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*Original Article*

# Comparison of clinicopathological features and *KRAS gene* mutation of left-sided and right-sided colon cancers

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**Abstract:** Colon cancer is the third most common cancer diagnosed in both men and women worldwide and one of the leading causes of cancer-related deaths. Based on where the cancer stared, we focused on the left-sided colon cancer and right-sided colon cancer in this study to reveal their pathological characteristics and their relationship to the KRAS gene mutation. From examining a total of 198 colorectal cancer specimens collected during 2013 and 2016 in Nan fang Hospital, we noticed that whether the cancer developed in left side or right side of the colon are highly relevant to the patients gender, age, tumor size, differentiation and distant metastasis. We found that right-sided colon cancers (RCC) are more likely to occur in women, be greater than 5 cm, and occur in patients older than 60 years, while left-sided colon cancers (LCC) are more prevalent in patients with poorly differentiated tumors and tumors with distant metastases. We then randomly selected 113 of the 198 samples and performed Sanger sequencing to examine their *KRAS* gene mutation. The result indicates that KRAS mutation is prominently higher in RCC compare to LCC, especially the mutation on the 12$^{th}$ codon GGT>GAT (G12D). In addition to the location in which the cancer developed, the *KRAS* mutation was also closely associated with the tumor size, degree of tumor differentiation, and lymph node metastasis. There was a significantly higher incidence of *KRAS* mutation in cases with RCC, poorly differentiated tumors, and non-lymph node metastasis compared to LCC with well- or moderately differentiated tumors with lymph node metastasis. Taken together, we determined that there are distinct differences in the clinicopathological characteristics of right-versus left-sided colon cancers and their correlation with the *KRAS* mutation. These differences suggest that practitioners need to diagnose and treat RCC and LCC differently.

**Keywords:** Colorectal cancer, left and right-side colon cancers, *kras* gene mutation, sanger sequencing, molecular diagnostics

## Introduction

Colon cancer is one of the most common malignant tumors of the digestive tract, and its incidence and mortality are increasing annually worldwide [1]. It has been established that mutations, amplification, and deletions of oncogenes are involved in the tumorigenesis and progression of colon cancer [2]. The *KRAS* gene mutation, an important downstream regulator of the epidermal growth factor receptor (EGFR) signaling pathway, is closely associated with colon cancer. The most recent colon cancer clinical practice guidelines from the National Comprehensive Cancer Network clearly indicate that all colorectal cancer patients should be tested for *KRAS*, and that only *KRAS* wild-type patients should receive EGFR inhibitor treatment, such as cetuximab and panitumumab [3].

For many years, differences in the biological behavior of malignant colon tumors of different pathogenic sites have been widely noted, but it was not until 1990 when Bufill suggested that left and right colon cancer are two different kinds of tumors. The differences of LCC and RCC are systematically expounded in terms of epidemiology, molecular characteristics, carcinogenesis and so on [4]. Subsequently, a large number of molecular biology studies and clinical retrospective analyses have confirmed the-

**Table 1.** Relationship between LCC and RCC and the clinicopathological features

| Clinicopathological features | RCC (n=55) | LCC (n=143) | $\chi^2$ value | *P* value |
|---|---|---|---|---|
| Gender | | | | |
| Male | 25 | 110 | 18.132 | 0.000* |
| Female | 30 | 33 | | |
| Age | | | | |
| <60 | 35 | 114 | 5.518 | 0.019* |
| ≥60 | 20 | 29 | | |
| Abdominal pain | | | | |
| Yes | 47 | 119 | 0.147 | 0.702 |
| No | 8 | 24 | | |
| Perianal hemorrhage | | | | |
| Yes | 32 | 105 | 4.331 | 0.037* |
| No | 23 | 38 | | |
| Tumor size (cm) | | | | |
| <5 | 33 | 110 | 5.671 | 0.017* |
| ≥5 | 22 | 33 | | |
| Degree of differentiation | | | | |
| Poor | 20 | 29 | 5.518 | 0.019* |
| Medium/high | 35 | 114 | | |
| T staging | | | | |
| T1+T2 | 14 | 32 | 0.211 | 0.646 |
| T3+T4 | 41 | 111 | | |
| N staging | | | | |
| N0 | 23 | 52 | 0.502 | 0.479 |
| N1+N2 | 32 | 91 | | |
| M staging | | | | |
| M0 | 39 | 78 | 4.400 | 0.036* |
| M1 | 16 | 65 | | |

* for P<0.05.

seviews [5, 6]. If the splenic flexure of colon is regarded as a point of differentiation, colon cancer can be classified into LCC and RCC.

