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CASE REPORT

ABOUT COVER

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AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 642 experts in gastroenterology and hepatology from 59 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

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ORIGINAL ARTICLE

Retrospective Study

PIK3CA and *TP53* mutations predict overall survival of stage Ⅱ/Ⅲ colorectal cancer patients

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Author contributions: Li AJ, Lin MB and Yin L designed the research; Li AJ, Li HG, Chen Y and Jiang HH performed the research; Li AJ, Wu W, Tang EJ and Li HG contributed to the analysis; Lin MB, Chen Y and Yin L supervised the study; Li AJ and Li HG wrote the paper.

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Abstract

AIM

To investigate the predictive value of *PIK3CA* and *TP53* mutation status in colorectal cancer (CRC) patients treated with 5-fluorouracil-based chemotherapy.

METHODS

In this study, a total of 315 patients with histologically proven CRC were enrolled from Yangpu Hospital affiliated to Shanghai Tongji University between 2007 and 2011. Of these patients, 241 with stage Ⅱ/Ⅲ CRC received 5-fluorouracil-based adjuvant chemotherapy. Formalin-fixed paraffin-embedded lesion samples of the patients with curatively resected CRC were collected.

Next-generation sequencing was performed to identify somatic gene mutations. The correlation of *PIK3CA* and *TP53* mutation status with overall survival (OS) was analyzed using a Cox proportional hazard model and the Kaplan-Meier method.

RESULTS

Among the 241 patients with stage Ⅱ/Ⅲ in this cohort, the *PIK3CA* and/or *TP53* mutation was detected in 177 patients, among which 54 patients had *PIK3CA* and *TP53* double mutations. The *PIK3CA* or *TP53* mutation was not significantly correlated with OS in univariate and multivariate analyses. Compared with patients without *PIK3CA* and *TP53* mutations, those with double *PIK3CA*-*TP53* mutations showed a significantly worse survival (univariate HR = 2.21; 95%CI: 1.15-4.24; multivariate HR = 2.02; 95%CI: 1.04-3.91). The *PIK3CA* mutation located in the kinase domain showed a trend toward a shorter OS compared with wild-type tumors (multivariate HR = 1.56; 95%CI: 1.00-2.44; P = 0.052). The Kaplan-Meier curve showed that patients harboring the *PIK3CA* mutation located in the kinase domain had a worse clinical outcome than those with wild-type status (Log-rank P = 0.041)

CONCLUSION

Double mutation of *PIK3CA* and *TP53* is correlated with a shorter OS in stage Ⅱ/Ⅲ CRC patients treated with 5-fluorouracil-based therapy.

Key words: Overall survival; Colorectal cancer; *PIK3CA*; *TP53*; 5-fluorouracil; Double mutation

Core tip: Targeted next-generation sequencing was used to detect gene mutations rather than mutational hotspots in the present study. This manuscript is by far the first to report the predictive value of the combined mutation status of *PIK3CA* and *TP53* in colorectal cancer patients receiving 5-FU-based adjuvant chemotherapy. Our data revealed that the double mutation of *PIK3CA* and *TP53* is an independent predictive factor for overall survival in stage Ⅱ/Ⅲ patients receiving 5-FU-based chemotherapy.

Li AJ, Li HG, Tang EJ, Wu W, Chen Y, Jiang HH, Lin MB, Yin L. *PIK3CA* and *TP53* mutations predict overall survival of stage Ⅱ/Ⅲ colorectal cancer patients. *World J Gastroenterol* 2018; 24(5): 631-640 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i5/631.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i5.631

INTRODUCTION

Colorectal cancer (CRC) is now the third most common cancer worldwide[1]. The genesis of colorectal cancer is a multi-step, multi-gene process, receiving a great deal of interactive influence from genetic and environmental factors. Attributed to advancements in surgical techniques and the popularization of chemotherapy and targeted therapy, the treatment of CRC has rapidly evolved over several decades. 5-fluorouracil (5-FU)-based adjuvant chemotherapy is regarded as a first-line chemotherapy in both adjuvant and palliative settings for advanced CRC. Regrettably, however, a considerable number of patients not only fail to show an objective response to first-line chemotherapy treatment but also suffer from side effects[2,3]. Hence, studies of the biomarkers related to clinical outcome are in urgent demand as a way to identify whether patients benefit from adjuvant chemotherapy.

