Deficient mismatch repair and *RAS* mutation in colorectal carcinoma patients: a retrospective study in Eastern China

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Objectives: To investigate the frequency and prognostic role of deficient mismatch repair (dMMR) and *RAS* mutations in Chinese patients with colorectal carcinoma.

Methods: Clinical and pathological information from 813 patients were reviewed and recorded and 114 of them were followed up. Expression of mismatch repair proteins was tested by immunohistochemistry. Mutation analyses for *RAS* gene were performed by real-time polymerase chain reaction. Correlations of mismatch repair status and *RAS* mutation status with clinicopathological characteristics and disease survival were determined.

Results: The overall percentage of dMMR was 15.43% (123/797). The proportion of dMMR was higher in patients <50 years old ($p < 0.001$) and in the right colon ($p < 0.001$). Deficient mismatch repair was also associated with mucinous production ($p < 0.001$), poor differentiation ($p < 0.001$), tumor stage ($p < 0.05$), and bowel wall invasion ($p < 0.05$). The overall *RAS* mutation rate was 45.88%, including 42.56% (346/813) *KRAS* mutation and 3.69% (30/813) *NRAS* mutation. *KRAS* mutation was significantly associated with mucinous production ($p < 0.05$), tumor stage ($p < 0.05$) and much higher in non-smokers ($p < 0.05$) and patients with colorectal carcinoma family history ($p < 0.05$). dMMR and *KRAS* mutation were not independent prognostic factors for colorectal carcinoma. Overall, 43.9% (54/123) dMMR tumors harbored *KRAS* mutation, however, dMMR tumors were less likely to have *NRAS* mutation.

Conclusions: This study confirms that the status of molecular markers, involving mismatch repair status and *RAS* mutation, reflects the specific clinicopathological characteristics of colorectal carcinoma.

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Keywords: colorectal cancer, deficient mismatch repair (dMMR), *KRAS* mutation, *NRAS* mutation, clinicopathological characteristics, prognosis

ABSTRACT

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higher in patients <50 years old ($p < 0.001$) and in the right colon ($p < 0.001$). Deficient mismatch repair was also associated with mucinous production ($p < 0.001$), poor differentiation ($p < 0.001$), tumor stage ($p < 0.05$), and bowel wall invasion ($p < 0.05$). The overall *RAS* mutation rate was 45.88%, including 42.56% (346/813) *KRAS* mutation and 3.69% (30/813) *NRAS* mutation. *KRAS* mutation was significantly associated with mucinous production ($p < 0.05$), tumor stage ($p < 0.05$) and much higher in non-smokers ($p < 0.05$) and patients with colorectal carcinoma family history ($p < 0.05$). dMMR and *KRAS* mutation were not independent prognostic factors for colorectal carcinoma. Overall, 43.9% (54/123) dMMR tumors harbored *KRAS* mutation, however, dMMR tumors were less likely to have *NRAS* mutation.

Conclusions: This study confirms that the status of molecular markers, involving mismatch repair status and *RAS* mutation, reflects the specific clinicopathological characteristics of colorectal carcinoma.

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common cancer in China, with 331,300 new cases and 159,300 disease-related deaths in 2012(Chen et al. 2016). The morbidity has increased steadily due to the growth of aging population and the change of lifestyle in recent years, however, the exact mechanism and related predicted biomarkers are largely unknown.

During the past decades, microsatellite instability (MSI) and *RAS* mutation have been well studied as two prevalent genetic biomarkers involved in colorectal carcinogenesis. The mismatch repair (MMR) system, which including the proteins of MLH1, MSH2, MSH6 and PMS2, can repair incorrect base-pairing or unmatched DNA loop to keep genomic stability. MSI is caused by the deficient mismatch repair (dMMR) system, which leads to a high rate of mutations in repeat sequences and accounts for approximately 15% of all CRCs as well as virtually all Lynch syndrome (LS) (Geiersbach & Samowitz 2011; Marra & Boland 1995; Zhang et al. 2016). Tumors with high level of microsatellite instability (MSI-H) which caused by germ line mutations or epigenetic silencing of MMR genes have unique clinicopathologic characteristics

(Cunningham et al. 2010). CRC patients with MSI-H demonstrated favorable prognosis compared to those with low level of microsatellite instability (MSI-L) and microsatellite stability (MSS) (Ribic et al. 2003; Sinicrope et al. 2011), however, these patients could not benefit from fluoropyrimidine-based adjuvant chemotherapy (Ribic et al. 2003; Sargent et al. 2010).

The *RAS* gene family, the other one significant biomarker, which including *KRAS*, *NRAS* and *HRAS*, is located downstream in the epidermal growth factor receptor (EGFR) signal pathway. Mutations in the *RAS* gene, which are thought to occur early in the adenoma-carcinoma continuum, have been proved to activate the *RAS* /MAPK pathway independent of EGFR activation, leading to poor response to EGFR inhibitors (Amado et al. 2008; Punt et al. 2016). Moreover, National Comprehensive Cancer Network (NCCN) clinical practice guidelines suggested that *KRAS* gene mutation should be detected for metastatic CRC (mCRC) patients before the treatment of Cetuximab and Panitumumab (Engstrom et al. 2009).

The status of dMMR and *RAS* mutation had been widely studied in western countries. The frequency of dMMR CRCs ranged from 15-20% (Giraldez et al. 2010; Sinicrope et al. 2011; Sinicrope et al. 2012), *KRAS* mutation ranged from 20-50% (De Roock et al. 2010; Naguib et al. 2010; Palomba et al. 2016; Rosty et al. 2013; Sasaki et al. 2016) and *NRAS* mutation was noted in less than 5% (De Roock et al. 2010; Palomba et al. 2016; Peeters et al. 2013; Russo et al. 2014). However, studies in China showed a lower frequency of dMMR compared with that in western populations, and the clinicopathologic characteristics were also inconsistent (Huang et al. 2010; Jin et al. 2008; Ye et al. 2015). Although several studies reported the frequency of *KRAS* mutation in Chinese CRC patients, the amount of samples was limited in most of these studies (Shen et al. 2011; Ye et al. 2015; Yunxia et al. 2010). Moreover, the information about *NRAS* mutation in Chinese CRC patients was limited. Little has been studied on the association between status of dMMR and *RAS* mutation. Therefore, in the present study, we collect the data of CRC patients in our department from 2013 to 2016 and analyze the status of dMMR and *RAS* mutation to evaluate possible associations between dMMR, *RAS* mutation and the clinicopathologic characteristics in colorecatal carcinoma and also attempt to explore the

prognostic role of dMMR and *RAS* mutation.

Materials and Methods

Eight hundred and thirteen formalin-fixed, paraffin-embedded tumor specimens from colorectal carcinoma patients who underwent primary surgical resection from 2013 to 2016 in the Affiliated Hospital of Qingdao University were selected for this study. Patients who had undergone preoperative radiotherapy, chemotherapy and/or EGFR-targeted therapy were not included in this study.