This comparative study of the clinicopathological features and *KRAS* gene mutations of RCC and LCC revealed the biological differences of tumors that originate in the different parts of the colon (the left versus right side) and suggests that RCC and LCC should follow different diagnosis and treatment processes, with the goal of achieving precise control and individualized treatment of tumors at the molecular level.

## Materials and methods

### *General material*

At the Nanfang hospital of Southern Medical University, 198 colon cancer specimens were collected after surgical resection during 2013-2016. All specimens were prepared with 10% neutral formalin for fixing and then paraffin embedded to prepare the standard formalin-fixed paraffin-embedded (FFPE) specimens. We randomly selected 113 of these specimens and performed Sanger sequencing method to examine their *KRAS* gene mutations.

### *DNA extraction*

The tissue DNA was extracted with the German QIAGEN paraffin tissue DNA Extraction Kit. HE (hematoxylin-eosin staining) sections were observed under the microscope by professional pathologists, and paraffin fragments of more than 80% of the tumor components were selected: 6 slices of 10 e used for *P* were used [6 slices were taken at 10 μm thickness], 1 of which was stained with HE, and the tumor tissue area was labeled. The remaining 5 slices were dewaxed, hydrated, and dried. The tumor tissue outside the marked tissue area was removed with a clean blade; then, the tumor area was scraped into a sterilized EP tube with the addition of lysis liquid (1-1.5 ml) and protease K (0.5-5 μ) at 65°C overnight for water bath digestion. The next day, the tissues were completely digested; the solution was clear and placed in 100°C water bath, to inactivate the protease k. In strict accordance with the reagent kit instructions, step-by-step [gradually] extraction of DNA was performed. The extract was frozen and stored at -20°C. After extraction, the DNA quality and concentration were measured by using a UV spectrophotometer to ensure the OD 260/280 value was 1.8-2.0. The unqualified specimens used for re-extraction of DNA.[Repetitive operation is performed for unqualified specimens. The DNA concentration was diluted to 10 ng/μl with 15 ng/μl as required.

**Table 2.** Analysis of the type of *KRAS* gene mutation in LCC and RCC

| Mutation type | Total (%) | RCC (%) | LCC (%) | $\chi^2$ value | *P* value |
|---|---|---|---|---|---|
| codon 12 GLY12ASP (GGT>GAT) | 16 (14.2%) | 10 (26.3%) | 6 (8.0%) | 6.961 | 0.008* |
| codon 12 GLY12VAL (GGT>GTT) | 7 (6.2%) | 3 (7.89%) | 4 (5.3%) | 0.285 | 0.594 |
| codon 12 GLY12ALA (GGT>GCT) | 4 (3.54%) | 2 (5.3%) | 2 (2.67%) | 0.498 | 0.480 |
| codon 12 GLY12CYS (GGT>TGT) | 4 (3.54%) | 2 (5.3%) | 2 (2.7%) | 0.498 | 0.480 |
| codon 13 GLY13ASP (GGC>GAC) | 8 (7.1%) | 3 (7.89%) | 5 (6.67%) | 0.058 | 0.810 |
| Total number | 39 (34.5%) | 20 (52.6%) | 19 (25.3%) | 4.186 | 0.041* |

* for P < 0.05.

