.0%)	0.839
N1	32/80 (40.0%)	14/44 (31.8%)		5/11 (45.5%)		8/21 (38.1%)		4/15 (26.7%)		4/8 (50.0%)	
N2	27/80 (33.8%)	20/44 (45.5%)		3/11 (27.3%)		10/21 (47.6%)		6/15 (40.0%)		2/8 (25.0%)	
Gross type#											
Swell type	13/80 (16.3%)	10/44 (22.7%)	0.587	3/11 (27.3%)	0.535	4/21 (19.0%)	0.954	4/15 (26.7%)	0,254	3/8 (37.5%)	0.293
Ulcer type	63/80 (78.8%)	31/44 (70.5%)		7/11 (63.6%)		16/21 (76.2%)		9/15 (60.0%)		5/8 (62.5%)	
Invasion type	4/80 (5.0%)	3/44 (6.8%)		1/11 (9.1%)		1/21 (4.8%)		2/15 (13.3%)		0/8 (0.0%)	
Pathological type											
Adenocarcinoma	75/86 (87.2%)	39/52 (75.0%)	0.067	13/14 (92.9%)	1*	17/23 (73.9%)	0.191*	13/18 (72.2%)	0.147*	10/11 (90.9%)	1*
Mucinous/rare adenocarcinoma	11/86 (12.8%)	13/52 (25.0%)		1/14 (7.1%)		6/23 (26.1%)		5/18 (27.8%)		1/11 (9.1%)	
Differentiation											
Well-moderate	45/86 (52.3%)	31/52 (59.6%)	0.404	10/14 (71.4%)	0.183	14/23 (60.9%)	0.465	13/18 (72.2%)	0.122	8/11 (72.7%)	0.335*
Poor	41/86 (47.7%)	21/52 (40.4%)		4/14 (28.6%)		9/23 (39.1%)		5/18 (27.8%)		3/11 (27.3%)	

(*Continued*)

Table 4 (Continued).

	All Wild Type	KRAS Codon 12 Mutation	P-value	KRAS Codon 13 Mutation	P-value	KRAS G12D Mutation	P-value	KRAS G12V Mutation	P-value	KRAS G13D Mutation	P-value
Location of metastasis											
Liver	55/86 (64.0%)	41/52 (78.8%)	0.065	7/14 (50.0%)	0.319	19/23 (82.6%)	0.089	13/18 (72.2%)	0.503	6/11 (54.5%)	0.530*
Lung	22/86 (25.6%)	15/52 (28.8%)	0.675	4/14 (28.6%)	0.754*	7/23 (30.4%)	0.640	6/18 (33.3%)	0.562*	3/11 (27.3%)	1*
Peritoneum	6/86 (7.0%)	11/52 (21.2%)	0.014	1/14 (7.1%)	1*	5/23 (21.7%)	0.052*	4/18 (22.2%)	0.068*	1/11 (9.1%)	0.582*
Distant lymph node	21/86 (24.4%)	6/52 (11.5%)	0.065	2/14 (14.3%)	0.512*	3/23 (13.0%)	0.242	3/18 (16.7%)	0.759*	2/11 (18.2%)	1*
Abdominal and pelvic implant	11/86 (12.8%)	11/52 (21.2%)	0.193	2/14 (14.3%)	1*	3/23 (13.0%)	1*	4/18 (22.2%)	0.288*	2/11 (18.2%)	0.639*
Single-site metastasis											
Liver-only metastasis	39/86 (45.3%)	17/52 (32.7%)	0.142	6/14 (42.9%)	0.862	8/23 (34.8%)	0.363	4/18 (22.2%)	0.070	5/11 (45.5%)	1*
Lung-only metastasis	4/86 (4.7%)	2/52 (3.8%)	1*	3/14 (21.4%)	0.055*	2/23 (8.7%)	0.604*	0/18 (0.0%)	1*	2/11 (18.2%)	0.137*
Peritoneum-only metastasis	2/86 (2.3%)	1/52 (1.9%)	1*	0/14 (0.0%)	1*	0/23 (0.0%)	1*	1/18 (5.6%)	0.438*	0/11 (0.0%)	1*
Distant lymph node-only metastasis	9/86 (10.5%)	0/52 (0.0%)	0.014*	0/14 (0.0%)	0.352*	0/23 (0.0%)	0.200*	0/18 (0.0%)	0.353*	0/11 (0.0%)	0.592*
Abdominal and pelvic implant-only metastasis	5/86 (5.8%)	3/52 (5.8%)	1*	2/14 (14.3%)	0.253*	0/23 (0.0%)	0.582*	1/18 (5.6%)	1*	2/11 (18.2%)	0.179*
Dual-site metastases											
Liver-Lung metastases	14/86 (16.3%)	12/52 (23.1%)	0.322	1/14 (7.1%)	0.687*	5/23 (21.7%)	0.544*	5/18 (27.8%)	0.313*	1/11 (9.1%)	1*
Liver-peritoneum metastases	1/86 (1.2%)	8/52 (15.4%)	0.002*	1/14 (7.1%)	0.262*	4/23 (17.4%)	0.007*	2/18 (11.1%)	0.077*	1/11 (9.1%)	0.215*
Lung-peritoneum metastases	1/86 (1.2%)	1/52 (1.9%)	1*	1/14 (7.1%)	0.262*	1/23 (4.3%)	0.379*	0/18 (0.0%)	1*	1/11 (9.1%)	0.215*
Number of metastases											
1	62/86 (72.1%)	23/52 (44.2%)	0.001	12/14 (87.5%)	0.346	10/23 (43.5%)	0.010	6/18 (33.3%)	0.002	9/11 (81.8%)	0.722*
≥1	24/86 (27.9%)	29/52 (55.8%)		2/14 (14.3%)		13/23 (56.5%)		12/18 (66.7%)		2/11 (18.2%)	