The clinical and pathologic variables were extracted from medical records and pathological reports, which include age, gender, primary locations of tumor, tumor diameter, histological characteristics, TNM stage, smoking status, drinking status and family medication history. 114 patients, who were diagnosed with stage I~ III colorectal carcinoma between September 2013 and May 2014, were followed up until May 2017, and the data concerning cancer recurrence and patient survival were collected.

Primary locations of tumors were divided into the right colon (from the cecum through the transverse colon), the left colon (from the splenic flexure through the rectosigmoid flexure) and rectum. Tumors were staged according to the criteria of the seventh edition of the American Joint Commission on Cancer (AJCC) TNM staging system. Mucinous adenocarcinoma and signet-ring cell carcinomas were recorded as mucin-producing tumors.

The study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (No.20130049) and all patients had signed the informed consent.

Immunohistochemistry for MMR proteins

As previously described by Song et al. (Lin et al. 2014b), all specimens were fixed in 10% neutral buffered formalin and embedded in paraffin blocks. 3 μm-thick tissue sections were used for immunohistochemical analysis. Immunohistochemical staining was performed using standard reagents and techniques on an Automated Staining System (BenchMark XT, Ventana Medical Systems, Inc. Arizona, USA) according to the manufacturer's instructions. The ready-to-use antibodies were used as follows: MLH1 (No.M1, Ventana Medical Systems Inc, Arizona, USA,

working solution), PMS2 (No.EPR3947, Ventana Medical Systems Inc, Arizona, USA, working solution), MSH2 (No.G219-1129, Ventana Medical Systems Inc, Arizona, USA, working solution), MSH6 (No.44, Ventana Medical Systems Inc, Arizona, USA, working solution).

The results were assessed by two pathologists who were blinded to the clinical data. Any tumor cell with nuclear staining was recorded as positive staining. Intact expression for all these proteins was regarded as proficient MMR (pMMR). Protein expression was defined as abnormal when nuclear staining of tumor cells was absent in the presence of positive staining in normal colonic epithelium and lymphocyte. Loss of expression of any MMR protein was regarded as dMMR.

Analysis of *KRAS* and *NRAS* gene mutations by ARMS-PCR

Formalin-fixed, paraffin-embedded tumor sections were deparaffinized and air dried, and DNA was extracted using the Tiangen Blood and Tissue Kit (TiangenInc, Beijing, China). *KRAS* (exons 2 and 3) and *NRAS* (exons 2 and 3) were detected by amplification refractory mutation system in multiple quantitative polymerasechain reaction (ARMS-multi-qPCR) analysis with the Human *KRAS* and *NRAS* Mutation Detection kit (YuanQi Bio-Pharmaceutical Co., Ltd. Shanghai, China). Codons of *RAS* were amplified as described previously (Dong et al. 2016). Briefly, 3 μl sample DNA was amplified in a 25 μl reaction system containing 9 μl of Mix1 and 13 μl of PCRMix3. Positive and negative controls for each sample were run simultaneously. The program for the PCR amplification flanking *KRAS* mutation site was as follows: 1 cycle at 42 °C for 5 min; 1 cycle at 94 °C for 3 min; 40 cycles at (94 °C for 15 sec; 60 °C for 60 sec). Fluorescence signals were collected at 60 °C. The program for the PCR amplification flanking *NRAS* mutation site was as follows: 1 cycle at 42 °C for 5 min; 1 cycle at 94°C for 3 min; 40 cycles at (94 °C for 45 sec; 60 °C for 80 sec). Fluorescence signals were collected at 60 °C. The mutations were identified with a specific probe labeled with Hydroxy fluorescein (FAM). Amplicons were detected using ABI7500 Fast Real-Time PCR System (ThermoFisher ScientificInc, MA, US).

Statistical analysis

Results were analyzed with SPSS 19.0 (SPSS, Inc, Chicago) software package. For comparison

of the frequencies among the groups, the Chi-square test and the Fisher exact test were used. Survival curves for disease free survival (DFS) and overall survival (OS) were estimated using the Kaplan–Meier method. Probability (p) value < 0.05 was considered as statistical significance.

RESULT

Patient characteristics

The main characteristics of the patients were summarized in the Table 1. There were 506 (62.24%) male and 307 (37.76%) female with a mean age of 64 years. The majority of the patients (87.7%) were older than 50 years. 11.69%, 40.84%, 37.15% and 10.33% of patients presented with stage I, Stage II, stage III and stage IV disease, respectively. The primary location was more common in rectum (54.49%).There were 248 (32.96%) patients and 165 (20.3%) patients with a smoking and alcohol in taking history, respectively. There were 133 (16.36%) patients with mucin-productive carcinoma.

MMR status and associations with clinicopathological characteristics

MMR status was successfully evaluated in 797 patients. 123 (15.43%) patients exhibited dMMR. The deficient rates of MLH1, PMS2, MSH2 and MSH6 in studied patients were 10.04% (80/797), 11.92% (95/797), 3.26% (26/797) and 4.14% (33/797), respectively. Loss expression of four proteins was detected in 2 patients, loss expression of any two proteins was detected in 105 patients, and loss expression of only one protein was found in 16 patients. The association of clinicopathological characteristics with MMR status was presented in Table 2. Patients with dMMR tumors were more likely to be younger ($p < 0.001$) and to have poorly differentiated tumors ($p < 0.001$), especially with a mucin production. A higher rate of dMMR was found in stage II (19.63%) versus stage I, stage III and stage IV(6.38%,13.62% and 15.79%, respectively) cancers ($p = 0.011$). dMMR was also found to be associated with localization of the tumor to the right colon ($p < 0.001$) and to have a higher propensity to bowel wall invasion ($p = 0.013$).

Although dMMR tumors were more often in patients with CRC family history, no significant difference (22.92% vs 13.43%, $p > 0.05$) was found in this study. The loss of MSH2/MSH6 expression was more often observed in patients with CRC family history (12.5%

vs 3.58%, p = 0.016). In other respects, the patients with tumors exhibiting dMMR were similar to those exhibiting pMMR.

RAS gene mutation and associations with clinicopathological characteristics

RAS status was detected for 813 patients. The mutation rates of *KRAS* and *NRAS* were 42.56% (346/813) and 3.69% (30/813), respectively. There were three patients demonstrating mutation in both *KRAS* and *NRAS*. Patients suffering from tumors with mucinous production had a higher incidence of *KRAS* mutation compared with those having tumors without mucinous production (54.89% vs 40.18%, p = 0.002). A higher rate of *KRAS* mutation was found in stage II (48.49%) compared with that in stage I, stage III and stage IV (36.84%, 40.45%, 34.52%, respectively) cancers (p = 0.023) and in non-smokers compared with smokers (45.32% vs 36.94%, p = 0.023). Patients with CRC family history also showed higher rate of *KRAS* mutation (54.17% vs 37.39%, p = 0.013). Tumors with *RAS* mutation showed lower propensity to lymph node metastasis (p = 0.006) and distant metastasis (p = 0.048). No significant associations between *KRAS* mutation and other clinicopathological characteristics were found in the present study. Meanwhile, *NRAS* mutation was not significantly associated with any clinicopathological characteristics (Table 3).

