Original Article

Unveiling the role of *KRAS* in Chinese colorectal cancer patients: a positive influence on tumor mutational burden

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Background: One of the main challenges associated with the development of therapeutic and diagnostic strategies for patients with colorectal cancer (CRC) is the establishment of minimally invasive and efficient biomarkers. Pertinent genes in CRC have been identified through their functions in systematic mutagenesis screens. *KRAS* is considered a dominant mutated oncogene that contributes to pathogenesis of CRC. This study aimed to explore the genomic alternations of *KRAS* in a CRC population.

Methods: Sequencing data of 94 Chinese patients with CRC were prospectively collected and analyzed using next-generation sequencing (NGS). The influence of *KRAS* and its associated subtype co-mutations on the expression level of the tumor mutational burden (TMB) was investigated. The objective of our study was to assess the potential prognostic significance of *KRAS* and other driving oncogenes in determining the clinical efficacy of immunotherapy.

Results: The gene mutation rates of *TP53*, *APC*, and *KRAS* were 81.91%, 71.28%, and 43.62%, respectively. Additionally, *KRAS* G12D displayed a relatively higher mutation rate than other *KRAS*-mutant subtypes. Increased TMB was observed in cases of *KRAS* and *BRAF* mutation combined with *APC* single mutation; furthermore, the expression of TMB in G12V was the highest, and G12D presented the lowest TMB in single *KRAS*-mutant subtypes or the combination with *APC* mutations.

Conclusions: The TMB driven by *KRAS* co-mutations may have the potential to be used as a key biomarker for prediction of treatment outcomes of immune checkpoint inhibitors (ICIs) in patients with CRC, especially with *APC* co-mutation.

Keywords: *KRAS*; mutant subtypes; tumor mutational burden (TMB); colorectal cancer (CRC); immune checkpoint inhibitors (ICIs)

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Introduction

Globally, colorectal cancer (CRC) is the third most increasingly common cancer, and it is associated with high mortality and morbidity rates in individuals aged >65 years (1). Extensive research has been performed on the subject, and the pathogenesis of CRC is known to be attributable to modifiable risk factors including diet, lifestyle, and genetic predisposition (2). In addition, it is noteworthy that China reports more than 600,000 new cases and 280,000 deaths cases of CRC in 2020, accounting for approximately 30% of the global deaths (3). The fact that colon epithelial cell growth achieves malignancy through the progressive accumulation of variations in genetic and epigenetic information is well known (4,5). Several molecular and genetic studies have attempted to confirm whether the involvement of genetic alternations affects CRC progression and development; mutations such as *TP53*, *KRAS*, and *APC* are the most widely reported (6-8).

Currently, the field of cancer research involving comprehensive analysis to elucidate mutations of key driver genes or genetic biomarkers related to immunotherapy has been progressively garnering increasing attention (9-11). As the most prevalent driver oncogene in CRC, *KRAS* mutation is noted in approximately 40–50% of CRC patients (12). *KRAS* mutations result in the activation of downstream signaling pathways, which facilitate uncontrolled cellular proliferation and confer resistance to specific targeted therapies, such as anti-epidermal growth factor receptor (EGFR) treatments (13). Additionally, the *KRAS* gene is known to have common mutant subtypes including G12C, G12V, G12A, and G13D; whereas the prognostic value in various tumor types remains incomplete and unclear (14,15). This may raise concerns regarding the actual prognostic value, which can be evaluated through the examination of additional mutant subtypes. Some studies have indicated a potential association between certain subtypes of *KRAS* mutations and increased levels of tumor mutational burden (TMB) in certain cancers, such as non-small cell lung cancer (NSCLC). There is evidence that *KRAS* G12C mutation has shown a high TMB and elevated expression of programmed cell death ligand 1 (PD-L1), which contributes to a higher likelihood of responding to immune checkpoint inhibitors (ICIs) (16). Further studies have proposed that *KRAS* G12D presents immunosuppressive features through driving immune checkpoint blockade and reducing response to ICIs in lung adenocarcinoma. This study reveals that the expression levels of TMB (median, G12V, 4.68 *vs.* G12D, 2.38) and PD-L1 are significantly elevated in the *KRAS* G12V subtype when compared to the *KRAS* G12D subtype (P<0.01). These findings imply a greater probability for *KRAS* G12V to derive benefits from immunotherapy in comparison to *KRAS* G12D (17-19). In fact, the presence of *KRAS* mutations may affect the immune response within the tumor microenvironment and potentially impact the efficacy of ICIs. *KRAS* mutation is associated with specific molecular subtypes of CRC, such as microsatellite stable (MSS) tumors, which generally have a lower TMB compared to microsatellite instability-high (MSI-H) tumors (20,21). It has been widely accepted that patients with CRC who have MSI-H or deficient DNA mismatch repair (dMMR) improves clinical outcomes when treated with ICIs (22).

