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ORIGINAL ARTICLE

Retrospective Study

KRAS and *BRAF* gene mutations and DNA mismatch repair status in Chinese colorectal carcinoma patients

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Abstract

AIM: To investigate gene mutations and DNA mismatch repair (MMR) protein abnormality in Chinese colorectal carcinoma (CRC) patients and their correlations with clinicopathologic features.

METHODS: Clinical and pathological information for 535 patients including 538 tumors was reviewed and recorded. Mutation analyses for exon 2 of *KRAS* gene and exon 15 of *BRAF* gene were performed by Sanger sequencing except that in 9 tumors amplification refractory mutation system PCR was used. Expression of MMR proteins including MHL1, MSH2, MSH6 and PMS2 was evaluated by immunohistochemistry. Correlations of *KRAS* and *BRAF* mutation status and the expression status of MMR proteins with age, gender, cancer stage, location, and histology were analyzed. Correlations between *KRAS* or *BRAF* mutations and MMR protein expression were also explored.

RESULTS: The overall frequencies of *KRAS* and *BRAF* mutations were 37.9% and 4.4%, respectively. KRAS mutations were more common in patients ⩾ 50 years old (39.8% *vs* 22% in patients < 50 years old, $P < 0.05$). The frequencies of *BRAF* mutants were higher in tumors from females (6.6% *vs* males 2.8%, $P < 0.05$), located in the right colon (9.6% *vs* 2.1% in the left colon, 1.8% in the rectum, $P < 0.01$), with mucinous differentiation (9.8% *vs* 2.8% without mucinous differentiation, $P < 0.01$), or being poorly differentiated (9.5% *vs* 3.4% well/moderately differentiated, $P < 0.05$). MMR deficiency was strongly associated with proximal location (20.5% in the right colon *vs* 9.2% in the left colon and 5.1% in the rectum, $P < 0.001$), early cancer stage (15.0% in stages Ⅰ-Ⅱ *vs* 7.7% in stages Ⅲ-Ⅳ, $P < 0.05$), and mucinous differentiation (20.2% *vs* 9.2% without mucin, $P < 0.01$). A higher frequency of MLH1/PMS2 loss was found in females (9.2% vs 4.4% in males, $P < 0.05$), and MSH2/MSH6 loss tended to be seen in younger (<50 years old) patients (12.0% *vs* 4.0% ⩾ 50 years old, $P < 0.05$). MMR deficient tumors were less likely to have *KRAS* mutations (18.8% *vs* 41.7% in MMR proficient tumors, $P < 0.05$) and tumors

with abnormal MLH1/PMS2 tended to harbor *BRAF* mutations (15.4% *vs* 4.2% in MMR proficient tumors, $P < 0.05$).

CONCLUSION: The frequency of sporadic CRCs having *BRAF* mutation, MLH1 deficiency and MSI in Chinese population may be lower than that in the Western population.

Key words: Colorectal carcinoma; *KRAS*; BRAF; DNA mismatch repair; MLH1; MSH2; MSH6; PMS2

Core tip: Tests for KRAS, BRAF and DNA mismatch repair protein status were important for clinical management of patients with colorectal carcinoma (CRC). Investigations from large samples for these molecular markers were limited in Chinese CRC patients. In the present study, we collected and summarized clinicopathological and molecular data of 535 CRC patients in our institution. These results would help to understand CRC molecular features and guide Lynch syndrome screening, CRC clinical management and individualized therapy in the Chinese population.

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INTRODUCTION

Colorectal carcinoma (CRC) is one of the most common malignancies in the world. Although CRC in China is not as common as that in most Western countries, its incidence has increased steadily in recent years due to living standard improvement and lifestyle change[1].

Based on current knowledge on the carcinogenesis of CRCs, individualized clinical managements have been recommended for CRC patients[2,3]. Testing tumor tissue for predictive or prognostic gene mutations to guide personalized therapy is a rapidly emerging field in pathology.