Notes: #25 patients without surgery were excluded here. *Two-sided Fischer's exact test, others are two-sided $\chi 2$ test.

with double *KRAS* mutations (2 of *G12D+G12S*, 2 of *G12A+G12S*, 1 of *G12V+D33E*). In addition, *NRAS* mutation was detected in 3% of mCRC patients and similar prevalence was obtained in other studies.[14,15] The majority of mutant subtypes in *NRAS* were *G12D* in *codon 12* and *Q61R* in *codon 61*, followed by *Q61K* in *codon 61*, *G60E* in *codon 60*. According to previous Western and Chinese studies, *BRAF* mutation could be detected in 8%-12% of all patients who suffered from CRC.[28,33–37] The most common subtype of *BRAF* mutation was *V600E*, which accounted for approximately 90% of mutant *BRAF*,[38] in addition, *non-V600 BRAF* mutations were considered as a special and uncommon category (they occurred in 2% of mCRC patients).[14] Certain differences between patients with *V600* and those with *non-V600 BRAF* mutations were reported in other studies,[39] which was not illustrated specifically in our report. In our study, the incidence of *BRAF* mutation was 11.5%, which was consistent with previous research.[18,19] *V600E* was the most frequent subtype of *BRAF* mutation. For *non-V600BRAF*mutation, *BRAF G466V* and *K601E* were found. Previous studies suggested that *KRAS* and *BRAF* mutations were mutually exclusive in mCRC.[40,41] However, in this study, we found one case harbored both *KRAS* and *BRAF* mutations, which demonstrated that *KRAS* and *BRAF* mutations were not mutually exclusive. The result was in line with that of Mao et al.[42]

Furthermore, we confirmed the strong relationship between *KRAS*-mutated carcinomas and right-sided colon, which was also confirmed by several pieces of research.[43–45] However, we could not find obvious significance among mucinous carcinoma, lung metastasis[2,15] and *KRAS* mutation, which might be due to small sample size in our study. In concordance with previous studies,[35,46,47] our study showed that *BRAF*-mutated colorectal cancers were more commonly located in right colon; histologically, the mucinous/rare type was more frequently associated with *BRAF* mutation; and in terms of distant metastases, tumors with mutated *BRAF* were more likely to metastasize to peritoneum and distant lymph nodes. Previous research[15] found that mucinous histology was less frequent in *NRAS* mutated tumors compared to all wild type, which was in line with our result.

For specific *KRAS* mutation, its connection with clinical features is still controversial. Li et al reported that both *KRAS codon 12* and *13* mutated carcinomas were more likely to be found in right-sided colon, and were more frequently mucinous histology type when compared with *KRAS/BRAF* wild-type carcinomas.[48] However, other studies[49,50] observed that *KRAS codon 12* mutation was closely related to mucinous differentiation and right-sided colon; *KRAS codon 13* mutations were more frequently located in right-sided colon, but there was no statistical linkage with mucinous differentiation. Our conclusion was similar to the latter.

