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Combined KRAS and TP53 mutation in patients with colorectal cancer enhance chemoresistance to promote postoperative recurrence and metastasis

YiMeng Tang[1] and Yao Fan[1*]

Abstract

The response of patients with colorectal cancer to chemotherapy is tightly correlated with their genomic variation. Among these, APC, TP53, KRAS, PIK3CA are the most frequently mutated genes in advanced colorectal cancer patients. However, the precise correlation between these mutations and the therapeutic effects of chemotherapy remains elusive. Here, we conducted genome sequencing to identify commonly mutated genes in colorectal cancer patients and comprehensively assessed their sensitivity to chemotherapy drugs by monitoring computer tomography (CT) scans and carcinoembryonic antigen (CEA) levels. Surprisingly, we discovered that the objective response rate to the standard first-line chemotherapy among patients harboring combined KRAS and TP53 mutations is dismal, and these patients are predisposed to recurrence and metastasis. Furthermore, advanced-stage patients with concurrent KRAS and TP53 mutations are susceptible to developing cancer-associated cachexia due to chemotherapy resistance or forced cessation of treatment. Our findings underscore the urgent need for the development of innovative and novel chemotherapeutic strategies to effectively manage colorectal cancer patients harboring combined KRAS and TP53 mutations.

Keywords Colorectal cancer, KRAS, TP53, Genetic mutation, Chemoresistance

Introduction

Colorectal cancer (CRC) has emerged as the third most prevalent form of cancer and the fourth leading cause of cancer-related fatalities, claiming approximately 700,000 lives globally each year, trailing only lung, liver, and gastric cancers [1]. The treatment of colorectal cancer is primarily determined by the stage (severity) of the disease, encompassing surgical intervention, neoadjuvant radiotherapy (primarily for rectal cancer patients), and adjuvant chemotherapy (primarily for patients with Stage III/IV colon cancer and those with high-risk Stage II disease). The five-year relative survival rate ranges from over 90% for those with Stage I disease to slightly above 10% for those with Stage IV disease [2]. Despite the effectiveness of anticancer drugs, nearly all metastatic patients ultimately succumb to chemoresistance [3]. Tumor evolution and the emergence of drug resistance are the primary culprits behind treatment failures and mortality in CRC patients [4]. The postoperative recurrence and chemoresistance of CRC pose significant challenges in improving patient survival rates. However, the lack of reliable clinical molecular markers to predict these

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recurrences remains a significant hurdle in the management of CRC patients.

The genetic predisposition of CRC in diverse populations has been the focus of numerous investigations. Among the most frequently observed genetic variations associated with this malignancy are mutations in the APC, TP53, RAS, PIK3CA, HER2, SMAD4, FBXW7 genes [5–9]. However, it is noteworthy that these genes exhibit comparable mutation frequencies in different populations, indicating a shared genetic basis for CRC across different ethnic and geographic groups. Genetic alterations are intricately linked to drug chemoresistance in cancer treatment [10]. Nevertheless, the scant literature available over the past decade has failed to provide a comprehensive overview of chemoresistance and its underlying mechanisms in CRC. the KRAS glycine-to-cysteine mutation at codon 12 (KRAS G12C) in colorectal cancer is associated with poorer overall survival in the first and second line when treated with chemotherapy [11, 12]. Although KRAS, BRAF, PIK3CA, APC, and TP53 mutations are frequently observed in colorectal cancer tissues [13–15], our comprehension of their intricate relationship with chemotherapy remains incomplete.

The implementation of CRC screening has significantly contributed to the decreasing trend in CRC incidence and mortality over the past two decades [16]. The emergence of evidence that cancer is a genetic disorder has revolutionized diagnosis and treatment strategies. Molecular profiling of colorectal tumors has enhanced our ability to pinpoint patients who are likely to benefit from targeted therapy [17]. This article delves into the intricate relationship between chemoresistance and prevalent gene mutations in colorectal cancer, specifically highlighting APC, TP53, KRAS, and PIK3CA. Our meticulous research revealed that the combined mutation of TP53 and KRAS significantly diminishes the responsiveness of colorectal cancer cells to the standard first-line chemotherapy. Notably, KRAS and TP53 mutations are indicative of a bleak prognosis in colorectal cancer (CRC) [4]. In addition, we embarked on a comprehensive literature review and rigorous analysis to unwrap the underlying mechanisms of chemoresistance in colorectal cancer patients harboring TP53 and KRAS mutations. This endeavor aims to pave the way for enhancing the prognosis and survival rates of these patients, offering them a ray of hope in their fight against this debilitating disease.

Material and method

Participants

The study received approval from the Ethics Committee of the affiliated traditional Chinese medicine hospital of southwest medical university. Written informed consent was obtain from inpatients between January 2018 and December 2022. The inclusion criteria for the study population were: (1) colorectal cancer was confirmed by hematoxylin and eosin (HE) staining and histological analysis; (2) inclusion of stage III patients according to American Joint Committee on Cancer (AJCC) guidelines; (3) patients were administrated the standard therapeutic regimens as first line according to NCCN guidelines for colorectal cancer. (4) genome sequencing were performed to identify gene status. (5) medical records and imaging information were complete preserved. The exclusions from the study were as follows: (1) patients with other tumors disease other than colorectal cancer; (2) other cases inconsistent with the above inclusion criteria.

