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# *KRAS* Gene Mutation Associated with Grade of Tumor Budding and Peripheral Immunoinflammatory Indices in Patients with Colorectal Cancer

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**Background:** The efficacy of targeted therapy for colorectal cancer (CRC) is affected by hub genes of epidermal growth factor receptor (EGFR) signaling pathways, such as *KRAS*. Immune cell infiltration may lead to gene mutation, but the relationship between *KRAS* status and peripheral immune-inflammatory indices has not been clarified in CRC.

**Methods:** Clinical records of CRC patients were collected. The relationship between *KRAS* status and clinicopathological characteristics, peripheral immune-inflammatory indices (pan-immune inflammation value (PIV) (monocyte×neutrophil×platelet/lymphocyte), systemic immune inflammation index (SII) (platelet×neutrophil/lymphocyte), and system inflammation response index (SIRI) (monocyte×neutrophil/lymphocyte)) were analyzed.

**Results:** 1033 CRC patients were collected, there were 514 (49.8%) patients with *KRAS* wild-type and 519 (50.2%) with *KRAS* mutation. Patients with *KRAS* mutation had higher proportions of female, III-IV stage, and lymph node metastasis and lower proportion of low grade of tumor budding (the presence of single tumor cells or small clusters of up to 5 cells in mesenchyma at the front of tumor invasion) than those with *KRAS* wild-type. The PIV, SII, and SIRI levels in *KRAS* mutation patients were significantly higher than those in *KRAS* wild-type patients. The proportion of aged ≥65 years old, dMMR, distant metastasis, and *KRAS* mutation were high in patients with high PIV, SII, and SIRI levels. Logistic regression analysis showed that non-low grade of tumor budding (odds ratio (OR): 1.970, 95% confidence interval (CI): 1.287–3.016, $p$=0.002), and high SII level (≥807.81 vs <807.81, OR: 1.915, 95% CI: 1.120–3.272, $p$=0.018) were independently associated with *KRAS* mutation.

**Conclusion:** Non-low grade of tumor budding, and high SII level were independently associated with *KRAS* mutation in CRC. It provides additional references for diagnosis and treatment options for patients with CRC.

**Keywords:** colorectal cancer, *KRAS*, systemic immune inflammation index, tumor budding

## Introduction

Colorectal cancer (CRC) is a cancer that occurs in the gut.[1] According to the statistics by the GLOBOCAN in 2020, the incidence and mortality of CRC in the world rank third and second respectively among malignant tumors.[2] Genetic factors,[3,4] bad diet and lifestyle habits,[5] obesity,[6] and low physical activity,[7] intestinal flora imbalance[8,9] are closely related to the development of CRC. With the rapid development of precision medicine, CRC has entered a targeted therapy mode with gene mutation status as a biomarker.[10] Tyrosine kinase inhibitors (TKI) have shown good efficacy in cancer patients with EGFR-activating mutations.[11]

EGFR as one of the main targets of targeted drugs, is a transmembrane tyrosine kinase receptor. The downstream of this signal transduction pathway mainly include RAS/RAF/MAPK, PI3K/AKT/mTOR pathways, and EGFR binding with ligands can cause the activation of the two downstream major pathways, thereby inducing cell proliferation,

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invasion, metastasis, and angiogenesis.[12] *Rat sarcoma (RAS)* gene is one of family of human proto-oncogenes, and *Kirsten rat sarcoma viral oncogene homologue (KRAS)* gene is the most studied *RAS* gene.[13,14] *KRAS* mutation leads to the continuous activation of EGFR-dependent RAS/RAF/MAPK pathway, causing excessive cell proliferation and differentiation, and thus inducing the progression of CRC.[15] More than 30% of CRC patients had *KRAS* gene activating mutations.[16] Immune inflammation is involved in the pathogenesis of many diseases.[17,18] The results of the study on the relationship between inflammatory response and tumor suggest that inflammatory microenvironment may promote the occurrence, development and distant metastasis of tumor.[19,20] Long-term inflammatory stimulation can lead to changes in tumor-related genes,[21] but there are very few studies on the correlation between inflammatory indicators and gene status.