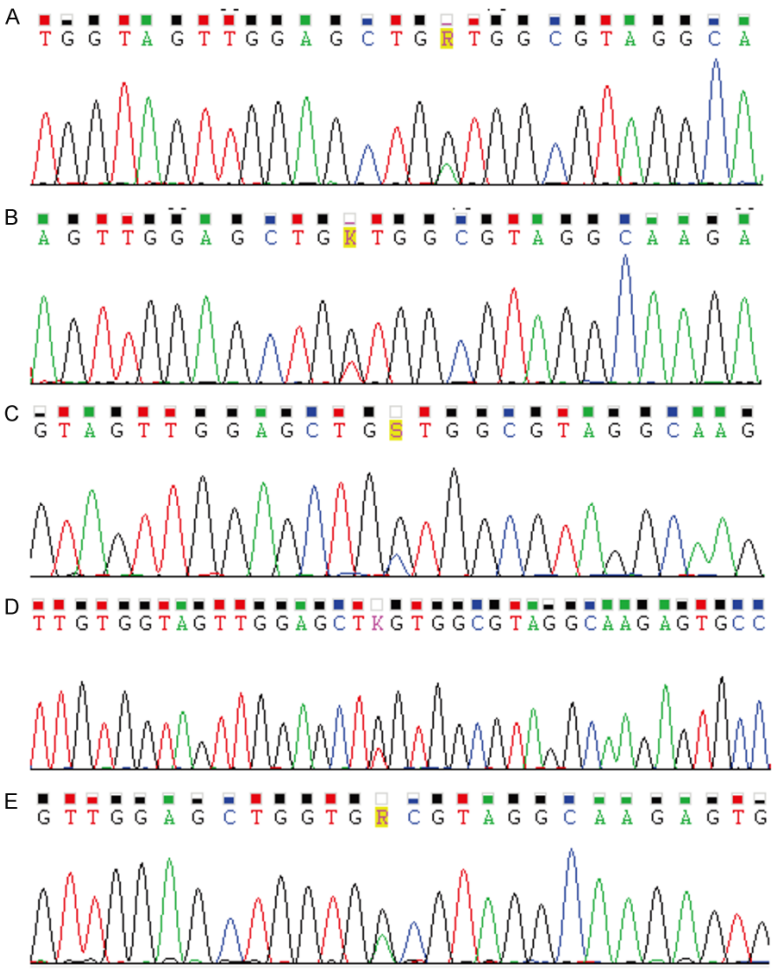


**Figure 1.** The sequencing diagram of *KRAS* gene by Sanger sequencing: A: Gly12ASP (GGT>GAT); B: Gly12VAL (GGT>GTT); C: Gly12ALA (GGT>GCT); D: Gly12CYS (GGT>TGT); E: Gly13ASP (GGC>GAC).

*Sanger DNA sequencing to detect KRAS gene mutation*

A total of 113 specimens were analyzed using the primers provided by Guangzhou baochuang, the primer biotechnology company, for the amplification of the second exon of the *KRAS* gene, as well as the twelfth and thirteenth codon regions: upstream primer: 5'-AGGCCTGCTGAAAATGACTG-3'; and downstream primer: 5'-TCAAAGAATGGTCCTGCACC-3'. The PCR reaction system was 25 ul: template DNA (100 mg/L) 1 ul, 10×ABI buffer 2.5 ul, MgCl2 (25 mmol/L) 1.5 ul, dNTP (2.5 mmol/L) 2 ul, ABI AmpliTaq Gold DNA polymerase 0.125 ul (5 u/ul), primer (10 umol/L), 1 ul, deionized water 15.875 ul. The PCR reaction conditions: 95 degrees Celsius, pre denatured 7 min, 95 degrees Celsius, 30 s, 60 degrees Celsius, 30 s, 72 degrees Celsius, 45 s, 40 cycles, and finally 72 degrees Celsius extended 10 min. The PCR amplification products were analyzed by routine bidirectional DNA sequencing on the ABI3500 DX gene analyzer.

*Statistical analysis*

The statistical software SPSS statistics 20.0 statistical software was used, and the $\chi^2$ test was used for analysis and comparison. The difference was statistically significant when $P<0.05$.

## Results

*Clinical and pathological characteristics of LCC and RCC*

Of the 198 patients with colon cancer, 135 were men (68.2%) and 63 were women (31.8%), with an age range of 24-86 years and an aver-