Genotyping for APC, TP53, KRAS, PIK3CA genes

Neoplastic tissue of patients were harvested by endoscopic biopsy, and were frozen in liquid nitrogen and then crushed using a mortar and pestle. DNA was extracted using the DNAzol™ reagent (invitrogen, America) according to the manufacturers' instructions. NanoDrop 2000 (Thermo Scientifc, America) was used to determine DNA concentration. When making measurements, the DNA samples should be diluted to 20 ng/μL, and the OD260/OD280 should be 1.8–2.0. Genetic testing using a high-throughput sequencing platform (Illumina HiSeq 4000, America). DNA library were constructed by using TruSeq DNA PCR-Free library Prep Kit (illumina, America) according to the manufacturers' instructions.

Chemotherapy regimen and valuation of tumor

All patients were diagnosed by endoscopic biopsy, and then underwent chest and full abdominal CT examination, and serum CEA determination to valuate the basic information of tumor. Following patients were administrated the based first-line chemotherapy according to mFOLFOX6 regime of NCCN guideline. After three months, CT examination and CEA test were performed to assess therapy-related differences.

Data collection and statistical analysis

Collection of clinical records including gender, age, tumor site, histologic type, maximum tumor diameter. SPSS statistical software version 20.0 (IBM Corporation, America) was used for data analysis. Descriptive statistics were used to summarize the clinicopathological characteristics of the patients. Categorical variables were compared using the $\chi 2$ test and Fisher's exact test. The test of significance was two-sided, with a P value < 0.05, and the difference was considered statistically significant.

Result

Characteristics of subjects

One hundred and sixty-three patients were enrolled in this study, including 54 (33.1%) female patients and 109

(66.9%) male patients. The age range of the patients was from 18 to 82 years old. Among them, 79 (48.5%) patients were younger than 60 years old, while 84 (51.5%) patients were 60 years old or older. In terms of tumor location, colon tumors were present in 55 (33.7%) patients, rectum tumors in 100 (61.3%) patients, and cecum tumors in 2 (1.2%) patients. Regarding tumor size, 65 patients (39.9%) had a tumor maximum diameter less than 5 cm, and 79 patients (48.5%) had a tumor maximum diameter of 5 cm or greater. In terms of tumor differentiation, well-differentiated tumors were detected in 8 (4.9%) cases, moderately differentiated tumors in 108 (66.3%) cases, and poorly differentiated tumors in 27 (16.7%) cases. All patients exhibited lymphatic vascular space invasion, but no distant metastases were observed. All cases were classified as stage III (Table 1).

Frequency and composition ratio of APC, TP53, KRAS, PIK3CA mutations

The genotyping analysis for KRAS (codons 12, 13, 59, 61, 117, and 146), APC (codons 8, 9, 14, and 16), TP53 (codons 5, 6, 7, and 8), and PIK3CA (codons 2, 5, 8, and 10) mutations was conducted in a cohort of patients (Fig. 1). The results indicate that mutations in at least one of these genes were detected in 58.9% of the patients, while 41.1% had no mutations tested. Specifically, KRAS, TP53, APC, and PIK3CA mutations were tested in 41.1%, 26.4%, 8%, and 7.4% of the samples, respectively. Simultaneous testing for mutations in KRAS and TP53, KRAS and APC, and KRAS and PIK3CA occurred in 11%, 3%, and 3% of the samples, respectively. Regarding the gender distribution of mutations, KRAS mutations were more prevalent in males (64.2%) compared to females (35.8%). Similarly, TP53 mutations were also more common in males (63%) than in females (37%).

In terms of the ranking of mutations based on composition, KRAS exon 2 mutations emerged as the most frequent, accounting for 35.6% of all mutations. Other notable mutations included KRAS exon 3 (4.9%), KRAS exon 34 (0.6%), various exons of TP53 ranging from 1.2 to 6.6%, APC exon 16 (8%), and various exons of PIK3CA ranging from 1.2 to 3.1%. It is worth noting that PIK3CA exon 22 mutations were detected in only 0.6% of the samples (Table 2).

Table 1 Baseline characteristics of CRC patients

Gender	
Male, *n* (%)	109(66.9%)
Female, *n* (%)	54(33.1%)
Age	
<60, *n* (%)	79(48.5%)
≥60, *n* (%)	84(51.5%)
Tumor site	
Colon	55(33.7%)
Rectum	98(60.1%)
Cecum	2(1.2%)
Rectal and sigmoid colon junction	8(5%)
Maximum diameter of tumor	
<5cm, *n* (%)	65(39.9%)
≥5cm, *n* (%)	79(48.5%)
Unknown, *n* (%)	19(11.6%)
Tumor differentiation	
Well, *n* (%)	8(4.9%)
Moderate, *n* (%)	108(66.2%)
Poor, *n* (%)	27(16.6%)
Unknown, *n* (%)	20(12.3%)
Lymphovascular invasion	
Present, *n* (%)	163(100%)
Disease stage at diagnosis	
III, *n* (%)	163(100.0%)
Distant metastasis	
No	0