The main factors affecting the choice of treatment and prognosis of CRC patients are the genetic mutation status of tumor patients after surgery. In clinical practice, there is an urgent need for preoperative noninvasive and easily accessible indicators to evaluate the therapeutic effect. Pan-immune inflammation value (PIV), systemic immune inflammation index (SII), and system inflammation response index (SIRI) are several comprehensive immune-inflammatory biomarkers based on complete blood counts.[22–25] PIV is associated with the clinical stage,[26,27] and prognosis[22] of CRC. SII has been proven to predict the therapeutic effect,[28] and prognosis[29,30] of CRC. SIRI index was related to the prognosis of several cancers.[31–33] The mutation status of *KRAS* gene, as well as the inflammation index PIV, SII and SIRI, which reflect the inflammation balance state of the body, have been proved to be effective in predicting the prognosis of tumor patients.

However, the relationship between *KRAS* gene status, levels of peripheral immune-inflammatory indices and clinicopathological features of CRC has not been fully studied. In addition, the relationship between *KRAS* gene mutation and the level of peripheral immune-inflammatory indices has not been reported. Therefore, in order to study the relationship between them, the relationship between *KRAS* status and clinicopathological characteristics, peripheral immune-inflammatory indices were analyzed. It should provide additional valuable reference data for diagnosis and treatment options for patients with CRC.

# Materials and Methods

## Participants

A total of 1033 CRC patients who were hospitalized in Meizhou People's Hospital, between January 2022 and January 2024. The inclusion criteria of the study as follows: (1) pathology confirmed the diagnosis of primary CRC; (2) patients undergoing radical surgery for CRC; (3) there were complete medical records. The exclusion criteria as follows: (1) CRC patients had other tumors; (2) CRC patients with severe organ dysfunction, severe infectious disease, and autoimmune disease; (3) clinical records were incomplete. This study was performed in accordance with the ethical standards of the Declaration of Helsinki and approved by the Human Ethics Committee of Meizhou People's Hospital.

## Data Collection

Clinicopathological features of the CRC patients including gender, age, vessel carcinoma embolus, grade of tumor budding, mismatch repair (MMR) status, clinical stage, lymph node metastasis, and distant metastasis. The patient's venous blood was collected before treatment, blood cell analysis was tested by Sysmex XE-2100 hematology analyzer (Sysmex Corporation, Japan).

*KRAS* gene mutation was detected by amplification refractory mutation system (ARMS)-PCR as previously described.[34] The genetic sites tested mainly included common mutations in exons 2, 3 and 4 of *KRAS* (codons 12, 13, 61, 117, and 146). The expressions of MutL homolog 1 (MLH1), MutS homolog 2 (MSH2), MutS homolog 6 (MSH6), and PMS1 homolog 2 (PMS2), which are related to MMR, were detected by immunohistochemical method. The deletion of any one or more mismatch repair proteins was identified as mismatch repair-deficient (dMMR), while the absence of any of the four mismatch repair proteins was identified as mismatch repair-proficient (pMMR). Cancer tissues were stained with hematoxylin and eosin (HE), and the number of tumor buds was observed under the microscope: 0–4 tumor buds per 0.785 $mm^2$ was classified as low grade, 5–9 tumor buds per 0.785 $mm^2$ was classified as intermediate grade, and ≥10 tumor buds per 0.785 $mm^2$ was classified as high grade.

## Data Analysis

PIV, SII, and SIRI were calculated according to the following formula:

PIV=monocyte×neutrophil×platelet/lymphocyte;

SII=platelet×neutrophil/lymphocyte;

SIRI=monocyte×neutrophil/lymphocyte.

The clinicopathological features were summarized with descriptive statistics. Categorical variables were compared using $\chi^2$ test or Fisher's exact test. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff values of PIV, SII, and SIRI to distinguish *KRAS* mutation. Gender, age, vessel carcinoma embolus, grade of tumor budding, MMR status, lymph node metastasis, distant metastasis, and levels of PIV, SII, and SIRI were selected as covariates in the multivariate logistic regression analysis for *KRAS* mutation, based on estimating the odds ratios (OR) and their 95% confidence intervals (CIs). $p<0.05$ was considered statistically significant.