Correlations between _RAS_ mutation and MMR status

RAS mutation rate was slightly higher in pMMR tumors than dMMR tumors, but it failed to reach a significant difference (46.44% vs 43.9%, p > 0.05). There was also no obvious correlation between MMR status and *KRAS* mutation (42.43% vs 43.9%, p > 0.05). No *NRAS* mutation was detected in dMMR tumors. pMMR was associated with *NRAS* mutation (p = 0.009, Table 4). The distribution of MMR and *KRAS* status was showed in supplement I. Correlation between *KRAS* gene mutation and clinicopathological characteristics in dMMR tumors was summarized in the Table 5. No significant associations between *KRAS* mutation and any clinicopathological characteristics were found in dMMR tumors.

Prognostic value of dMMR and _KRAS_ mutation

Of the 114 followed-up patients (23 with dMMR and 43 with *KRAS* mutation), 13 deaths occurred. The stage of these patients was 16 in stage I, 58 in stage II and 40 in stage III. dMMR

was not independent prognostic factor for 3-year DFS and OS (p = 0.911 and 0.858) (Figure.1A and Figure.1B, respectively). *KRAS* mutation was also not independent prognostic factor for 3-year DFS and OS (p = 0.418 and 0.427) (Figure.1C and Figure.1D, respectively).

DISCUSSION

As prognostic and predictive biomarkers, MMR deficiency and *RAS* mutation are important for clinical treatments and prognosis of CRC patients. Compared with pMMR patients, dMMR CRCs are reported to have unique clinicopathological characteristics such as poor differentiation, early stage, increased tumor-infiltrating lymphocytes and better clinical outcome (Brenner et al. 2014; Korphaisarn et al. 2015; Ribic et al. 2003). The *RAS* gene is a predictive biomarker for the resistance to anti-EGFR monoclonal antibodies (MoAbs) treatment in mCRCs (Amado et al. 2008; Punt et al. 2016). However, geographic and racial differences between Chinese and other countries were reported (Huang et al. 2010; Ismael et al. 2017; Kim et al. 2007; Vasovcak et al. 2011; Ye et al. 2015), which need to be validated with large sample amounts. Furthermore, data regarding *RAS* mutation frequency and dMMR colorectal carcinoma is not consistent in China. Thus, we designed this study in the Chinese population aiming to explore the relationship between the *RAS* mutation, MMR status and clinicopathological parameters, also expecting to find some prognostic and predictive biomarkers for colorectal cancer.

Our results demonstrated that an overall MMR deficiency rate was 15.43%, which was within the established range of 15-21% (Giraldez et al. 2010; Sinicrope et al. 2012; Carethers et al. 2004; Cushman-Vokoun et al. 2013), but slightly higher than that reported from other Chinese population (Huang et al. 2010; Jin et al. 2008; Ye et al. 2015). Reports from Korea (Jung et al. 2012) and Japan (Kadowaki et al. 2015) which using PCR-based MSI testing also showed that the frequencies of MSI-H CRCs were around 10%. This discrepancy can be elucidated by the differently detective methods to some extent. Compared with PCR-based MSI testing examination, immunohistochemistry is thought to be easily available and time-saving. Furthermore, immunohistochemistry may detect MMR-deficient cases that can potentially missed by PCR-based MSI testing (Shia 2008).

Correlations between dMMR status and clinicopathologic characteristics were controversial (Ismael et al. 2017; Jin et al. 2008; Ribic et al. 2003; Sinicrope et al. 2011). Reports from three independent Chinese groups (Huang et al. 2010; Jin et al. 2008; Ye et al. 2015) indicated that dMMR were associated with gender, tumor location and mucin production, but not with age. In a study including 1063 CRCs, Lin et al (Lin et al. 2014a) observed that MSI were not only associated with gender, tumor location and mucin production, but also with tumor differentiation and tumor stage. In our current study, we found patients younger than 50 tended to be with dMMR. These diverse findings may attribute to different criteria for age division, ethnicities environmental factors as well as the specificity and sensitivity of the detective methods.

In our study, there was a correlation between MSH2/MSH6 deficiency and family history of colorectal cancer, but not MLH1/PMS2 deficiency. This implicated that different gene mutation in dMMR deficiency might show different clinicalpathologic characteristics. In addition, according to the Bethesda criteria (Burt et al. 2010), 12 CRCs were diagnosed LS. In MSH2/MSH6 deficiency CRCs, 33.3% (6/18) was LS and the percentage was 14% (6/43) in MLH1/PMS2 defective cases, suggesting MSH2/MSH6 deficiency patients had higher opportunity to diagnosis LS. Some of the recent studies may help to explain this finding: the majority dMMR CRCs were caused by inactivation of MLH1 and more than 70% MLH1 deficiency was caused by primarily MLH1 promoter hypermethylation (Hampel et al. 2005), which could distinguish sporadic dMMR CRCs from LS cases, therefore, most MLH1 defective tumors were sporadic CRC. Another interesting phenomenon in our investigation is that we found most patients' family medical history was unclear and they did not know whether other family members had polyps removed, moreover, many cancers might be prevented by early stage colonoscopy, so the family history may be deceptive (Hampel 2014). Therefore, screening strategy based on family history may be improper, all patients with newly diagnosed CRC should be screened for Lynch syndrome (Hampel 2014). Inconsistent with previous studies, which indicated that patients with dMMR tumors had significantly better survival than that of pMMR patients (Des Guetz et al. 2009; Korphaisarn et al. 2015; Lanza et al. 2006), our study showed

that there was no significant difference in 3-year DFS and OS between dMMR and pMMR patients, although the incidence of dMMR in stage III/IV disease was lower, suggesting that dMMR tumors had lower propensity to metastasize.

In the present study, the mutation rates of *KRAS* and *NRAS* are 42.56% and 3.69%, respectively. The *KRAS* mutation rate is significantly higher than the value of 20.7% among 314 CRC patients from Taiwan, China (Liou et al. 2011), 22% among 202 CRC patients from the England (Naguib et al. 2010), 30.1% among 392 CRC patients from Switzerland (Zlobec et al. 2010), but similar to that previously reported in Guangzhou, China (43.9%, 25/57) (Mao et al. 2012). Several factors may lead to such differences, such as sample size, dietary and lifestyle, as well as racial and/or environmental differences. Furthermore, we detected the coding sequence of exon 2 and exon 3 of *KRAS* and exon 2 and exon3 of *NRAS*, which may help to explain the higher percentage of *RAS* mutation than those detected exon 2 only. Except for exon 2 and exon 3, recent studies have showed 5-10% of tumors harbored exon 4 mutation (Janakiraman et al. 2010; Lin et al. 2014a), which would also result in the resistance to anti-EGFR inhibitors. Therefore, extending detective spectrum of *RAS* might help to optimize the selection of the CRC patients to receive anti-EGFR MoAbs.