The epidermal growth factor receptor (EGFR) is a major therapeutic target in CRCs[4]. Activating mutations of the *KRAS* gene is thought to stimulate the RAS/RAF/MAPK pathway independent of EGFR activation, therefore CRCs with *KRAS* mutations are resistant to EGFR inhibitors[5]. Although the predictive value of BRAF mutation status for response to EGFR inhibitors is still uncertain[6,7], its prognostic value for CRCs is widely accepted, *i.e.*, patients with the *BRAF* V600E mutation tend to have a poor prognosis[8-10]. Moreover, the presence of the BRAF V600E mutation in a MLH1 deficient CRC indicates that it is a sporadic rather than a Lynch syndrome associated carcinoma with high level of microsatellite instability (MSI-H)[11]. MSI-H CRCs are either caused by germ line mutations or epigenetic silencing of DNA mismatch repair (MMR) genes[2] and have distinct clinical and pathological features. Detection of mismatch repair protein deficiency or MSI status is not only useful for screening Lynch syndrome but also can serve as a prognostic marker for favorable outcome. In addition, it is also a negative predictive marker for fluoropyrimidine-based chemotherapy in patients with stage Ⅱ disease[12,13]. From 2010, mutation analysis for *KRAS* and *BRAF* as well as MSI/MMR testing has been suggested to be performed for CRC patients by the National Comprehensive Cancer Network (NCCN) clinical practice guidelines[14].

Frequencies of *KRAS* and *BRAF* mutations and MSI-H in CRCs have been widely studied in Western populations. Among them, *KRAS* mutations are the most frequent molecular changes, with a frequency ranging from 22% to 46.7%[15-20], while *BRAF* mutations are less frequent, with a frequency ranging from 5.0% to 21.8%[15,21-25]. Several studies have reported the frequencies of *KRAS* and *BRAF* mutations in Chinese CRC patients in the English literature. However, most of the studies performed with limited sample size and the results were controversial, with a frequency of *KRAS* mutations ranging from 19.7% to 43.9% and that of *BRAF* mutations ranging from 1.7% to 25.4%[26-32]. MSI CRCs account for approximately 15-20% of all CRCs in Western countries[17,19,20,33-35]. Limited reports from China show a frequency of MSI CRCs in Chinese patients (ranging from 9.6% to 13%) lower than that in Western populations but close to reports from Korea[36-40].

Information from previous studies raises the possibility that geographic and/or racial differences may present between Chinese and Western populations. Therefore, more data are needed to further clarify the characteristics of these important molecular changes in Chinese CRC patients. In the present study, we collected the data of CRC patients treated from 2010 to 2013 in our department and hope to provide more information about CRC in Chinese patients.

MATERIALS AND METHODS

Patients and tissues

We searched the pathology database of the Department of Pathology of Peking University Third Hospital from 2010 to 2013 for primary or metastatic colorectal adenocarcinomas. Five hundred and thirty-five patients with 538 tumors tested for *KRAS* and *BRAF* mutations or MMR protein expression

Table 1 Primary antibodies for immunohistochemical staining for mismatch repair proteins

Antibody	Supplied by	Clone No.	Positive signal localization	Dilution	Incubation temperature (℃)	Incubation period (h)
MLH1	Novocastra, UK	14	Nuclear	1:50	37	2
MSH2	Novocastra, UK	FE11	Nuclear	1:20	37	2
MSH6	Novocastra, UK	BC/44	Nuclear	1:80	37	2
PSM2	Novocastra, UK	EP51	Nuclear	1:30	37	2

Table 2 Primer sequences for *KRAS* exon 2 and *BRAF* exons 15, annealing temperature and size of expected PCR products

Exon	Primer No.	Primer sequence 5'-3'	AT (℃)	Product size (bp)
KRAS 2	2-F	TAGTCACATTTTCATTATTTTTAT	55	160
	2-R	AGATTTACCTCTATTGTTGGAT		
BRAF 15	15-F	ATCTACTGTTTTCCTTTACTTACT	55	160
	15-R	ATTCTTACCATCCACAAAATG		

AT: Annealing temperature; F: Forward; R: Reverse.

were collected. The pathology records and clinical charts were reviewed to obtain the following information: patient gender, age, anatomic site of tumor, morphological characteristics (histologic type, tumor grade, depth of tumor penetration, lymph node involvement, lymphatic or vascular invasion, perineural invasion), and history of metastasis or other tumors. Base on clinical data, primary locations of tumors were defined as the right colon (from the cecum through the transverse colon), left colon (from the splenic flexure through the rectosigmoid flexure) and rectum (15 cm above the anal verge). Tumors were staged according to the seventh edition of the American Joint Commission on Cancer (AJCC) TNM staging system. Well to moderately differentiated tumors were grouped together and tumors diagnosed as mucinous adenocarcinomas, signet-ring cell carcinomas and adenocarcinomas with mucinous or signet-ring cell differentiation were recorded as mucin-producing tumors.

Data and tissue collection was approved by the Ethics Committee of Peking University Health Science Center, following the ethical guidelines of the 1975 Declaration of Helsinki.

