Immunogenetics: Open Access

OPEN ACCESS Freely available online

Research Article

Differences in Gene Mutation Profiles of Colon Cancer Patients with or without a *KRAS* Mutation

Shujian Chang[1*], Yudan Zhou[2], Ruirong Wu[1], Xiaosong Ge[1], Yong Pu[3]

[1]Department of Oncology, Affiliated Hospital of Jiangnan University, Wuxi 214122, Jiangsu, China;[2]Wuxi School of Medicine, Jiangnan University, Wuxi 214125, Jiangsu, China;[3]Department of Pathology, Affiliated Hospital of Jiangnan University, Wuxi 214122, Jiangsu, China

ABSTRACT

In order to compare the gene mutation profiles of patients with or without a *KRAS* mutation, the clinicopathological data of 858 patients and NGS test results of 1697 patients with colorectal cancer were used in this analysis. In 858 patients, only 2 out of 349 (0.5%) *KRAS* mutant patients had a *BRAF* mutation, while 25 out of 422 (5.9%) *KRAS* wild-type patients had a *BRAF* mutation ($p<0.0001$). The NGS results showed that in RAS mutant patients, genes with a high mutation rate mainly included *APC*, *TP53*, *PIK3CA*, *Smad4*, and *Fbxw7*, and in RAS wild-type patients, genes with a high mutation rate mainly included *TP53*, *APC*, *LRP1B*, *MYC*, and *BRAF*. The mutation rates of *BRAF* and *EGFR* in the RAS wild-type group were 15% and 9%, respectively, while they were only 3% in the RAS mutant group. The mutation rate of *PIK3CA* in the RAS mutant group was 31%, while that in the RAS wild-type group was 14%. The mutation rate of *APC* was 72.2% (i.e., 687/952). The mutation rate of the gene, *RNF43*, in the *APC* wild-type group was 5.23 times higher than that in the *APC* mutant group, and the gene, NSD1, in the *APC* wild-type group was 0.07 times higher than that in the *APC* mutant group. The mutation rate of *TP53* was 78.2% (i.e., 744/952). The mutation rate of *MLH1* in the *TP53* wild-type group was 8.42 times higher than that in the *TP53* mutant group, which was significantly higher than that in the *TP53* mutant group. Generally, the gene mutation profiles were significantly different between *KRAS* mutation and wild-type colorectal cancer patients. A single gene mutation may be sufficient to cause the dysfunction of a signal transduction pathway, and *APC*, *TP53*, or RAS are not necessary for the carcinogenesis of sporadic colorectal cancer.

Keywords: Colon cancer; *KRAS* mutation; Patients; Chemotherapy

INTRODUCTION

The incidence of Colorectal Cancer (CRC) in China is rising despite significant advances in its diagnosis and management. According to the National Cancer Center of China, it is the fifth most common cancer and the leading cause of cancer-related deaths in China [1]. Cancer development is commonly regarded as a multistep process involving an initial mutagenic event called tumor initiation. In colorectal cancer, the adenoma-carcinoma sequence is regarded as a classic model of sporadic colon cancer [2]. In this process, many signal transduction pathways are involved such as Mitogen-Activated Protein Kinase (MAPK), Wnt/β-catenin signaling cascade, and apoptosis signaling cascade [3]. APC gene mutation was found to be the initiating event in the classic adenoma-carcinoma sequence [4]. In cells harboring a mutated *APC* gene, due to the absence of the inhibitory effect exerted by Wnt signaling, β-catenin accumulates, and after its translocation in the nucleus, acts as a co-activator of T-Cell Factor (*TCF*)-Lymphocyte Enhancer Factor (LEF). The β-catenin/*TCF-LEF* complex acts, in turn, as a transcriptional activator of the key cell cycle regulatory genes, cyclin D1 and c-Myc, to promote tumor genesis [5]. In this model, *APC* suppression determines the formation of adenomas in the colon (i.e., intestine); then, in the presence of additional mutations, such as in the *TP53* and *KRAS* genes, these tumors are induced to progress into colon cancers [6-8].

In 1987, using a combination of DNA hybridization analyses and tissue sectioning techniques, researchers demonstrated that RAS

Correspondence to: Shujian Chang, Department of Oncology, Affiliated Hospital of Jiangnan University, Wuxi 214122, Jiangsu, China, E-mail: 9812015210@jiangnan.edu.cn

Received: 22-Jul-2022, Manuscript No. IGOA-22-18538; **Editor assigned:** 25-Jul-2022, Pre QC No. IGOA-22-18538 (PQ); **Reviewed:** 08-Aug-2022, QC No. IGOA-22-18538; **Revised:** 15-Aug-2022, Manuscript No. IGOA-22-18538 (R); **Published:** 22-Aug-2022, DOI: 10.35248/IGOA.22.7.175.

Citation: Chang S, Zhou Y, Wu R, Ge X, Pu Y (2022) Differences in Gene Mutation Profiles of Colon Cancer Patients with or without a *KRAS* Mutation. Immunogenet Open Access.7:175.

gene mutations occurred in over one-third of human colorectal cancers and that the mutations usually preceded the development of malignancy [9]. Subsequent studies further proved that the prevalence of *KRAS* mutations ranged from 25% to 52%, and these mutations were typically located at codons 12 and 13 in exon 2 of the coding region of the *KRAS* gene [10]. Single base substitutions in codons 12 and 13 affect glycine residues in the GTP-binding pocket critical for GTPase function; thus, these *KRAS* mutations lead to stabilization of protein in their prolonged active state, thereby amplifying the downstream signaling pathways [11]. The main downstream signaling pathways are the MAPK and AKT pathways, which allow tumor cells to proliferate in the absence of growth factors and increase their survival [12]. In addition, *KRAS* can stimulate Wnt signaling through inhibition of GSK-3beta, regulate vascular endothelial growth factor (VEGF) gene expression, and promote tumor progression by cooperating with Wnt signaling [13].