Accordingly, we performed a comprehensive genomic landscape analysis on samples from 94 patients diagnosed with CRC using next-generation sequencing (NGS) technology. In this study, we conducted an analysis of the mutational signature associated with various driver genes and investigated the expression level of TMB in relation to co-occurring genomic alterations and single gene mutation.

Highlight box

Key findings

- Tumor mutational burden (TMB) driven by *KRAS* co-mutations may have the potential to be used as a key biomarker for treatment of colorectal cancer (CRC) populations.
- *KRAS*-mutant subtypes can have varying effects on TMB, which may have a more favorable response to immune checkpoint inhibitors (ICIs).
- Patients with *KRAS* G12V/*APC* might have a more favorable response to ICIs, whereas those with *KRAS* G12D/*APC* mutations may not derive significant benefits from ICIs.

What is known and what is new?

- Different *KRAS*-mutant subtypes can have varying effects on TMB and subsequent treatment outcomes of various cancers.
- The potential of TMB as a predictive biomarker for ICIs treatment, and the specific impact of different *KRAS* mutant subtypes on the efficacy of ICIs remains unclear.
- Our data indicated that the potential advantages of ICIs immunotherapy can be observed in CRC patients with specific *KRAS* subtypes and co-mutation genes.

What is the implication, and what should change now?

- The potential predictive value of *KRAS* mutation to achieve clinical benefits of immunotherapy in CRC requires elucidation.
- Further exploration of the molecular mechanism of immunosuppression in the population with *KRAS*, *BRAF*, *APC* co-mutation is warranted and will provide an efficient strategy toward achieving the clinical benefits of ICIs.

In addition, we analyzed the impact of different *KRAS*-mutant subtypes on TMB and explored the potential of *KRAS* mutations as biomarkers to indirectly predict the efficacy of ICIs by impacting TMB. We present this article in accordance with the STROBE reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-600/rc).

Methods

Patients and samples

A total of 103 patients with metastatic CRC (mCRC) were recruited between June 2019 and January 2020 at the Sun Yat-sen University Cancer Center. However, 9 of these patients were excluded from all testing procedures due to the fact that less than 20% of their tumors were present on formalin-fixed paraffin-embedded (FFPE) sections. Consequently, the data considered for further analysis were derived from the sequencing results of the remaining 94 patients. The main inclusion criteria were as follows: adults aged ≥18 years; complete clinicopathological information; and confirmed diagnosis of CRC using imaging techniques, including endoscopy, ultrasound, magnetic resonance imaging, and computed tomography, among others. Clinical response and tumor burden were evaluated according to the Response Evaluation Criteria in Solid Tumors, V.1.1 (23). All data were publicly available and patients with data deficiency were excluded. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by Sun Yat-sen University Cancer Center Ethics Committee (B2019-031-01, Guangdong, China) and informed consent was provided by all individual participants.

DNA extraction, library construction, and targeted sequencing

All tumor samples were reviewed by in-house pathologists and only samples with 30% or more of tumor cellularity were accepted for analysis. DNA from fresh tissue samples and FFPE tumor tissue samples were extracted using the EasyPure® Genomic DNA Kit (Beijing TransGen Biotech, Beijing, China) and QIAamp DNA FFPE Kit (QIAGEN, Valencia, CA, USA), respectively. DNA fragments and circulating tumor DNA (ctDNA) were used to construct a library by using the KAPA Library Preparation kit (Kapa Biosystems, Inc., Wilmington, MA, USA). Hybridization-based target enrichment was performed using a HaploX pan-cancer panels, a validated customized panel targeted 680 cancer-related genes (table available at https://cdn.amegroups.cn/static/public/TCR-24-600-1.docx). Library fragment size and DNA sequencing were performed by using the Agilent 4200 TapeStation system (Agilent Technologies, Santa Clara, CA, USA) and Illumina Novaseq 6000 system (Illumina, San Diego, CA, USA). Sequencing data were filtered by fastp version 0.18.0 (https://github.com/OpenGene/fastp) and aligned to the hg19 genome (GRch37) (24). Data quality control was performed using the following criteria: the ratio of remaining data filtered by fastq in raw data was ≥85%; the proportion of Q30 bases was ≥85%; the ratio of reads on the reference genome was ≥85%; target region coverage of ≥98%; and the mean tissue sequencing depth of ≥500×.

