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Original Article

P53 mutations occur more commonly than *KRAS* mutations in colorectal adenoma

Wen-Feng Yan[1], Gang Wu[1], Pei-Chun Sun[1], Dong Qiu[2]

[1]*Department of General Surgery, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou 450003, Henan Province, P. R. China;* [2]*Department of Clinical Laboratory, Henan Medical College, Zhengzhou 451191, Henan Province, P. R. China*

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Abstract: *TP53* and *KRAS* mutations are commonly found in colorectal tumors. The rates of mutation of these two genes in colorectal carcinoma were compared to better understand their contribution to the disease. Here, colorectal tissue samples were obtained from 49 patients with colorectal adenoma, 90 patients with single primary colorectal carcinoma, 32 patients with multiple primary colorectal carcinoma, and 50 healthy individuals. Real-time PCR was used to amplify exons 5-8 of *TP53* and codons 12-13 (exon 1) of *KRAS* from each sample. Clinical and pathological features of tumor samples were recorded, and these features were compared against mutation status using multivariate logistic regression. The proportions of samples with mutations of *KRAS* and/or *TP*53 were significantly different between control individuals and those with colorectal lesions ($P < 0.05$). Indeed, more than 80% of carcinoma samples were positive for either a *KRAS* or *TP53* mutation. Further, mutations in *KRAS* and/or *TP53* were significantly more common among the two groups with confirmed carcinoma than in individuals with colorectal adenoma ($P < 0.05$). Interestingly, *TP53* mutations were significantly more frequent than *KRAS* mutations in the colorectal adenoma group ($P < 0.01$). However, no associations were observed for the frequency of *KRAS* or *TP53* mutations between well-differentiated and poorly-differentiated tumors, different tumor stages, or other clinical and pathological features like age, sex, family history, tumor location, and stage and grade of differentiation. In conclusion, *KRAS* and *TP53* mutations are important contributors to colorectal cancer, and *TP53* mutation appears to occur earlier than *KRAS* mutation.

Keywords: Colorectal cancer, KRAS, TP53, p53, mutation

Introduction

Colorectal cancer (CRC) is diagnosed in approximately one million people annually, and it has one of the highest cancer-related mortalities, with 694,000 deaths reported globally in 2012 [1, 2]. The precancerous lesion known as colorectal adenoma often develops into CRC, with a higher probability of malignancy the younger the patient. Later, CRC is classified based on the number of sites of origin as either primary colorectal carcinoma (PCC) or multiple primary colorectal carcinoma (MPCC). MPCC has 2 or more separate primary foci that occur in the colon and rectum [3]. Although routine screening has been implemented for people over age 50 to improve diagnosis, its high-and increasing-incidence, low complete resection rate, and high postoperative relapse rate, make CRC one of the most serious malignancies [4]. New insights to the etiology of CRC are needed to aid in the diagnosis and treatment of the disease to improve patient outcomes.

A number of factors, including age, family history, diet, alcohol consumption, and smoking have been associated with CRC risk [5]. Heritable gene mutations, usually those that inactivate tumor suppressor genes or activating oncogenes, can result in the development of colorectal adenomas [6, 7]. In particular, mutations of the *TP53* tumor suppressor gene and *KRAS* oncogene contribute to more than 80% of cases of CRC [8]. *TP53* encodes the tumor suppressor protein p53, which is a cell cycle regulator; mutations in *TP53* leading to CRC commonly occur in exons 5-8 [9]. *KRAS* encodes a GTPase that recruits other protein in mitogen-

activated protein kinase signaling; mutations in *KRAS* related to CRC are commonly found in codons 12 and 13 within exon 1 [10]. Understanding the contributions of these mutations to CRC etiology can aid in the development of tailored therapies to combat this malignancy.

To better understand the contributions of *TP53* and *KRAS* mutations to CRC, we selected sequenced *KRAS* codons 12 and 13 and *TP53* exons 5-8 in samples of normal colorectal tissue, colorectal adenoma, primary colorectal carcinoma, and multiple primary colorectal carcinomas. The mutation rates of these genes were compared across samples and analyzed for any associations with clinical or pathological features.