The frequency of *KRAS* mutation has been reported to be associated with age, gender, differentiation and tumor stage (Gao et al. 2012; Li et al. 2011; Ye et al. 2015; Yunxia et al. 2010; Zhu et al. 2012). In our study, there was no association between *KRAS* mutation and age, gender, tumor differentiation or tumor location. However, *RAS* mutated tumors showed lower propensity to lymph node and distant metastasis. Moreover, we found that *KRAS* mutation was associated with mucin production, tumor stage, no-smoking and family history of CRC, which was similar with the results reported by Liou et al (Liou et al. 2011). The detailed mechanism about no-smoking CRC patients tended to harbor *KRAS* mutation needs further epidemiological investigation. No convincing evidence demonstrates that *KRAS* mutation is an independent prognostic factor for CRC (Jin et al. 2008; Palomba et al. 2016; Russo et al. 2014; Yunxia et al. 2010). In the present study, we found no associations of *KRAS* mutation with OS in CRC.

Further studies based on longer follow-up time and larger sample size were needed to conform this conclusion.

In our study, the percentage of the four subgroups tumors, including dMMR/KRAS mutation, dMMR/KRAS wild, pMMR/KRAS mutation and pMMR/KRAS wild tumors was 6.78%, 8.66%, 35.88%, 48.68%, respectively, which was similar to the data reported by study from Beijing, China(Ye et al. 2015). According to the recent reports (Nash et al. 2009; Roth et al. 2010), patients with MSS/*KRAS* mutant tumor had the worst survival than other three groups. Therefore, dMMR and *KRAS* markers may provide foundation for developing a molecular prognostic scoring system for colorectal cancer patients in the future.

Previous studies have shown that pMMR patients tended to harbor more *KRAS* mutation than dMMR patients (Naguib et al. 2010; Ye et al. 2015). One hypothesis for this result is that *BRAF* and *KRAS* mutations were almost mutually exclusive in colorectal cancer and MSI tumor harbor more *BRAF* mutation, so MSS tumor might harbor more *KRAS* mutation (Naguib et al. 2010). However, in the present study, we didn't find any differences in *KRAS* mutation between pMMR and dMMR patients, and further studies based on larger sample size were needed to explore this controversy in Chinese CRCs.

Additionally, our study provided an opportunity to investigate the status of *KRAS* mutation in Chinese dMMR patients. *KRAS* mutation presented in 43.9% dMMR patients in our study, similar to previous studies in western countries (Cushman-Vokoun et al. 2013; Oliveira 2004). All of these results indicate that *KRAS* mutation could be quite common in dMMR tumors. There were no association between *KRAS* mutation and clinicopathologic characteristics in dMMR tumor. A study conducted by Nash et al, indicated *KRAS* status was an independent prognostic factor in MSI CRC patients (Nash et al. 2009). Moreover, MSI patients with wild-type *KRAS* and *BRAF* tumors have more favorable prognosis than patients with mutated *KRAS* or *BRAF* tumors (de Cuba et al. 2016; Phipps et al. 2015).

NRAS gene, as one of the *RAS* family, showed close relations with *KRAS*. Unlike *KRAS*, *NRAS* mutation was rarely in CRC patients. In our study, the mutation rate of *NRAS* was 3.69%,

similar to the previous reports (Chang et al. 2016; Irahara et al. 2010; Palomba et al. 2016; Peeters et al. 2013). Moreover, we observed 25 out of 388 *KRAS* wild-type tumors with *NRAS* mutation, which can partially help to explain the anti-EGFR MoAbs resistance in *KRAS* wild-type patients. Considering the heavy financial burden in MoAbs treatment in CRC patients, *NRAS* mutation should be tested before MoAbs treatment in *KRAS* wild-type tumors. Another interesting phenomenon is that no *NRAS* mutation was detected in dMMR patients, which suggested *NRAS* mutation might be mutually exclusive with dMMR. Meanwhile, *NRAS* mutation was not significantly associated with any clinicopathologic characteristics in our study.

However, our study findings should be elucidated with consideration of its limitations: first, the samples size was relatively small, rendering some of our findings inconclusive; second, we used commercially available kit authenticated by China Food and Drug Administration (CFDA) and the mutation subgroups were uncertain. A study conducted by Lin et al (Lin et al. 2014a). Therefore, the subgroup of mutation codons should be carefully explored in future; third, we did not collect the data on clinical management, therefore, the influence of clinical treatment for survival was uncertain.

Conclusion

In conclusion, this was an exploratory analysis of correlations between *RAS* mutation and MMR status and the clinicopathologic characteristics in Eastern Chinese CRC patients. The status of these molecular markers, which involving MLH1/PMS2, MSH2/MSH6, *KRAS* and *NRAS* mutation, reflects the specific clinicalpathologic characteristics of colorectal carcinoma. More comprehensive molecular classification and survive analysis should be explored in our future experiments.

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None

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, Juan T, Sikorski R, Suggs S, Radinsky R, Patterson SD, and Chang DD. 2008. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 26:1626-1634. 10.1200/JCO.2007.14.7116

Brenner H, Kloor M, and Pox CP. 2014. Colorectal cancer. *Lancet* 383:1490-1502. 10.1016/s0140-6736(13)61649-9

Burt RW, Barthel JS, Dunn KB, David DS, Drelichman E, Ford JM, Giardiello FM, Gruber SB, Halverson AL, Hamilton SR, Ismail MK, Jasperson K, Lazenby AJ, Lynch PM, Martin EW, Jr., Mayer RJ, Ness RM, Provenzale D, Rao MS, Shike M, Steinbach G, Terdiman JP, and Weinberg D. 2010. NCCN clinical practice guidelines in oncology. Colorectal cancer screening. *J Natl Compr Canc Netw* 8:8-61.

Carethers JM, Smith EJ, Behling CA, Nguyen L, Tajima A, Doctolero RT, Cabrera BL, Goel A, Arnold CA, Miyai K, and Boland CR. 2004. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* 126:394-401. 10.1053/j.gastro.2003.12.023

Chang YY, Lin PC, Lin HH, Lin JK, Chen WS, Jiang JK, Yang SH, Liang WY, and Chang SC. 2016. Mutation spectra of RAS gene family in colorectal cancer. *Am J Surg* 212:537-544.e533. 10.1016/j.amjsurg.2016.02.013

Chen W, Zheng R, Zuo T, Zeng H, Zhang S, and He J. 2016. National cancer incidence and mortality in China, 2012. *Chin J Cancer Res* 28:1-11. 10.3978/j.issn.1000-9604.2016.02.08

Cunningham D, Atkin W, Lenz H-J, Lynch HT, Minsky B, Nordlinger B, and Starling N. 2010.

Colorectal cancer. *Lancet* 375:1030–1047.