# Results

## Clinicopathological Features of the CRC Patients

Among 1033 CRC patients were included, 648 (62.7%) were male and 385 (37.3%) were female. There were 517 (50.0%) cases aged <65 years old and 516 (50.0%) cases with aged ≥65 years old. There were 192 (18.6%), 40 (3.9%), and 40 (3.9%) patients with vessel carcinoma embolus, high grade of tumor budding, and dMMR, respectively. And 615 (59.5%) patients had lymph node metastasis, and 203 (19.7%) had distant metastasis. In this study, the *KRAS* gene mutation rate was 50.2% (519/1033). The level of PIV, SII, and SIRI in these patients was 302.31 (167.80, 562.76), 735.75 (493.98, 1219.63), and 1.15 (0.73, 1.95), respectively (Table 1).

**Table 1** The Clinicopathological Features of the CRC Patients

| Clinicopathological Features | CRC (n=1033) |
|---|---|
| Gender | |
| Male, n (%) | 648 (62.7%) |
| Female, n (%) | 385 (37.3%) |
| Age (years) | |
| <65, n (%) | 517 (50.0%) |
| ≥65, n (%) | 516 (50.0%) |
| Vessel carcinoma embolus | |
| No, n (%) | 818 (79.2%) |
| Yes, n (%) | 192 (18.6%) |
| Unknown, n (%) | 23 (2.2%) |
| Grade of tumor budding | |
| Low, n (%) | 169 (16.4%) |
| Intermediate, n (%) | 245 (23.7%) |
| High, n (%) | 40 (3.9%) |
| Unknown, n (%) | 579 (56.1%) |
| MMR | |
| pMMR, n (%) | 974 (94.3%) |
| dMMR, n (%) | 40 (3.9%) |
| Unknown, n (%) | 19 (1.8%) |
| TNM stage | |
| I-II, n (%) | 362 (35.0%) |
| III-IV, n (%) | 671 (65.0%) |

(*Continued*)

**Table 1** (Continued).

| Clinicopathological Features | CRC (n=1033) |
|---|---|
| Lymph node metastasis | |
| No, n (%) | 400 (38.7%) |
| Yes, n (%) | 615 (59.5%) |
| Unknown, n (%) | 18 (1.7%) |
| Distant metastasis | |
| No, n (%) | 729 (70.6%) |
| Yes, n (%) | 203 (19.7%) |
| Unknown, n (%) | 101 (9.8%) |
| *KRAS mutation* | |
| No, n (%) | 514 (49.8%) |
| Yes, n (%) | 519 (50.2%) |
| Indexes of immune-nutritional status | |
| PIV, median (P25, P75) | 302.31 (167.80, 562.76) |
| SII, median (P25, P75) | 735.75 (493.98, 1219.63) |
| SIRI, median (P25, P75) | 1.15 (0.73, 1.95) |

**Abbreviations**: CRC, colorectal cancer; MMR, mismatch repair; PIV, pan-immune-inflammation value; SII, systemic immune-inflammatory index; SIRI, systemic inflammatory response index; P25, 25th percentile; P75, 75th percentile.

## Comparison of Clinicopathological Features in CRC Patients with or Without KRAS Mutation

514 (49.8%) CRC patients with *KRAS* wild-type and 519 (50.2%) with *KRAS* mutation. The proportion of CRC patients with *KRAS* mutation who were female (40.3% vs 34.2%, $p$=0.046), III-IV stage (68.8% vs 61.1%, $p$=0.011), had lymph node metastasis (63.6% vs 55.4%, $p$=0.010) was higher than that in CRC patients with *KRAS* wild-type, respectively, whereas the proportion of low grade of tumor budding (11.4% vs 21.4%, $p$<0.001) lower than that in CRC patients with *KRAS* wild-type. PIV, SII, and SIRI levels in *KRAS* mutation CRC patients were significantly higher than those in *KRAS* wild-type CRC patients (all $p$<0.05). There was no difference in age distribution and proportion of vessel carcinoma embolus, dMMR, and distant metastasis between those with and without *KRAS* mutation (Table 2).