At present, the response rate of CRC patients receiving 5-FU-based adjuvant thermotherapy is approximately 40%-50%[3-7], and despite much research on the prediction of response, it has not been possible to identify a predictive biomarker as an indicator of the likely benefit in patients receiving 5-FU-based adjuvant chemotherapy. Thus, an early, quick, efficient, and accurate method is of great clinical significance and value to identify new predictive markers.

It is widely known that the multi-drug resistance (MDR) to anticancer drugs is the main cause of chemotherapy failure. Recent studies have indicated that activation of the PI3K/AKT signaling pathway can result in disturbance of cellular growth, proliferation, and survival in a variety of solid tumors[8] and can cause MDR of cancer cells through multiple mechanisms[9]. It is well documented that the effect of the aberrant regulation of the PI3K/AKT signaling pathway on cell growth and apoptosis induced by anti-cancer drugs was observed *in vitro* and *in vivo*[10-14]. Given the role of the PI3K/AKT signaling pathway in the development of resistance to anticancer drugs, it is conceivable that genetic mutations (such as *PIK3CA*) in the molecules of the PI3K/AKT signaling pathway could be a promising predictive biomarker for chemotherapy efficacy. *TP53*, the widely studied tumor suppressor gene which has an intimate connection with the occurrence and progression of many tumors in humans, mainly regulates several cellular processes, including cell cycle regulation, DNA repair, and apoptosis[15,16]. Multiple studies have bolstered the notion that *TP53* is correlated with the drug resistance of tumor cells and could emerge as a biomarker with predictive value and potential clinical utility[17-20]. Thus, we proposed that *PIK3CA* and *TP53* mutation status is likely well associated with clinical outcome in patients undergoing chemotherapy.

In the present study, we evaluated the predictive value of *PIK3CA* and *TP53* mutation status in CRC patients undergoing 5-FU-based chemotherapy after curative surgery and identified subgroups of patients

who greatly benefited from specific treatment regimens.

MATERIALS AND METHODS

Study population

In this study, a total of 315 patients with histologically proven CRC were enrolled from Yangpu Hospital affiliated to Shanghai Tongji University between 2007 and 2011. Among these patients, 241 with stage Ⅱ/Ⅲ CRC received 5-FU-based adjuvant chemotherapy as a first-line treatment for at least six cycles. Tumor staging was strictly abided by the TNM classification of the American Joint Committee on Cancer. Formalin-fixed paraffin-embedded (FFPE) lesion samples of the patients with curatively resected CRC were collected from the pathology department where the primary colorectal tumors from the patients in this study were preserved. Histopathological diagnosis by an experienced pathologist was performed for all tissue samples, and at least 70% of tumor cells could be observed in the whole section through light microscopy. Any case that did not meet the experimental standard was excluded from this study. The clinicopathological features, including pathologic stage, tumor location, and date of diagnosis and death, were collected. The definition of proximal colon cancer included the cecum and ascending and transverse colon, while tumors located in the splenic flexure and descending and sigmoid colon were characterized as distal colon cancer, and the rectum was defined from the rectosigmoid junction (the end of the sigmoid colon) to the dentate line. Written informed consent was obtained from all participants at the time of study enrollment. This study was approved by the Medical Ethics Committee of Yangpu Hospital.

DNA extraction and targeted next-generation sequencing

Genomic DNA was extracted from FFPE sections of the 315 tumors. The 10-μm-thick sections were subjected to standard deparaffinization procedures and proteinase K digestion overnight. Genomic DNA was isolated using a QIAamp DNA FFPE Tissue kit (Qiagen). All extracted DNA was quantified with a Qubit 3.0 fluorometer (Thermo Fisher Scientific) and Bioanalyzer 2100 (Agilent) before targeted next-generation sequencing[21,22].