Samples and methods

Samples

Tissue specimens were obtained from 171 patients undergoing surgical treatment for colorectal lesions in Henan Province People's Hospital between January 2003 and June 2013. Of these, 49 patients had colorectal adenoma, 90 had primary colorectal carcinoma, and 32 had multiple primary colorectal carcinoma. For the control group, paraffin-embedded samples of normal colorectal tissues were obtained from the Department of Pathology during same period; these specimens were obtained from 50 patients who received enteroscopic biopsy but were confirmed to have normal mucosa. Participants in the control group were free of genetic disorders. Cancer patients did not receive any preoperative anti-cancer therapy including chemotherapy, radiotherapy, or biological treatment, and their diagnoses were confirmed by pathologists. This study was approved by the Ethics Committee of Henan Province People's Hospital.

Clinical records

Clinical and demographic data were collected from all participants. These records included age, sex, family history of colorectal cancer, tumor site(s), degree of tumor differentiation, and tumor stage. Patients with family history of colorectal cancer were defined, based on published guidelines, as those whose relatives on a direct paternal or maternal line had a primary malignant tumor of the digestive tract [11].

DNA extraction and amplification of target genes

DNA was extracted from the specimens strictly according to the instructions of the DNA extraction kit (Bioleaf Biotech, Shanghai, China). Methods for synthesizing primers specific to *KRAS* codons 12 and 13 and for amplifying *TP53* exons 5-8 were performed according to the methods of Herring et al [12]. All primers was designed and synthesized by Sangon Biotech (Shanghai). Primer sequences used and anticipated products were as follows: *TP53* exon 5, TCCCCTGCCCTCAACAAGAT (sense strand), TCACCATCGCTATCTGAGCA (antisense), 185 bp; *TP53* exon 6, TCCTCACTGATTGCTCTTAG (sense), AGTTGCAAACCAGACCTCAG (antisense), 148 bp; *TP53* exon 7, GCCTGTGTTATCTCCTAGGT (sense), CAAGTGGCTCCTGACCTGGA (antisense) 143 bp; *TP53* exon 8, CCTATCCTGAGTAGTGGTAA (sense), GGTGAGGCTCCCCTTTCTT (antisense), 121 bp; *KRAS* codons 12 and 13, AGGCCTGCTGAAAATGACTG (sense), CTATTGTTGGATCATATTCG (antisense), 180 bp. Positive control template was the DNA product extracted from normal tissues, and negative control template was sterile deionized water. Reactions were performed in a total of volume of 20 mL, which contained 2 mL of 10x PCR Buffer, 0.1 mL of Taq DNA polymerase, 2 mL of dNTP mix (10 mM), 12.9 mL of sterile deionized water, 1 mL of template DNA, 1 mL of upstream primer, and 1 mL of downstream primer. The negative control received the same amount of sterile deionized water in place of DNA. *KRAS* was amplified under the following conditions: 55 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s; and extension at 72°C for 5 min. *TP53* was amplified under the following conditions: 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min; and extension at 72°C for 10 min. PCR products were stained with bromophenol blue (4 mL:1 mL) and isolated by agarose gel electrophoresis for detection with a UV analyzer. Products were submitted to Sangon Biotech for sequencing. If one or more mutations was identified in *KRAS* codons 12-13 or *TP53* exons 5-8 within one sample, then the sample was considered positive for a mutation in that gene.

Table 1. Clinical and pathological features in the study population [$\bar{x}\pm s$ or n (%)]

Characteristic	Control (n=50)	Colorectal adenoma (n=49)	Single primary colorectal cancer (n=90)	Multiple primary colorectal cancer (n=32)	*F* or χ^2	*P*
Age, years	60.48±8.15	61.15±8.30	62.34±8.47	62.10±8.43	2.11	0.113
Sex					1.14	0.768
Male	26 (52.00)	21 (42.86)	46 (51.11)	15 (46.88)		
Female	24 (48.00)	28 (57.14)	44 (48.89)	17 (53.13)		
Family history					0.40	0.819
Yes	/	16 (32.65)	25 (27.78)	10 (31.25)		
No	/	33 (67.35)	65 (72.22)	22 (68.75)		
Tumor location					0.46	0.793
Colon	/	19 (38.78)	39 (43.33)	12 (37.50)		
Rectal colon	/	30 (61.22)	51 (56.67)	20 (62.50)		
Differentiation					0.04	0.849
Poor	/	/	69 (76.67)	24 (75.0)		
Well	/	/	21 (23.33)	8 (25.00)		
Stage					0.30	0.587
I-II	/	/	18 (20.00)	5 (15.63)		
III-IV	/	/	72 (80.00)	27 (84.38)		

Table 2. Proportion of samples with *KRAS* or *TP53* mutation by group [n (%)]

Group	*KRAS*	*TP53*	χ^2	*P*
Control (n=50)	0 (0.00)	0 (0.00)	/	/
Colorectal adenoma (n=49)	9 (18.37)*	22 (44.90)*	7.97	0.005
Single primary colorectal cancer (n=90)	37 (41.11)*,#	38 (42.22)*	0.02	0.880
Multiple primary colorectal cancer (n=32)	16 (50.00)*,#	14 (43.75)*	0.25	0.616

Note: *vs Control $P < 0.05$; #vs Colorectal adenoma $P < 0.05$.