**Table 2** Comparison of Clinicopathological Features Among CRC Patients with or Without KRAS Mutation

| Clinicopathological Features | *KRAS* wild-type (n=514) | *KRAS* mutation (n=519) | *p* values |
|---|---|---|---|
| Gender | | | |
| Male, n (%) | 338 (65.8%) | 310 (59.7%) | 0.046 |
| Female, n (%) | 176 (34.2%) | 209 (40.3%) | |
| Age (years) | | | |
| <65, n (%) | 255 (49.6%) | 262 (50.5%) | 0.804 |
| ≥65, n (%) | 259 (50.4%) | 257 (49.5%) | |
| Vessel carcinoma embolus | | | |
| No, n (%) | 403 (78.4%) | 415 (80.0%) | 0.873 |
| Yes, n (%) | 96 (18.7%) | 96 (18.5%) | |
| Grade of tumor budding | | | |
| Low, n (%) | 110 (21.4%) | 59 (11.4%) | <0.001 |
| Intermediate, n (%) | 117 (22.8%) | 128 (24.7%) | |
| High, n (%) | 15 (2.9%) | 25 (4.8%) | |

(*Continued*)

**Table 2** (Continued).

| Clinicopathological Features | *KRAS* wild-type (n=514) | *KRAS* mutation (n=519) | *p* values |
|---|---|---|---|
| MMR | | | |
| pMMR, n (%) | 483 (94.0%) | 491 (94.6%) | 0.749 |
| dMMR, n (%) | 21 (4.1%) | 19 (3.7%) | |
| TNM stage | | | |
| I-II, n (%) | 200 (38.9%) | 162 (31.2%) | 0.011 |
| III-IV, n (%) | 314 (61.1%) | 357 (68.8%) | |
| Lymph node metastasis | | | |
| No, n (%) | 219 (42.6%) | 181 (34.9%) | 0.010 |
| Yes, n (%) | 285 (55.4%) | 330 (63.6%) | |
| Distant metastasis | | | |
| No, n (%) | 374 (72.8%) | 355 (68.4%) | 0.234 |
| Yes, n (%) | 94 (18.3%) | 109 (21.0%) | |
| Indexes of immune-nutritional status | | | |
| PIV, median (P25, P75) | 277.27 (154.70, 462.30) | 329.14 (181.37, 632.29) | <0.001 |
| SII, median (P25, P75) | 693.22 (462.86, 1054.41) | 819.93 (543.25, 1403.34) | <0.001 |
| SIRI, median (P25, P75) | 1.08 (0.68, 1.78) | 1.23 (0.77, 2.21) | 0.004 |

**Abbreviations**: CRC, colorectal cancer; MMR, mismatch repair; PIV, pan-immune-inflammation value; SII, systemic immune-inflammatory index; SIRI, systemic inflammatory response index; P25, 25th percentile; P75, 75th percentile.

## Clinicopathological Characteristics Were Compared in Different Levels of PIV, SII, and SIRI

Cutoff values of PIV, SII, and SIRI to distinguish *KRAS* mutation were determined by ROC analysis, the critical value of PIV was 431.72 (sensitivity 40.1%, specificity 73.7%, area under the ROC curve (AUC)=0.572), the SII cutoff value was 807.81 (sensitivity 50.9%, specificity 61.9%, AUC=0.584), and the SIRI cutoff value was 1.995 (sensitivity 28.7%, specificity 80.2%, AUC=0.552) (Figure 1).

The proportion of aged ≥65 years old, dMMR, distant metastasis, and *KRAS* mutation in patients with PIV, SII, and SIRI ≥ cutoff value was higher than those in patients with < cutoff value, respectively (all $p<0.05$). There was no