Cushman-Vokoun AM, Stover DG, Zhao Z, Koehler EA, Berlin JD, and Vnencak-Jones CL. 2013. Clinical utility of KRAS and BRAF mutations in a cohort of patients with colorectal neoplasms submitted for microsatellite instability testing. *Clin Colorectal Cancer* 12:168-178. 10.1016/j.clcc.2013.04.005

de Cuba EMV, Snaebjornsson P, Heideman DAM, van Grieken NCT, Bosch LJW, Fijneman RJA, Belt E, Bril H, Stockmann HBAC, Hooijberg E, Punt CJA, Koopman M, Nagtegaal ID, Coupé VHM, Carvalho B, and Meijer GA. 2016. Prognostic value ofBRAFandKRASmutation status in stage II and III microsatellite instable colon cancers. *Int J Cancer* 138:1139-1145. 10.1002/ijc.29855

De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M, and Tejpar S. 2010. 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* 11:753-762. 10.1016/s1470-2045(10)70130-3

Des Guetz G, Schischmanoff O, Nicolas P, Perret G-Y, Morere J-F, and Uzzan B. 2009. Does microsatellite instability predict the efficacy of adjuvant chemotherapy in colorectal cancer? A systematic review with meta-analysis. *Eur J Cancer* 45:1890-1896. 10.1016/j.ejca.2009.04.018

Dong YU, Ren W, Qi J, Jin BO, Li Y, Tao H, Xu R, Li Y, Zhang Q, and Han B. 2016. EGFR, ALK, RET, KRAS and BRAF alterations in never-smokers with non-small cell lung cancer. *Oncol Lett* 11:2371-2378. 10.3892/ol.2016.4235

Engstrom aF, Arnoletti JP, III ABB, Chen Y-J, Choti MA, Cooper HS, Covey A, Dilawari RA,

Early DS, Enzinge PC, Fakih MG, Fleshman J, Fuchs C, Grem JL, Kiel K, Knol JA, Leong LA, Lin E, Mulcahy MF, Rao S, Ryan DP, Saltz L, Shibata D, Skibber JM, Sofocleous C, Thomas J, Venook AP, and Willett C. 2009. The NCCN Colon Cancer Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 7:778-831.

Gao J, Sun ZW, Li YY, and Shen L. 2012. [Mutations of KRAS and BRAF in Chinese patients with colorectal carcinoma: analyses of 966 cases]. *Zhonghua Bing Li Xue Za Zhi* 41:579-583. 10.3760/cma.j.issn.0529-5807.2012.09.002

Geiersbach KB, and Samowitz WS. 2011. Microsatellite instability and colorectal cancer. *Arch Pathol Lab Med* 135:1269-1277. 10.5858/arpa.2011-0035-RA

Giraldez MD, Balaguer F, Bujanda L, Cuatrecasas M, Munoz J, Alonso-Espinaco V, Larzabal M, Petit A, Gonzalo V, Ocana T, Moreira L, Enriquez-Navascues JM, Boland CR, Goel A, Castells A, and Castellvi-Bel S. 2010. MSH6 and MUTYH deficiency is a frequent event in early-onset colorectal cancer. *Clin Cancer Res* 16:5402-5413. 10.1158/1078-0432.CCR-10-1491

Hampel H. 2014. NCCN increases the emphasis on genetic/familial high-risk assessment in colorectal cancer. *J Natl Compr Canc Netw* 12:829-831.

Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Nakagawa H, Sotamaa K, Prior TW, Westman J, Panescu J, Fix D, Lockman J, Comeras I, and de la Chapelle A. 2005. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med* 352:1851-1860. 10.1056/NEJMoa043146

Huang YQ, Yuan Y, Ge WT, Hu HG, Zhang SZ, and Zheng S. 2010. Comparative features of colorectal and gastric cancers with microsatellite instability in Chinese patients. *J Zhejiang Univ Sci B* 11:647-653. 10.1631/jzus.B1000198

Irahara N, Baba Y, Nosho K, Shima K, Yan L, Dias-Santagata D, Iafrate AJ, Fuchs CS, Haigis KM, and Ogino S. 2010. NRAS mutations are rare in colorectal cancer. *Diagn Mol Pathol* 19:157-163. 10.1097/PDM.0b013e3181c93fd1

Ismael NEHS, El Sheikh SA, Talaat SM, and Salem EM. 2017. Mismatch Repair Proteins and

406 Microsatellite Instability in Colorectal Carcinoma (MLH1, MSH2, MSH6 and PMS2):
407 Histopathological and Immunohistochemical Study. *Open Access Maced J Med Sci* 5:9.
408 10.3889/oamjms.2017.003
409 Janakiraman M, Vakiani E, Zeng Z, Pratilas CA, Taylor BS, Chitale D, Halilovic E, Wilson M,
410 Huberman K, Ricarte Filho JC, Persaud Y, Levine DA, Fagin JA, Jhanwar SC,
411 Mariadason JM, Lash A, Ladanyi M, Saltz LB, Heguy A, Paty PB, and Solit DB. 2010.
412 Genomic and Biological Characterization of Exon 4 KRAS Mutations in Human Cancer.
413 *Cancer Res* 70:5901-5911. 10.1158/0008-5472.can-10-0192
414 Jin HY, Liu X, Li VK, Ding Y, Yang B, Geng J, Lai R, Ding S, Ni M, and Zhao R. 2008.
415 Detection of mismatch repair gene germline mutation carrier among Chinese population
416 with colorectal cancer. *BMC Cancer* 8:44. 10.1186/1471-2407-8-44
417 Jung SB, Lee HI, Oh HK, Shin IH, and Jeon CH. 2012. Clinico-pathologic Parameters for
418 Prediction of Microsatellite Instability in Colorectal Cancer. *Cancer Res Treat* 44:179-
419 186. 10.4143/crt.2012.44.3.179
420 Kadowaki S, Kakuta M, Takahashi S, Takahashi A, Arai Y, Nishimura Y, Yatsuoka T, Ooki A,
421 Yamaguchi K, Matsuo K, Muro K, and Akagi K. 2015. Prognostic value of KRAS and
422 BRAF mutations in curatively resected colorectal cancer. *World J Gastroenterol*
423 21:1275-1283. 10.3748/wjg.v21.i4.1275
424 Kim GP, Colangelo LH, Wieand HS, Paik S, Kirsch IR, Wolmark N, Allegra CJ, and National
425 Cancer I. 2007. Prognostic and predictive roles of high-degree microsatellite instability in
426 colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel
427 Project Collaborative Study. *J Clin Oncol* 25:767-772. 10.1200/JCO.2006.05.8172
428 Korphaisarn K, Pongpaibul A, Limwongse C, Roothumnong E, Klaisuban W, Nimmannit A,
429 Jinawath A, and Akewanlop C. 2015. Deficient DNA mismatch repair is associated with
430 favorable prognosis in Thai patients with sporadic colorectal cancer. *World J*
431 *Gastroenterol* 21:926-934. 10.3748/wjg.v21.i3.926
432 Lanza G, Gafa R, Santini A, Maestri I, Guerzoni L, and Cavazzini L. 2006.