KRAS not only plays a key role in the occurrence and development of colorectal cancer, but it also affects the treatment of colorectal cancer. *KRAS* gene mutations are important predictors of response to cetuximab or panitumumab therapy in patients with colorectal cancer [14-17]. Patients bearing a mutated *KRAS* cannot benefit from cetuximab or panitumumab, whereas patients with wild-type *KRAS* can benefit from these drugs [18,19]. Meanwhile, some studies reported that *KRAS* mutation was a negative prognostic factor for Overall (OS) and Recurrence Free Survival (RFS) [20].

It is obvious that *KRAS* plays an important role in the carcinogenesis of colorectal epithelial cells and the treatment of patients with colorectal cancer; however, *KRAS* mutations are only present, at most, in approximately 50% of patients. In other words, approximately 50% of patients have the wild type. It can be speculated that patients with mutant or wild-type *KRAS* may have different gene mutation spectrums and, thus, show different biological characteristics. Therefore, comparing the gene mutation profiles of patients with or without a *KRAS* mutation may help researchers better understands the occurrence, development, and treatment of colorectal cancer.

MATERIALS AND METHODS

Patients' data

Two data sets of patients with colorectal cancer were included in this study. The first data set consisted of the clinical and pathological data of 858 patients from the Affiliated Hospital of Jiangnan University, and the second data set was composed of the NGS tests of 1697 patients from the Data Bank of the GENE+ Company (Beijing, China).

Data set 1

From May 2012 to December 2015, 858 colorectal cancer patients underwent colorectal resection of their primary cancer at the Affiliated Hospital of Jiangnan University (Jiangsu Province, China). The medical records of these colorectal cancer patients were carefully reviewed. The following data were collected retrospectively: age, sex, stage, site, and gene mutation status (*KRAS*, B-raf, Her-2, Ki-67, and *MMR*). Mutational analyses on *KRAS* and *BRAF* were performed using genomic DNA extracted from microdisected tumor tissue with the DNA Mini Kit (Qiagen), and gene mutation status was analyzed using the ADx-ARMSTM mutation test kit (Xiamen). The *KRAS* mutation status was analyzed using the Human *KRAS* Gene 7 Mutations Fluorescence Polymerase Chain Reaction (PCR) Diagnostic Kit (ADx-ARMSTM); testing for the *BRAF*V600E hotspot mutation in exon 15 was performed using the Human *BRAF* (V006E) Gene Mutations Fluorescence Polymerase Chain Reaction (PCR) Diagnostic Kit (ADx-ARMSTM). The expression of *MMR* (*MLH1*, *PMS2*, *MSH2*, and *MSH6*), Her-2, and KI-67 were analyzed by immunohistochemistry according to routine methods. These analyses involved an initial hematoxylin and eosin slide review by a pathologist to confirm the diagnosis, delineate the percentage of tumor present, and demarcate tumor from normal tissue. Specimens were required to contain at least 50% tumor within the sample.

Data set 2

A total of 1697 patients with colorectal cancer were enrolled in this analysis. There were 798 cases with wild-type RAS and 899 cases with a mutant RAS (i.e., *KRAS*: 864 and *NRAS*: 35). Six hundred and thirty-seven patients underwent chemotherapy, 952 patients did not accept chemotherapy, and 108 patients' treatment histories were unknown. Details on the methods of DNA extraction and quality control, target capture and next-generation sequencing, and sequencing data analysis can be found in Reference [21]. Briefly, based on second-generation sequencing technology, four types of mutations (including point mutation, small fragment insertion or deletion, copy number variation, and currently known fusion genes) of 1021 genes related to tumor genesis and development were detected. Among them, all exon regions of 312 genes, introns, primers and fusion regions of 38 genes, and partial exon regions of 709 genes were detected.

Statistical analysis

The chi-square test was used to investigate the relationships between *KRAS* mutation status and other clinicopathological factors. To estimate the effect of *KRAS* on overall survival, Kaplan-Meier curves were plotted and compared using the log-rank test. All reported p-values were two-sided, and $p<0.05$ was considered statistically significant.

RESULTS

Clinical characteristics

In 858 patients in the first data set, 436 patients had a mutant *KRAS* and 422 patients had wild-type *KRAS*. The mutation rate of *KRAS* was 50.8% (i.e., 436/858). Of the 858 patients, a total of 771 patients were tested for *BRAF*. Among them, only 2 out of 349 (0.5%) *KRAS* mutant patients had a *BRAF* mutation, while 25 out of 422 (5.9%) *KRAS* wild-type patients had a *BRAF* mutation. The difference between them was highly statistically significant ($p<0.0001$). In addition, 432 patients were tested for *NRAS*. Among them, 5 out of 196 (2.6%) *KRAS* mutant patients had a *NRAS* mutation, while 18 out of 236 *KRAS* wild-type patients had a *NRAS* mutation. The difference between them was statistically significant ($p=0.0193$). With the exception of *BRAF* and *NRAS*, there were no differences in the mutation rate of *KRAS* in patients with different clinicopathological characteristics (Table 1).

Table 1: *KRAS* mutation status in different clinic pathologic factors of 858 patients.

	Number of patients		P-value
	KRAS (mutation)	*KRAS* (wild type)	
Gender			0.8448
male	245	256	
female	177	180	
Year			0.2893
<60	139	129	
>=60	283	307	
Stage			0.1535
I	58	59	
II	172	148	
III	143	164	
IV	49	65	
Site			0.8845
Right colon	118	95	
Left colon	361	284	
NRAS a			0.0193
wild	191	218	
mutation	5	18	
BRAF(V600E)			<0.0001 b
wild	347	397	
mutation	2	25	
MMR			0.8983
dMMR	40	52	
PMMR	159	213	
Her-2			0.8132
Negative (-, +)	90	123	
Positive (++, +++)	95	124	
Ki-67(%)			0.2262
<=60	24	42	
> 60	176	221	

Note: a: *NRAS*, *BRAF*, HER-2, dMMR, and Ki-67 were not tested in all 858 patients, so the analysis was done only in patients who received test. b: Fisher test.