KRAS Combined with TP53 mutation in colorectal cancer promotes chemoresistance

To explore the correlation between chemoresistance in colorectal cancer (CRC) and the genomic profiles of APC, TP53, KRAS, and PIK3CA, we conducted a longitudinal study focusing on patients undergoing chemotherapy. Over a three-year period, we regularly monitored the patients' progress through computed tomography (CT) scans and carcinoembryonic antigen (CEA) measurements, taken at three-month intervals (Table 3). Concurrently, we conducted a comprehensive analysis of gene mutations and their impact on the effectiveness of chemotherapy. Remarkably, our findings revealed that KRAS and TP53 are the most frequently mutated genes across CRC cases. Furthermore, the presence of a double mutation in both KRAS and TP53 was significantly associated with a higher likelihood of liver metastasis and/or an upregulation of CEA levels. These observations suggest that the mutational status of these genes may play a crucial role in determining the response to chemotherapy and the overall prognosis of CRC patients (Fig. 2).

Discussion

Postoperative adjuvant chemotherapy remains the standard treatment strategy for locally advanced colorectal cancer, yet the response to therapy varies significantly among patients [18]. Despite receiving adjuvant chemotherapy after surgery, some individuals still face challenges such as distant metastasis, enlarged lymph nodes, or elevated CEA levels. Consequently, the emergence of drug resistance poses a significant obstacle to improving the prognosis of CRC [19]. Currently, we lack a precise screening method to identify the subset of colorectal cancer patients who are likely to benefit from postoperative adjuvant chemotherapy. This study reveals that the