433 Immunohistochemical Test for MLH1 and MSH2 Expression Predicts Clinical Outcome
434 in Stage II and III Colorectal Cancer Patients. *J Clin Oncol* 24:2359-2367.
435 10.1200/jco.2005.03.2433
436 Li HT, Lu YY, An YX, Wang X, and Zhao QC. 2011. KRAS, BRAF and PIK3CA mutations in
437 human colorectal cancer: relationship with metastatic colorectal cancer. *Oncol Rep*
438 25:1691-1697. 10.3892/or.2011.1217
439 Lin C-C, Lin J-K, Lin T-C, Chen W-S, Yang S-H, Wang H-S, Lan Y-T, Jiang J-K, Yang M-H,
440 and Chang S-C. 2014a. The prognostic role of microsatellite instability, codon-specific
441 KRAS, and BRAFmutations in colon cancer. *J Surg Onco* 110:451-457.
442 10.1002/jso.23675
443 Lin S, YuJun L, XiaoMing X, and WenWen R. 2014b. Expression and significance of leptin
444 receptor, p-STAT3 and p-AKT in diffuse large B-cell lymphoma. *Acta Histochem*
445 116:126-130. 10.1016/j.acthis.2013.06.003
446 Liou JM, Wu MS, Shun CT, Chiu HM, Chen MJ, Chen CC, Wang HP, Lin JT, and Liang JT.
447 2011. Mutations in BRAF correlate with poor survival of colorectal cancers in Chinese
448 population. *Int J Colorectal Dis* 26:1387-1395. 10.1007/s00384-011-1229-1
449 Mao C, Zhou J, Yang Z, Huang Y, Wu X, Shen H, Tang J, and Chen Q. 2012. KRAS , BRAF
450 and PIK3CA Mutations and the Loss of PTEN Expression in Chinese Patients with
451 Colorectal Cancer. *PLoS One* 7:e36653. 10.1371/journal.pone.0036653.t001
452 Marra G, and Boland CR. 1995. Hereditary nonpolyposis colorectal cancer: the syndrome, the
453 genes, and historical perspectives. *J Natl Cancer Inst* 87:1114-1125.
454 Naguib A, Mitrou PN, Gay LJ, Cooke JC, Luben RN, Ball RY, McTaggart A, Arends MJ, and
455 Rodwell SA. 2010. Dietary, lifestyle and clinicopathological factors associated with
456 BRAF and K-ras mutations arising in distinct subsets of colorectal cancers in the EPIC
457 Norfolk study. *BMC Cancer* 10:99. 10.1186/1471-2407-10-99
458 Nash GM, Gimbel M, Cohen AM, Zeng Z-S, Ndubuisi MI, Nathanson DR, Ott J, Barany F, and
459 Paty PB. 2009. KRAS Mutation and Microsatellite Instability: Two Genetic Markers of

Early Tumor Development That Influence the Prognosis of Colorectal Cancer. *Ann Surg Oncol* 17:416-424. 10.1245/s10434-009-0713-0

Oliveira C. 2004. Distinct patterns of KRAS mutations in colorectal carcinomas according to germline mismatch repair defects and hMLH1 methylation status. *Hum Mol Genet* 13:2303-2311. 10.1093/hmg/ddh238

Palomba G, Doneddu V, Cossu A, Paliogiannis P, Manca A, Casula M, Colombino M, Lanzillo A, Defraia E, Pazzola A, Sanna G, Putzu C, Ortu S, Scartozzi M, Ionta MT, Baldino G, Sarobba G, Capelli F, Sedda T, Virdis L, Barca M, Gramignano G, Budroni M, Tanda F, and Palmieri G. 2016. Prognostic impact of KRAS, NRAS, BRAF, and PIK3CA mutations in primary colorectal carcinomas: a population-based study. *J Transl Med* 14. 10.1186/s12967-016-1053-z

Peeters M, Oliner KS, Parker A, Siena S, Van Cutsem E, Huang J, Humblet Y, Van Laethem JL, Andre T, Wiezorek J, Reese D, and Patterson SD. 2013. Massively parallel tumor multigene sequencing to evaluate response to panitumumab in a randomized phase III study of metastatic colorectal cancer. *Clin Cancer Res* 19:1902-1912. 10.1158/1078-0432.CCR-12-1913

Phipps AI, Limburg PJ, Baron JA, Burnett-Hartman AN, Weisenberger DJ, Laird PW, Sinicrope FA, Rosty C, Buchanan DD, Potter JD, and Newcomb PA. 2015. Association Between Molecular Subtypes of Colorectal Cancer and Patient Survival. *Gastroenterology* 148:77-87.e72. 10.1053/j.gastro.2014.09.038

Punt CJA, Koopman M, and Vermeulen L. 2016. From tumour heterogeneity to advances in precision treatment of colorectal cancer. *Nat Rev Clin Oncol* 14:235-246. 10.1038/nrclinonc.2016.171

Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE, Tu D, Redston M, and Gallinger S. 2003. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 349:247-257. 10.1056/NEJMoa022289

487 Rosty C, Young JP, Walsh MD, Clendenning M, Walters RJ, Pearson S, Pavluk E, Nagler B,
488 Pakenas D, Jass JR, Jenkins MA, Win AK, Southey MC, Parry S, Hopper JL, Giles GG,
489 Williamson E, English DR, and Buchanan DD. 2013. Colorectal carcinomas with KRAS
490 mutation are associated with distinctive morphological and molecular features. *Mod*
491 *Pathol* 26:825-834. 10.1038/modpathol.2012.240
492 Roth AD, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, Dietrich D, Biesmans B,
493 Bodoky G, Barone C, Aranda E, Nordlinger B, Cisar L, Labianca R, Cunningham D, Van
494 Cutsem E, and Bosman F. 2010. Prognostic role of KRAS and BRAF in stage II and III
495 resected colon cancer: results of the translational study on the PETACC-3, EORTC
496 40993, SAKK 60-00 trial. *J Clin Oncol* 28:466-474. 10.1200/JCO.2009.23.3452
497 Russo AL, Borger DR, Szymonifka J, Ryan DP, Wo JY, Blaszkowsky LS, Kwak EL, Allen JN,
498 Wadlow RC, Zhu AX, Murphy JE, Faris JE, Dias-Santagata D, Haigis KM, Ellisen LW,
499 Iafrate AJ, and Hong TS. 2014. Mutational analysis and clinical correlation of metastatic
500 colorectal cancer. *Cancer* 120:1482-1490. 10.1002/cncr.28599
501 Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, French AJ, Kabat
502 B, Foster NR, Torri V, Ribic C, Grothey A, Moore M, Zaniboni A, Seitz JF, Sinicrope F,
503 and Gallinger S. 2010. Defective mismatch repair as a predictive marker for lack of
504 efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol* 28:3219-
505 3226. 10.1200/JCO.2009.27.1825
506 Sasaki Y, Akasu T, Saito N, Kojima H, Matsuda K, Nakamori S, Komori K, Amagai K,
507 Yamaguchi T, Ohue M, Nagashima K, and Yamada Y. 2016. Prognostic and predictive
508 value of extendedRASmutation and mismatch repair status in stage III colorectal cancer.
509 *Cancer Sci* 107:1006-1012. 10.1111/cas.12950
510 Shen H, Yuan Y, Hu HG, Zhong X, Ye XX, Li MD, Fang WJ, and Zheng S. 2011. Clinical
511 significance of K-ras and BRAF mutations in Chinese colorectal cancer patients. *World J*
512 *Gastroenterol* 17:809-816. 10.3748/wjg.v17.i6.809
513 Shia J. 2008. Immunohistochemistry versus microsatellite instability testing for screening

colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. *J Mol Diagn* 10:293-300. 10.2353/jmoldx.2008.080031

