Original Paper

Comprehensive Analysis of the Relationship Between RAS and RAF Mutations and MSI Status of Colorectal Cancer in Northeastern China

Wenqi Li^a Huining Li^{b,c} Ruiqi Liu^d Xinxin Yang^a Yuhui Gao^a
Yangyang Niu^a Jiashi Geng^e Yingwei Xue^f Xiaoming Jin^c
Qi You^f Jingshu Geng^{a,c} Hongxue Meng^{a,b,c}

^aDepartment of Pathology, Harbin Medical University Cancer Hospital, Harbin, ^bDepartment of Pathology, The First Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, ^cDepartment of Pathology, Harbin Medical University, Harbin, ^dDepartment of Radiation Oncology, State Key Laboratory of Oncology in South China, Collaborative innovation Center for Cancer Medicine, Sun Yat-Sen University Cancer Center, Guangzhou, ^eDepartment of Radiology, Harbin Medical University Cancer Hospital, Harbin, ^fDepartment of Gastroenterology, Harbin Medical University Cancer Hospital, Harbin, China

Key Words

Ras • RAF • MSI • Colorectal cancer • Northeastern China • Race

Abstract

Background/Aims: Colorectal cancer (CRC) is mainly caused by chromosomal instability (CIN) and microsatellite instability (MSI). The *RAS* and *RAF* genes are essential components of the CIN pathway, and several studies have found that *RAS* and *RAF* mutations are associated with MSI status in CRC. Here, we examined these three factors in CRC in Northeast China and aimed to reveal new details of the relationship between these mutations and MSI status. **Methods:** This study involved 290 patients with CRC who had *RAS* or *RAF* gene mutation detected using fluorescence-based allele-specific polymerase chain reaction or Sanger sequencing. The majority of the identified patients were found to harbor MSI (MSI status). Accurate molecular detection was carried out using formalin-fixed paraffin-embedded tissue or blood samples. **Results:** The rates of *RAS* and *RAF* mutations were 58.5% and 4.1%, respectively. The prevalence of *RAS* mutation in CRC was clearly higher and that of *RAF* mutation was lower in Northeast China compared with previously reported cohorts in other locations. High MSI level (MSI-H status) was more complex, at around 10%. This was consistent with previous data from China. However, compared with data reported from other continents, MSI-H was higher than that of Japan or South Korea in Asia, and lower than that of Europe or the United States. **Conclusion:**

W. Li, H. Li and R. Liu contributed equally to this work.

Hongxue Meng
and Qi You

Department of Pathology, Harbin Medical University Cancer Hospital
150 Haping Road, Harbin (China)
E-Mail menghongxue15@163.com; youqi318@126.com

RAS/RAF mutations and MSI status in CRC are closely associated with tumor location and ethnicity. Further studies investigating the relationship between these three factors can help in the development of treatment strategies for patients with CRC.

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Introduction

As the third most common cancer worldwide [1], colorectal cancer (CRC) mainly develops through two major genetic pathways: chromosomal instability (CIN) and microsatellite instability (MSI) [2]. The CIN pathway of CRC accounts for about 80–85% of these tumors [3]. Besides this pathway, the RAS-RAF-MEK-ERK-MAPK pathway is the most well-known pathway in the pathogenesis of CRC. Mutations in this pathway have been reported in about 50% of patients with CRC [4]. Both *RAS* and *RAF* are proto-oncogenes and can be divided into two different types: wild-type and mutant. When a gene mutation occurs, it may activate the adenoma-carcinoma sequence, causing mucosal hyperplasia, which can lead to the development of adenoma, and eventually carcinoma [5]. Furthermore, once *RAS* or *RAF* mutations occur, the downstream pathway is no longer regulated by the upstream signal. The whole pathway continues to be activated and this promotes tumor formation.