Previous laboratory studies[50–52] suggested that the presence of mutation in *KRAS codon 12* confers substantially greater oncogenic potential as compared with *codon 13* mutation. Regulation of *RAS* involves binding of GTP, which activates the protein. Activation of *RAS* enables high affinity interactions with downstream effectors such as *RAF-MAPK* and phosphoinositide 3-kinase. Subsequently, slow intrinsic GTPase activity leads to *RAS* functional in activation. This on and off switch regulation is tightly controlled by ARHGAP (Rho-GTPase activating proteins) and RAPGEF (Rap guanine-nucleotide exchange factors). Interestingly, *RAS* mutants are resistant to ARHGAP-mediated GTPase activation, leading to elevated cellular levels of *RAS*-GTP.[52] Guerrero et al[50] found that *KRAS codon 12* mutation, by altering the threshold for induction of apoptosis, confers a more aggressive tumor phenotype than *codon 13* mutation. This suggests that *codon 12* mutation results in greater resistance to ARHGAP-mediated GTPase activation than *codon 13* mutation. Several research[53,54] has also confirmed that *KRAS* mutation in *codon 12*, rather than in *codon 13*, is a negative factor of survival outcome, when compared with all wild type. In a word, these experimental and clinical data are consistent with our observations that *KRAS codon 12* mutation may be associated with more aggressive tumor behavior to metastasize to peritoneum and liver-peritoneum, which more frequently present with multi-organ metastases.

Despite some positive findings observed in the present study, our study still has some limitations. First of all, owing to the nature of retrospective research, there is unavoidable selection bias in our outcomes. Secondly, based on a relatively small sample size, the amount of samples was not enough to examine other less common mutations, like *KRAS* mutation in *codon 59*, *61*, *117* and *146*. Finally, survival analysis was not performed due to the short follow-up for patients. In further study, we will continuously collect the survival data and therapy regimens for further investigations.

Conclusion

Our study suggests that clinicopathological characteristics (specifically for metastasis) are related to *KRAS/NRAS/*

BRAF mutations in therapy-naïve synchronous mCRC population in China. We demonstrated that distant lymph node-only metastasis is visibly linked with all wild-type tumors. We found that patients with any gene mutations, *KRAS* mutation are more likely to carry peritoneal metastasis, liver-peritoneum metastases and multi-organ metastases than those with all wild type. After stratification, *KRAS codon 12* mutation, but not *codon 13* mutation, was remarkably associated with peritoneal metastasis, liver-peritoneum metastases and multi-organ metastases compared to all wild type. These results may be useful for aiding in the prediction of prognosis and choosing the appropriate regimens for therapy.

Disclosure

The authors report no conflicts of interest for this work.