Immunohistochemistry for MMR proteins

Sections of one representative block of each tumor were incubated with antibodies to MLH1, MSH2, MSH6 and PMS2. Standard heat-induced epitope retrieval in EDTA solution (pH 9.0 for MLH1, PMS2, and MSH2, pH 6.0 for MSH6) and immunostaining and signal detection using the Dako EnVision Detection System and a Dako Autostainer (Dako North America Inc., California, United States) were performed. The sources, dilutions, and incubation time of each primary antibody are listed in Table 1. Any tumor cell with nuclear staining was defined as positive for that marker. Positive staining for all these proteins was regarded as proficient MMR (pMMR). Negative staining for any of these four proteins was regarded as deficient MMR (dMMR) (Figure 1). Since family history and genetic information were unavailable, no attempt was made to further classify patients into with Lynch syndrome or sporadic MSI-H CRCs.

Analysis of KRAS or BRAF gene mutations by Sanger sequencing

As previously described by Zhong *et al*[41] and Liu *et al*[42], genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue sections with the Qiagen Blood and Tissue Kit (QiagenInc, Valencia, CA) according to the manufacturer's protocol. The *KRAS* exon 2 and BRAF exon 15 were amplified by polymerase chain reaction (PCR) using PromegaGoTaq Hot Start Colorless Master Mixes (Promega Corporation, Madison, WI, United States). The specific primers and sizes of the expected amplicons are presented in Table 2. Genomic DNA of 50-100 ng was amplified in a 50 μL reaction system containing 25 μL of Hot Start Colorless Master Mix and 5 μL of 10 μmol/L primer mix. The PCR reaction conditions consisted of 2 min at 95 ℃, 40 cycles of 94 ℃ for 30 s, 55 ℃ for 40 s and 72 ℃ for 1 min, and 72 ℃ for 7 min. Five microliters of the PCR product was analyzed on a 1.2% agarose gel with 100 to 600 bp DNA marker. Gels were visualized on a BioRad Gel Doc 2000TM system and Quantity One software (BioRad, Hercules, CA, United States). The resulting PCR amplicons were purified and sequenced in both directions using the BigDye Terminator kit and an ABI Prism 3500 DNA Analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Mutant cases were validated by a second independent PCR and sequencing. The sequencing results were observed with ABI Sequence Scanner software and compared with the reference sequences of the *BRAF* and *KRAS* genes from NCBI database to mark the position of nucleotide change.

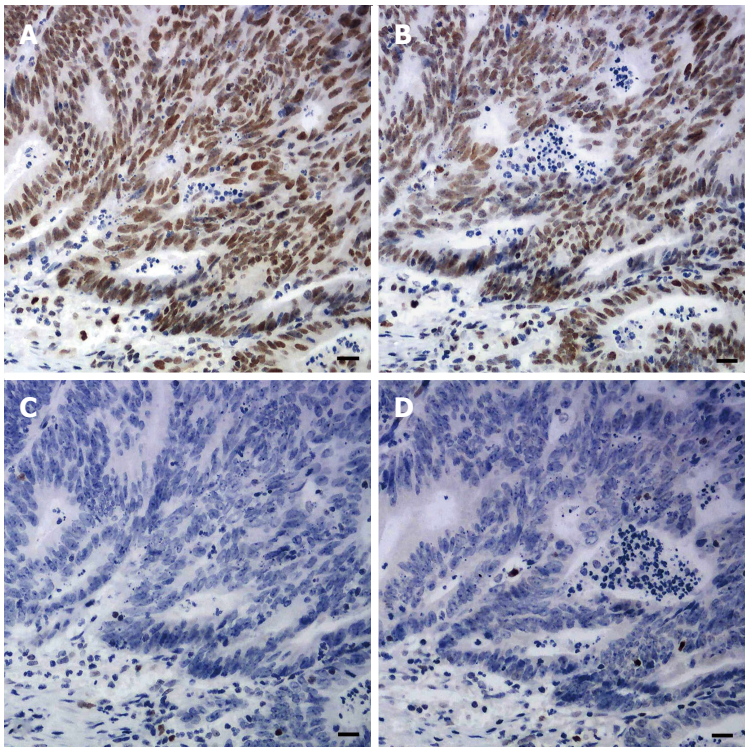

Figure 1 Immunohistochemical staining for DNA mismatch repair proteins in one case of colorectal carcinoma. Tumor cells with retained MLH1 (A) and PMS2 (B) expression, and with absent MSH2 (C) and MSH6 (D) expression, which were regarded as deficient MMR. Note stromal cells and lymphocytes serving as internal positive controls.