Sinicrope FA, Foster NR, Thibodeau SN, Marsoni S, Monges G, Labianca R, Kim GP, Yothers G, Allegra C, Moore MJ, Gallinger S, and Sargent DJ. 2011. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. *J Natl Cancer Inst* 103:863-875. 10.1093/jnci/djr153

Sinicrope FA, Foster NR, Yoon HH, Smyrk TC, Kim GP, Allegra CJ, Yothers G, Nikcevich DA, and Sargent DJ. 2012. Association of obesity with DNA mismatch repair status and clinical outcome in patients with stage II or III colon carcinoma participating in NCCTG and NSABP adjuvant chemotherapy trials. *J Clin Oncol* 30:406-412. 10.1200/JCO.2011.39.2563

Vasovcak P, Pavlikova K, Sedlacek Z, Skapa P, Kouda M, Hoch J, and Krepelova A. 2011. Molecular genetic analysis of 103 sporadic colorectal tumours in Czech patients. *PLoS One* 6:e24114. 10.1371/journal.pone.0024114

Ye JX, Liu Y, Qin Y, Zhong HH, Yi WN, and Shi XY. 2015. KRAS and BRAF gene mutations and DNA mismatch repair status in Chinese colorectal carcinoma patients. *World J Gastroenterol* 21:1595-1605. 10.3748/wjg.v21.i5.1595

Yunxia Z, Jun C, Guanshan Z, Yachao L, Xueke Z, and Jin L. 2010. Mutations in epidermal growth factor receptor and K-ras in Chinese patients with colorectal cancer. *BMC Med Genet* 11:34. 10.1186/1471-2350-11-34

Zhang C-M, Lv J-F, Gong L, Yu L-Y, Chen X-P, Zhou H-H, and Fan L. 2016. Role of Deficient Mismatch Repair in the Personalized Management of Colorectal Cancer. *Inter J Env Res Pub Heal* 13:892. 10.3390/ijerph13090892

Zhu XL, Cai X, Zhang L, Yang F, Sheng WQ, Lu YM, Du X, and Zhou XY. 2012. [KRAS and BRAF gene mutations in correlation with clinicopathologic features of colorectal carcinoma in Chinese]. *Zhonghua Bing Li Xue Za Zhi* 41:584-589.

10.3760/cma.j.issn.0529-5807.2012.09.003

Zlobec I, Bihl MP, Schwarb H, Terracciano L, and Lugli A. 2010. Clinicopathological and protein characterization of BRAF- and K-RAS-mutated colorectal cancer and implications for prognosis. *Int J Cancer* 127:367-380. 10.1002/ijc.25042

Table 1(on next page)

Clinicopathological information of the studied patients (n=813)

1 Table 1. Clinicopathological information of the studied patients (n=813).

Characteristics	Number	(%)
Gender		
Male	506	62.24
Female	307	37.76
Age		
< 50	100	12.3
≥ 50	713	87.7
Location		
Right colon	181	22.26
Left colon	189	23.25
Rectum	443	54.49
Mucin production		
With	133	16.36
Without	680	83.64
Tumor differentiation		
Poor	138	16.97
moderate	599	73.68
Well	33	4.06
Unknown	43	5.29
Tumor stage		
Ⅰ	95	11.69
Ⅱ	332	40.84
Ⅲ	302	37.15
Ⅳ	84	10.33
Bowel wall invasion (T)		
T1	21	2.58

T2	104	12.79
T3	336	41.33
T4	352	43.3
Lymph node metastasis (N)		
N0	458	56.33
N1	203	24.97
N2	152	18.7
Distant metastasis (M)		
M0	729	89.67
M1	84	10.33
Lymphovascular invasion		
Yes	339	41.7
No	462	56.83
Unknown	12	1.47
Alcohol intake		
Ever	165	20.3
Never	648	79.7
Smoking		
Ever	283	34.81
Never	530	65.19
Colorectal family history		
Yes	48	5.9
No	337	41.45
Unknown	428	52.65

Table 2(on next page)

Correlations between mismatch repair protein deficiency and clinicopathological characteristics (n=797)

Table2. Correlations between mismatch repair protein deficiency and clinicopathological characteristics (n=797)

Characteristics	Number	dMMR		MLH1/ PMS2		MSH2/MSH6	
		Defective (%)	P value	Defective (%)	P value	Defective (%)	P value
Gender							
Male	495	73 (14.75)	0.493	55 (11.11)	0.241	21 (4.24)	0.853
Female	302	50 (16.56)		42 (13.91)		12 (3.97)	
Age							
< 50	99	29 (29.29)	< 0.001	24 (24.24)	< 0.001	6 (6.06)	0.284*
≥ 50	698	94 (13.47)		73 (10.46)		27 (3.87)	
Location							
Right colon	173	62 (35.84)	< 0.001	48 (27.75)	< 0.001	18 (10.4)	< 0.001
Left colon	185	26 (14.05)		21 (11.35)		7 (3.78)	
Rectum	439	35 (7.97)		28 (6.38)		8 (1.82)	
Mucin production							
With	131	36 (27.48)	< 0.001	27 (20.61)	< 0.001	11 (8.4)	0.007
Without	666	87 (13.06)		70 (10.51)		22 (3.3)	
Tumor differentiation							
Poor	134	36 (26.87)	< 0.001	29 (21.64)	< 0.001	12 (8.96)	0.012*
moderate	589	73 (12.39)		55 (9.34)		19 (3.23)	
Well	31	4 (12.9)		4 (12.9)		1 (3.23)	
Unknown	43						
Tumor stage							
Ⅰ	94	6 (6.38)	0.011	5 (5.32)	0.025	1 (1.06)	0.288*
Ⅱ	326	64 (19.63)		52 (15.95)		17 (5.21)	
Ⅲ	301	41 (13.62)		32 (10.63)		11 (3.65)	
Ⅳ	76	12 (15.79)		8 (10.52)		4 (5.26)	

Bowel wall invasion (T)							
T1	20	3 (15)	0.013	2 (10)	0.069	1 (5)	0.067*
T2	102	5 (4.9)		5 (4.9)		0 (0)	
T3	334	61 (18.26)		49 (14.67)		15 (4.49)	
T4	341	54 (15.83)		41 (12.02)		17 (4.98)	
Lymph node metastasis (N)							
N0	445	76 (17.08)	0.155	61 (13.68)	0.275	20 (4.49)	0.583
N1	200	31 (15.5)		24 (12)		9 (4.5)	
N2	152	16 (10.53)		12 (7.95)		4 (2.63)	
Distant metastasis (M)							
M0	721	112 (15.53)	0.808	89 (12.34)	0.645	30 (4.16)	0.929*
M1	76	11 (14.47)		8 (10.53)		3 (3.95)	
Lymphovascular invasion							
Yes	335	48 (14.33)	0.473	40 (11.94)	0.894	12 (3.58)	0.481
No	457	74 (16.19)		56 (12.25)		21 (4.59)	
Unknown	5						
Alcohol intake							
Ever	162	19 (11.72)	0.144	14(8.64)	0.124	6 (3.7)	0.755
Never	635	104 (16.38)		83 (13.07)		27 (4.25)	
Smoking							
Ever	263	35 (13.31)	0.244	26 (9.89)	0.166	11 (4.18)	0.967
Never	534	88 (16.48)		71 (13.3)		22 (4.12)	
Colorectal family history							
Yes	48	11 (22.92)	0.082	6 (12.5)	0.765	6 (12.5)	0.016*
No	335	45 (13.43)		37 (11.04)		12 (3.58)	
Unknown	414						