References

1. Siegel R, DeSantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin*. 2014;64:104–117. doi:10.3322/caac.21220
2. Bonnot PE, Passot G. RAS mutation: site of disease and recurrence pattern in colorectal cancer. *Chin Clin Oncol*. 2019;8:55. doi:10.21037/cco.2019.08.11
3. Jass JR, Whitehall VL, Young J, Leggett BA. Emerging concepts in colorectal neoplasia. *Gastroenterology*. 2002;123:862–876. doi:10.1053/gast.2002.35392
4. Ogino S, Goel A. Molecular classification and correlates in colorectal cancer. *J Mol Diagn*. 2008;10:13–27. doi:10.2353/jmoldx.2008.070082
5. Barault L, Veyrie N, Jooste V, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. *Int J Cancer*. 2008;122:2255–2259. doi:10.1002/ijc.23388
6. Cengel KA, Voong KR, Chandrasekaran S, et al. Oncogenic K-ras signals through epidermal growth factor receptor and wild-type H-ras to promote radiation survival in pancreatic and colorectal carcinoma cells. *Neoplasia*. 2007;9:341–348. doi:10.1593/neo.06823
7. Sartore-Bianchi A, Martini M, Molinari F, et al. PIK3CAMutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Res*. 2009;69:1851–1857. doi:10.1158/0008-5472.can-08-2466
8. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med*. 2004;351:337–345. doi:10.1056/NEJMoa033025
9. Van Cutsem E, Peeters M, Siena S, et al. Open-Label Phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J Clin Oncol*. 2007;25:1658–1664. doi:10.1200/jco.2006.08.1620
10. Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. *N Engl J Med*. 2005;352:5. doi:10.1056/NEJMra040958
11. Bokemeyer C, Bondarenko I, Hartmann JT, et al. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann Oncol*. 2011;22:1535–1546. doi:10.1093/annonc/mdq632
12. Normanno N, Tejpar S, Morgillo F, et al. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. *Nat Rev Clin Oncol*. 2009;6:519–527. doi:10.1038/nrclinonc.2009.111
13. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br J Cancer*. 2001;85:692–696. doi:10.1054/bjoc.2001.1964
14. Afrăsânie V-A, Marinca MV, Alexa-Stratulat T, et al. KRAS, NRAS, BRAF, HER2 and microsatellite instability in metastatic colorectal cancer – practical implications for the clinician. *Radiol Oncol*. 2019;53:265–274. doi:10.2478/raon-2019-0033
15. Schirripa M, Cremolini C, Loupakis F, et al. Role of NRAS mutations as prognostic and predictive markers in metastatic colorectal cancer. *Int J Cancer*. 2015;136:83–90. doi:10.1002/ijc.28955
16. De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol*. 2010;11:753–762. doi:10.1016/s1470-2045(10)70130-3
17. Au H-J, Karapetis CS, O'Callaghan CJ, et al. Health-related quality of life in patients with advanced colorectal cancer treated with cetuximab: overall and KRAS -specific results of the NCIC CTG and AGITG CO.17 trial. *J Clin Oncol*. 2009;27:1822–1828. doi:10.1200/jco.2008.19.6048
18. Tol J, Nagtegaal ID, Punt CJ. BRAF mutation in metastatic colorectal cancer. *N Engl J Med*. 2009;361:98–99. doi:10.1056/NEJMc0904160
19. Chen D, et al. BRAFV600E mutation and its association with clinicopathological features of colorectal cancer: a systematic review and meta-analysis. *PLoS One*. 2014;9:e90607. doi:10.1371/journal.pone.0090607
20. Cantwell-Dorris ER, O'Leary JJ, Sheils OM. BRAFV600E: implications for carcinogenesis and molecular therapy. *Mol Cancer Ther*. 2011;10:385–394. doi:10.1158/1535-7163.MCT-10-0799
21. Tol J, Dijkstra JR, Klomp M, et al. Markers for EGFR pathway activation as predictor of outcome in metastatic colorectal cancer patients treated with or without cetuximab. *Eur J Cancer*. 2010;46:1997–2009. doi:10.1016/j.ejca.2010.03.036
22. Qiu L-X, Mao C, Zhang J, et al. Predictive and prognostic value of KRAS mutations in metastatic colorectal cancer patients treated with cetuximab: a meta-analysis of 22 studies. *Eur J Cancer*. 2010;46:2781–2787. doi:10.1016/j.ejca.2010.05.022
23. Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology Provisional Clinical Opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti–epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol*. 2009;27:2091–2096. doi:10.1200/JCO.2009.21.9170
24. Van Cutsem E, Cervantes A, Adam R, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol*. 2016;27:1386–1422. doi:10.1093/annonc/mdw235
25. Yuan ZX, et al. The prognostic role of BRAF mutation in metastatic colorectal cancer receiving anti-EGFR monoclonal antibodies: a meta-analysis. *PLoS One*. 2013;8:e65995. doi:10.1371/journal.pone.0065995
26. Foltran L, Maglio GD, Pella N, et al. Prognostic role of KRAS, NRAS, BRAF and PIK3CA mutations in advanced colorectal cancer. *Future Oncol*. 2015;11:629–640. doi:10.2217/fon.14.279
27. Rimbert J, Tachon G, Junca A, et al. Association between clinicopathological characteristics and RAS mutation in colorectal cancer. *Modern Pathol*. 2018;31:517–526. doi:10.1038/modpathol.2017.119
28. Tie J, Gibbs P, Lipton L, et al. Optimizing targeted therapeutic development: analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. *Int J Cancer*. 2011;128:2075–2084. doi:10.1002/ijc.25555
29. Franko J, Shi Q, Meyers JP, et al. Prognosis of patients with peritoneal metastatic colorectal cancer given systemic therapy: an analysis of individual patient data from prospective randomised trials from the Analysis and Research in Cancers of the Digestive System (ARCAD) database. *Lancet Oncol*. 2016;17:1709–1719. doi:10.1016/s1470-2045(16)30500-9