Analysis of KRAS and BRAF gene mutations by amplification refractory mutation system PCR

For nine cases, mutations in *KRAS* exon 2 and *BRAF* exon 15 were identified by amplification refractory mutation system (ARMS)-PCR. Briefly, FFPE tissues were digested using 20 mg/mL proteinase K in ATL buffer (Qiagen) overnight at 56 ℃. DNA isolation was performed with the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's protocol. *KRAS* and *BRAF* mutation status was assessed with Human *KRAS* Gene 7 Mutations Fluorescence Polymerase Chain Reaction Diagnostic Kit and Human *BRAF* Gene V600E Mutations Fluorescence Polymerase Chain Reaction Diagnostic Kit (Amoy Diagnostics Co. Ltd, Xiamen, China) on the Agilent-Stratagene M × 3000P Q-PCR System (Agilent Technologies, Santa Clara, CA), according to the manufacturers' instructions. The 7 most common *KRAS* mutations (p.G12D, p.G12V, p.G12A, p.G12C, p.G12S, p.G12R, and p.G13D) in CRCs were detected. The reaction conditions included 1 cycle at 95 ℃ for 5min; 15 cycles at 95 ℃ for 25 s, 64 ℃ for 20 s, 70 ℃ for 20 s; 31 cycles at 93 ℃ for 25 s, 60 ℃ for 35 s, 72 ℃ for 20 s. Fluorescence signals were collected at 60 ℃.

Statistical analysis

Clinical and pathological characteristics are summarized as percentages. Mutation rates of *KRAS* and *BRAF* and overall or each MMR protein expression deficiency were also calculated. Clinical and pathological characteristics were compared across different subgroups and analyzed with χ^2 test. Correlations between *KRAS* or *BRAF* mutations and dMMR status were also explored. *P* values < 0.05 were considered statistically significant.

RESULTS

Patient characteristics

Of these 535 patients, males were slightly more than females with a male to female ratio of 1.34:1. Patient age at presentation ranged from 21 to 95 years (median, 65 years) with 10.7% of patients < 50 years at diagnosis. Most patients (61.2%) presented with stage Ⅱ or Ⅲ disease. Almost all patients had solitary primary tumor except that three patients had synchronous tumors (two patients had two tumors confined in the left colon, one had two tumors with one in the right colon and the other in the rectum). In 6 patients, only metastatic lesions were available for testing, and 3 of them received neoadjuvant therapy. Both preoperative biopsy and radical specimens were tested for *KRAS* and *BRAF* mutations in two patients, and both primary and metastatic lesions were tested in 5 patients. Since there was no discrepancy between biopsy and radical resection specimens or between primary and metastatic tumors, the results from two different tests were recorded as once. Clinical information of all studied patients is summarized in Table 3.

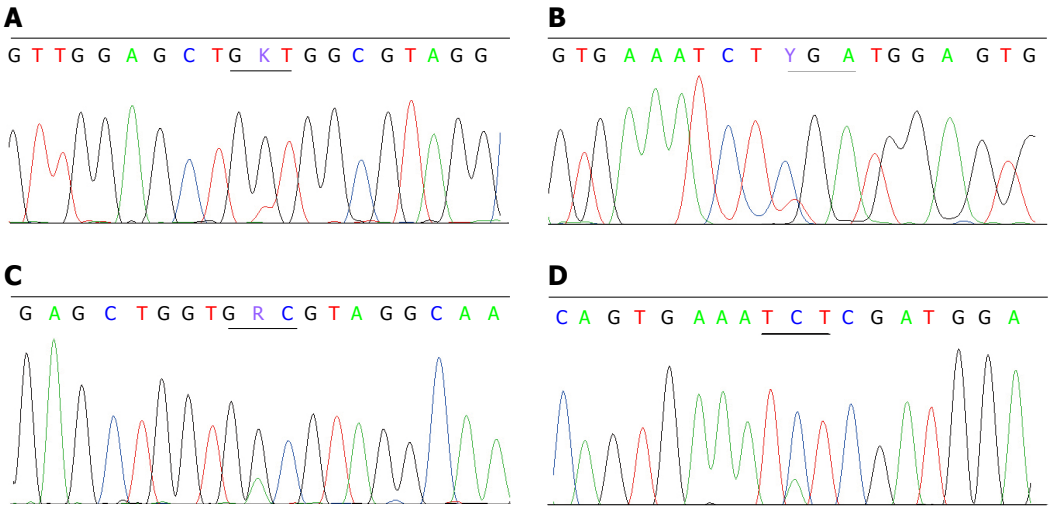

Figure 2 Sequence chromatograms displaying concomitant mutations of *KRAS* and *BRAF* found in this study. Concomitant mutations of *KRAS* G12V (A) and *BRAF* R603stop were detected in colorectal carcinoma tissue of a 71-year-old woman (B). Another patient, a 62-year-old woman, had mutations of both KRAS G13D (C) and BRAF S602Y (D). The amino acid changes corresponding to the codon alternations are as follows: G12V (GGT→GTT) and G13D (GGC→GAC) in exon 2 of the KRAS gene, S602Y (TCT→TAT) and R603stop (CGA→TGA) in exon 15 of the *BRAF* gene.