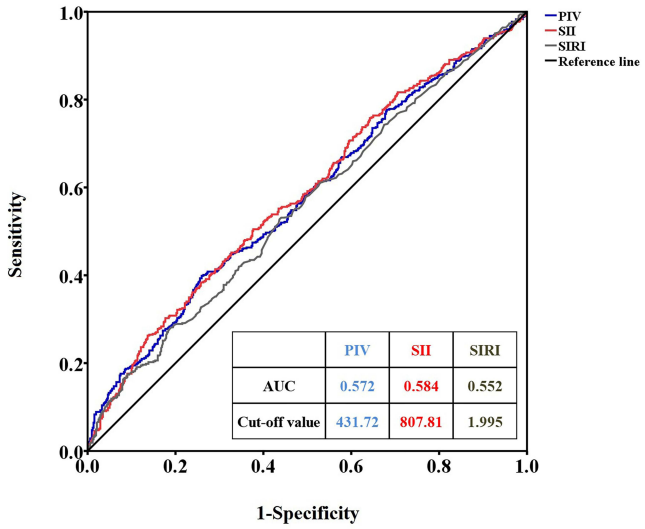


**Figure 1** The ROC curve of PIV, SII, and SIRI based on *KRAS* mutation.
**Abbreviations**: PIV, Pan-immune inflammation value; SII, systemic immune inflammation index; SIRI, system inflammation response index.

difference in the distributions of gender and grade of tumor budding, and the proportions of vessel carcinoma embolus, III-IV stage, lymph node metastasis in different levels of PIV, SII, and SIRI (Table 3).

## Logistic Regression Analysis of the Relationship Between KRAS Mutation, Tumor Budding and Clinicopathological Characteristics

In univariate analysis, gender (male vs female, odds ratio (OR): 0.772, 95% confidence interval (CI): 0.600–0.995, $p$=0.045), high and intermediate grade of tumor budding (high plus intermediate vs low, OR: 2.161, 95% CI: 1.459–3.201, $p$<0.001), lymph node metastasis (OR: 1.401, 95% CI: 1.088–1.804, $p$=0.009), high PIV level (≥431.72 vs <431.72, OR: 1.878, 95% CI: 1.443–2.444, $p$<0.001), high SII level (≥807.81 vs <807.81, OR: 1.680, 95% CI: 1.311–2.152, $p$<0.001), and high SIRI level (≥1.995 vs <1.995, OR: 1.627, 95% CI: 1.219–2.170, $p$=0.001) were associated with *KRAS* mutation. And *KRAS* mutation (yes vs no, OR: 2.161, 95% CI: 1.459–3.201, $p$<0.001), and lymph node metastasis (yes vs no, OR: 3.352, 95% CI: 1.849–6.078, $p$<0.001) were associated with high and intermediate grade of tumor budding (Table 4).

In multivariate regression logistic analysis, high and intermediate grade of tumor budding (high plus intermediate vs low, OR: 1.970, 95% CI: 1.287–3.016, $p$=0.002), and high SII level (≥807.81 vs <807.81, OR: 1.915, 95% CI: 1.120–3.272, $p$=0.018) were independently associated with *KRAS* mutation. And *KRAS* mutation (yes vs no, OR: 1.950, 95% CI: 1.275–2.983, $p$=0.002), and lymph node metastasis (yes vs no, OR: 3.347, 95% CI: 1.738–6.445, $p$<0.001) were independently associated with high and intermediate grade of tumor budding (Table 4).

# Discussion

CRC is the most common gastrointestinal malignancies.[1] In recent decades, the research and clinical application of molecular basis and targeted therapy in CRC have developed rapidly.[35–37] The efficacy of targeted therapy is influenced by some genes status downstream of the EGFR signaling pathway,[38] and the mutation status of these genes should be identified before targeted therapy.[37] *KRAS* is an important oncogenic gene in EGFR-mediated RAS/RAF/MAPK signaling pathway. There is a certain proportion of *KRAS* mutations in CRC, *KRAS* mutation frequency was about 35.0–50.0% in Chinese CRC patients.[39–43] In this study, *KRAS* gene mutations in 1033 tumor samples of CRC patients were analyzed, and the results showed that the total *KRAS* mutation rate was 50.2%. Our results are generally consistent with those reported.