Damaging variation, calculation of TMB, and definition of driver genes

Damaging or neutral variants were defined and predicted according to the records and rules of the Catalogue Of Somatic Mutations In Cancer (COSMIC) database (25). TMB calculation was performed by dividing the total number of tissue non-synonymous single nucleotide polymorphism (SNP) and insertion/deletion (indel) variations (allele frequency, ≥5%) by the size of the coding region of the 680 panels (26-28).

The definition of MSS and MSI

Based on the recommendations of the National Cancer Institute (NCI), 5 microsatellite sites—including NR21, NR24, NR27, BAT25, and BAT26 were used to determine microsatellite instability (MSI) (29). The tumors were classified as follows: 1 of the 5 sites is altered, low microsatellite instability (MSI-L); 2 or more of the 5 sites are altered, MSI-H; none of the 5 microsatellite sites is altered, MSS (30).

Statistical and data analyses

Statistical analysis was performed and figures were plotted using the GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). Student's *t*-test was performed when comparing 2 groups, and analysis of variance (ANOVA) and post hoc tests were performed when comparing 3 or more groups. Mutation spectrum figures were designed using the R software (https://www.r-project.org/) (*, P<0.05; **, P<0.01; ***, P<0.001).

Table 1 Clinical characteristics of patients with CRC

Patients characteristics	Number (%)
Gender	
Female	43 (41.75)
Male	60 (58.25)
Age (years)	
Below 50	16 (15.53)
50 to 59	26 (25.24)
60 to 69	37 (35.92)
70 and above	24 (23.30)
TNM stage	
T3	29 (28.2)
T4	74 (71.8)
Family history	
Yes	46 (44.66)
No	42 (40.78)
Unknown	15 (14.56)
DNA types	
Tissue	96 (93.2)
Blood	7 (6.8)
MSI	
MSI-H	4 (4.26)
MSI-L	7 (7.45)
MSS	83 (88.30)
Localization of tumor	
Colon	55 (53.40)
Rectum	28 (27.18)
Unknown	20 (19.42)
TMB	
TMB-H	4 (4.26)
TMB-L	90 (95.74)

CRC, colorectal cancer; TNM, tumor-node-metastasis; MSI, microsatellite instability; MSI-H, high microsatellite instability; MSI-L, low microsatellite instability; MSS, microsatellite stable; TMB, tumor mutational burden; TMB-H, tumor mutational burden-high; TMB-L, tumor mutational burden-low.

Results

Patient clinical characteristics

A total of 94 patients, with ages ranging from 30 to 88 years and a median age of 59 years, were enrolled in this study. Detailed clinical characteristics pertaining to CRC, specifically colon cancer, rectal cancer, and mCRC, were recorded for each patient (*Table 1*). In the population of participants, the proportion of males was 58%, whereas 44.66% of patients presented a family history. Patients were diagnosed with CRC along with varying degrees of lung, hepatic, and renal metastases. Among these patients, 53.40% had colon cancer, whereas 27.18% had rectal cancer. Approximately 7.45% of patients exhibited tumors with MSI-L status, whereas 4.26% of tumors were determined to have MSI-H status.

Somatic mutational signature of driver genes

In this study, a total of 311 genes exhibiting frequent genomic alterations were analyzed through targeted NGS. Overall, pathogenic mutations were observed across all somatic chromosomes in which 3 important mutation types were identified: synonymous (silent mutation), non-synonymous (nonsense, missense, and frameshift), and splice-site mutations. Furthermore, 257 non-silent and 10 synonymous mutations were determined, respectively. The top mutated genes were *TP53* (81.91%, n=77), *APC* (71.28%, n=67), *KRAS* (43.62%, n=41), *SYNE1* (22.34%, n=21), *LRP1B* (18.09%, n=17), *PIK3CA* (17.02%, n=16), *SMAD4* (12.77%, n=12), *BRAF* (9.57%, n=9), and *ATM* (6.38%, n=6) in 94 samples (*Figure 1* & table available at https://cdn.amegroups.cn/static/public/TCR-24-600-2.docx). The *NRAS* (1.06%, n=1) mutation as a key prognostic hotspot biomarker was also detected. The majority of the variants were classified as frequent single nucleotide variants (SNVs) (98.50%, 263/267), whereas a small proportion of indel mutations were observed (1.50%, 4/267). Notably, a significant association was observed between MSI-H and TMB-high (TMB-H) in the majority of analyzed tumors.