Table 3 Clinicopathological information of the studied patients *n* (%)

Clinical feature		Total cases
Gender	Male	306 (57.2)
	Female	229 (42.8)
Age (yr)	< 50	57 (10.7)
	⩾ 50	478 (89.3)
Location	Right colon	177 (32.9)
	Left colon	165 (30.7)
	Rectum	196 (36.4)
Tumor differentiation	Poor	94 (17.5)
	Well-moderate	444 (82.5)
Tumor stage	I	95 (17.7)
	II	168 (31.2)
	III	215 (40.0)
	IV	50 (9.3)
	Not available	10 (1.9)

KRAS gene mutations and correlations with clinicopathological features

KRAS status was ascertained for 485 patients including 488 tumors. The over-all mutation rate was 37.9% (185/488). *KRAS* mutations identified in codon 12 included G12D (*n* = 90, 18.4%), G12V (*n* = 37, 7.6%), G12C (*n* = 9, 1.8%), G12A (*n* = 8, 1.6%), G12S (*n* = 7, 1.4%), and G12R (*n* = 1, 0.2%). Mutations in *KRAS* codon 13 included G13D (*n* = 31, 6.4%), G13C (*n* = 1, 0.2%) and G13G (silent mutation) (*n* = 1, 0.2%). One patient was identified with concomitant *KRAS* mutations in codon 12 and codon 33 (G12D and D33N). Compared with patients < 50 years old, *KRAS* mutations in patients ⩾ 50 years old were more common (39.8% *vs* 22%, $P < 0.05$). The rate of *KRAS* mutations was not significantly associated with gender, tumor location, tumor differentiation, stage or mucin production (Table 4).

Table 4 Correlations between *KRAS* and *BRAF* status and clinicopathological features *n* (%)

Clinicopathological feature	*KRAS*		*BRAF*	
	Mutant/tested cases	*P* value	Mutant/tested cases	*P* value
Gender				
Male	100/277 (36.1)	0.336	7/254 (2.8)	0.048
Female	84/208 (40.4)		13/196 (6.6)	
Age (yr)				
< 50	11/50 (22)	0.014	3/47 (6.4)	0.758
⩾ 50	173/435 (39.8)		17/403 (4.2)	
Location				
Right colon	67/161 (41.6)	0.318	14/146 (9.6)	0.001
Left colon	50/150 (33.3)		3/167 (2.1)	
Rectum	68/177 (38.4)		3/140 (1.8)	
Mucin production				
With	41/108 (38)	0.990	10/102 (9.8)	0.006
Without	144/380 (37.9)		10/351 (2.8)	
Tumor differentiation				
Poor	25/84 (29.8)	0.091	7/74 (9.5)	0.045
Well-moderate	160/404 (39.6)		13/379 (3.4)	
Tumor stage				
I - II	85/232 (36.6)	0.553	10/218 (4.6)	0.918
III - IV	97/247 (39.3)		10/228 (4.4)	

BRAF mutations and correlations with clinicopathological features

Twenty (4.4%) mutations were detected in 453 CRCs from 450 patients. Sixteen mutations were V600E and the other four were D594G, D594N, R603stop and S602Y. There were no concomitant *KRAS* and *BRAF* V600E mutations, but one patient had concomitant mutations of *KRAS* G12V (Figure 2A) and BRAF R603stop (Figure 2B), and one with KRAS G13D (Figure 2C) and BRAF S602Y (Figure 2D). The proportion of mutant *BRAF* was higher in females (6.6% *vs* 2.8% in males, $P < 0.05$), in tumors

Table 5 Correlations between DNA mismatch repair protein deficiency and clinicopathological features *n* (%)