Epidermal growth factor receptor (EGFR) is an upstream factor in the RAS-RAF-MEK-ERK-MAPK pathway [6]. As a result, mutations in the downstream factors, will affect response to EGFR treatment in CRC. Members of the RAS family include *KRAS*, *NRAS*, and *HRAS* [7]. Therefore, *RAS* gene mutations are heterozygous. The most important mutation occurs in *KRAS*, most commonly in codon 12 and 13. Based on previous statistical analyses, *KRAS* codon 12/13 mutations account for 80% of all *RAS* mutations. Patients who have been found to have *KRAS* mutations in codon 12/13 should not receive anti-EGFR drugs [8]. Similarly, a high-quality meta-analysis has previously indicated that there is insufficient evidence to suggest that CRC patients with *RAS* mutations can benefit from vascular endothelial growth factor therapy [9]. However, in many cases, patients with wild-type *KRAS* codon 12/13 are still unable to benefit from traditional targeted therapies, suggesting that there may be other mutations in the signaling pathway, such as *NRAS* and *HRAS* mutations.

BRAF, a serine/threonine-specific protein kinase, shares a high degree of sequence homology with *CRAF* and *ARAF* [10]. It binds receptors and RAS proteins on the cell surface via the nuclear transcription factors, MEK and ERK, and then activates several other factors. *BRAF* participates in the regulation of various biological processes, such as cell growth, differentiation, and apoptosis. The hotspot for mutations in *BRAF* is at codon 600 (V600E), with very few mutations occurring in other loci. Hence, the presence of *RAS* or *RAF* mutations can lead to drug resistance with poor prognosis.

The MSI pathway accounts for the development of a lesser proportion (around 15%) of tumors in CRC [3]. However, its role cannot be ignored. The majority of CRCs caused by MSI are sporadic. Others include hereditary nonpolyposis colorectal cancer/Lynch syndrome [11]. MSI has been reported to be caused by defects in DNA mismatch repair (MMR) genes, such as *MLH1*, *MSH2*, *PMS2*, and *MSH6* [3]. MSI is commonly due to high-level CpG island methylation of *MLH1* promoter and of gene silencing [12]. MSI caused by MMR defects is conserved in the genome through DNA replication and cell division. It may increase the instability of other genes, leading to genomic instability. This also results in abnormal cell proliferation and differentiation. Several possible factors promote the occurrence of tumors. However, although there is a risk of inducing cancer, patients with high levels of MSI (MSI-H) CRC have a favorable prognosis. MSI-H status inhibits the tumor-promoting effects of PD1–PD-L1 interaction, blocks the signaling pathway, and causes cancer cell death [13].

In recent years, a large number of studies on *RAS*, *RAF*, and MSI have been conducted. However, the relationship between these three factors remains to be explored. This has prompted us to examine two conundrums. The first involves one individual, in whom the mutation frequency of the three factors in different parts of the large bowel is examined, along with the relationship with the clinical features and pathological findings. The second

is to analyze the molecular differences in CRC among different races and compare the latest data from China with previously reported data from various other continents.

Regarding the different parts of the large bowel in the same cohort, although the colon and rectum seem to be a continuous entity, it can be subdivided into three parts: right colon, left colon, and rectum. Each part has different features with different molecular characteristics, clinical manifestations, treatment responses, and outcomes [14]. In order to explore their specific differences and similarities, they may be regarded as three separate parts of the body.

Over time, diagnostic standards and technological innovations may lead to changes in data for the same race. It is also possible that genotypic or phenotypic traits change as a species evolves. For different races, there are few studies on the differences in CRC molecules in different countries so far. Exploring the occurrence and development of CRC in each race, and the changes in CRC molecules in different countries, will help us further understand susceptibility to cancer, vulnerable populations, and adverse factors.

It is extremely difficult to conduct a cross-continent clinical case study due to the high costs and long time required, as well as differences in test standards. However, it is possible to analyze the relationship between the three CRC factors in various countries and regions by extensively reviewing experimental data from various countries.