In order to acquire a more systematic perspective of *KRAS* mutation, this study focused on analyzing the frequency signature of *KRAS*, *TP53*, *BRAF*, *PIK3CA*, and *APC*. The co-mutation of *KRAS*/*APC* (31.92%, n=30) exhibited the highest frequency of occurrence, followed by *KRAS*/*BRAF* (29.79%, n=28), *KRAS*/*TP53* (28.72%, n=27), and *KRAS*/*PIK3CA* (27.66%, n=26) (*Figure 2A-2D*). Mutated subtypes including several common allosteric sites of *KRAS* such as G12D, G12C, G12V, G12S, G13D, and rare mutations were also confirmed. We conducted a detailed analysis of the co-occurring *KRAS* subtypes in conjunction with *APC* mutations. The most frequently detected co-positive rate with *APC* was observed in patients carrying the G12D mutation (12.77%), followed by G12V/*APC* (5.32%) and G13D/*APC* (5.32%) (*Figure 2D*). In line with previous

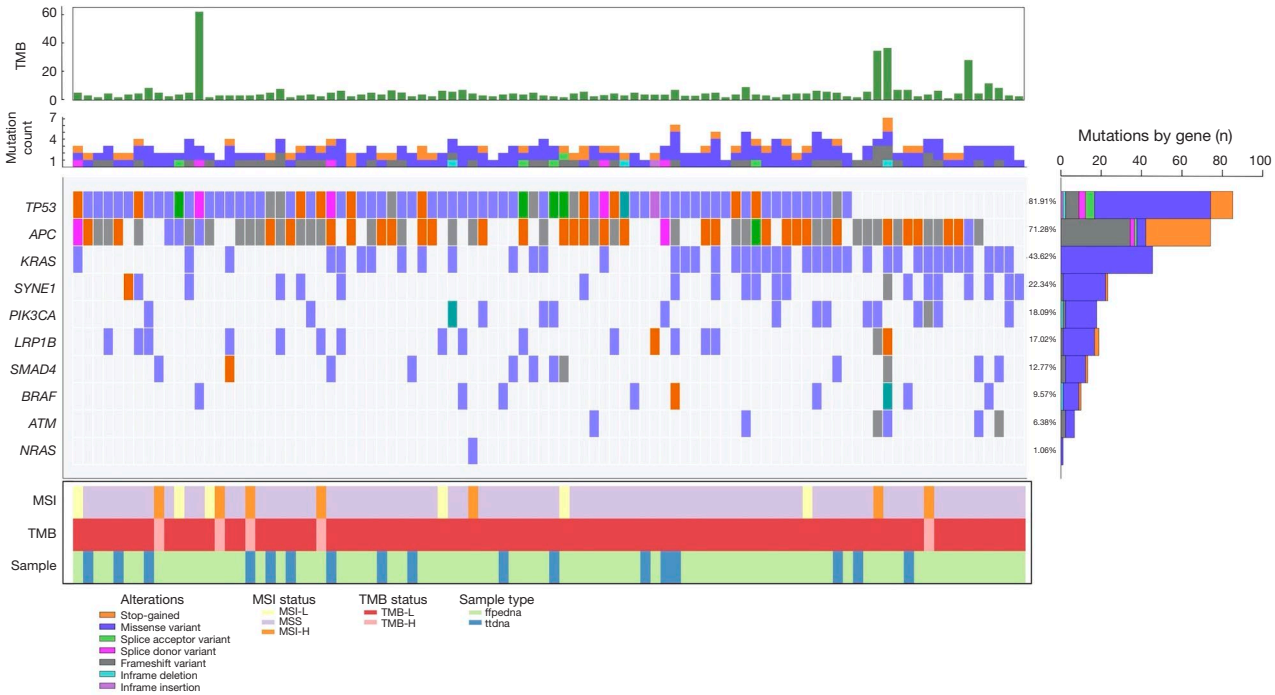

Figure 1 Genomic landscape and mutational hotspots in CRC patients. Mutational landscape of patients with CRC for all cases involved in this study. All obtained genes were aligned according to mutational frequency, in which SNV, indel, TMB, and MSI status were also presented. Mutational frequency was positively correlated with color. TMB, tumor mutational burden; MSI, microsatellite instability; MSI-L, low microsatellite instability; MSS, microsatellite stable; MSI-H, high microsatellite instability; TMB, tumor mutational burden; TMB-L, tumor mutational burden-low; TMB-H, tumor mutational burden-high; CRC, colorectal cancer; SNV, single nucleotide variant; indel, insertion/deletion.

studies, patients with G12D showed a higher frequency of mutations than the other *KRAS* mutant subtypes (31,32).