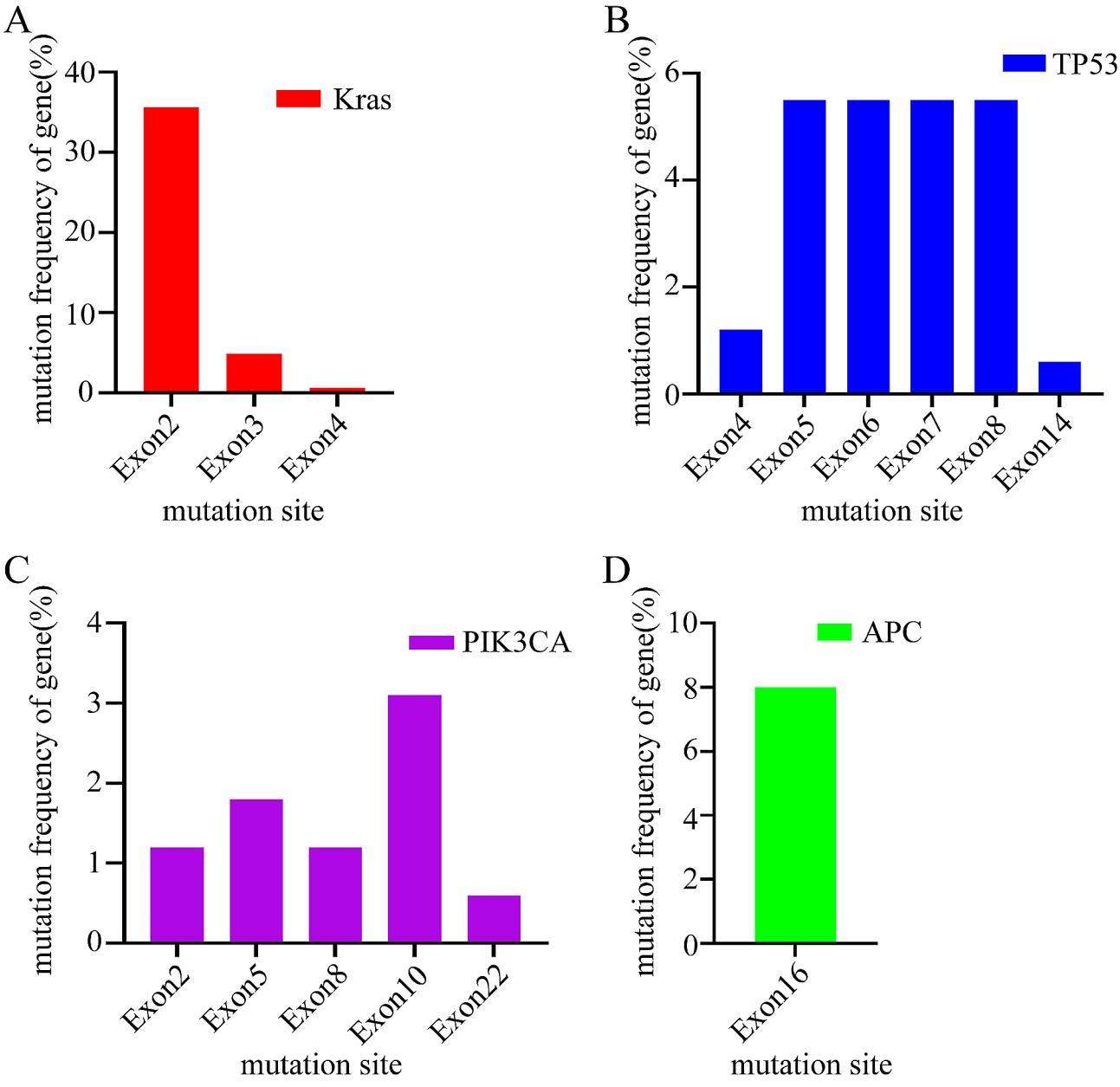

Fig. 1 The gene mutation sites and frequency for KRAS, APC, TP53, PIK3CA were analyzed in patient with colorectal cancer. **(A)** Exon 2, 3 and 4 were tested for KRAS gene. **(B)** Exon 4, 5, 6, 7, 8 and 14 were tested for TP53 gene. **(C)** Exon 2, 5, 8,10 and 22 were tested for PIK3CA gene. **(D)** Exon 16 was tested for APC gene

combined presence of KRAS and TP53 mutations diminishes the sensitivity to first-line chemotherapy drugs in colorectal cancer. Furthermore, patients in the late stages of the disease who harbor both KRAS and TP53 mutations are predisposed to developing cancer-associated cachexia, often due to chemotherapy resistance or the forced cessation of treatment.

The RAS pathway stands out as a crucial target for the metastatic treatment of colorectal cancer, as numerous specific mutations within the RAS family have been linked to the progression of this malignancy [20]. The mutational status of KRAS is recognized as a pivotal biomarker indicating resistance to EGFR-targeted therapy [21]. Cancer cells harboring oncogenic alleles of RAS typically rely on the persistent expression of the mutant allele for their survival [22]. Notably, approximately 30% of human malignancies are resistant to effective treatment due to the presence of mutant RAS oncogenes [23]. KRAS mutations are frequently observed in around 40% of CRC patients, emphasizing its central role in colorectal cancer [24]. Cancer cells frequently become dependent on oncogenic signals, underscoring the need for RAS/

Table 2 Frequency and composition ratio of KRAS/TP53/APC/PIK3CA mutation in CRC patients

Gene Name	Exon	Number and Frenquency of mutation, *n*(%)	Composition Ratio of Mutations
KRAS	Exon2	58 (35.6%)	86.6%
	Exon3	8(4.9%)	11.9%
	Exon4	1(0.6%)	1.5%
TP53	Exon4	2(1.2%)	4.7%
	Exon5	9(5.5%)	20.9%
	Exon6	9(5.5%)	20.9%
	Exon7	9(5.5%)	20.9%
	Exon8	9(5.5%)	20.9%
	Exon14	1(0.6%)	2.3%
APC	Exon16	13(8%)	100%
PIK3CA	Exon2	2(1.2%)	16.7%
	Exon5	3(1.8%)	25%
	Exon8	1(1.2%)	8.3%
	Exon10	5(3.1%)	41.7%
	Exon22	1(0.6%)	8.3%
Total		135(82.8%)	

Table 3 Incidence of disease progression are associated with mutations in various types of genes

Gene Name	Number of mutation	Number of progression	Incidence (*p* value)
No mutation	28	1	3.57%
KRAS	67	11	16.42% (0.10)
TP53	39	7	17.95% (0.13)
APC	13	3	23.08% (0.08)
PIK3CA	12	2	16.67% (0.21)
KRAS and TP53	18	12	66.67% (< 0.001)

BRAF status testing as a prognostic stratification criterion [23]. A significant proportion of patients harboring these mutations fail to respond to major drug combination regimens, including those involving monoclonal antibodies [25]. KRAS functions downstream of EGFR in the RAS/MAPK signaling pathway, and its mutation, independent of EGFR status, activates the pathway, leading to resistance [26]. Additionally, epigenetic regulatory genes are frequently deregulated in cancer, increasing the likelihood of their contribution to resistance mechanisms [27].

The tumor suppressor p53 functions as a transcription factor that gets activated in response to DNA damage, triggering the expression of genes involved in halting cancer cell growth or inducing apoptosis [28]. The inactivation of p53 is a common trait observed in human cancer cells, often resulting from mutations or deletions of the p53 gene [29]. CRC, a heterogeneous disease with intricate genetic and biochemical components, frequently exhibits dysregulation in crucial intracellular signaling pathways, including Wnt/β-cyclin signaling, Ras signaling, and p53 signaling [30]. Recent evidence suggests that mutant p53 plays a pivotal role in oncogenic properties, such as sustained cellular proliferation, resistance to cell death, invasion and metastasis, as well as tumor-induced inflammation [31]. The activation of wild-type p53 in response to genotoxic stress occurs through diverse mechanisms, including alterations in protein conformation, posttranslational modifications, and nuclear localization, ultimately leading to DNA binding to specific promoters [28].