Materials and Methods

Participants and sample collection

In this study, we investigated 303 patients who had been diagnosed with primary CRC at the Harbin Medical University Cancer Hospital, from January 2016 to November 2017. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissues for PCR analysis or Sanger sequencing. All patients were found to harbor *RAS* and/or *RAF* gene mutations (*RAS* and/or *RAF* gene status). Based on this, majority of the patients were tested for MSI status. Among these, 290 patients with CRC were eligible, and their DNA was extracted from FFPE tissues for further analysis.

In this study, we collected detailed patient information before performing any analysis. Hence, clinical data of all patients were retrospectively reviewed, including sex, age, lymph node metastasis, pathologic stage, mutational status, and treatment and chemotherapy response, in addition to the treatment course for each patient.

We accumulated cancer cases including 70 cases in the right colon, 80 cases in the left colon, and 140 cases in the rectum for our study. Compared with left- or right-sided colon cancer, a greater proportion of patients had rectal cancer. The position of the tumor, which with the location on the border of the two sides of the colon, was regarded as the major location for the purpose of classification of the final position. All patients underwent radical resection of the primary tumor without preoperative radiotherapy and chemotherapy.

Ethical approval

This study was carried out according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants, and this study was approved by the Institutional Ethics Committee of Harbin Medical University Cancer Hospital (Protocol KY-27).

RAS and RAF detection

Detection of *RAS* and *RAF* genes depends mainly on PCR amplification. DNA was extracted from the patient's blood sample or from FFPE tissue using the DNA FFPE Tissue Kit (Applied Biosystems, Foster City, CA). The recommended concentration of DNA template to be used was 1–3 ng/L. The samples were tested using positive and negative controls. The samples were then subjected to PCR with a 7500 Fast Real-Time PCR System (Applied Biosystems). Amplification conditions were as follows: Pre-denaturation at 95°C for 5 min; followed by 15 cycles at 95°C for 25 s, 64°C for 45 s, 72°C for 20 s; and then 31 cycles at 93°C for 25 s, 60°C for 35 s, and 72°C for 20 s. After separation and purification, *KRAS* (exon 2, 3, 4), *NRAS* (exon 2, 3, 4), *HRAS* (exon 2), and *BRAF* genes were detected with PCR using the ABI 7500 real-time fluorescence system

(Applied Biosystems). The mutation status of *RAS* and *RAF* was analyzed based on the amplification curves of FAM and HEX signals.

MSI detection

MSI status was determined using a 3730 sequencer (Life Technologies, Carlsbad, CA). For this purpose, whole blood samples (1 μ L) or prepared FFPE tissue were diluted to 2 ng/ μ L or 20 ng/ μ L, respectively, followed by addition of 2.8 μ L ddH₂O, 4 μ L 2.5 $\times$ Buffer A, 2 μ L 5 $\times$ MSI Primer Mix, and 0.2 μ L Taq DNA Polymerase I. PCR amplification was carried out as follows: Pre-denaturation at 95°C for 5 min; followed by 30 cycles at 94°C for 30 s, 60°C for 1 min, 70°C for 1 min; and then final extension at 60°C for 30 min. Finally, the temperature was reduced to 15°C, and samples were centrifuged at 3000 rpm for 1 min. NR-21 and BAT-26 were labeled with a blue fluorescent dye, NR-27 and BAT-25 with a green dye, and NR-24 and MONO-27 with a yellow dye. Tumors were termed MSI-H if two or more markers showed instability, MSI-L if only one marker was unstable, and MSS in case of no mutation.

Statistical analysis

All statistical analyses were carried out using the SPSS17.0 software. The independent sample *t*-test was used to compare the relationship between *RAS* and *RAF* mutations and MSI status based on age, site, sex, and other factors. *P* < 0.05 was considered statistically significant.

Results

Sample selection

In our study, 290 eligible samples were included, (177 male, 113 female). The average age of patients was 59.8 (range 31–87) years. According to the clinical records, there were 80 cases of left colon cancer, 70 cases of right colon cancer, and 140 cases of rectal cancer. We obtained and analyzed the samples over a period of 2 years.