Impact of driver genes co-mutations on TMB

The correlation between genomic alternations and TMB expression was explored by analyzing individual mutated genes and mutation combinations. Co-occurring mutations of key genes were categorized into 5 subgroups, as follows: *KRAS*/*PIK3CA*, *KRAS*/*BRAF*, *KRAS*/*APC*, *KRAS*/*TP53*, and *BRAF*/*APC*. The highest expression level of TMB was observed in cases where the *KRAS*/*BRAF* mutation occurred (median, 9.54). This was followed by cases with *BRAF*/*APC* co-mutation (median, 7.24), and *KRAS* mutation (median, 6.25) and *BRAF* mutation (median, 5.26) (*Figure 3*). Notably, TMB exhibited a considerably higher level in patients harboring co-mutations of *KRAS*/*BRAF*, as opposed to patients with *KRAS* mutation alone (P<0.001). The *APC* mutation showed similar characteristics when combined with either *KRAS* or *BRAF* mutation (*KRAS*/*APC*; median, 5.26), demonstrating a significantly higher TMB compared to cases with a single *APC* mutation (median, 3.95) (*KRAS*/*APC*, P=0.003; *BRAF*/*APC*, P=0.003).

KRAS subtypes increased TMB driven by APC mutation

In order to examine the impact of *KRAS* subtypes on the TMB of *APC* mutation, an analysis was conducted on several significant *KRAS* subtypes, including G12D, G12C, and G12V, as well as rare subtypes. We found that when *KRAS* and *APC* mutations were present in combination, the samples exhibited a higher TMB compared to samples with only the *APC* mutation (*Figure 4A*). The median TMB was most significantly impacted by the combination of *APC* and *KRAS* mutant subtypes, with the highest effect observed in rare mutation group (median, 5.92), followed by G12V (median, 5.26), G12C (median, 4.61), and G12D (median, 4.61). Our results demonstrated that the TMB of patients