Median overall survival (OS) of patients with or without a *KRAS* mutation

Patients were treated according to NCCN guidelines. In short, patients in stage I were mainly treated with surgery alone; patients in stage II and III were treated with capecitabine monotherapy, 5-Fu/LV, or a FOLFOX regimen according to their tumor stage, risk classification and MSI status after surgery; patients in stage IV were treated with a FOLFOX/FOLFIRI+/- Bevacizumab or Cetuximab regimen (left colon, RAS and *BRAF* wild type). The deadline for follow up was September 30, 2019, and the median follow-up time was 38.6 months for all 858 patients. Seventy-seven patients were lost to follow up. The median OS of 436 patients with a mutant *KRAS* (56 patients lost to follow up) was 83.47 months, and the median OS of 422 patients with wild-type *KRAS* (21 patients lost to follow up) was not reached. The hazard ratio (log-rank) was 0.8147 (95% CI: 0.6271-1.051; p=0.1153) (Figure 1).

Differences in the gene mutation profiles of patients with or without an RAS mutation by NGS test

To rule out the effects of chemotherapy on gene mutation, we only compared the gene mutation profiles of patients who had not received chemotherapy. A total of 952 patients did not receive chemotherapy at the time of the NGS test, of which 487 had wild-type RAS and 465 had a mutant. The RAS mutation rate was 48.8% (i.e., 465/952). In RAS mutant patients, genes with a high mutation rate mainly included *APC*, *TP53*, *PIK3CA*, *Smad4*, and *Fbxw7* (Table 2 and Figure 2). In RAS wild-type patients, genes with a high mutation rate mainly included *TP53*, *APC*, LRP1B, *Myc*, and *BRAF* (Table 2 and Figure 3). Comparing the mutation rate of genes between RAS mutation and wild-type patients, there were 40 genes with different mutation rates (defined as a difference in the mutation rate between the two groups greater than 1%) (Table 3 and Figure 4). There was no difference in the mutation rate of *APC* between the two groups, but genes, such as *BRAF*, *EGFR*, *PIK3CA*, *SOX9*, and *SMAD4*, were significantly different between the two groups. The mutation rates of *BRAF* and *EGFR* in the RAS wild-type group were 15% and 9%, respectively, while they were only 3% in the RAS mutant group. The mutation rate of *PIK3CA* in the RAS mutant group was 31%, while that in the RAS wild-type group was 14% (Table 3).

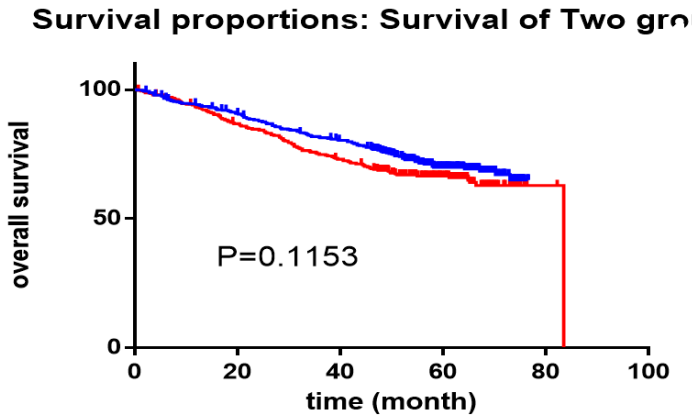

Figure 1: The overall survival (OS) of colorectal cancer patients with or without a *KRAS* mutation. Note: (—) *kras* (-), (—) *kras* (+).

Table 2: Genes with mutation rate in the top 10 in patients with a mutant (RAS-MUT) or wild-type (RAS-WT) *KRAS*.

	All patients		Patients without chemothreapy		Patients with chemothreapy	
	RAS-MUT	*RAS-WT*	*RAS-MUT*	*RAS-WT*	*RAS-MUT*	*RAS-WT*
number of patients	899	798	465	487	379	258
No.1	*Tp53*	*Tp53*	*Tp53*	*Tp53*	*Tp53*	*Tp53*
No.2	*apc*	*apc*	*apc*	*apc*	*apc*	*apc*
No.3	*Pik3ca*	*Lrp1b*	*Pik3ca*	*Lrp1b*	*Smad4*	*Lrp1b*
No.4	*Smad4*	*Braf*	*Smad4*	*Braf*	*Pik3ca*	*Braf*
No.5	*Fbxw7*	Myc	*Fbxw7*	*Pik3ca*	*Fbxw7*	Myc
No.6	*Tcf7l2*	*Fbxw7*	*Tcf7l2*	*Fbxw7*	*Tcf7l2*	*Fbxw7*
No.7	*Lrp1b*	*Smad4*	*Sox9*	*Tcf7l2*	*Lrp1b*	*Smad4*
No.8	*Sox9*	*Pik3ca*	*Lrp1b*	*Mll3*	*Sox9*	*Rnf43*
No.9	*Fam123b*	*Tcf7l2*	*Fam123b*	*Arid1a*	*Fat2*	*Mll3*
No.10	*Arid1a*	*Mll3*	*Arid1a*	*Smad4*	*Fam123b*	*Pik3ca*

Note: RAS-MUT is mutant RAS; RAS-WT is wild-type RAS.

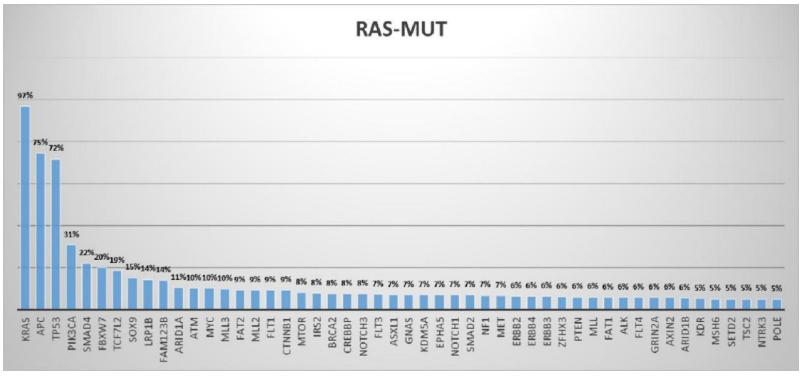

Figure 2: Gene mutation profiling in untreated colorectal patients with a mutant RAS (Only the top 50 genes are listed).