**Table 3.** Correlation analysis between *KRAS* gene mutation and clinicopathological features

| Clinicopathological parameters | Total number (n) | Mutation number (n) | Mutation rate (%) | *P* value |
|---|---|---|---|---|
| Primary site | | | | |
| RCC | 38 | 20 | 52.6 | 0.004* |
| LCC | 75 | 19 | 25.3 | |
| Gender | | | | |
| Male | 76 | 26 | 34.2 | 0.923 |
| Female | 37 | 13 | 35.1 | |
| Age | | | | |
| <60 | 65 | 22 | 33.8 | 0.862 |
| ≥60 | 48 | 17 | 35.4 | |
| Tumor size (cm) | | | | |
| <5 | 72 | 29 | 40.2 | 0.088 |
| ≥5 | 41 | 10 | 24.4 | |
| Degree of differentiation | | | | |
| Poor | 27 | 15 | 55.6 | 0.008* |
| Medium/high | 86 | 24 | 27.9 | |
| T staging | | | | |
| T1+T2 | 14 | 5 | 35.7 | 0.920 |
| T3+T4 | 99 | 34 | 34.3 | |
| N staging | | | | |
| N0 | 55 | 24 | 43.6 | 0.047* |
| N1+N2 | 58 | 15 | 25.9 | |
| M staging | | | | |
| M0 | 90 | 29 | 32.2 | 0.311 |
| M1 | 23 | 10 | 43.5 | |

* for P< 0.05.

age age of 53.2 years. Statistical analysis showed that the location of the tumor was closely related to the patient's gender, age, tumor size, level of tumor differentiation, and distant metastasis (**Table 1**). RCC was more prevalent in women, older patients (over age 60), and patients with tumors with a diameter greater than or equal to 5 cm, while patients with LCC had a higher proportion of anal bleeding/fecal occult blood admission, and tumors with low differentiation levels with distant metastasis (M staging) ($P$<0.05). There were no significant differences considering the tumor site, abdominal pain, T staging, and N staging ($P$>0.05).

*Analysis of the type of KRAS gene mutation in LCC and RCC*

Analysis of the mutations of the *KRAS* gene in 113 cases of colon cancer (**Table 2**) showed that there were five mutation types in codon 12, 13 of exon 2 (**Figure 1**), and the total mutation rate of *KRAS* gene was 34.5% (39/113). The RCC mutation rate of 52.6% (20/55) was significantly higher than that of LCC (25.3%, 19/143) ($P$<0.05). Further analysis of the *KRAS* gene in codon 12 for GLY12ASP mutations (GGT>GAT) showed that the mutation rate was higher in RCC than in LCC ($P$<0.05), while the mutation rate of the other mutations that were compared showed no significant difference. In this test, no single mutation types except 5 kinds of *KRAS* mutations were found, and no *KRAS* mutations were observed in different codons.

*Relationship between KRAS gene mutation and clinicopathological features*

*KRAS* gene mutation analysis of 113 colon cancer specimens found that the mutation rate of the *KRAS* gene in primary tumors are closely related with tumor site, tumor differentiation and lymph node status ($P$<0.05) (**Table 3**). In colon cancer with poor-differentiation and no-lymph node metastasis (N0) (**Figure 2**), the mutation rate of *KRAS* was significantly higher than that of colon cancer with medium/high differentiation and lymph node metastasis (N1+N2) (**Figure 3**), respectively.

## Discussion

Data from the United States Surveillance, Epidemiology, and End Results program (SEER) database during 1992-2005 show more female patients in RCC [6]. This study analyzed the relationship between the primary site and the pathological parameters of 198 cases of colon cancer, and found that RCC was more common in women and elderly patients, consistent with previous studies. Studies have shown that this may be due to the role of female hormones, which affect cholesterol metabolism

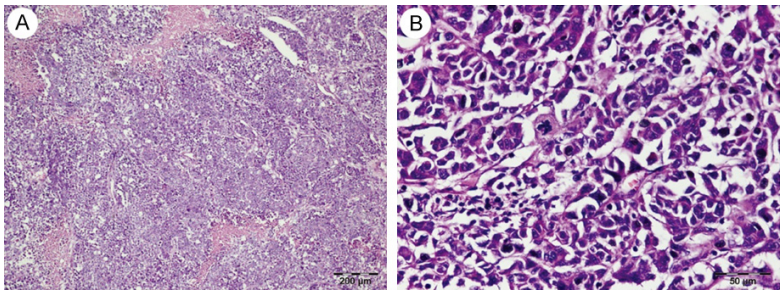


**Figure 2.** Representative pictures of HE staining. A. Poorly differentiated colon cancer with *KRAS* mutation (200 μm). B. Poorly differentiated colon cancer with *KRAS* mutation (50 μm).