RAS and TP53 mutations frequently occur in various types of human cancers, including colorectal cancer (CRC), pancreatic ductal adenocarcinoma (PDAC), and non-small cell lung cancer (NSCLC) [32–34]. Studies have shown that patients with co-alteration in RAS and TP53 have poorer overall survival and progression-free survival rates following colorectal liver metastasis resection compared to patients with only one alteration or no alteration in these genes [35]. Additionally, combined KRAS/TP53 mutations in locally advanced rectal cancer are independently associated with a decreased response to neoadjuvant therapy and higher rates of lymph node metastasis [36]. KRAS/TP53 double mutations may weaken antitumor immunity by reducing the energy metabolism of T- and myeloid cells and remodeling cellular interactions to promote immunosuppression [37]. Tumors harboring KRAS codon 13 mutations tend to have a higher incidence of concurrent TP53 mutations compared to tumors with other KRAS mutations, and the specific KRAS codon mutation may differentially affect resistance to neoadjuvant chemoradiation therapy (nCRT) in rectal cancer. This variable resistance could be linked to the varying frequencies of TP53 mutations among KRAS-mutant tumors [38]. Furthermore, tumors that simultaneously carry mutations in TP53 and either KRAS or NRAS have a poorer prognosis than those with a wild-type TP53/KRAS/NRAS genotype [39]. Concomitant RAS and TP53 mutations are also associated with decreased survival rates following resection of colorectal liver metastases [40].

Addressing chemoresistance and prolonging the efficacy of EGFR-targeted therapies.

are greatly important to improve the disease-free survival (DFS) and overall survival (OS) for CRC [41, 42]. Tumors harboring RAS/TP53 mutations often exhibit a heightened sensitivity to adavosertib due to their G1 checkpoint dysfunction, replication stress, and increased reliance on intra-S phase and G2/M checkpoints for cell cycle progression [43]. Combining irinotecan with CHEK1 inhibitors has demonstrated synergistic effects in KRAS-TP53 double-mutant colon cancer cells, triggering apoptosis and suppressing tumor xenograft growth [44]. While p53 mutations are associated with resistance to traditional chemotherapy, they also confer an abundance of neoantigens, potentially making these tumors more responsive to immunotherapy [45]. The coexistence of

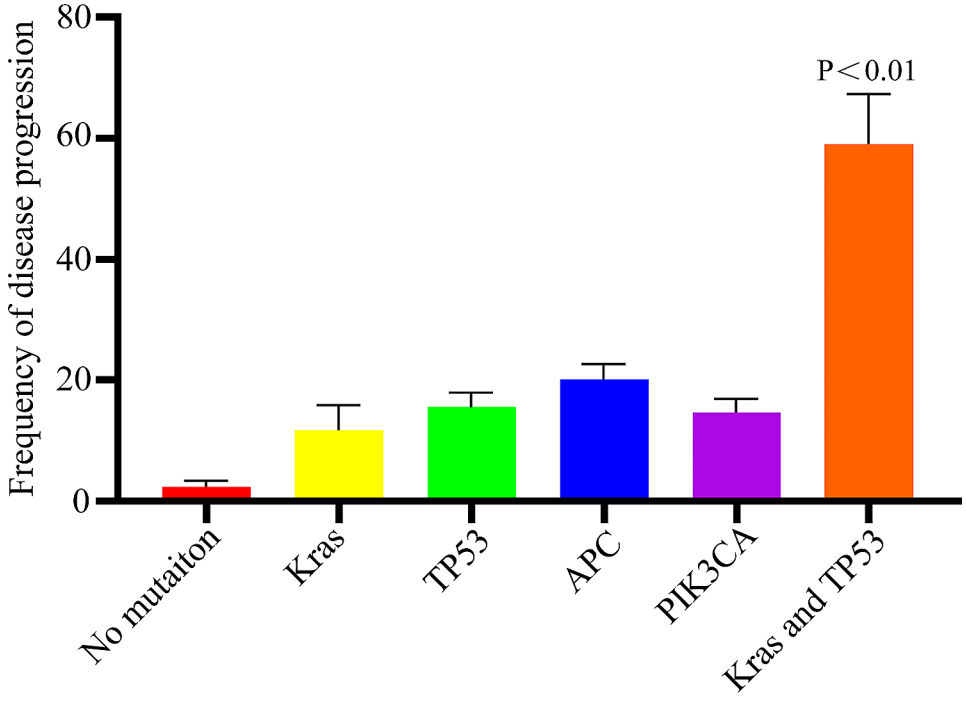

Fig. 2 Frequency of disease progression were analyzed by monitoring CT and CEA levels. In comparison to the non-mutation group, the progression of the disease did not exhibit any significant disparities in the case of KRAS, APC, TP53, or PIK3CA mutations alone, but the patients with combined KRAS and TP53 mutations are predisposed to recurrence and metastasis ($P<0.01$)

KRAS and TP53 mutations creates a "hot" tumor microenvironment, which is conducive to a stronger response to immunotherapy [32]. Additionally, novel agents like pradimicin-IRD, a polycyclic antibiotic, have exhibited antitumor effects in colon cancer cells after extended exposure, offering another potential therapeutic option [46]. Collectively, these promising findings suggest that colorectal cancer patients with KRAS and TP53 co-mutations who have failed conventional treatments may benefit from combination therapies or immunotherapy approaches.