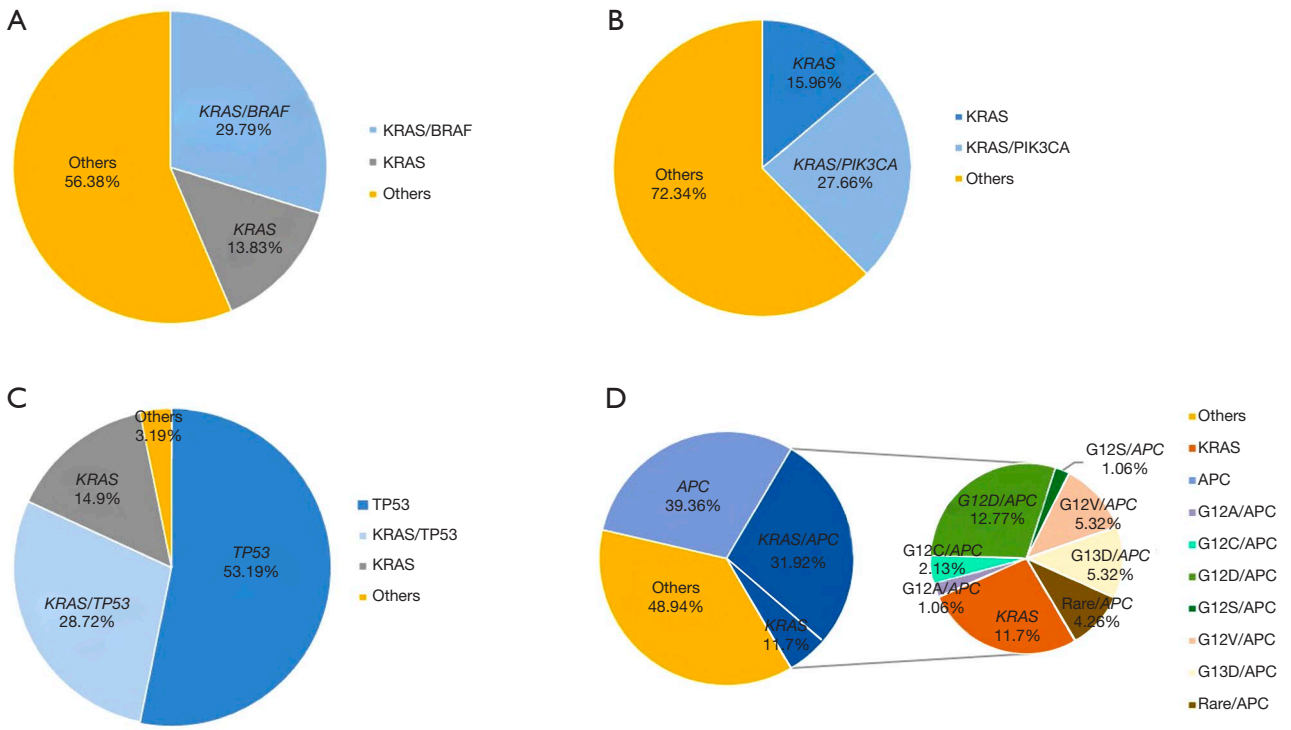

Figure 2 Co-occurring rate of *KRAS* and other key regulated genes in CRC. (A-D) The frequency of *KRAS*, *BRAF*, *APC*, and *PIK3CA* mutation was in 94 samples. (D) The mutational frequency of the combination between different *KRAS* mutant subtypes and *APC* was determined in this study. CRC, colorectal cancer.

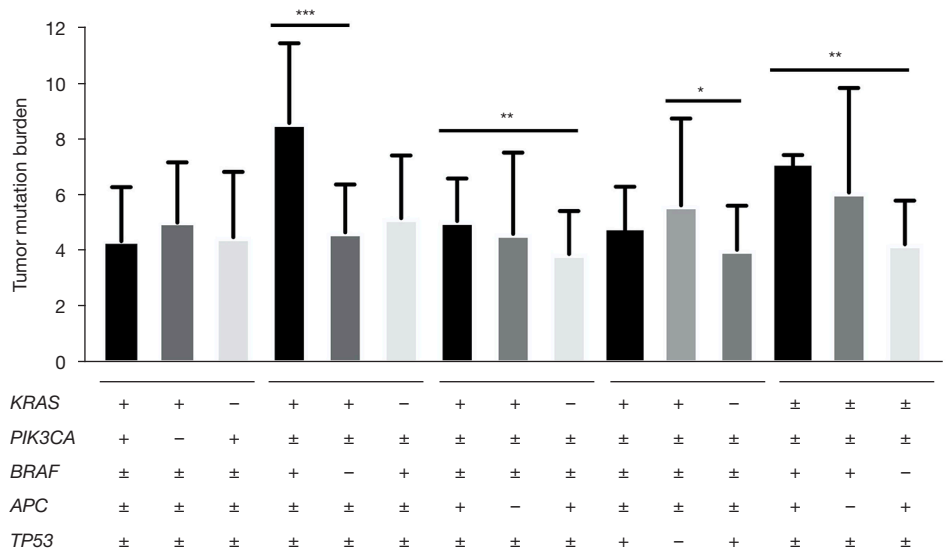

Figure 3 Evaluation of TMB driven by oncogenes. The impact of *KRAS*, *PIK3CA*, *BRAF*, *APC*, and *TP53* mutations and co-mutations (*KRAS*/*BRAF*, *KRAS*/*APC*, *KRAS*/*TP53*, and *BRAF*/*APC*) on TMB. Values are presented as mean ± standard error mean. *, P<0.05; **, P<0.01; ***, P<0.001. (+) represented single gene mutation presence; (−) represented mutation absence; (±) represented mutations possibly presence. TMB, tumor mutational burden.

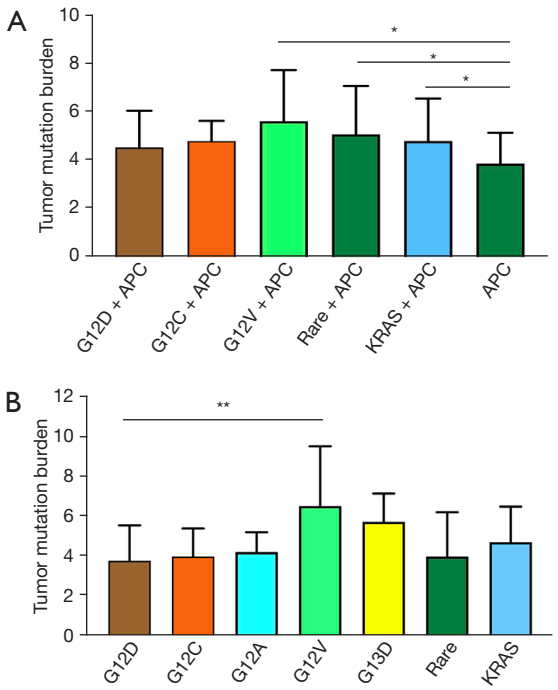

Figure 4 TMB affected by *KRAS* mutant subtypes. (A) TMB driven by *KRAS* and *APC* co-mutations. (B) TMB was affected by *KRAS*-specific mutant subtypes. *, P<0.05; **, P<0.01. TMB, tumor mutational burden.

with G12V/*APC* co-mutations was significantly higher compared to those with only *APC* mutation (P=0.01).