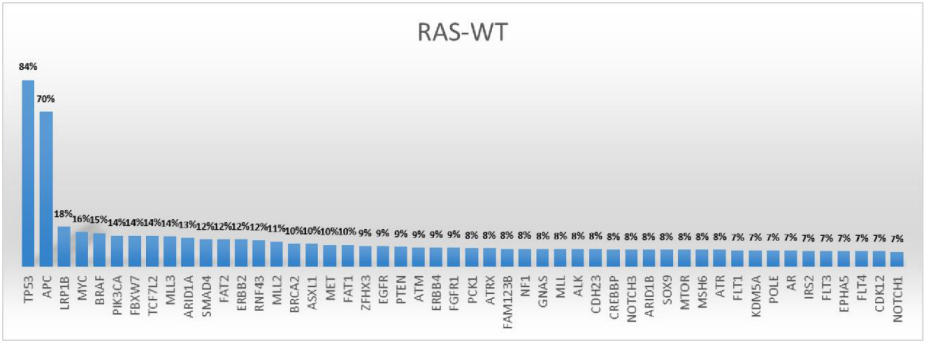

Figure 3: Gene mutation profiling in untreated colorectal patients with a mutant RAS (Only the top 50 genes are listed).

Table 3: Genes with different mutation rates in mutant (RAS-MUT) and wild-type (RAS-WT) RAS patients who did not receive chemotherapy (only genes with mutation rates greater than 5% are listed).

Gene	Mutation rate (%)		Times
	RAS-MUT	*RAS-WT*	*WT/MUT*
BRAF	3	15	5
EGFR	3	9	3
BCL2L1	2	6	3
TOP2A	2	6	3
PTCH1	2	5	2.5
PTCH2	2	5	2.5
BRCA1	2	5	2.5
CSF1R	2	5	2.5
RNF43	5	12	2.4
FGFR1	4	9	2.25
ERBB2	6	12	2
PCK1	4	8	2
ATRX	4	8	2
ATR	4	8	2
IGF1R	3	6	2
AXL	3	6	2
AR	4	7	1.75
FAT1	6	10	1.67
ABL1	3	5	1.67
MSH3	3	5	1.67
MYC	10	16	1.6
CDH23	5	8	1.6
TP53	72	84	1.17
FBXW7	20	14	0.7
FAM123B	14	8	0.57
SMAD2	7	4	0.57
SMAD4	22	12	0.55
SOX9	15	8	0.53
PIK3CA	31	14	0.45

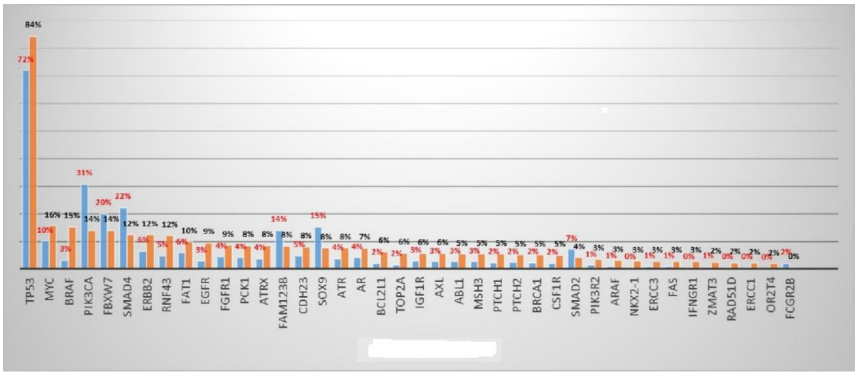

Figure 4: 40 genes whose mutation rate is different between RAS mutant (RAS-MUT) and wild-type (RAS-WT) patients. **Note:** ( ) RAS-MUT ( ) RAS-WT.

Differences in the gene mutation profiles of patients with or without an *APC* mutation

Among the 952 patients who did not receive chemotherapy, 265 had wild-type *APC* and 687 had an *APC* mutant. The mutation rate of *APC* was 72.2% (i.e., 687/952). There were 28 genes in which the mutation rate was different between the two groups (defined as a difference in the mutation rate between the two groups greater than 1%) (Figure 5 and Table 4). As shown in Table 4, the mutation rate of the gene, *RNF43*, in the *APC* wild-type group was 5.23 times higher than that in the *APC* mutant group (Table 4). On the contrary, the mutation rate of the gene, NSD1, in the *APC* wild-type group was 0.07 times higher than that in the *APC* mutant group, which was significantly lower than that in the *APC* mutant group.

Differences in the gene mutation profiles of patients with or without a TP53 mutation

Among the 952 patients who did not receive chemotherapy, 208 had wild-type *TP53* and 744 had a *TP53* mutant. The mutation rate of *TP53* was 78.2% (i.e., 744/952). There were differences in the mutation rates of 102 genes between the two groups (defined as a difference in the mutation rate between the two groups greater than 1%) (Figure 6 and Table 5). As shown in Table 5, *MLH1*, HDAC4, TGFBR2, RAD50, and MSH3 were the top five genes in the ratio of the two groups. Two of them, *MLH1* and MSH3, were mismatch repair genes (MMR). The mutation rate of *MLH1* in the *TP53* wild-type group was 8.42 times higher than that in the *TP53* mutant group, which was significantly higher than that in the *TP53* mutant group (Table 5).