In this study, grade of tumor budding (high and intermediate), and SII positive (≥807.81 vs <807.81) were independently associated with *KRAS* mutation. The tumor budding is a single tumor cell or a cluster of less than 5 tumor cells scattered in the mesenchyma at the front of tumor invasion, which is an independent prognostic factor for many solid tumors.[44,45] Several studies found that high-grade tumour budding was associated with *KRAS* mutation.[46–48] Prall et al suggested that in sporadic primary CRC, there was a significant increase in tumor budding in tumors with *KRAS* gene mutations.[49] The study performed by Anne Trinh et al revealed that tumor budding is a poor prognostic factor for CRC and is associated with *KRAS* mutation.[50] Tumor budding is one of the poor prognostic indicators in patients with CRC. The results of the association between grade of tumor budding and *KRAS* mutation suggest that patients with *KRAS* mutation need to be concerned about the risk of poor prognosis. It provides additional reference data for CRC patients' clinical diagnosis and treatment.

In terms of molecular mechanisms, epithelial-to-mesenchymal transition (EMT) is considered to be the key mechanism of malignant phenotype and invasive transformation of epithelial cells.[51] Tumor budding prior to CRC invasion is a poor prognostic indicator associated with EMT.[52] Tumor budding is considered to be the morphologic manifestation of cancer cells after EMT.[51] Maffeis et al suggested that RAS signaling pathway is involved in tumor spread caused by initiation of EMT in CRC.[53] However, some studies suggested that there may be other mechanisms of tumor budding besides EMT.[54,55] Therefore, the relationship between *KRAS* gene activation mutation and tumor budding still needs more clinical and basic studies to confirm.

The main components of tumor microenvironment including tumor cells, stromal cells and various inflammatory cells, which play an important role in tumor growth, invasion, metastasis and treatment.[56] There are currently relatively

**Table 3** Clinicopathological Characteristics Were Compared According to the Different Levels of PIV, SII, and SIRI in CRC Patients

| Clinicopathological Features | PIV | | *p* values | SII | | *p* values | SIRI | | *p* values |
|---|---|---|---|---|---|---|---|---|---|
| | <431.72 (n=690) | ≥431.72 (n=343) | | <807.81 (n=573) | ≥807.81 (n=460) | | <1.995 (n=782) | ≥1.995 (n=251) | |
| Gender | | | | | | | | | |
| Male, n (%) | 425(61.6%) | 223(65.0%) | 0.306 | 367(64.0%) | 281(61.1%) | 0.332 | 480(61.4%) | 168(66.9%) | 0.116 |
| Female, n (%) | 265(38.4%) | 120(35.0%) | | 206(36.0%) | 179(38.9%) | | 302(38.6%) | 83(33.1%) | |
| Age (years) | | | | | | | | | |
| <65, n (%) | 366(53.0%) | 151(44.0%) | 0.007 | 309(53.9%) | 208(45.2%) | 0.006 | 419(53.6%) | 98(39.0%) | <0.001 |
| ≥65, n (%) | 324(47.0%) | 192(56.0%) | | 264(46.1%) | 252(54.8%) | | 363(46.4%) | 153(61.0%) | |
| Vessel carcinoma embolus | | | | | | | | | |
| No, n (%) | 542(78.6%) | 276(80.5%) | 0.200 | 446(77.8%) | 372(80.9%) | 0.090 | 612(78.3%) | 206(82.1%) | 0.048 |
| Yes, n (%) | 137(19.9%) | 55(16.0%) | | 118(20.6%) | 74(16.1%) | | 157(20.1%) | 35(13.9%) | |
| Grade of tumor budding | | | | | | | | | |
| Low, n (%) | 114(16.5%) | 55(16.0%) | 0.302 | 95(16.6%) | 74(16.1%) | 0.404 | 123(15.7%) | 46(18.3%) | 0.095 |
| Intermediate, n (%) | 180(26.1%) | 65(19.0%) | | 154(26.9%) | 91(19.8%) | | 196(25.1%) | 49(19.5%) | |
| High, n (%) | 26(3.8%) | 14(4.1%) | | 24(4.2%) | 16(3.5%) | | 27(3.5%) | 13(5.2%) | |
| MMR | | | | | | | | | |
| pMMR, n (%) | 657(95.2%) | 317(92.4%) | 0.004 | 548(95.6%) | 426(92.6%) | 0.003 | 741(94.8%) | 233(92.8%) | 0.025 |
| dMMR, n (%) | 18(2.6%) | 22(6.4%) | | 13(2.3%) | 27(5.9%) | | 24(3.1%) | 16(6.4%) | |
| TNM stage | | | | | | | | | |
| I-II, n (%) | 252(36.5%) | 110(32.1%) | 0.166 | 213(37.2%) | 149(32.4%) | 0.116 | 274(35.0%) | 88(35.1%) | 1.000 |
| III-IV, n (%) | 438(63.5%) | 233(67.9%) | | 360(62.8%) | 311(67.6%) | | 508(65.0%) | 163(64.9%) | |
| Lymph node metastasis | | | | | | | | | |
| No, n (%) | 278(40.3%) | 122(35.6%) | 0.274 | 234(40.8%) | 166(36.1%) | 0.271 | 300(38.4%) | 100(39.8%) | 0.405 |
| Yes, n (%) | 407(59.0%) | 208(60.6%) | | 337(58.8%) | 278(60.4%) | | 476(60.9%) | 139(55.4%) | |
| Distant metastasis | | | | | | | | | |
| No, n (%) | 517(74.9%) | 212(61.8%) | <0.001 | 431(75.2%) | 298(64.8%) | <0.001 | 574(73.4%) | 155(61.8%) | <0.001 |
| Yes, n (%) | 103(14.9%) | 100(29.2%) | | 85(14.8%) | 118(25.7%) | | 130(16.6%) | 73(29.1%) | |
| *KRAS mutation* | | | | | | | | | |
| No, n (%) | 379(54.9%) | 135(39.4%) | <0.001 | 318(55.5%) | 196(42.6%) | <0.001 | 412(52.7%) | 102(40.6%) | 0.001 |
| Yes, n (%) | 311(45.1%) | 208(60.6%) | | 255(44.5%) | 264(57.4%) | | 370(47.3%) | 149(59.4%) | |