30. Liu J, Zeng W, Huang C, et al. Predictive and prognostic implications of mutation profiling and microsatellite instability status in patients with metastatic colorectal carcinoma. *Gastroenterol Res Pract*. 2018;2018:4585802. doi:10.1155/2018/4585802
31. Neumann J, Zeindl-Eberhart E, Kirchner T, Jung A. Frequency and type of KRAS mutations in routine diagnostic analysis of metastatic colorectal cancer. *Pathol Res Pract*. 2009;205:858–862. doi:10.1016/j.prp.2009.07.010
32. Vaughn CP, Zobell SD, Furtado LV, Baker CL, Samowitz WS. Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. *Genes Chromosomes Cancer*. 2011;50:307–312. doi:10.1002/gcc.20854
33. Maughan TS, Adams RA, Smith CG, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised Phase 3 MRC COIN trial. *The Lancet*. 2011;377:2103–2114. doi:10.1016/s0140-6736(11)60613-2
34. Yokota T, Ura T, Shibata N, et al. BRAF mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer. *Br J Cancer*. 2011;104:856–862. doi:10.1038/bjc.2011.19
35. Tran B, Kopetz S, Tie J, et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *Cancer*. 2011;117:4623–4632. doi:10.1002/cncr.26086
36. Richman SD, Seymour MT, Chambers P, et al. KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. *J Clin Oncol*. 2009;27:5931–5937. doi:10.1200/JCO.2009.22.4295
37. Souglakos J, Philips J, Wang R, et al. Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. *Br J Cancer*. 2009;101:465–472. doi:10.1038/sj.bjc.6605164
38. Wan PT, Garnett MJ, Roe SM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell*. 2004;116:855–867. doi:10.1016/s0092-8674(04)00215-6
39. Zarkavelis G, et al. Current and future biomarkers in colorectal cancer. *Ann Gastroenterol*. 2017;30:613–621. doi:10.20524/aog.2017.0191
40. Li WQ, et al. BRAF mutations are associated with distinctive clinical, pathological and molecular features of colorectal cancer independently of microsatellite instability status. *Mol Cancer*. 2006;5:2. doi:10.1186/1476-4598-5-2
41. Rajagopalan H, Bardelli A, Lengauer C, et al. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. *Nature*. 2002;418:934. doi:10.1038/418934a
42. Mao C, Zhou J, Yang Z, et al. KRAS, BRAF and PIK3CA mutations and the loss of PTEN expression in Chinese patients with colorectal cancer. *PLoS One*. 2012;7:e36653. doi:10.1371/journal.pone.0036653
43. Tong JH, Lung RW, Sin FM, et al. Characterization of rare transforming KRAS mutations in sporadic colorectal cancer. *Cancer Biol Ther*. 2014;15:768–776. doi:10.4161/cbt.28550
44. Gonsalves WI, Mahoney MR, Sargent DJ, et al. Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147. *J Natl Cancer Inst*. 2014;106. doi:10.1093/jnci/dju106
45. Yaeger R, Cowell E, Chou JF, et al. RAS mutations affect pattern of metastatic spread and increase propensity for brain metastasis in colorectal cancer. *Cancer*. 2015;121:1195–1203. doi:10.1002/cncr.29196
46. Kambara T. BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. *Gut*. 2004;53:1137–1144. doi:10.1136/gut.2003.037671
47. Yaeger R, Cercek A, Chou JF, et al. BRAF mutation predicts for poor outcomes after metastasectomy in patients with metastatic colorectal cancer. *Cancer*. 2014;120:2316–2324. doi:10.1002/cncr.28729
48. Li W, et al. Colorectal carcinomas with KRAS codon 12 mutation are associated with more advanced tumor stages. *BMC Cancer*. 2015;15:340. doi:10.1186/s12885-015-1345-3
49. Yoon HH, Tougeron D, Shi Q, et al. KRAS codon 12 and 13 mutations in relation to disease-free survival in BRAF-wild-type stage III colon cancers from an adjuvant chemotherapy trial (N0147 alliance). *Clin Cancer Res*. 2014;20:3033–3043. doi:10.1158/1078-0432.CCR-13-3140
50. Guerrero S, et al. K-ras codon 12 mutation induces higher level of resistance to apoptosis and predisposition to anchorage-independent growth than codon 13 mutation or proto-oncogene overexpression. *Cancer Res*. 2000;60:6750–6756.
51. Bollag G, McCormick F. Intrinsic and GTPase-activating protein-stimulated Ras GTPase assays. *Methods Enzymol*. 1995;255:161–170. doi:10.1016/s0076-6879(95)55020-8
52. Karnoub AE, Weinberg RA. Ras oncogenes: split personalities. *Nat Rev Mol Cell Biol*. 2008;9:517–531. doi:10.1038/nrm2438
53. Russo A, Bazan V, Agnese V, Rodolico V, Gebbia N. Prognostic and predictive factors in colorectal cancer: kirsten Ras in CRC (RASCAL) and TP53CRC collaborative studies. *Ann Oncol*. 2005;16 Suppl 4:iv44–49. doi:10.1093/annonc/mdi907
54. Imamura Y, Morikawa T, Liao X, et al. Specific mutations in KRAS codons 12 and 13, and patient prognosis in 1075 BRAF wild-type colorectal cancers. *Clin Cancer Res*. 2012;18:4753–4763. doi:10.1158/1078-0432.CCR-11-3210