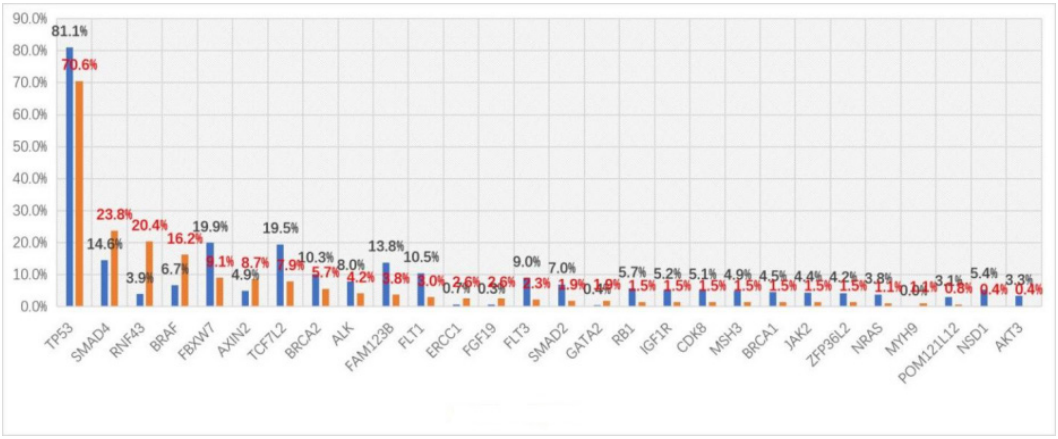

Figure 5: 28 genes whose mutation rate is different between patients with a mutant (APC-mut) and a wild-type. **Note:** APC (APC-wt) ( ) APC-mut ( ) APC-wt.

Table 4: Genes with different mutation rates in mutant (APC-MUT) and wild-type (APC-WT) APC patients (only genes with mutation rates greater than 5% are listed).

Gene	Mutation rate (%)		Times
	APC-MUT	*APC-WT*	*WT/MUT*
RNF43	3.9	20.4	5.23
BRAF	6.7	16.2	2.42
SMAD4	14.6	23.8	1.63
TP53	81.1	70.6	0.87

BRCA2	10.3	5.7	0.55
ALK	8	4.2	0.53
FBXW7	19.9	9.1	0.46
TCF7L2	19.5	7.9	0.41
CDK8	5.1	1.5	0.29
IGF1R	5.2	1.5	0.29
FLT1	10.5	3	0.29
FAM123B	13.8	3.8	0.28
SMAD2	7	1.9	0.27
RB1	5.7	1.5	0.26
FLT3	9	2.3	0.26
NSD1	5.4	0.4	0.07

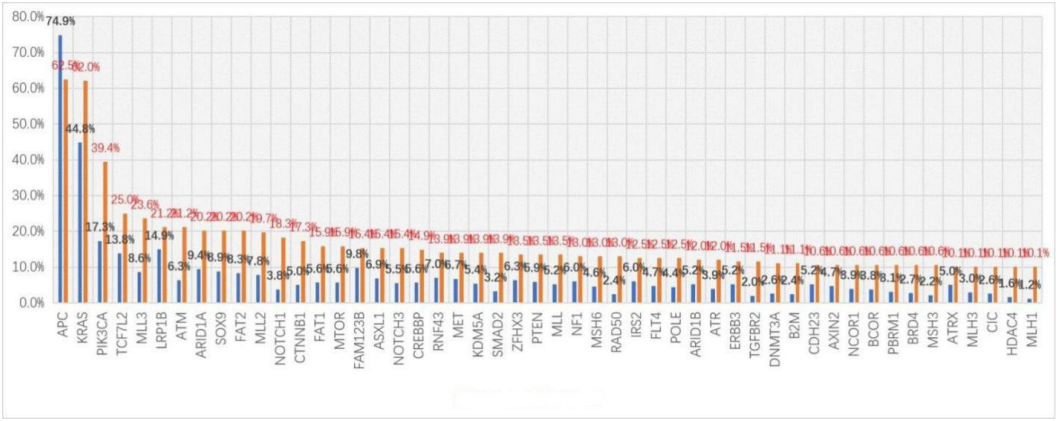

Figure 6: Genes with different mutation rate between TP53 mutant (TP53-mut) and wild-type (*TP53*-wt) patients (Only top 50 genes are shown). **Note:** (■) *TP53*-mut (■) *TP53*-wt.

Table 5: Genes with different mutation rates in mutant (*TP53*-MUT) and wild-type (*TP53*-WT) *TP53* patients who did not receive chemotherapy (only genes with mutation rates greater than 5% are listed).

Gene	Mutation rate (%)		Times
	TP53-*MUT*	TP53-*WT*	*WT/MUT*
MLH1	1.2	10.1	8.42
HDAC4	1.6	10.1	6.31
TGFBR2	2	11.5	5.75
RAD50	2.4	13	5.42
MSH3	2.2	10.6	4.82
NOTCH1	3.8	18.3	4.82
B2M	2.4	11.1	4.63
SMAD2	3.2	13.9	4.34
DNMT3A	2.6	11.1	4.27
BRD4	2.7	10.6	3.93
CIC	2.6	10.1	3.88
CTNNB1	5	17.3	3.46

PBRM1	3.1	10.6	3.42
MLH3	3	10.1	3.37
ATM	6.3	21.2	3.37
ATR	3.9	12	3.08
POLE	4.4	12.5	2.84
FAT1	5.6	15.9	2.84
MTOR	5.6	15.9	2.84
MSH6	4.6	13	2.83
NOTCH3	5.5	15.4	2.8
BCOR	3.8	10.6	2.79
MLL3	8.6	23.6	2.74
NCOR1	3.9	10.6	2.72
CREBBP	5.6	14.9	2.66
FLT4	4.7	12.5	2.66
MLL	5.2	13.5	2.6
KDM5A	5.4	13.9	2.57
MLL2	7.8	19.7	2.53
FAT2	8.3	20.2	2.43
ARID1B	5.2	12	2.31
PTEN	5.9	13.5	2.29
PIK3CA	17.3	39.4	2.28
SOX9	8.9	20.2	2.27
AXIN2	4.7	10.6	2.26
ASXL1	6.9	15.4	2.23
ERBB3	5.2	11.5	2.21
NF1	6	13	2.17
ARID1A	9.4	20.2	2.15
ZFHX3	6.3	13.5	2.14
IRS2	6	12.5	2.08
MET	6.7	13.9	2.07
CDH23	5.2	10.6	2.04
ATRX	5	10.1	2.02
RNF43	7	13.9	1.99
TCF7L2	13.8	25	1.81
FAM123B	9.8	15.4	1.57
LRP1B	14.9	21.2	1.42
KRAS	44.8	62	1.38
APC	74.9	62.5	0.83

DISCUSSION

It is widely known that RAS plays an important role in the occurrence of colorectal cancer. This study demonstrated that, as shown in Table 1, in patients with colorectal cancer, the mutation rate of the *KRAS* gene was not significantly different according to gender, age, tumor stage, tumor location, or other clinicopathological features. This suggests that there is no inevitable correlation between *KRAS* gene mutation and the above clinicopathological features. However, it is worth noting that the BRAF gene mutation rate was only approximately 0.6% (i.e., 2/349) in patients with a *KRAS* mutation, while it was 5.9% (i.e., 25/422) in patients with wild-type *KRAS* (Table 1). The mutation rate of the *BRAF* gene in the wild-type *KRAS* group was significantly higher than that in the KRAS mutation group.