**Abbreviations**: CRC, colorectal cancer; MMR, mismatch repair; PIV, pan-immune-inflammation value; SII, systemic immune-inflammatory index; SIRI, systemic inflammatory response index.


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**Table 4** Logistic Regression Analysis of the Relationship Between KRAS Mutation, Tumor Budding and Clinicopathological Characteristics in CRC Patients

| Variables | *KRAS* Mutation | | | | Tumor Budding | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate | | Multivariate | | Univariate | | Multivariate | |
| | OR (95% CI) | *p* values | OR (95% CI) | *p* values | OR (95% CI) | *p* values | OR (95% CI) | *p* values |
| Gender (Male vs Female) | 0.772 (0.600–0.995) | 0.045 | 1.125 (0.744–1.702) | 0.577 | 0.946 (0.639–1.400) | 0.780 | 1.023 (0.661–1.583) | 0.920 |
| Age (≥65 vs <65, years old) | 0.966 (0.757–1.233) | 0.780 | 1.178 (0.782–1.774) | 0.434 | 0.748 (0.509–1.100) | 0.140 | 0.823 (0.535–1.265) | 0.374 |
| Vessel carcinoma embolus (Yes vs No) | 0.971 (0.709–1.330) | 0.855 | 0.918 (0.523–1.611) | 0.765 | 1.742 (0.978–3.103) | 0.060 | 1.313 (0.702–2.457) | 0.394 |
| Grade of tumor budding (High + Intermediate vs Low) | 2.161 (1.459–3.201) | <0.001 | 1.970 (1.287–3.016) | 0.002 | – | – | – | – |
| *KRAS* mutation (Yes vs No) | – | – | – | – | 2.161 (1.459–3.201) | <0.001 | 1.950 (1.275–2.983) | 0.002 |
| MMR (dMMR vs pMMR) | 0.890 (0.473–1.676) | 0.718 | 0.752 (0.259–2.186) | 0.601 | 1.089 (0.395–3.001) | 0.869 | 1.353 (0.428–4.279) | 0.607 |
| Lymph node metastasis (Yes vs No) | 1.401 (1.088–1.804) | 0.009 | 1.187 (0.701–2.011) | 0.523 | 3.352 (1.849–6.078) | <0.001 | 3.347 (1.738–6.445) | <0.001 |
| Distant metastasis (Yes vs No) | 1.222 (0.894–1.669) | 0.208 | 1.283 (0.507–3.244) | 0.598 | 1.279 (0.510–3.209) | 0.600 | 1.066 (0.382–2.972) | 0.903 |
| PIV (≥431.72 vs <431.72) | 1.878 (1.443–2.444) | <0.001 | 0.914 (0.417–2.005) | 0.822 | 0.795 (0.526–1.202) | 0.276 | 1.139 (0.504–2.575) | 0.755 |
| SII (≥807.81 vs <807.81) | 1.680 (1.311–2.152) | <0.001 | 1.915 (1.120–3.272) | 0.018 | 0.772 (0.524–1.136) | 0.189 | 0.796 (0.455–1.393) | 0.424 |
| SIRI (≥1.995 vs <1.995) | 1.627 (1.219–2.170) | 0.001 | 1.052 (0.486–2.277) | 0.897 | 0.743 (0.479–1.155) | 0.187 | 0.948 (0.425–2.112) | 0.896 |