To determine the prerequisite for the impact of *KRAS* co-mutations on TMB, an analysis was conducted on the effects of common *KRAS* subtypes on TMB (*Figure 4B*). Further findings emerged from our study, indicating that *KRAS* G13D presented the highest TMB (median, 6.58), followed by G12V (median, 5.26), G12D (median, 4.61), G12C (median, 3.62), and G12A (median, 3.95), respectively. Notably, TMB levels were significantly higher in patients with *KRAS* G12V mutation than those with G12D mutation (P=0.009). The expression of TMB in G12V was the highest, whereas G12D presented the lowest TMB both when considered individually or in combination with *APC* mutation. In contrast to the previous findings regarding the higher TMB observed in lung adenocarcinoma associated with *KRAS* subtypes and *TP53* mutation (19), our study was unable to establish a similar relationship between TMB and the combination of *KRAS* subtypes and *TP53* in CRC (Figure S1). *TP53* may potentially reduce the TMB of *KRAS* G12V and G12C, whereas the combination between G12D and *TP53* mutation has been observed to increase the TMB.

Discussion

The current study found that the majority of patients were men (58.25%), and the occurrence of CRC was more common in individuals aged over 50 years (84.46%). In addition, family history of CRC was observed in 46 patients, accounting for 44.66% of the total samples. As mentioned in the literature review, CRC incidence and mortality in the early prenatal to adolescent periods of life progressively increase each year, influenced by age, gender, and physical inactivity (33,34).

In this study, a targeted NGS panel was employed to conduct comprehensive genomic profiling on CRC samples. We determined a large number of candidate mutant genes such as *APC*, *TP53*, *KRAS*, *SYNE1*, *LRP1B*, *PIK3CA*, *FBXW7*, and *ATM*, which exhibited a higher frequency of mutations. Among the mutations observed, the genes *KRAS*, *APC*, and *TP53* were selected for further analysis due to their high frequency of occurrence. It is estimated that approximately 80% of sporadic colorectal tumors and almost all cases of familial adenomatous polyposis (FAP) have *APC* gene mutations. The *APC* mutation plays a significant role in the development of adenomas in the intestinal mucosal epithelium, leading to CRC in individuals who carry this high penetrance mutation (5). *TP53* is a stress-inducible transcription factor, the mutation of which disrupts the normal function of p53, resulting in the dysregulation of downstream genes involved in cell cycle control, apoptosis, and DNA repair. Notably, the *TP53* mutation is observed in 50% of sporadic cases of CRC (35). Totally, mutations in these genes result in the disruption of normal cell cycle regulation and DNA repair mechanisms in CRC cells, leading to uncontrolled tumor growth and progression. Importantly, genomic biomarker testing such as RAS family, *BRAF* and mismatch repair (MMR) are recommended for testing by relevant guidelines and laboratories for the treatment of CRC population (36,37). *KRAS* mutation is a highly significant biomarker that has been reported in approximately 20% of NSCLC patients and 45% of CRC patients (38,39). *KRAS* reportedly modulates the immune response, in cases of CRC and pancreatic cancer in particular. Studies have shown that patients with *KRAS*, *NRAS*, or *BRAF* mutations may exhibit varied responses to treatments (40-42). In addition to the identification of molecular biomarkers, there are also

low-incidence markers such as MSI and TMB that can provide guidance for making therapeutic decisions in CRC patients. Previous studies have investigated an association between the mutational analysis of driver genes, including *KRAS*, *APC*, *TP53*, and *BRAF* as well as their respective mutant subtypes, with TMB levels. TMB is an influential independent predictive factor that refers to the cumulative number of mutations detected in the DNA of a tumor cell. Higher TMB is accompanied by the production of neoantigens, which are helpful for tumor recognition and enhancing the effectiveness of ICIs. The TMB level holds promise as a potential biomarker for predicting the response to ICIs in various cancer types (43-45).