As is well known, the RAF gene codes for cytoplasmic serine/threonine kinases that are regulated by binding RAS, and the vast majority of RAF mutations represent a single nucleotide change in T-A at nucleotide 1796, resulting in a valine to glutamic acid change at residue 599 within the activation segment of *BRAF* [22]. RAS function is not required for the growth of cancer cell lines with the *BRAF* V599E mutation [23]. RAF and RAS mutations are rarely concurrently present in the same cancer, and cancer types with *BRAF* mutations are similar to those with RAS mutations. This explains why the *BRAF* mutation rate is low in *KRAS* mutant patients but significantly higher in *KRAS* wild-type patients in this study.

Studies have shown that *KRAS* mutation is negatively correlated with the overall survival of patients with colorectal cancer [24,25]. In this study, it was also observed that the median survival of *KRAS* mutant patients was worse than that of *KRAS* wild-type patients, although the difference was not statistically significant.

Although *KRAS* plays an important role in the occurrence of colorectal cancer, up to 50% of patients had wild-type *KRAS*. Therefore, in order to further understand the differences in the biological behavior between *KRAS* mutation and *KRAS* wild-type colon cancer, we compared the results of the NGS test in patients with *KRAS* mutation and *KRAS* wild-type colon cancer. The results showed that there were significant differences in the gene mutation profiles between the two groups. Interestingly, in the RTK–RAS–MAPK pathway, NGS detection showed that the mutation rates of *BRAF* and *EGFR* in RAS wild-type patients were significantly higher than those in the RAS mutant group, and the mutation rates of *BRAF* and *EGFR* in the RAS wild-type group were five times and three times higher than those in the RAS mutant group, respectively (Table 3). This suggests that *KRAS* mutation and *BRAF* or *EGFR* mutation may be mutually exclusive in colorectal cancer [26] and that in the process of colorectal mucosal cell carcinogenesis, a single gene mutation may be sufficient to cause the dysfunction of a signal pathway. For example, the RAS gene mutation may be enough to cause the dysfunction of the RTK–RAS-MAPK pathway, and there is no need for *EGFR* or *BRAF* mutations at the same time. Accordingly, if there are multiple gene mutations in a signal pathway in patients with colorectal cancer, which is rare, blocking these genes at the same time may be a good treatment option. Many therapeutic studies targeting multiple signaling molecules in the RTK-RAS-MAPK pathway have achieved positive results *in vitro* [27,28] and *in vivo* [29-33].

Colorectal cancers are believed to arise *via* the gradual stepwise accumulation of mutations [34,35], and there are many mutations in oncogenes and/or tumor suppressor genes during this process. In the classic adenoma cancer pathway of colorectal cancer, *APC* gene mutation in the Wnt/β-catenin pathway is considered to be the initiating event of adenoma [36]. On this basis, deregulated WNT signaling is often followed by mutations in *KRAS/NRAS*, the TGFβ pathway, *PIK3CA*, *TP53*, or any combination of several of these alterations [37]. The mutations of *TP53*, RAS, and other genes further promote the occurrence and development of colon cancer. This study showed that there was no difference in the mutation rate of *APC* between RAS mutant and wild-type patients, suggesting that *APC* plays an equally important role in the occurrence of colorectal cancer, whether the presence of a RAS mutation or not. However, the problem is that not all patients with sporadic colorectal cancer harbor an *APC* mutation, and in this study, approximately 30% of patients had wild-type *APC*. Similarly, approximately 20% of patients had wild-type *TP53*, and nearly 50% of patients had wild-type *KRAS*. On the other hand, in the *APC* wild-type patients, the mutation rate of the tumor suppressor gene, *RNF43*, was 5.23 times higher than that in the *APC* mutation group, and the mutation rate of *Smad4* was 1.63 times higher than that in the *APC* mutation group (Table 4). Similarly, the *BRAF* mutation rate in *KRAS* wild-type patients was five times higher than that in *KRAS* mutation patients (Table 3). In *TP53* wild-type patients, the mutation rates of the mismatch repair genes, *MLH1* and MSH3, were 8.42 and 4.82 times higher than those in the mutation group, respectively (Table 5). Therefore, although some genes, such as *APC*, *TP53*, and *KRAS*, have a very high mutation frequency in colorectal cancer, none of them are necessary for the occurrence of sporadic colorectal malignancies. For example, in patients with wild-type oncogene *KRAS*, the mutation of oncogene *BRAF* or *EGFR* may play a role in RAS gene mutation; similarly, *RNF43* mutations may play a role in *APC* mutations in patients with wild-type *APC*, as *RNF43* mutation could contribute to the activation of Wnt signaling in colorectal carcinoma [38,39] and cancer-associated mutations that abrogate *RNF43* phosphorylation and cooperate with active RAS to promote tumorigenesis by abolishing the inhibitory function of *RNF43* in Wnt signaling. [40].