**Abbreviations**: CRC, colorectal cancer; OR, odds ratio; CI, confidence interval; MMR, mismatch repair; PIV, pan-immune-inflammation value; SII, systemic immune-inflammatory index; SIRI, systemic inflammatory response index.

few studies on the role of inflammatory markers in CRC. Zhao et al found that PIV is associated with the tumor stage of CRC patients and is one of the possible indicators for preoperative adjuvant assessment of CRC.[26] Pre-treatment inflammatory indexes are potential biomarkers for predicting efficacy and survival in CRC patients.[57,58] Several studies had showed that PIV can be used as a valuable prognostic marker for CRC patients.[22,27,59–61] Research by Shuji Nakamoto et al showed that SII may be a valuable indicator for predicting recurrence in CRC patients.[62] Yuji Miyamoto et al found that *KRAS* genotype significantly influenced the prognostic impacts by SII index in patients with metastatic CRC.[63] In terms of molecular mechanisms, neutrophils become tumor-associated neutrophils (TAN) under the recruitment of related chemokines in the tumor microenvironment, and involved in the development of tumors.[64] Monocytes can kill tumor cells through direct action, and can also secrete various pro-inflammatory factors to mediate inflammation and exert anti-tumor immune properties.[65] Platelets can provide a series of pro-angiogenesis related factors to stimulate tumor growth and protect tumor cells from normal immune response.[66,67] The imbalance of the proportion of inflammatory cells in these local tumor microenvironments ultimately leads to the disharmony between the tumor-promoting and tumor-inhibiting effects, ultimately leading to the occurrence and progression of tumors. Systemic inflammation plays a critical role in the development, invasion, and metastasis of cancer. Due to the advantages of convenient sampling of blood samples and low threshold of detection technology, some hematologic inflammation indices have been used as biomarkers for the diagnosis and treatment of CRC, and can be used as auxiliary evaluation indices for some high-risk patients.

The study has some limitations. First, this was a single-center study. Multi-center studies are needed in the future. Second, this was a retrospective study, and some other factors (such as lymphocyte subsets and other inflammatory markers) not included in this study may be related to the clinicopathological characteristics of patients, the reliability of the results may be biased. In addition, the optimal diagnostic cutoff values for peripheral immune-inflammatory indices used in different studies varied widely. The optimal critical value of SII in this study is 807.81, which needs to be verified by further research.

## Conclusion

Non-low grade of tumor budding and high SII level were independently associated with *KRAS* mutation. Importantly, CRC patients with *KRAS* mutations were more likely to have intermediate-to-high grade tumor budding. Changes in tumor-related genes may be related to an imbalance in the proportion of inflammatory cells. It provides additional reference data for CRC patients' clinical diagnosis and treatment. Given the limitations of this study, the results of this study need to be confirmed by more researches. In addition, more and more in-depth studies are needed to explore the mechanism of immune-inflammatory response affecting *KRAS* mutation and the relationship between *KRAS* mutation and grade of tumor budding.

## Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Ethics Approval

All participants were informed on the study procedures and goals and the study obtained written informed consent from all the participants. The study was performed under the guidance of the Declaration of Helsinki and approved by the Ethics Committee of Medicine, Meizhou People's Hospital.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare that they have no competing interests.

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