Next-generation mutational analysis was performed using an Ion Torrent platform (Thermo Fisher Scientific) in CRC and matched normal tissues of 315 patients to identify somatic gene mutation profile. DNA library was generated using an Ion AmpliSeq DNA library kit (Thermo Fisher Scientific). According to the manufacturer's protocols, 10 ng of DNA was used as the template to amplify targets with sequencing primer panel (Thermo Fisher Scientific). Amplified targets were digested with Fupa enzyme and subsequently ligated with adapters. The library was quantified with a quantitative PCR kit and loaded into chips in Ion Chef (Thermo Fisher Scientific). The chips were loaded into an Ion S5 XL sequencer (Thermo Fisher Scientific) for sequencing. Sequencing data were processed and analyzed on a bioinformatics analysis server termed as Ion Reporter (Thermo Fisher Scientific)[23].

Statistical analysis

The study endpoint was overall survival (OS), which was calculated from pathologic diagnosis to death, regardless of cause. All statistical analyses were performed using Stata software (version 14.2). We used Cox proportional hazards models to estimate the hazard ratios (HRs) of survival adjusted for baseline patient variables. The Kaplan-Meier method was performed to generate a survival curve, with significance evaluated using a log-rank test. Where appropriate, categorical and continuous variables were estimated by the χ^2 test. $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

Altogether, 315 CRC patients were enrolled in this study; 14.6% (n = 46) of them had stage I CRC, while 40.6% (n = 128), 35.9% (n = 113), and 8.9% (n = 28) had stages Ⅱ, Ⅲ, and Ⅳ CRC, respectively. The clinicopathological features of the study population are summarized in Table 1. Genomic DNA from FFPE lesion samples of the patients with curatively resected CRC was screened for somatic mutations in the *PIK3CA* and *TP53* genes. Among the 315 patients, the incidence of *PIK3CA* and *TP53* mutations was 38.4% (n = 121) and 65.1% (n = 205), respectively, while wild type was detected in 61.6% (n = 194) and 34.9% (n = 110), respectively. In the study cohort, *PIK3CA*-mutated tumors were significantly correlated with proximal location (P = 0.036), while *TP53*-mutated tumors were not significantly associated with any examined clinical features. The frequency of *PIK3CA* mutations in the proximal, distal, and rectum location was 50% (39/78), 38.7% (29/75), and 32.7% (53/162), respectively.

Among the 241 patients with stage Ⅱ/Ⅲ disease in this cohort, the *PIK3CA* and/or *TP53* mutation was detected in 177 patients, of whom 54 had *PIK3CA* and *TP53* double mutations. As reported in Table 1, mutation status of the *PIK3CA* and *TP53* genes was comparable according to baseline characteristics. No significant difference was observed between these variables and baseline characteristics (Table 1).

For individual sites of *PIK3CA* mutation, the most frequent mutation in CRC was the mutation of Glu545Lys (5.1%), followed by Glu542Lys (2.2%). The most frequent *TP53* mutations included: Arg175His (5.1%), Arg282Trp (3.5%), Gly245Ser (2.9%), and Arg248Gln (2.9%) (Table 2).

Predictive value of PIK3CA or TP53 mutation in stage Ⅱ/Ⅲ CRC patients

All stage Ⅱ/Ⅲ CRC patients (n = 241) received 5-FU-

Table 1 Patient demographics and disease characteristics *n* (%)