Mismatch Repair (MMR) proteins, especially *MLH1*, are closely related to apoptosis induced by alkylating agents [41]. Alkylating agents can induce both apoptosis and phosphorylation of the Ser-15 site of *TP53* in a *MLH1*-dependent manner. [42]. *MLH1* deficiency is a prominent signal during carcinogenesis of colorectal mucosal cells, but does not appear to be an absolute requirement or sufficient to cause colon cancer alone [43]; therefore, the mutant *MLH1* may promote tumorigenesis by inhibiting the apoptosis of cells through impeding the phosphorylation of *TP53* in patients with wild-type *TP53*. HDAC4 is an important regulator of proliferation of colon cancer cells, and it can promote the growth of colon cancer cells *via* the repression of p21 [44]. Furthermore, there is a complex interaction between *TP53* and HDAC4. *Tp53* can induce HDAC4 cytoplasmic translocation and phosphorylation, and DAC4 phosphorylation and translocation promotes autophagy through the transcription of ATG3 [45]. Thus, mutations in the mismatch repair gene, *MLH1*, and in histone deacetylase 4 (HDAC4) may play an important role, albeit *via* a mechanism that is not yet fully understood, in *TP53* wild-type colon cancer.

CONCLUSION

The gene mutation spectrum differs significantly between patients with *KRAS* mutant and wild-type colorectal cancer. Among *KRAS* wild-type patients, the mutation rate of oncogenes, such as *BRAF* and *EGFR*, was significantly higher than that in *KRAS* mutant patients, and the mutation rate of *PIK3CA* was significantly lower than that in *KRAS* mutant patients. In addition, a single gene mutation may be sufficient to cause the dysfunction of a signal transduction pathway, and *APC*, *TP53*, or RAS are not necessary for the carcinogenesis of sporadic colorectal cancer. Colorectal cancers are believed to arise *via* the gradual stepwise accumulation of mutations, and there are many mutations in oncogenes and/or tumor suppressor genes during this process. Therefore, in order to further understand the differences in the biological behavior between *KRAS* mutation and *KRAS* wild-type colon cancer, we compared the results of the NGS test in patients with *KRAS* mutation and *KRAS* wild-type colon cancer.

AVAILABILITY OF DATA AND MATERIALS

We reached an agreement of all participants in this study to publish this document.

COMPETING INTERESTS

The authors declared no conflicts of interest.

FUNDING

Shujian Chang was supported by the clinical research and translational medicine research project of the Affiliated Hospital of Jiangnan University (Project No.: lcyj202240).

AUTHORS' CONTRIBUTIONS

Shujian Chang conceptualized the study, analyzed the data and wrote the manuscript; Yudan Zhou and Ruirong Wu collected the clinical data and prepared the figures and tables. Xiaosong Ge and Yong Pu collected the pathological and NGS data. All authors have reviewed the manuscript.

ACKNOWLEDGEMENTS

Not applicable.

REFERENCES

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China. CA Cancer J Clin, 2016; 66(2): 115-132.
2. Nguyen LH, Goel A, Chung DC. Pathways of colorectal carcinogenesis. Gastroenterol. 2020; 158(2): 291-302.
3. Ishaque N, Abba ML, Hauser C, Patil N, Paramasivam N, Huebschmann D, et al. Whole genome sequencing puts forward hypotheses on metastasis evolution and therapy in colorectal cancer. Nat Commun. 2018, 9(1): -4781-4782.
4. Fearon E R. Molecular genetics of colorectal cancer. Annu Rev Pathol. 2011; 6(3): 479-507.
5. Schuijers J, Mokry M, Hatzis P, Cuppen E, Clevers H. Wnt-induced transcriptional activation is exclusively mediated by TCF/LEF. Embo J. 2014; 33(2): 146-56.
6. Hashimoto K, Yamada Y, Semi K, Yagi M, Tanaka A, Itakura F, et al. Cellular context-dependent consequences of Apc mutations on gene regulation and cellular behavior. Proc Natl Acad Sci USA. 2017; 114(4): 758-763.
7. Phelps RA, Chidester S, Dehghanizadeh S, Phelps J, Sandoval I T, Rai K, et al. A two-step model for colon adenoma initiation and progression caused by APC loss. Cell. 2009; 137(4): 623-634.
8. Wu X, Tu X, Joeng KS, Hilton MJ, Williams DA, Long F. Rac1 activation controls nuclear localization of beta-catenin during canonical Wnt signaling. Cell, 2008; 133(2): 340-353.
9. Bos JL, Fearon ER, Hamilton SR, Vries MV, van Boom JH, vander Eb A J, et al. Prevalence of ras gene mutations in human colorectal cancers. Nature, 1987. 327(6120): 293-297.
10. Imamura Y, Morikawa T, Liao X, Lochhead P, Kuchiba A, Yamauchi M, et al. Specific mutations in KRAS codons 12 and 13, and patient prognosis in 1075 BRAF wild-type colorectal cancers. Clin Cancer Res. 2012; 18(17): 4753-4763.
11. László L, Kurilla A, Takács T, Kudlik G, Koprivanacz K, Buday L, et al. Recent updates on the significance of KRAS mutations in Colorectal Cancer Biology. Cells. 2021; 10(3).
12. Goel S, Huang J, Klampfer L. K-Ras. Intestinal homeostasis and colon cancer. Curr Clin Pharmacol. 2015; 10(1): 73-81.
13. Li J, Mizukami Y, Zhang X, Jo W S, Chung D C. Oncogenic K-ras stimulates Wnt signaling in colon cancer through inhibition of GSK-3beta. Gastroenterology. 2005; 128(7): 1907-1918.
14. Roth AD, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: Results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol. 2010; 28(3): 466-474.
15. Karapetis CS, Ford SK, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med. 2008; 359(17): 1757-1765.
16. Tejpar S, Celik I, Schlichting M, Sartorius U, Bokemeyer C, Van Cutsem E. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. J Clin Oncol. 2012; 30(29): 3570-3577.
17. Dahabreh I J, Terasawa T, Castaldi P J, Trikalinos T A. Systematic review: Anti-epidermal growth factor receptor treatment effect modification by KRAS mutations in advanced colorectal cancer. Ann Intern Med. 2011; 154(1): 37-49.
18. Ibrahim EM, Zekri JM, Bin Sadiq BM. Cetuximab-based therapy for metastatic colorectal cancer: A meta-analysis of the effect of K-ras mutations. Int J Colorectal Dis. 2010; 25(6): 713-721.
19. Jonker DJ, Karapetis CS, Harbison C, O'Callaghan CJ, Tu D, Simes RJ, et al. Epiregulin gene expression as a biomarker of benefit from cetuximab in the treatment of advanced colorectal cancer. Br J Cancer. 2014; 110(3): 648-655.
20. Tsilimigras DI, Stathopoulos IN, Bagante F, Moris D, Cloyd J, Spartalis E, et al. Clinical significance and prognostic relevance of KRAS, BRAF, PI3K and TP53 genetic mutation analysis for resectable and unresectable colorectal liver metastases: A systematic review of the current evidence. Surg Oncol. 2018; 27(2): 280-288.
21. Li L, Zhou W, Li Q, Li P, Yang L, Xia X, et al. Tumor-derived mutations in postoperative plasma of colorectal cancer with microsatellite instability. Transl Oncol. 2021; 14(1): 1009-1045.
22. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002, 417(6892): 949-954.
23. Mercer KE, Pritchard CA. Raf proteins and cancer: B-Raf is identified as a mutational target. Biochim Biophys Acta, 2003; 1653(1): 25-40.
24. Marques RP, Godinho AR, Heudtlass P, Pais HL, Quintela A, Martins AP. Cetuximab versus bevacizumab in metastatic colorectal cancer: A comparative effectiveness study. J Cancer Res Clin Oncol. 2020; 146(5): 1321-34.
25. Alonso MD, Moreno FM, Sanz RG, García BM, Merino EO, Molina R, et al. Prognostic value of KRAS gene mutation on survival of patients with peritoneal metastases of colorectal adenocarcinoma. Int J Surg Oncol. 2021: 6394-6875.