Clinicopathological feature		dMMR		MLH1/PMS2		MSH2/MSH6	
		Defective/tested	*P* value	Defective/tested	*P* value	Defective/tested	*P* value
Total		55/481 (11.4)		31/481 (6.4)		24/481 (5.0)	
Gender	Male	25/272 (9.2)	0.095	12/272 (4.4)	0.034	13/272 (4.8)	0.970
	Female	29/206 (14.1)		19/206 (9.2)		10/206 (4.9)	
Age (yr)	< 50	9/50 (18.0)	0.114	3/50 (6.0))	1.000	6/50 (12.0)	0.031
	⩾ 50	45/428 (10.5)		28/428 (6.5)		17/428 (4.0)	
Location	Right colon	33/161 (20.5)	0.000	23/161 (14.3)	0.000	10/161 (6.2)	0.446
	Left colon	13/142 (9.2)		5/142 (3.5)		8/142 (5.6)	
	Rectum	9/178 (5.1)		3/178 (1.7)		6/178 (3.4)	
Mucin production	With	20/99 (20.2)	0.002	11/99 (11.1)	0.034	9/99 (9.1)	0.065
	Without	35/382 (9.2)		20/382 (5.2)		15/382 (3.9)	
Tumor differentiation	Poor	13/79 (16.5)	0.125	6/79 (7.6)	0.649	7/79 (8.9)	0.148
	Well-moderate	42/402 (10.4)		25/402 (6.2)		17/402 (4.2)	
Tumor stage	Ⅰ-Ⅱ	37/246 (15.0)	0.012	20/246 (8.1)	0.127	17/246 (6.9)	0.049
	Ⅲ-Ⅳ	18/234 (7.7)		11/234 (4.7)		7/234 (3.0)	

Table 6 Correlations between DNA mismatch repair protein expression deficiency and KRAS or BRAF status *n* (%)

MMR status	KRAS (*n* = 432)		BRAF (*n* = 424)	
	Mutant/tested cases	*P* value	Mutant/tested cases	*P* value
dMMR	9/48 (18.8)	0.002[1]	4/43 (9.3)	0.264
MLH1/PMS2 deficiency	3/29 (10.3)	0.001[2]	4/26 (15.4)	0.037[3]
MSH2/MSH6 deficiency	6/19 (31.6)	0.383[3]	0/17 (0)	0.817[3]
pMMR	160/384 (41.7)		16/381 (4.2)	

[1]dMMR *vs* pMMR; [2]MLH1/PMS2 deficiency *vs* pMMR; [3]MSH2/MSH6 *vs* pMMR. MMR: DNA mismatch repair.

located in the right colon (9.6% *vs* 2.1% in the left colon and 1.8% in the rectum, $P < 0.05$), with poor differentiation (9.5% *vs* 3.4% with well-moderate differentiation, $P < 0.05$) and with mucinous appearance (9.8% *vs* 2.8% without mucin, $P < 0.05$). *BRAF* mutations were not related with patient's age or tumor stage (Table 4).

MMR protein expression and correlations with clinicopathological features

Overall, 11.4% of tumors showed loss of expression for at least one MMR protein, and the deficient rates of MLH1, MSH2, MSH6 and PMS2 in studied patients were 5.8% (28/481), 2.7% (13/481), 3.8% (18/480) and 6.5% (19/293), respectively. The proportion of dMMR tumors varied by site with a significantly higher rate (20.5%) in tumors located in the right colon compared to those in the left colon (9.2%) and rectum (5.1%, $P < 0.001$). There were more stages Ⅰ-Ⅱ dMMR tumors than stages Ⅲ-Ⅳ dMMR tumors (15.0% *vs* 7.7%, $P < 0.05$) and dMMR tumors tended to show mucinous differentiation. In females, a higher frequency of MLH1/PMS2 deficiency was found (9.2% *vs* 4.4% in males, $P < 0.05$). Loss of MSH2/MSH6 expression was more frequent in patients < 50 years old than in those ⩾ 50 years old (12.0% *vs* 4.0%, $P < 0.05$). Although dMMR tumors were more often in tumors with poor differentiation than in those with well-moderate differentiation, it did not show a significant difference (16.5% *vs* 10.4%, $P > 0.05$) (Table 5).

Correlations between KRAS and BRAF mutations and dMMR status

Less *KRAS* mutants were seen in dMMR tumors than in pMMR tumors (18.8% *vs* 41.7%, $P < 0.05$). *BRAF* mutation rate was higher in dMMR tumors than in pMMR tumors, although it did not show a significant difference (9.3% *vs* 4.2%, $P > 0.05$). Nevertheless, tumors with defected MLH1/PMS2 tended to harbor *BRAF* mutations compared with pMMR tumors (15.4% *vs* 4.2%, $P < 0.05$). No *BRAF* mutation was detected in tumors with MSH2/MSH6 deficiency (Table 6).