Clinicopathological feature	*PIK3CA*		*P* value	*TP53*		*P* value	Both *PIK3CA* and *TP53* wild-type	Both *PIK3CA* and *TP53* mutation	Others	*P* value
	Wild-type	Mutation		Wild-type	Mutation					
Age (yr)										
< 60	52 (26.80)	29 (23.97)		31 (28.18)	50 (24.39)		26 (31.33)	22 (28.57)	33 (21.29)	
60-70	47 (24.23)	33 (27.27)		28 (25.45)	52 (25.37)		20 (24.10)	18 (23.38)	42 (27.10)	
⩾ 70	95 (48.97)	59 (48.76)	0.776	51 (46.36)	103 (50.24)	0.733	37 (44.58)	37 (48.05)	80 (51.61)	0.500
Sex										
Male	107 (55.15)	73 (60.33)		67 (60.91)	113 (55.12)		47 (56.63)	45 (58.44)	88 (56.77)	
Female	87 (44.85)	48 (39.67)	0.367	43 (39.09)	92 (44.88)	0.322	36 (43.37)	32 (41.56)	67 (43.23)	0.965
Tumor location										
Rectum	109 (56.19)	53 (43.80)		53 (48.18)	109 (53.17)		48 (57.83)	41 (53.25)	73 (47.10)	
Proximal	39 (20.10)	39 (32.23)		32 (29.09)	46 (22.44)		17 (20.48)	19 (24.68)	42 (27.10)	
Distal	46 (23.71)	29 (23.97)	0.036	25 (22.73)	50 (24.39)	0.426	18 (21.69)	17 (22.08)	40 (25.81)	0.601
Stage T										
T1-T2	34 (17.53)	26 (21.49)		20 (18.18)	40 (19.51)		15 (18.07)	16 (20.78)	29 (18.71)	
T3	38 (19.59)	19 (15.70)		21 (19.09)	36 (17.56)		17 (20.48)	11 (14.29)	29 (18.71)	
T4	122 (62.89)	76 (62.81)	0.539	69 (62.73)	129 (62.93)	0.923	51 (61.45)	50 (64.94)	97 (62.58)	0.884
Stage N										
N0	118 (60.82)	70 (57.85)		68 (61.82)	120 (58.54)		53 (63.86)	45 (58.44)	90 (58.06)	
N1	55 (28.35)	35 (28.93)		28 (25.45)	62 (30.24)		20 (24.10)	23 (29.87)	47 (30.32)	
N2	21 (10.82)	16 (13.22)	0.785	14 (12.73)	23 (11.22)	0.656	10 (12.05)	9 (11.69)	18 (11.61)	0.889
Stage										
Ⅰ	23 (11.86)	23 (19.01)		16 (14.55)	30 (14.63)		12 (14.46)	14 (18.18)	20 (12.90)	
Ⅱ	86 (44.33)	42 (34.71)		47 (42.73)	81 (39.51)		37 (44.58)	28 (36.36)	63 (40.65)	
Ⅲ	70 (36.08)	43 (35.54)		38 (34.55)	75 (36.59)		27 (32.53)	26 (33.77)	60 (38.71)	
Ⅳ	15 (7.73)	13 (10.74)	0.166	9 (8.18)	19 (9.27)	0.948	7 (8.43)	9 (11.69)	12 (7.74)	0.774

Table 2 Top ten mutations of *PIK3CA* and *TP53* in this study

PIK3CA	*n* (%)	*TP53*	*n* (%)
Glu545Lys	16 (5.1)	Arg175His	16 (5.1)
Glu542Lys	7 (2.2)	Arg282Trp	11 (3.5)
Val105Ile	6 (1.9)	Gly245Ser	9 (2.9)
Met1004Ile	6 (1.9)	Arg248Gln	9 (2.9)
His1047Arg	6 (1.9)	Arg273His	8 (2.5)
Glu218Lys	6 (1.9)	Arg273Cys	7 (2.2)
Trp552Ter	5 (1.6)	Arg248Trp	7 (2.2)
Ser438Phe	5 (1.6)	Ser260Phe	6 (1.9)
Pro835Leu	5 (1.6)	Glu358Lys	6 (1.9)
Asp1029Asn	5 (1.6)	Pro153Ser	5 (1.6)

based adjuvant chemotherapy for at least six cycles as first-line treatment after operation. In univariate and multivariate analyses, neither *PIK3CA* nor *TP53* mutation was significantly correlated with patient survival (Table 3). The Kaplan-Meier curve showed that patients harboring the *TP53* mutation had a worse clinical outcome than patients with wild-type status (Log-rank *P* = 0.046; Figure 1A), and no association was found between *PIK3CA* and clinical outcome (Log-rank *P* = 0.150; Figure 1B).