Frequency of *RAS* mutation

RAS status was evaluated in 53 cases using matched normal and tumor FFPE tissue or blood samples (Fig. 1, 2). Almost half the patients were male and the remaining were female (26 vs 27) (Table 1). *RAS* mutations are heterozygous, with many kinds of mutations, but are relatively concentrated on specific codons. The majority of the *RAS* mutations are at *KRAS* exon 2 (codon 12/13). In our specimens, *RAS* genes were mutated in 31 cases (58.5%), and 90% (28/31) of these mutations were *KRAS* mutations. It is worth mentioning that mutations in *KRAS* exon 2 (codon 12/13) accounted for the vast majority, about 89% (25/28), of all *KRAS* mutations. The mutation rate of *KRAS* exon 2 (codon 61) was 1.9% (1/53), the same as that of *HRAS* exon 2 (codon 12/13) (Table 2). Similarly, the mutation rates of *KRAS* exon 4 (codon 146) and *NRAS* exon 3 (codon 61) were the same, around 3.8% (2/53). The linear relationship between *RAS* mutation status and CRC is discontinuous, being highest in the right semicolon, second highest in the rectum, and lowest in the left semicolon. Sex had very little effect on the status of *RAS* mutation. In our study, the majority of the patients with *RAS* mutations were less than 50 years of age, suggesting the presence of *RAS* mutations in patients with early-onset CRC (*P* = 0.028).

Frequency of *RAF* mutation

In contrast to *RAS* mutations, *RAF* mutations are homozygous. In our study, all *RAF* mutations were in *BRAF*^{V600E} (Fig. 1, 2). The *RAF* gene was detected in 269 patients with CRC, including 166 males and 103 females (Table 1), and was mutated in 11 patients with a mutation rate of 4.1%. Moreover, analysis based on sex showed a *RAF* mutation rate of in female patients (5.8%), nearly 2 times higher than that in male patients (3%); there was no statistically significant difference. Next, we aimed to determine the effect of *RAF* mutations in different locations on CRC. The mutation rates of *RAF* in the right colon, left semicolon, and rectum were 7.7%, 3.8%, and 2.4%, respectively (Table 2). This suggested a linear

Fig. 1. ARMS-PCR to detect RAS and RAF genes, and comparison of mutant and wild-type samples. Example of mutant (left) and wild-type (right) KRAS and RAF gene expression using PCR.

Fig. 2. Sanger sequencing to detect RAS and RAF genes, and comparison of mutant and wild-type samples. Example of mutant (left) and wild-type (right) RAS and RAF gene expression using Sanger sequencing.

Table 1. Clinical, pathological, molecular, and biological characteristics of patients based on RAS, RAF, and MSI expression in CRC