DISCUSSION

As predictive and/or prognostic biomarkers, *KRAS* and *BRAF* mutation tests are important for predicting response to EGFR-targeted therapy and prognosis of CRC patients. However, regional and racial differences in mutation rates may be present[15-19,38,43,44]. Marked differences between Chinese population and other countries were also observed[26-32], which need to be further confirmed in studies based on large sample size.

In the present study, we found an overall *KRAS* mutation rate of 37.9% in CRCs, which was close to most of the previous reports either about Chinese or other ethnicities[15-18,20,26-30,43,45-49]. Studies based on clinical practices or CRC cell line models showed that

CRCs with *KRAS* G13D mutation responded partly to cetuximab and panitumumab[50,51]. Therefore, the subtype of KRAS mutations may also have clinical implications. In our cases, the major mutant types were G12D, G12V and G13D, accounting for 85% of all mutations. About 6.4% of CRC patients in our group were found to have *KRAS* G13D mutation, which was also consistent with other reports[15,16,52]. One uncommon finding in our cases was concomitant *KRAS* mutations in codon 12 (G12D) and codon 33 (D33N). The patient was a 59-year-old male with a poorly differentiated tumor located in the left colon. To our knowledge, the latter mutation is the first identified mutant in CRCs. Other non-hot mutations were also found in previous reports, however, clinical impact of these mutations is unknown[53-55]. CRCs or colorectal cancer cell lines with special *KRAS* mutations seemed to have different malignant potential and proliferative ability[50,56], which may lead to different responses to anti-EGFR agents. However, detailed mechanism needs further exploration.

Correlations between *KRAS* status and clinicopathological features are controversial. Some of the previous reports showed that the frequency of *KRAS* mutation was in association with age, gender, tumor grade or stage but some did not[15,16,19,26,28,30]. Reports from four independent Chinese groups[28,30,45,52] showed that *KRAS* mutations were associated with patient gender, but not with patient age. In a study including 966 CRCs, Gao *et al*[57] observed that *KRAS* mutations were not only associated with patient gender and age, but also with tumor differentiation. Similar inconsistent results were also presented in studies on other ethnicities[46,55]. In our series of cases, we could only find that tumors in patients older than 50 years tended to harbor mutant KRAS. These diverse findings suggest that the difference in *KRAS* mutation rates in different groups is too minute to be declared with limited samples. The different criteria for age division might also be a cause[46,58].

BRAF mutation rates are significantly different in previous studies of non-Chinese population[15,21-25]. The lowest mutation rate came from a study on Japanese CRC patients (3%), followed by reports from Russia and Israel (4.1% and 5%, respectively)[24,43,44]. On the contrary, reports about European and Americans showed significantly higher rates of *BRAF* mutation, which were mostly around 15%[19,20,23,59]. In most of the studies about Chinese population[30,31,48], low percentage of CRCs were found to harbor mutant BRAF, ranging from 1.7% to 7%. The only exception is a report from Mao *et al*[32], which showed that the frequency of *BRAF* mutations was as high as 25.4% in a group of Chinese CRC patients. However, their report also showed that 24% of *KRAS* mutant cases had concomitant *BRAF* V600E mutation. This phenomenon was inconsistent with the general opinion that concomitant *KRAS* and *BRAF* V600E mutations were rare if not mutually exclusive[15,45,60,61]. Considering the relatively limited number of cases included in their study (69 cases), the result may be not representative. In our study, we also observed a low *BRAF* mutation rate of 4.4%, which was consistent with most of other reports in Chinese and was similar to that in Japanese[44]. Our finding together with other reports supports the opinion that the frequency of *BRAF* mutation varies among different races and/or regions[21,31,61]. In addition, no patient was found to have concomitant mutations for *KRAS* and *BRAF* V600E in our cases. However, of the four cases with non-V600E *BRAF* mutation, two had concomitant *KRAS* mutation. Although the significance for these uncommon mutations was uncertain[22], our findings further confirmed the rarity of concomitant *KRAS* and *BRAF* V600E mutations in CRCs. Also in accordance with previous reports[21,61], we found *BRAF* mutation to be more common in females, in proximally located poorly differentiated tumors, or in mucin-producing tumors, although no significant association was found between *BRAF* mutations and patient age or tumor stage. This may be partially because the number of BRAF mutant cases is limited in our study.