Statistical analysis

Double data entry was performed using EpiData version 3.1 to create a data bank, and logic checks were performed. SAS 9.2 (SAS Institute) was used to analyze the data by analysis of variance, chi-square test, and unconditional logistic regression. $P < 0.05$ was considered to indicate that a difference was statistically significant.

Results

Clinical features of study population

Clinical and demographic features of the study population are presented in **Table 1**. No statistical differences were observed for participant age or sex between the control group, the colorectal adenoma group, the primary colorectal carcinoma group, and the multiple primary colorectal carcinoma group. Further, no differences were detected between the non-control groups (adenoma, PCC, MPCC) for family history or tumor site (each *P* value > 0.05), and no differences were detected between the two cancer groups in terms of the degree of tumor differentiation and the stage of tumor (each *P* value > 0.05).

Mutation status of KRAS and TP53 in colorectal tissue samples

Sequencing of *KRAS* codons 12-13 and *TP53* exons 5-8 identified was performed for samples in each group. The proportion of samples positive for a mutation in either gene was determined for each group (**Table 2**). The proportion of samples with a mutation in *KRAS* increased across groups, from 0% of normal colorectal tissues to 50% of multiple primary colorectal carcinomas; these differences were statistically significant (each *P* value < 0.05). Further, the proportion of *KRAS*-positive samples was significantly higher in both primary colorectal carcinomas and multiple primary colorectal carcinomas than in colorectal adenomas (each *P* value < 0.05). However, no significant difference was detected in proportion of positive samples between primary colorectal carcino-

Table 3. The proportion of *KRAS* or *TP53* mutations by tumor features of single primary colorectal cancer [n (%)]

Characteristic	*KRAS*	χ^2	P	*TP53*	χ^2	*P*
Differentiation		0.36	0.55		0.31	0.580
Poor (n=69)	28 (40.58)			31 (44.93)		
Well (n=21)	7 (33.33)			8 (38.10)		
Stage		0.42	0.519		0.05	0.831
I-II (n=18)	6 (33.33)			8 (44.44)		
III-IV (n=72)	30 (41.67)			30 (41.67)		

Table 4. The proportion of *KRAS* or *TP53* mutations by tumor features of multiple primary colorectal cancers [n (%)]

Characteristic	*KRAS*	χ^2	*P*	*TP53*	χ^2	*P*
Differentiation			0.681*			0.433*
Poor (n=24)	7 (29.17)			8 (33.33)		
Well (n=8)	3 (37.50)			4 (50.00)		
Stage			0.626*			0.631*
I-II (n=5)	1 (20.00)			3 (60.00)		
III-IV (n=27)	11 (40.74)			11 (40.74)		

Note: *Fishers' exact test.

mas and multiple primary colorectal carcinomas (*P* > 0.05). Similar results were observed for mutation in *TP53*, although the differences between colorectal adenomas and primary colorectal carcinomas or multiple primary colorectal carcinomas were not statistically significant (each *P* value > 0.05). In colorectal adenomas, *TP53* mutation was significantly more common than *KRAS* mutation (P < 0.01). In contrast, no differences in mutation rates between these genes were found in any other group.

KRAS and TP53 mutations in various stages of colorectal cancer

An analysis was performed to determine whether the mutation rates of *KRAS* and *TP53* genes in primary colorectal carcinomas and multiple primary colorectal carcinomas varied by tumor features such as differentiation degree or tumor stage (**Tables 3**, **4**). No significant differences in mutation rate of either gene were observed between well differentiated and poorly differentiated tumors, or between stage I/II and stage III/IV tumors.

Correlation between clinical factors and KRAS and TP53 mutations

Due to a limited sample size of multiple primary colorectal carcinoma specimens, correlation between various clinical factors and *KRAS*/*TP53* mutations were only analyzed for adenoma and primary colorectal carcinoma (**Tables 5**, **6**). Logistic regression analysis failed to identify any significant correlation between patients' age, sex, family history, or tumor sites and *KRAS* and *TP53* mutations in adenoma and primary colorectal carcinoma (each *P* value > 0.05). Further, the differentiation degree and stage of tumor were not risk factors for *KRAS* and *TP53* mutations in primary colorectal carcinoma (each *P* value > 0.05).