Characteristics	RAS Mutant n (%)	Wild-type n (%)	RAF Mutant n (%)	Wild-type n (%)	MSI MSI-H n (%)	MSS n (%)
Age (years)						
<40	1 (3.2)	1 (4.5)	0	8 (3.1)	1 (4.2)	6 (2.8)
40–49	9 (29.0)	1 (4.5)	3 (27.3)	27 (10.5)	5 (20.8)	21 (9.7)
50–59	8 (25.8)	10 (45.5)	6 (54.5)	78 (30.2)	8 (33.3)	65 (30.1)
≥60	13 (41.9)	10 (45.5)	2 (18.1)	145 (56.2)	10 (41.7)	124 (57.4)
Sex						
Male	15 (48.4)	11 (50)	5 (45.5)	161 (62.4)	14 (58.3)	139 (64.4)
Female	16 (51.6)	11 (50)	6 (54.5)	97 (37.6)	10 (41.7)	77 (35.6)
MSI status						
MSS/MSI-L	0	53 (100)	9 (81.8)	236 (91.5)	/	/
MSI	0	0	2 (18.1)	22 (8.5)	/	/
Tumor location						
Proximal colon	3 (9.7)	8 (36.4)	3 (27.3)	124 (48.1)	4 (16.7)	69 (31.9)
Distal colon	6 (19.4)	1 (4.5)	3 (27.3)	75 (29.1)	17 (70.8)	44 (20.4)
Rectum	22 (71.0)	13 (59.1)	5 (45.5)	59 (22.9)	3 (12.5)	103 (47.7)
Type						
Mucinous adenocarcinoma	4 (12.9)	1 (4.5)	1 (9.1)	24 (9.3)	3 (12.5)	19 (8.8)
Adenocarcinoma	27 (87.1)	21 (95.5)	10 (90.9)	234 (90.7)	21 (87.5)	197 (91.2)
Histological grade (adenocarcinoma)						
High	0	0	0	9 (3.5)	1 (4.2)	9 (4.2)
Medium	25 (80.6)	18 (81.8)	5 (45.5)	200 (77.5)	13 (54.2)	170 (78.7)
Low	2 (6.5)	3 (13.6)	5 (45.5)	25 (9.7)	7 (29.2)	18 (8.3)
Disease stage						
II	22 (71.0)	14 (63.6)	5 (45.5)	224 (86.8)	24	188 (87.0)
III	9 (29.0)	8 (36.4)	6 (54.5)	32 (12.4)	0	26 (12.0)
IV	0	0	0	2 (0.8)	0	2 (0.9)
T stage						
2	0	1 (4.5)	1 (9.1)	5 (1.9)	0	6 (2.8)
3	31 (100)	21 (95.5)	10 (90.9)	253 (98.1)	24	210 (97.2)
LN metastasis						
0	22 (71)	14 (63.6)	5 (45.5)	226 (87.6)	24	190 (88.0)
1	4 (12.9)	4 (18.2)	5 (45.5)	22 (8.5)	0	21 (9.7)
2	5 (16.1)	4 (18.2)	1 (9.1)	10 (3.9)	0	5 (2.3)
Metastasis						
0	31 (100)	22 (100)	11 (100)	256 (99.2)	24	214 (99.1)
1	0	0	0	2 (0.8)	0	2 (0.9)

relationship between *RAF* mutation and tumor location, a continuous downward trend from the cecum to rectum (Table 1). In summary, we can conclude that *RAF* mutations are closely associated with sex and the proximal colon. In addition, the number of lymph nodes involved was higher in patients with *RAF* mutations (54.5% vs 12.4%; $P = 0.031$), and there were more mutations in middle-aged patients than in patients aged less than 60 years ($P = 0.020$). The risk of poorly differentiated tumors in patients with *RAF* mutations was significantly increased ($P = 0.049$). Well-differentiated tumors were found in patients with *BRAF* wild-type, but no such case was found in patients with *RAF* mutant.

Table 2. Relationship between RAS and RAF mutations and MSI status in different parts of the large bowel

MSI status (240)	n (%)	Left	Right	Rectum
MSI-H	10% (24)	4	17	3
MSI-L	0 (0)	0	0	0
MSS	90% (216)	69	44	103
RAF mutations (269)				
Positive	4.1% (11)	3	3	5
Negative	95.9% (258)	124	75	59
RAS mutations (53)				
Positive	58.5% (31)	3	6	22
Negative	41.5% (22)	8	1	13
KRAS	52.8% (28)	2	5	21
KRAS exon 2 (codon 12/13)	47.2% (25)	2	3	20
KRAS exon 2 (codon 61)	1.9% (1)	0	0	1
KRAS exon 4 (codon 164)	3.8% (2)	0	2	0
NRAS exon 3 (codon 61)	3.8% (2)	1	0	1
HRAS exon 2 (codon 12/13)	1.9% (1)	0	1	0
Sex				
Male	61% (177)	51	43	83
Female	39.0% (113)	29	27	57
Age (years)				
<40	3.1% (9)	1	3	5
40–49	12.1% (35)	9	11	15
50–59	31.4% (91)	27	16	47
≥60	53.4% (155)	43	40	71