Predictive value of double PIK3CA-TP53 mutations in stage Ⅱ/Ⅲ CRC patients

We assessed the predictive value of double *PIK3CA*-*TP53* mutations for survival in stage Ⅱ/Ⅲ CRC patients treated with 5-FU-based chemotherapy according to the statistical results of Cox proportional hazards and Kaplan-Meier analyses. Compared with concomitant *PIK3CA* and *TP53* wild-type tumors, double *PIK3CA*-*TP53* mutations were a significantly poor predictive factor for OS (univariate HR = 2.21; 95%CI: 1.15-4.24; multivariate HR = 2.02; 95%CI: 1.04-3.91) (Table 3). In contrast, no association was found between the mutational status of a single gene, either *PIK3CA* or *TP53*, and OS (*P* = 0.629; Table 3). The Kaplan-Meier curve showed a shorter OS in patients harboring double *PIK3CA* and *TP53* mutations compared with concomitant *PIK3CA* and *TP53* wild-type patients (Log-rank *P* = 0.034; Figure 1C).

Association of PIK3CA mutation in a functional domain with clinical outcome in stage Ⅱ/Ⅲ CRC patients

In the multivariable analysis of the association of *PIK3CA* functional domain mutation with OS, no significant difference was observed. The results of multivariable analysis are shown in Table 4. As suggested by the results, the *PIK3CA* mutation located in the kinase domain showed a trend toward a shorter OS compared with wild-type tumors (multivariate HR = 1.56; 95%CI: 1.00-2.44; *P* = 0.052). The Kaplan-Meier curve showed that patients harboring the *PIK3CA* mutation located in the kinase domain had a worse clinical outcome than those with wild-type status (Log-rank *P* = 0.041; Figure 2).

DISCUSSION

CRC is one of the most common malignancies. Despite much research on biomarkers in patients with cancer, the number of biomarkers with predictive value and potential clinical utility is pitifully small[24-27]. Moreover, co-occurring genetic alterations have been detected

Table 3 Univariate and multivariate analyses (Cox proportional hazards model) of OS for patients with stage Ⅱ/Ⅲ CRC treated with 5-FU-based chemotherapy according to *PIK3CA* and/or *TP53* mutation status *n* (%)

	Alive	Dead	Univariate HR (95%CI)	*P* value	Multivariate HR (95%CI)	*P* value
PIK3CA						
Wild-type	114 (73.08)	42 (26.92)	1 (Ref.)		1 (Ref.)	
Occasional	5 (55.56)	4 (44.44)	1.93 (0.69-5.39)	0.208	1.40 (0.49-4.05)	0.530
Recurrent	48 (63.16)	28 (36.84)	1.50 (0.93-2.42)	0.096	1.29 (0.79-2.11)	0.314
TP53						
Wild-type	63 (74.12)	22 (25.88)	1 (Ref.)		1 (Ref.)	
Occasional	32 (80.00)	8 (20.00)	0.80 (0.35-1.79)	0.583	0.84 (0.37-1.94)	0.687
Recurrent	72 (62.07)	44 (37.93)	1.65 (0.99-2.76)	0.055	1.68 (0.98-2.87)	0.057
PIK3CA and *TP53*						
Both *PIK3CA* and *TP53* Wild-type	49 (76.56)	15 (23.44)	1 (Ref.)		1 (Ref.)	
Both *PIK3CA* and *TP53* Mutation	31 (57.41)	23 (42.59)	2.21 (1.15-4.24)	0.017	2.02 (1.04-3.91)	0.037
Others	87 (70.73)	36 (29.27)	1.31 (0.72-2.40)	0.376	1.16 (0.63-2.16)	0.629

Occasional mutation was defined as a single tumor with mutation of *PIK3CA* or *TP53*, while recurrent was mutations detected in two or more tumors. HR: Hazard ratio; 95%CI: 95% confidence interval; OS: Overall survival; CRC: Colorectal cancer.