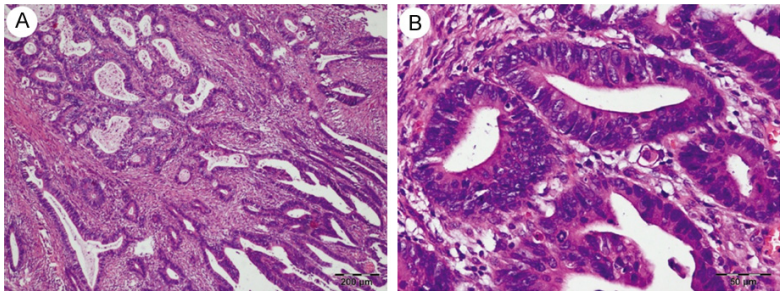


**Figure 3.** Representative pictures of HE staining. A. Medium/highly differentiated colon cancer with *KRAS* mutation (200 μm). B. HE staining of medium/highly differentiated colon cancer with *KRAS* mutation (50 μm).

and reduce plasma levels of insulin-like growth factors, and these processes are more closely related to the distribution of RCC, while unhealthy habits more prevalent in men, such as smoking and consuming alcohol, are more closely related to LCC distribution. In addition, elderly patients were more prone to RCC, which is Correspond to the results reported in NAWA et al [7].

A large number of studies [6, 8-11] showed that, in RCC, tumor volume is larger, the mass is more easily palpable, pain symptoms are more common, tumors have a lower degree of differentiation, and metastasis is rare; LCC tumors tend to be smaller in size, less palpable, lead to less pain, and more highly differentiated. The statistical results of the study also showed that the larger tumor size and low differentiation seen in RCC is probably due to the fact that tumors tend to grow to larger extent and medical treatment or surgery later because wide lumen and thin wall of the right colon.

The *KRAS* gene is closely related to the occurrence and development of human colon tumors; the major tumorigenesis changes are point mutation and gene amplification. Point mutations occurred in exon 12, codon 13, and exon 2. Under normal circumstances, the *KRAS* protein regulates downstream signaling pathways through GDP/GTP conversion; *KRAS* protein mutation does not depend on the higher signal stimulus will continue, in conjunction with the GTP, leading to activation of downstream signaling pathways, promoting the growth and reproduction of tumor cells. The mutation rate of *KRAS* gene was 30-40% [12, 13]. In this study, the mutation status of the *KRAS* gene in 113 cases of colon cancer was examined, and the mutation rate was 34.5%, which is consistent with rates reported in the literature. Different site mutations indicate different clinicopathological features. The most common mutation site was codon 12, especially among the GLY12ASP mutation types. This study is consistent with other literature reports [14, 15]. Another study showed that overall survival in patients who received first-line cetuximab combined with chemotherapy in the treatment of *KRAS* gene GLY12ASP mutation was significantly longer than that of patients with other types of point mutations, but survival compared with the wild type *KRAS* was shorter [16].

The relationship between RCC and LCC with the mutation of *KRAS* is still uncertain. XuJianming and other studies [17] showed that there was no difference in the rate of *KRAS* gene mutation between RCC and LCC in sporadic colon cancer; while two small samples [18, 19] found that the *KRAS* mutation was associated with the occurrence of RCC. This study showed that the total mutation rate of *KRAS* gene in RCC was significantly higher than that in LCC, and the mutation was mainly in the twelfth codon GLY13ASP mutation of *KRAS* gene. The conclusions of this study require further verification with a larger number of cases, and the molecu-

lar biological mechanism also need further study.

In summary, the pathological characteristics of left and right colon cancer in are different, with RCC more prevalent in women and in elderly patients, the incidence of late onset, large tumor size, and tumor differentiation; LCC patients are more prone to anal bleeding/fecal occult blood symptom and distant metastasis. The mutation rate of *KRAS* gene was higher in RCC cases, poorly differentiated and patients with no -lymph node metastasis. Differences in gene expression and mutation also affect the efficacy of chemotherapy and molecular targeted drugs, and mutations may be a cause of differences in patient treatment and prognosis. In this age of precision medicine, the diagnosis and treatment of RCC and LCC should be considered individually, providing more accurate medical services for the patients.

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## Disclosure of conflict of interest

None.

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