Frequency of MSI status

We determined the MSI status in samples obtained from a total of 240 patients. Results showed that 10% of the patients had MSI-H status and the remaining 90% had MSS status (Table 1). Interestingly, not even one patient with MSI-L was detected. There was a clear relationship between MSI-H status and tumor location (Table 2). MSI-H status in right colon cancer patients was found to be 27.9% (17/61), as shown by a small peak. Compared to this, MSI-H status in left colon and rectal cancer patients was lower: 5.5% (4/73) and 2.8% (3/106), respectively. A descending linear relationship, similar to that of *RAF* mutation, was observed. The incidence of MSI-H CRC in the right colon was nearly 10 times higher than that in the rectum. Further review of cases showed that patients with MSI-H status were found to have deeper tumor invasion, and MSI-H status was closely associated with poorly differentiated carcinoma or moderately to poorly differentiated carcinoma ($P = 0.030$). In addition, no case of mutation in lymph node in MSI-H patients was found in our study. There was a significant correlation between MSI status and lymph node metastasis ($P < 0.001$).

Discussion

RAS is one of the earliest discovered factors that can affect the development of CRC. In this study, the *RAS* mutation rate was 58.5%. Among the *RAS* mutations, majority of the mutations were in *KRAS* exon 2 (codon 12/13), accounting for 47.2%. The rest of the mutations were in the specific *RAS* gene, which accounted for 19.3% of the total number of mutations. Two mutations each occurred in *KRAS* exon 2 (codon 164) and *NRAS* exon 3 (codon 61), while one mutation each occurred in *KRAS* exon 2 (codon 61) and *HRAS* exon 2 (codon 12/13). Our data suggested that *KRAS* is the most important member of the *RAS* family. Compared with previous research data from China, the total *RAS* mutation or the specific *RAS* mutation is higher in this study.

By comparing the mutation rates of *KRAS* in different countries [15-42] (Table 3), the following conclusions were drawn: the *KRAS* mutation rate among Americans is the highest [15-19], followed by Europeans [33-42], and then Asians [20-32]. *KRAS* is the most important member of the *RAS* family. It conforms to the digital relationship of *RAS* in different countries. *NRAS* mutation has been the focus of research in recent years. In Asia, the mutation rate seems to be lower than that in other regions [21, 24, 31], but the difference is not significant. There was no significant difference in *NRAS* mutation rates in other countries, where the mutation rate was around 2-4%.

In our study, the *RAS* mutation rate of was significantly higher in cancer located in the proximal colon (85.7%) than in the distal colon cancer (27.2%). In rectal cancer, the rate was 62.8%. The mutation frequency curve was not a straight linear [15, 40], which is consistent with the results of most of the countries. In addition, some scholars believe

Table 3. Frequency of RAS mutation in different continents

Continent	Country	KRAS Mutation rate (%)	NRAS Mutation rate (%)	Title	Year
North America	America	48.5	4	Loree et al. [15]	2017
		50.8		Pershad et al. [16]	2017
		49.5		Taieb et al. [17]	2016
		52.6		Gong et al. [18]	2016
South America	Brazil	46.8	3.3	Yamane et al. [19]	2014
		38.8		Lin et al. [20]	2014
		37		Chang et al. [21]	2013
		36.2		Lee et al. [22]	2015
Asia	Korea	35.3	1.2	Kim et al. [23]	2014
		44.1		Sasak et al. [24]	2016
		38.4		Kadowaki et al. [25]	2015
		38.6		Okada et al. [26]	2014
	Vietnam	27.9		Soeda et al. [27]	2012
		33.3		Miwata et al. [28]	2014
		37.4		Mahbooben et al. [29]	2008
		31.8		Suhaimi et al. [30]	2015
	India	23	2	Bagadi et al. [31]	2012
		21.8		Chaiyapan et al. [32]	2013
		39.7		Birgisson et al. [33]	2015
		35.4		Frouws et al. [34]	2017
Europe	The Netherlands	40	2.4	Morkel et al. [35]	2015
		42.2		Roa et al. [36]	2013
		35.6		Rako et al. [37]	2012
		35.6		Palomba et al. [38]	2016
	England	45.6	4.4	Douillard et al. [39]	2013
		36.6		Samara et al. [40]	2015
		41.3		Negru et al. [41]	2014
		39.2		Negru et al. [41]	2014
	Romania	39.2		Negru et al. [41]	2014
		35.6		Oleg et al. [42]	2015