26. Testa U, Pelosi E, Castelli G. Colorectal cancer: Genetic abnormalities, tumor progression, tumor heterogeneity, clonal evolution and tumor-initiating cells. Med Sci (Basel). 2018; 6(2).

27. Subramanian R R, Yamakawa A. Combination therapy targeting Raf-1 and MEK causes apoptosis of HCT116 colon cancer cells. Int J Oncol. 2012; 41(5): 1855-1862.

28. Kim KO, Park WJ, Jung Y, Lee WS. Chemotherapeutic effects of MEK kinase inhibitor and BRAF kinase inhibitor on KRAS-mutated human colon cancer cell lines with different microsatellite instability. J Chemother. 2020, 32(8): 437-444.

29. Corcoran RB, André T, Atreya CE, Schellens JHM, Yoshino T, Bendell JC, et al. Combined BRAF, EGFR, and MEK inhibition in patients with BRAF(V600E)-mutant colorectal cancer. Cancer Discov. 2018; 8(4): 428-443.

30. Yaeger R, Cercek A, O'Reilly EM, Reidy DL, Kemeny N, Wolinsky T, et al. Pilot trial of combined BRAF and EGFR inhibition in BRAF-mutant metastatic colorectal cancer patients. Clin Cancer Res. 2015; 21(6): 1313-1320.

31. Kopetz S, Grothey A, Yaeger R, Van Cutsem E, Desai J, Yoshino T, et al. Encorafenib, binimetinib, and cetuximab in BRAF V600E-mutated colorectal cancer. N Engl J Med. 2019; 381(17): 1632-1643.

32. Hong DS, Morris VK, El Osta B, Sorokin AV, Janku F, Fu S, et al. Phase IB study of vemurafenib in combination with irinotecan and cetuximab in patients with metastatic colorectal cancer with BRAFV600E mutation. Cancer Discov. 2016, 6(12): 1352-1365.

33. Van Geel R, Tabernero J, Elez E, Bendell J C, Spreafico A, Schuler M, et al. A phase Ib dose-escalation study of encorafenib and cetuximab with or without alpelisib in metastatic BRAF- omutant clorectal cancer. Cancer Discov. 2017; 7(6): 610-9.

34. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development [J]. N Engl J Med. 1988; 319(9): 525-532.

35. Hanahan D, Weinberg R A. Hallmarks of cancer: The next generation. Cell. 2011; 144(5): 646-674.

36. Miyoshi Y, Nagase H, Ando H, Horii A, Ichii S, Nakatsuru S, et al. Somatic mutations of the APC gene in colorectal tumors: Mutation cluster region in the APC gene. Hum Mol Genet. 1992; 1(4): 229-233.

37. Nagase H, Nakamura Y. Mutations of the APC (adenomatous polyposis coli) gene. Hum Mutat. 1993; 2(6): 425-434.

38. Serra S, Chetty R. Rnf43. J Clin Pathol, 2018; 71(1): 1-6.

39. Zhao M, Mishra L, Deng CX. The role of TGF-Ⅱ/SMAD4 signaling in cancer. Int J Biol Sci, 2018; 14(2): 111-123.

40. Tsukiyama T, Zou J, Kim J, Ogamino S, Shino Y, Masuda T, et al. A phospho-switch controls RNF43-mediated degradation of Wnt receptors to suppress tumorigenesis. Nat Commun. 2020; 11(1): 45-86.

41. Rikitake M, Fujikane R, Obayashi Y, Oka K, Ozaki M, Hidaka M. MLH1-mediated recruitment of FAN1 to chromatin for the induction of apoptosis triggered by O(6)-methylguanine. Genes Cells. 2020, 25(3): 175-186.

42. Yanamadala S, Ljungman M. Potential role of MLH1 in the induction of p53 and apoptosis by blocking transcription on damaged DNA templates. Mol Cancer Res. 2003; 1(10): 747-754.

43. Pussila M, Törönen P, Einarsdottir E, Katayama S, Krjutškov K, Holm L, et al. Mlh1 deficiency in normal mouse colon mucosa associates with chromosomally unstable colon cancer. Carcinog. 2018; 39(6): 788-797.

44. Wilson AJ, Byun DS, Nasser S, Murray LB, Ayyanar K, Arango D, et al. HDAC4 promotes growth of colon cancer cells via repression of p21. Mol Biol Cell. 2008; 19(10): 4062-4075.

45. Zhang X, Qi Z, Yin H, Yang G. Interaction between p53 and Ras signaling controls cisplatin resistance via HDAC4- and HIF-1α-mediated regulation of apoptosis and autophagy Theranostics. 2019; 9(4): 096-114.