Discussion

KRAS and *TP53* mutations are well known for their contributions to colorectal cancer; any number of mutations in these genes can initiate tumorigenesis, not only in colorectal tissues, but in other organs as well [13-15]. The GTPase KRAS acts like a molecular switch regulating signal transduction pathways; however, mutations that upregulate KRAS result in excessive cell growth and proliferation, promoting oncogenesis [16, 17].

The tumor suppressor p53 regulates the cell cycle to prevent uncontrolled cell growth and proliferation; mutations in *TP53* can result in overexpression of p53 that results in tumorigenesis. *TP53* mutations are less common in well-differentiated adenomas than in colorectal cancer, suggesting an important contribution of p53 to the etiology of colorectal cancer [18]. Indeed, Mohamadkhani et al. demonstrate that p53 can induce p21 expression to inhibit the growth of tumor cells, but that mutations in TP53 prohibit this interaction [19].

The current study found that *KRAS* and *TP53* exhibited mutations in precancerous colorectal adenoma tissues. Mutations were more common in primary colorectal carcinomas and multiple primary colorectal carcinomas. These findings underscore the importance of mutations in *KRAS* and *TP53* in the etiology colorectal cancer. Moreover, our findings are consistent with a previous study indicating that *TP53* mutations occur in 50-80% of colorectal can-

Table 5. Logistic regression analysis to detect association of *KRAS* or *TP53* mutation with the clinicopathologic features of colorectal adenomas

Characteristic	*KRAS* mutation				*TP53* mutation			
	β	S_x	*P*	*OR*	*β*	S_x	*P*	*OR*
Age	-0.209	1.130	0.932	0.801	0.038	0.804	0.981	1.106
Sex	-0.214	1.126	0.917	0.798	0.684	0.796	0.572	1.810
Family history	0.313	1.129	0.855	1.273	0.715	0.846	0.404	2.245
Tumor location	-0.201	0.994	0.893	0.911	0.402	0.799	0.708	1.630

Table 6. Logistic regression analysis to detect association of *KRAS* or *TP53* mutationwith the clinicopathologic features of single primary colorectal cancer

Variables	*KRAS* mutation				*TP53* mutation			
	β	S_x	*P*	*OR*	*β*	S_x	*P*	*OR*
Age	0.021	0.547	0.990	1.104	0.125	0.509	0.836	1.173
Sex	-0.003	0.439	0.995	0.107	-0.194	0.507	0.692	0.880
Family history	0.198	0.561	0.695	0.814	-0.241	0.552	0.679	0.823
Tumor location	0.176	0.503	0.743	1.203	0.031	0.505	0.980	1.134
Differentiation	0.158	0.592	0.837	1.181	0.030	0.612	0.987	1.132
Stage	0.186	0.639	0.799	1.251	-0.010	0.613	0.992	0.998

cers, but that there is no correlation between mutation and histological types of adenocarcinoma [19]. Similar to findings from another study [20], in our population *TP53* mutation was more common in precancerous lesions than *KRAS* mutation. This suggests that detection of p53 expression may help predict a tendency toward malignancy in patients with colorectal adenomas.

In our study, no difference in mutation rate of *KRAS* or *TP53* was detected between well differentiated and poorly differentiated tumors or between stage I/II and stage III/IV tumors. Further, patient sex, age, family history, and tumor site were not correlated with *KRAS* and *TP53* mutations in adenoma and primary colorectal carcinoma, and the tumor differentiation degree and stage were not risk factors for *KRAS* and *TP53* mutations in primary colorectal carcinoma. The lack of difference in mutation rates between primary colorectal carcinomas and multiple primary colorectal carcinomas is consistent with the findings of Katsuhiko et al. [21].

In sum, this study confirmed that *KRAS* and *TP53* mutations are frequent in colorectal cancer. Further, *TP53* mutations appear to occur earlier than *KRAS* gene mutations, as indicated by more frequent *TP53* mutations in precancerous tissues. This suggests that p53 expression may be a useful early biomarker for malignancy. The clinical application of gene mutations in colorectal cancer must be further explored.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Dong Qiu, Department of Clinical Laboratory, Henan Medical College, 8 Xinzhenglonghu Zhenshuanghudadao, Zhengzhou 451191, Henan Province, P. R. China. E-mail: DongQiu451191@163.com

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