that for *RAS* mutations the frequency curve should be a straight-linear type, descending from the right colon to rectum [25]. On the other hand, most scholars believe that *RAS* mutations are associated with sex, and the mutation rate in females is significantly higher than that in males [38, 42], which is different from our findings.

RAF, a gene that functions downstream of EGFR, also has some effects on EGFR treatment. *RAF* mutations are quite common, although the frequency is very low. The *RAF* mutation rate in the total population is only 4.1%, and is closely associated with right-sided tumors, female sex, and lower age. Hence, it is lower than other cohorts previously reported in China (Table 4). In Western European countries [33-40, 43-44], most studies demonstrated that the *RAF* mutation rate was over 10%. In the United States, only a few studies demonstrated a higher mutation rate of *RAF* [15-17, 45]. In Asia, the mutation rate is the lowest, ranging between 4-9% [24-28, 45-47].

According to previously obtained data, the mutation rate of *RAF* decreases from the right colon to rectum, which is in accordance with our data. The frequency of *RAF* mutation in various countries is 3-8 times higher in colon cancer than in rectal cancer [23, 44]. The ratio of involvement is almost 2-5 times on the right compared to the left [43]. Besides, *BRAF* has an obvious relationship with tumor position and sex, and is also associated with high-grade tumors, mucous adenocarcinoma, and lymph node metastases.

In our study, MSI-H status was more complex and much higher than current values in China. However, compared with previously reported data from various continents (Table 5), MSI-H status was higher than that of Japan or South Korea in Asia, and lower than that of Europe or the United States. MSI-H status has a great influence on CRC prognosis. The frequency of MSI-H in the right colon cancer was very high, while that in the left colon and rectum was extremely low (27.9 vs 5.5 vs 2.8, respectively). Previous studies have suggested that MSI-H status in patients with rectal cancer may be associated with hereditary factors, but our study did not come to this conclusion. In addition, MSI-H status is also related to

Table 4. Frequency of *RAF* mutation in different continents

Continent	Country	Mutation rate (%)	RAF Title	Year
North America	America	5	Loree et al. [15]	2017
		9.9	Taieb et al. [17]	2016
South America	Brazil	8.7	Yamane et al. [19]	2014
		8.6	Hurtado et al. [43]	2015
Asia	China	5.8	Lin et al. [20]	2014
		9.7	Kim ST et al. [44]	2017
		9.6	Kim B et al. [23]	2014
	Japan	4	Sasak et al. [24]	2016
		4.9	Kadowaki et al. [25]	2015
		5.4	Kawazoe et al. [45]	2015
		8.3	Okada et al. [26]	2014
		4.7	Soeda et al. [27]	2012
		6.3	Takane et al. [28]	2012
	Singapore	11.3	Suhaimi et al. [30]	2015
Europe	Sweden	23	Birgisson et al. [33]	2015
	The Netherlands	17	Frouws et al. [34]	2017
	Finland	12.3	Seppä la et al. [46]	2015
		8.5	Rako et al. [37]	2012
	Germany	11.6	Modest et al. [47]	2012
	England	8.3	Douillard et al. [39]	2013
	Greece	9	Samara et al. [40]	2015
	Romania	8.3	Negru et al. [41]	2014
		6.2	Negru et al. [41]	2014