CRCs with deficient MMR exhibit high frequency MSI and have distinctive clinicopathological features, biological behavior and clinical treatments compared to CRCs with pMMR, which in most cases are microsatellite stable (MSS)[2]. dMMR CRCs accounted for about 15%-20% of all CRCs in reports from Western countries[12,13,17,19,20,33,34,62], and are more common in stage Ⅱ tumors (up to 22%) than in stage Ⅲ tumors (up to 14%)[15,35]. Information about dMMR CRCs in Chinese population is limited. Huang *et al*[36] and Jin *et al*[37] using PCR-based MSI testing showed that the frequencies of MSI-H CRCs in their cases from Southeast China were 11.9% to 13%, which were slightly lower than most of the reports from Western populations[15,17,19,20,33-35]. Detecting MMR protein loss by IHC showed similar efficiency to more complex gene analysis for MSI or MMR gene mutations[63,64]. The current study is one of the largest series that analyzed the MMR status by IHC in Chinese CRCs. Again, our data showed a low frequency of dMMR in Chinese CRCs. The overall frequency of dMMR CRCs and the frequencies of dMMR CRCs in early stage (stage Ⅰ-Ⅱ) and advanced stage (stage Ⅲ-Ⅳ) CRCs were 11.4%, 15.4% and 7.7%, respectively. Our findings, together with Huang *et al*[36]'s and Jin *et al*[37]'s reports, suggest that CRCs in Chinese might have different genetic background from that in Western populations, and were less likely to have MSI.

Of note, the majority of dMMR CRCs are sporadic and caused by inactivation of MLH1 (about 95%). Lynch syndrome, which is caused by germ-line mutations of MMR genes, accounts for about 20%

of the dMMR CRCs, and MLH1 mutation is found in approximately 40% of the cases[65]. Therefore, the vast majority of dMMR CRCs should have MLH1 deficiency. By using IHC, our study was able to inform which MMR proteins were lost in an MSI CRC, and provide more information than previous reports in Chinese CRCs. Surprisingly, our data showed only 50.9% of dMMR CRCs with MLH1 loss, which was much lower than expected. Moreover, *BRAF* mutations have been reported in 33% to 60% of MSI-H tumors particularly in tumors with methylation of the MLH1 promoter[20,35,66,67]. Among our dMMR cases, only 15.4% had *BRAF* mutations, which was also much lower than previous reports. These findings suggest that the low frequency of dMMR CRCs in our series is likely caused by including less sporadic dMMR CRCs. Our finding further indicated that ethnic and geographic differences might be present in Chinese dMMR CRCs, although further investigations, such as germ-line mutation analysis of MMR genes and/or analysis of MLH1 promoter methylation, are needed to clarify this possibility. Since Hampel *et al*[68]'s study has showed that the widely used Amsterdam or Bethesda screening guideline may miss as many as 22% of patients with Lynch syndrome, feasible and economic IHC technique was suggested to perform on all newly diagnosed CRCs to screen Lynch syndrome and guide clinical management for MSI patients[69,70]. In this case, there will be more and more data coming out to provide detailed information of Chinese CRCs.

In summary, our results show a low frequency of *BRAF* mutations and MMR deficiency, especially less MLH1 deficiency, in a large series of Chinese CRC patients. It suggests that CRCs are less likely to have MSI in Chinese populations, and it is probably caused by the fact that there are less sporadic MMR deficient CRCs in Chinese. However, additional epidemiologic data and genetic investigations are needed to confirm the difference.

COMMENTS

Background

Mutation analyses for *KRAS* and *BRAF* as well as microsatellites instability/DNA mismatch repair (MSI/MMR) testing have been suggested to be performed for patients with colorectal carcinomas (CRCs). Information from previous studies raises the possibility that geographic and/or racial differences may be present between Chinese and Western populations. Investigations from large samples for these molecular markers were limited in Chinese CRC patients.

Research frontiers

The current study demonstrated that a low frequency of *BRAF* mutations and MMR deficiency, especially MLH1 deficiency, in a large series of Chinese CRC patients.

Innovations and breakthroughs

Results in the present study suggest that CRCs are less likely to have MSI in Chinese populations, and it is probably caused by the fact that there are less sporadic MMR deficient CRCs in Chinese.

Applications

These results would help to understand CRC molecular features and guide Lynch syndrome screening, CRC clinical management and individualized therapy in the Chinese population.

Peer-review

The study reports *KRAS* and *BRAF* gene mutations and MMR protein expression status in large number of Chinese CRC patients. The results corroborate the earlier findings and show high rates of mutations in the *KRAS* gene. Associations of these genetic markers with various clinicopathological parameters have also been made. The study also shows less MMR defects in these patients.

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