Although the combination of *KRAS* inhibitors with ICIs or other immunotherapies presents potential therapeutic opportunities for NSCLC, there remains a significant need to address other *KRAS* mutant subtypes. Different *KRAS* mutant subtypes can have varying effects on TMB and subsequent treatment outcomes of various cancers. For example, the G12C mutation plays a crucial role in increasing TMB, thereby providing a pivotal advantage in utilizing immunotherapeutic strategies that target neoantigens. The *KRAS* G12D mutation has been reported to be associated with a lower TMB, resulting in limited response to ICIs in cases of lung adenocarcinoma (19). Consistent with previous studies, our study also found that *KRAS* G12D, G12C and G12V variants were the most prevalent in CRC population. Although several studies have emphasized the potential of TMB as a predictive biomarker for ICIs treatment, the specific impact of different *KRAS* mutant subtypes on the efficacy of ICIs remains unclear (46,47). Our study demonstrated that co-mutation of *KRAS* G12V and *APC* contributed to a greater increase in TMB compared to single *APC* mutation in CRC. It was strongly implied that patients with G12V/*APC* might have a more favorable response to ICIs, whereas those with G12D/*APC* mutations may not derive significant benefits from ICIs. Our data indicated that the potential advantages of ICIs immunotherapy were observed in CRC patients with specific *KRAS* subtypes and co-mutation of the *APC* gene. Other studies have provided corroboration for a decreased prevalence of TMB-H attributed to *KRAS* subtypes in mCRC as opposed to NSCLC. Nevertheless, there is currently no established standard for the threshold of TMB in various cancers (48,49). However, mutations in *KRAS* subtypes, including G12V and G12D, continue to serve as significant independent prognostic indicators in individuals with CRC. The *KRAS* G12V mutation specifically triggers the *KRAS* signaling pathway, thereby fostering tumorigenesis (50). The study reveals significant differences in median survival rates among patients with *KRAS* G12V and G12D mutations. Specifically, individuals with the G12V mutation exhibits a notably lower median survival of 31.7 months, whereas those with the G12D mutation display a comparatively higher median survival of 49.2 months (51). Mutations in *KRAS* G12V have been shown to impact the effectiveness of anti-EGFR therapy and contribute to poor survival rate (52). Furthermore, T-cell receptor (TCR)-recognized *KRAS*-G12V neoantigens have been identified as a promising therapeutic approach for tumor immunotherapy (53). Hence, these findings underscore the potential prognostic variability associated with the *KRAS* G12D/G12V alternation, thereby suggesting implications for the design and implementation of future clinical investigations.

Meanwhile, we revealed that the combination of *BRAF* mutations, along with a single *APC* mutation, significantly elevated the TMB. Indeed, the *BRAF* mutation and TMB are currently generally accepted as independent predictive factors for recurrence-free survival (54). The majority of *BRAF* mutation in this study was the V600E subtype (36.36%, n=4), and considering the limited screening of other mutant subtypes, analyzing the impact of a single *BRAF* subtype on TMB may present some challenges. Importantly, some co-mutations of *KRAS* and *TP53* exhibited a noticeable reduction in TMB compared to case with only *KRAS* mutation, such as G12V and G12C. A possible explanation for this difference is that *TP53* mutations can modulate the impact of *KRAS* mutations on tumor progression and response to therapy. It is hypothesized that *TP53* mutations, in combination with specific *KRAS* subtypes, further impair DNA repair mechanisms and increase genomic stability, leading to a decrease in the accumulation of mutations and subsequently reducing TMB levels (55). The combination between G12D and *TP53* presented an opposite trend compared to the single G12D mutation. Furthermore, the presence of *KRAS* mutations across various subtypes did not yield a significant impact on TMB expression of *TP53*. It might be influenced by various independent factors, including the type of cancer and the individual condition. Therefore, further validation of the correlation between *KRAS* and *TP53* is essential in large cohorts of patients diagnosed with CRC.

It is important to note that the relationship between *KRAS* mutant subtypes and ICIs is still an area of active research, and more studies are necessary to fully understand

the complexities of this interaction. The response to ICIs is influenced by several additional factors, including the tumor microenvironment, overall tumor burden, and the presence of other genetic alterations. Further exploration of the molecular mechanism of immunosuppression in the population with *KRAS*, *BRAF*, and *APC* co-mutation is warranted and will provide an efficient strategy toward achieving the clinical benefits of ICIs.

Conclusions

Our study provides evidence to support the potential use of *KRAS* as promising therapeutic targets and a key predictive biomarker for immunotherapy ICIs in patients with CRC. Since TMB is upregulated in cases with *KRAS* G12V/*APC* co-mutation, there could be an enhanced response to ICIs. Our data strongly suggest the potential benefits of immunotherapy with ICIs in CRC patients with specific *KRAS* subtypes and co-mutation groups. However, further validation in larger clinical research datasets is necessary to confirm these findings.

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Footnote

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Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-600/dss

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by Sun Yat-sen University Cancer Center Ethics Committee (B2019-031-01, Guangdong, China) and informed consent was provided by all individual participants.

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