*Fisher's exact test was used

Table 3(on next page)

Correlations between *RAS* gene mutations and clinicopathological characteristics (n=813)

Table 3. Correlations between *RAS* gene mutations and clinicopathological characteristics (n=813)

Characteristics	Number	*RAS*		*KRAS*		*NRAS*	
		Mutation(%)	P value	Mutation (%)	P value	Mutation (%)	P value
Gender							
Male	506	221 (43.68)	0.105	204 (40.32)	0.097	19 (3.75)	0.9
Female	307	152 (49.51)		142 (46.25)		11 (3.58)	
Age							
< 50	100	38 (38)	0.091	37 (37)	0.23	1 (1)	0.161*
≥ 50	713	335 (46.98)		309 (43.34)		29 (4.07)	
Location							
Right colon	181	91 (50.28)	0.178	88 (48.62)	0.097	3 (1.66)	0.164
Left colon	189	77 (40.74)		71 (37.57)		6 (3.17)	
Rectum	443	205 (46.28)		187 (42.21)		21 (4.74)	
Mucin production							
With	133	74 (55.64)	0.014	73 (54.89)	0.002	1 (0.75)	0.087
Without	680	299 (43.97)		273 (40.18)		29 (4.22)	
Tumor differentiation							
Poor	138	55 (39.86)	0.315	54 (39.13)	0.604	1 (0.72)	0.093
moderate	599	276 (46.08)		251 (41.9)		28 (4.67)	
Well	33	17 (51.52)		16 (48.48)		1 (3.03)	
Unknown	43						
Tumor stage							
Ⅰ	95	41 (43.16)	0.031	35 (36.84)	0.023	6 (6.32)	0.18*
Ⅱ	332	170 (51.2)		161 (48.49)		9 (2.71)	
Ⅲ	302	133 (44.04)		122 (40.4)		14 (4.64)	

Ⅳ	84	29 (34.52)		28 (34.52)		1 (1.19)	
Bowel wall invasion (T)							
T1	21	9 (42.86)	0.36	8 (38.1)	0.158	1 (4.76)	0.36*
T2	104	40 (38.46)		34 (32.69)		6 (5.77)	
T3	336	154 (45.83)		146 (43.45)		9 (2.68)	
T4	352	170 (48.3)		158 (44.89)		14 (3.98)	
Lymph node metastasis (N)							
N0	458	224 (48.91)	0.006	209 (45.63)	0.079	15 (3.28)	0.265
N1	203	88 (43.35)		83 (40.89)		6 (2.96)	
N2	152	61 (40.13)		54 (35.53)		9 (5.92)	
Distant metastasis (M)							
M0	729	343 (47.05)	0.048	317 (43.48)	0.116	29 (3.98)	0.353*
M1	84	30 (35.71)		29 (34.52)		1 (1.19)	
Lymphovascular invasion							
Yes	339	157 (46.31)	0.763	145 (42.77)	0.825	14 (4.13)	0.623
No	462	209 (45.24)		194 (41.99)		16 (3.46)	
Unknown	12						
Alcohol intake							
Ever	165	67 (40.61)	0.128	63 (38.18)	0.203	5 (3.03)	0.615
Never	648	306 (47.22)		283 (43.67)		25 (3.86)	
Smoking							
Ever	283	109 (38.52)	0.002	99 (34.98)	0.001	10 (3.53)	0.863
Never	530	264 (49.81)		247 (46.6)		20 (3.77)	
Colorectal family history							
Yes	48	28 (58.33)	0.017	26 (54.17)	0.013	3 (6.25)	0.178*
No	337	135(40.95)		126 (37.39)		9 (2.67)	

Unknown	428

*Fisher's exact test was used

Table 4(on next page)

Correlations between DNA mismatch repair protein expression deficiency and *RAS* status (n =797)

Table 4. Correlations between DNA mismatch repair protein expression deficiency and *RAS* status (n =797)

MMR status	*RAS*		*KRAS*		*NRAS*	
	Mutant/tested cases (%)	P value	Mutant/tested cases (%)	P value	Mutant/tested cases (%)	P value
dMMR	54/123 (43.9)	0.623	54/123 (43.9)	0.742	0/123 (0)	0.009*
MHL1/PMS2 deficiency	41/97 (42.26)	0.456	41/97 (42.26)	0.994	0/97 (0)	0.066*
MSH2/ MSH6 deficiency	15/33 (45.45)	0.924	15/33 (45.45)	0.721	0/33 (0)	0.391*
pMMR	313/674 (46.44)		286/674 (42.43)		30/674 (4.45)	

*Fisher's exact test was used

Table 5(on next page)

Correlations between *KRAS* gene mutations and clinicopathological characteristics in dMMR tumors (n=123)

Table5. Correlations between *KRAS* gene mutations and clinicopathological characteristics in dMMR tumors (n=123)

Characteristics	Number	*KRAS* Mutation (%)	P value
Gender			
Male	73	31 (42.47)	0.698
Female	50	23 (46)	
Age			
< 50	29	11 (37.93)	0.459
≥ 50	94	43 (45.74)	
Location			
Right colon	62	26 (41.94)	0.545
Left colon	26	12 (46.15)	
Rectum	35	16 (45.71	
Mucin production			
With	36	20 (55.56)	0.094
Without	87	34 (39.08)	
Tumor differentiation			
Poor	36	10 (27.78)	0.112*
Moderate	73	35 (47.95)	
Well	4	2 (50)	
Unknown	10		
Tumor stage			
Ⅰ	6	2 (33.33)	0.357*
Ⅱ	64	33 (51.5)	
Ⅲ	41	15 (36.59)	
Ⅳ	12	4(33.33)	

Bowel wall invasion (T)			
T1	3	2 (66.67)	0.156*
T2	5	0 (0)	
T3	61	26 (42.62)	
T4	54	26 (48.15)	
Lymph node metastasis (N)			
N0	76	38 (50)	0.07
N1	31	13 (41.94)	
N2	16	3 (18.75)	
Distant metastasis (M)			
M0	112	50 (44.64)	0.754*
M1	11	4 (36.36)	
Lymphovascular invasion			
Yes	48	21 (43.75)	0.956
No	74	32 (43.24)	
Unknown	1		
Alcohol intake			
Ever	19	9(47.37)	0.807
Never	104	46(43.4)	
Smoking			
Ever	35	14 (40)	0.582
Never	88	40 (45.45)	
Colorectal family history			
Yes	11	5 (45.45)	0.742
No	45	24 (53.33)	
Unknown	67		

3 *Fisher's exact test was used

Figure 1

Kaplan-Meier curves for disease free survival and overall survival according to dMMR or *KRAS* status

A: Disease free survival (DFS) according to dMMR status; B: Overall survival (OS) according to dMMR status; C: DFS according to *KRAS* status; D: OS according to *KRAS* status.