Table 5. MSI status in different continents

Continent	Country	Mutation rate (%)	MSI Title	Year
North America	America	11	Taieb et al. [17]	2016
South America	Brazil	10.6	Yamane et al. [19]	2014
Asia	China	11.5	Yan et al. [48]	2016
		11.9	Lin et al. [20]	2014
	Korea	3.9	Kim ST et al. [44]	2017
		7.7	Kim WG et al. [49]	2017
		9.4	Kim YB et al. [50]	2016
	Japan	4.3	Kim B et al. [23]	2014
		8.3	Kadowaki et al. [25]	2015
		7.7	Miwata et al. [28]	2014
		22.2	Miwata et al. [28]	2014
	Iran	24	Mahbooben et al. [29]	2008
Europe	Sweden	15	Ghanipour et al. [51]	2016
	Finland	20	Birgisson et al. [33]	2015
		14.6	Seppä la et al. [46]	2015

deep invasion, older age, less lymph node metastasis (or no lymph node metastasis as in our study), and distant metastasis. All of these may contribute to better prognosis.

The frequency of MSI-H status is similar to that of *BRAF* mutation. Firstly, they show linear relations of linear descent. A right colon mutation is an absolute advantage. As shown in data from different countries [48-51], the frequency of MSI-H status is 10 times higher in colon cancer than in rectal cancer and 2–5 times higher in the right colon than in the left colon. In Europe, MSI-H occurs in about 15–20% of all CRCs [33, 43, 51]. In the United States, it occurs in about 10–15% of all CRCs. In Asia, it occurs in only 5–10% of all CRCs [17, 19], which is far less than that in other countries [48-50].

In our study, there were two distinct mutation samples, in which *BRAF* mutations occurred in CRCs with MSI-H status, and both were obtained from older female patients with right colon cancer. We also found that the *BRAF* mutation rate in MSI-H CRC was 2 times higher than that in MSS CRC. However, previous studies have reported that the mutation rate of *BRAF* in MSI-H tumors is 5, or even 10, times higher than that in MSS tumors.

These two samples were further analyzed. *BRAF* mutation was associated with younger age and MSI-H status was associated with older age. However, *BRAF* mutations in MSI-H CRCs occurred in all older patients, and thus we speculate that the role of MSI-H status in CRC is more crucial than that of *BRAF* mutation. As for the relationship between *RAS* and *RAF*, previous studies have not reached the same conclusion. Mainstream research indicates that *RAS* and *RAF* are mutually exclusive [52]. However, a few large-scale experiments have shown that the coexistence of *RAS* and *RAF* mutations is mostly between 0.5–1.4% [23, 53].

Finally, there was a unique feature seen in 290 cases of CRC. Among all cases, there were 26 cases of mucinous adenocarcinoma. There were four cases of lymph node metastasis, all of which occurred in the rectum. We speculated that patients with mucinous adenocarcinoma were at a lower risk of metastatic colon cancer.

There are several differences between the data of our current research and that of previous research. These may be attributed to differences in diagnostic criteria, genome evolution, or racial differences.

The molecular status of cancer plays a significant role in the detection of tumors, and differences in the cancer status in each country may be due to various reasons. First, it may be due to race differences, because different genes cause molecular differences in the human body. Second, lifestyle differences, such as eating habits, may also have an impact. Third, it may be caused by environmental factors, such as climate and temperature. The exact reasons still need to be identified by further studies. Exploring the molecular mechanisms that contribute to mutations may help develop strategies for the prevention and treatment of tumors.

Cancer treatment is increasingly relying on tumor markers to predict prognosis and choose a suitable therapeutic plan for patients. Although research in the field of biomarkers is growing, its clinical application is rather limited. A more standardized system of therapeutic markers should be applied in clinical practice, so as to clearly define treatment plans, standardize treatment options, and take full advantage of gene technology.

Conclusion

RAS, *RAF*, and MSI status all affect the prognosis and treatment of CRC. We found that the frequency of *RAS* mutation in CRC was obviously higher, while that of *RAF* mutation was lower than that previously reported in China, and both were lower than the global average. MSI-H status is more complex and is much higher than the current values in China. However, compared with previously reported data from various continents, MSI-H rate was higher than that of Japan or South Korea in Asia, and lower than that of Europe or the United States. These discrepancies may be due to a variety of reasons.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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