Recently, accumulating evidence suggests that the concurrent presence of KRAS and TP53 mutations significantly contributes to oncogenic processes, including metastasis and drug resistance. As such, it is imperative to take into accounts this molecular signature when administering traditional chemotherapy regimens to patients with colorectal cancer (CRC). Notably, numerous promising therapeutic approaches emerging from fundamental and preclinical research, including drug combinations, immunotherapies, and innovative drug strategies, present viable options. Our study has indeed revealed a notable trend where patients harboring combined mutations in TP53 and KRAS genes exhibit a higher likelihood of relapse following surgical intervention. Nevertheless, the limited sample size of our investigation necessitates further validation through a multi-center study to solidify this observation. Additionally, the precise mechanism underlying how the combined mutation of TP53 and KRAS promotes drug resistance remains elusive, and further fundamental investigations are necessary to elucidate this intricate roles and regulatory mechanisms, ultimately aiming to enhance the efficacy of therapeutic interventions. In summary, our investigation indicates the existence of possible genetic predispositions that may contribute to the recurrence of colorectal cancer following surgical intervention.

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Author contributions
F.Y and Y.M.T collected data and wrote this manuscript.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Consent for publication
Authors agree that the journal of BMC cancer may publish the article in print and electronic formats, and that it may be made available to the public online.

Competing interests
The authors declare no competing interests.

Ethical approval and consent to participate
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References

1. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodriguez Yoldi MJ. Colorectal carcinoma: a General Overview and Future perspectives in Colorectal Cancer. Int J Mol Sci 2017, 18(1).
2. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet (London England). 2014;383(9927):1490–502.
3. Hsu HH, Chen MC, Baskaran R, Lin YM, Day CH, Lin YJ, Tu CC, Vijaya Padma V, Kuo WW, Huang CY. Oxaliplatin resistance in colorectal cancer cells is mediated via activation of ABCG2 to alleviate ER stress induced apoptosis. J Cell Physiol. 2018;233(7):5458–67.
4. Picco G, Cattaneo CM, van Vliet EJ, Crisafulli G, Rospo G, Consonni S, Vieira SF, Rodríguez IS, Cancelliere C, Banerjee R, et al. Werner Helicase is a Synthetic-Lethal vulnerability in Mismatch Repair-Deficient Colorectal Cancer Refractory to targeted therapies, Chemotherapy, and Immunotherapy. Cancer Discov. 2021;11(8):1923–37.
5. Afrăsânie VA, Marinca MV, Gafton B, Alexa-Stratulat T, Rusu A, Froicu EM, Sur D, Lungulescu CV, Popovici L, Lefter AV et al. Clinical, pathological and molecular insights on KRAS, NRAS, BRAF, PIK3CA and TP53 mutations in metastatic colorectal Cancer patients from Northeastern Romania. Int J Mol Sci 2023, 24(16).
6. Peng X, Zhang T, Jia X, Wang T, Lin H, Li G, Li R, Zhang A. Impact of a haplotype (composed of the APC, KRAS, and TP53 genes) on colorectal adenocarcinoma differentiation and patient prognosis. Cancer Genet. 2022;268–269:115–23.
7. Dos Santos W, Dos Reis MB, Porto J, de Carvalho AC, Matsushita M, Oliveira G, Syrjänen K, Reis RM, Guimarães DP. Somatic targeted mutation profiling of colorectal cancer precursor lesions. BMC Med Genom. 2022;15(1):143.
8. Matsumoto K, Urabe Y, Oka S, Inagaki K, Tanaka H, Yuge R, Hayashi R, Kitadai Y, Arihiro K, Shimamoto F, et al. Genomic Landscape of early-stage colorectal neoplasia developing from the Ulcerative Colitis Mucosa in the Japanese Population. Inflamm Bowel Dis. 2021;27(5):686–96.
9. Wang HW, Yan XL, Wang LJ, Zhang MH, Yang CH, Wei L, Jin KM, Bao Q, Li J, Wang K, et al. Characterization of genomic alterations in Chinese colorectal cancer patients with liver metastases. J Translational Med. 2021;19(1):313.
10. Vaquero J, Pavy A, Gonzalez-Sanchez E, Meredith M, Arbelaiz A, Fouassier L. Genetic alterations shaping tumor response to anti-EGFR therapies. Drug Resist Updates: Reviews Commentaries Antimicrob Anticancer Chemother. 2022;64:100863.
11. Ros J, Vaghi C, Baraibar I, Saoudi González N, Rodríguez-Castells M, García A, Alcaraz A, Salva F, Tabernero J, Elez E. Targeting KRAS G12C Mutation in Colorectal Cancer, a review: new arrows in the Quiver. Int J Mol Sci 2024, 25(6).
12. van de Haar J, Ma X, Ooft SN, van der Helm PW, Hoes LR, Mainardi S, Pinato DJ, Sun K, Salvatore L, Tortora G, et al. Codon-specific KRAS mutations predict survival benefit of trifluridine/tipiracil in metastatic colorectal cancer. Nat Med. 2023;29(3):605–14.
13. Alizadeh-Sedigh M, Mahmoodzadeh H, Fazeli MS, Haddadi-Aghdam M, Teimoori-Toolabi L. The potential of PIK3CA, KRAS, BRAF, and APC hotspot mutations as a non-invasive detection method for colorectal cancer. Mol Cell Probes. 2022;63:101807.
14. Jauhri M, Bhatnagar A, Gupta S, Bp M, Minhas S, Shokeen Y, Aggarwal S. Prevalence and coexistence of KRAS, BRAF, PIK3CA, NRAS, TP53, and APC mutations in Indian colorectal cancer patients: next-generation sequencing-based cohort study. Tumour Biology: J Int Soc Oncodevelopmental Biology Med. 2017;39(2):1010428317692265.
15. Derbel O, Wang Q, Desseigne F, Rivoire M, Meeus P, Peyrat P, Stella M, Martel-Lafay I, Lemaistre AI, de La Fouchardière C. Impact of KRAS, BRAF and PI3KCA mutations in rectal carcinomas treated with neoadjuvant radiochemotherapy and surgery. BMC Cancer. 2013;13:200.
16. Simon K. Colorectal cancer development and advances in screening. Clin Interv Aging. 2016;11:967–76.
17. Jacobs SA, Lee JJ, George TJ, Wade JL 3rd, Stella PJ, Wang D, Sama AR, Piette F, Pogue-Geile KL, Kim RS, et al. Neratinib-Plus-Cetuximab in Quadruple-WT (KRAS, NRAS, BRAF, PIK3CA) metastatic colorectal Cancer resistant to Cetuximab or Panitumumab: NSABP FC-7, a phase ib study. Clin cancer Research: Official J Am Association Cancer Res. 2021;27(6):1612–22.
18. Li JY, Huang XZ, Gao P, Chen XW, Song YX, Lv XE, Fu Y, Xiao Q, Wang ZN. Postoperative Adjuvant Treatment Strategy for Locally Advanced Rectal Cancer after Neoadjuvant Treatment. *BioMed research international* 2021, 2021:8852699.
19. Wang Q, Shen X, Chen G, Du J. Drug Resistance in Colorectal Cancer: from mechanism to Clinic. Cancers (Basel) 2022, 14(12).
20. Piawah S, Venook AP. Targeted therapy for colorectal cancer metastases: a review of current methods of molecularly targeted therapy and the use of tumor biomarkers in the treatment of metastatic colorectal cancer. Cancer. 2019;125(23):4139–47.
21. Rivera M, Fichtner I, Wulf-Goldenberg A, Sers C, Merk J, Patone G, Alp KM, Kanashova T, Mertins P, Hoffmann J, et al. Patient-derived xenograft (PDX) models of colorectal carcinoma (CRC) as a platform for chemosensitivity and biomarker analysis in personalized medicine. Neoplasia (New York NY). 2021;23(1):21–35.
22. Shao DD, Xue W, Krall EB, Bhutkar A, Piccioni F, Wang X, Schinzel AC, Sood S, Rosenbluh J, Kim JW, et al. KRAS and YAP1 converge to regulate EMT and tumor survival. Cell. 2014;158(1):171–84.
23. Sun C, Hobor S, Bertotti A, Zecchin D, Huang S, Galimi F, Cottino F, Prahallad A, Grernrum W, Tzani A, et al. Intrinsic resistance to MEK inhibition in KRAS mutant lung and colon cancer through transcriptional induction of ERBB3. Cell Rep. 2014;7(1):86–93.
24. Lu X, Li Y, Li Y, Zhang X, Shi J, Feng H, Yu Z, Gao Y. Prognostic and predictive biomarkers for anti-EGFR monoclonal antibody therapy in RAS wild-type metastatic colorectal cancer: a systematic review and meta-analysis. BMC Cancer. 2023;23(1):1117.
25. Yagdi Efe E, Mazumder A, Lee JY, Gaigneaux A, Radogna F, Nasim MJ, Christov C, Jacob C, Kim KW, Dicato M, et al. Tubulin-binding anticancer polysulfides induce cell death via mitotic arrest and autophagic interference in colorectal cancer. Cancer Lett. 2017;410:139–57.
26. Lièvre A, Ouine B, Canet J, Cartier A, Amar Y, Cacheux W, Mariani O, Guimbaud R, Selves J, Lecomte T, et al. Protein biomarkers predictive for response to anti-EGFR treatment in RAS wild-type metastatic colorectal carcinoma. Br J Cancer. 2017;117(12):1819–27.
27. Bajpe PK, Prahallad A, Horlings H, Nagtegaal I, Beijersbergen R, Bernards R. A chromatin modifier genetic screen identifies SIRT2 as a modulator of response to targeted therapies through the regulation of MEK kinase activity. Oncogene. 2015;34(4):531–6.
28. Garufi A, Ubertini V, Mancini F, D'Orazi V, Baldari S, Moretti F, Bossi G, D'Orazi G. The beneficial effect of zinc(II) on low-dose chemotherapeutic sensitivity involves p53 activation in wild-type p53-carrying colorectal cancer cells. J Experimental Clin cancer Research: CR. 2015;34(1):87.
29. Hata AN, Rowley S, Archibald HL, Gomez-Caraballo M, Siddiqui FM, Ji F, Jung J, Light M, Lee JS, Debussche L, et al. Synergistic activity and heterogeneous acquired resistance of combined MDM2 and MEK inhibition in KRAS mutant cancers. Oncogene. 2017;36(47):6581–91.
30. Li XL, Zhou J, Chen ZR, Chng WJ. P53 mutations in colorectal cancer - molecular pathogenesis and pharmacological reactivation. World J Gastroenterol. 2015;21(1):84–93.
31. Solomon H, Dinowitz N, Pateras IS, Cooks T, Shetzer Y, Molchadsky A, Charni M, Rabani S, Koifman G, Tarcic O, et al. Mutant p53 gain of function underlies high expression levels of colorectal cancer stem cells markers. Oncogene. 2018;37(12):1669–84.
32. Xu M, Zhao X, Wen T, Qu X. Unveiling the role of KRAS in tumor immune microenvironment. Biomed Pharmacotherapy = Biomedecine Pharmacotherapie. 2024;171:116058.
33. Yakar D, Bozkırlı BO, Ceyhan GO. Genetic landscape of pancreatic cancer: a narrative review. Chin Clin Oncol. 2022;11(1):5.
34. Otegui N, Houry M, Arozarena I, Serrano D, Redin E, Exposito F, Leon S, Valencia K, Montuenga L, Calvo A. Cancer Cell-intrinsic alterations Associated with an immunosuppressive Tumor Microenvironment and Resistance to Immunotherapy in Lung Cancer. Cancers (Basel) 2023, 15(12).
35. Diener MK, Fichtner-Feigl S. Biomarkers in colorectal liver metastases: rising complexity and unknown clinical significance? Annals Gastroenterological Surg. 2021;5(4):477–83.
36. Chow OS, Kuk D, Keskin M, Smith JJ, Camacho N, Pelossof R, Chen CT, Chen Z, Avila K, Weiser MR, et al. KRAS and Combined KRAS/TP53 mutations in locally advanced rectal Cancer are independently Associated with decreased response to Neoadjuvant Therapy. Ann Surg Oncol. 2016;23(8):2548–55.
37. Liu X, Xu X, Wu Z, Shan Q, Wang Z, Wu Z, Ding X, Huang W, Wang Z. Integrated single-cell RNA-seq analysis identifies immune heterogeneity

associated with KRAS/TP53 mutation status and tumor-sideness in colorectal cancers. Front Immunol. 2022;13:961350.
38. Duldulao MP, Lee W, Nelson RA, Li W, Chen Z, Kim J, Garcia-Aguilar J. Mutations in specific codons of the KRAS oncogene are associated with variable resistance to neoadjuvant chemoradiation therapy in patients with rectal adenocarcinoma. Ann Surg Oncol. 2013;20(7):2166–71.
39. De Mattia E, Roncato R, Palazzari E, Toffoli G, Cecchin E. Germline and somatic pharmacogenomics to refine rectal Cancer patients selection for Neo-adjuvant Chemoradiotherapy. Front Pharmacol. 2020;11:897.
40. Chun YS, Passot G, Yamashita S, Nusrat M, Katsonis P, Loree JM, Conrad C, Tzeng CD, Xiao L, Aloia TA, et al. Deleterious effect of RAS and Evolutionary High-risk TP53 double mutation in Colorectal Liver metastases. Ann Surg. 2019;269(5):917–23.
41. Sartore-Bianchi A, Loupakis F, Argilés G, Prager GW. Challenging chemoresistant metastatic colorectal cancer: therapeutic strategies from the clinic and from the laboratory. Annals Oncology: Official J Eur Soc Med Oncol. 2016;27(8):1456–66.
42. Sforza V, Martinelli E, Ciardiello F, Gambardella V, Napolitano S, Martini G, Della Corte C, Cardone C, Ferrara ML, Reginelli A, et al. Mechanisms of resistance to anti-epidermal growth factor receptor inhibitors in metastatic colorectal cancer. World J Gastroenterol. 2016;22(28):6345–61.
43. Seligmann JF, Fisher DJ, Brown LC, Adams RA, Graham J, Quirke P, Richman SD, Butler R, Domingo E, Blake A, et al. Inhibition of WEE1 is effective in TP53- and RAS-Mutant metastatic colorectal Cancer: a Randomized Trial (FOCUS4-C) comparing Adavosertib (AZD1775) with active monitoring. J Clin Oncology: Official J Am Soc Clin Oncol. 2021;39(33):3705–15.
44. Jaaks P, Coker EA, Vis DJ, Edwards O, Carpenter EF, Leto SM, Dwane L, Sassi F, Lightfoot H, Barthorpe S, et al. Effective drug combinations in breast, colon and pancreatic cancer cells. Nature. 2022;603(7899):166–73.
45. Michel M, Kaps L, Maderer A, Galle PR, Moehler M. The role of p53 dysfunction in Colorectal Cancer and its implication for Therapy. Cancers (Basel) 2021, 13(10).
46. Almeida LC, Bauermeister A, Rezende-Teixeira P, Santos EAD, Moraes LAB, Machado-Neto JA, Costa-Lotufo LV. Pradimicin-IRD exhibits antineoplastic effects by inducing DNA damage in colon cancer cells. Biochem Pharmacol. 2019;168:38–47.

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