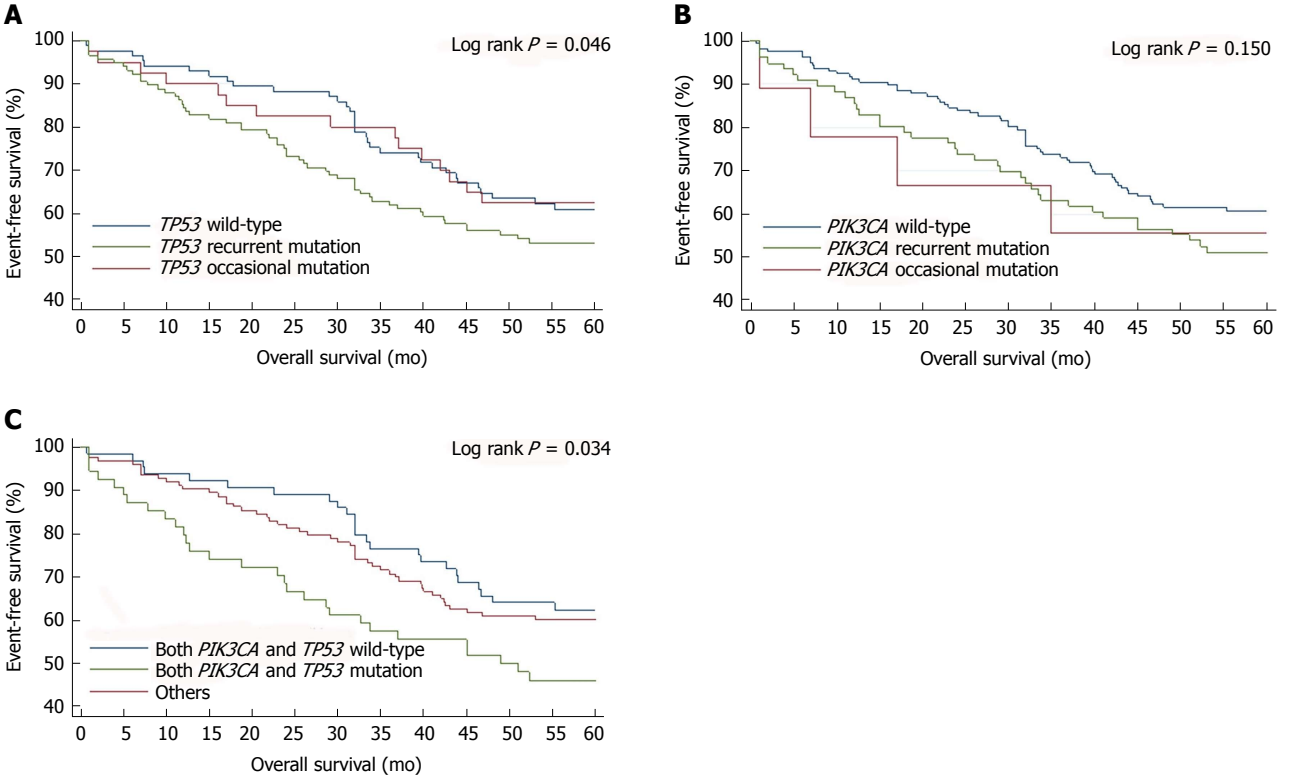

Figure 1 Kaplan-Meier survival analysis for overall survival in patients with stage Ⅱ/Ⅲ colorectal cancer according to *TP53* mutation status (A); *PIK3CA* mutation status (B); and *PIK3CA* and *TP53* mutation status (C).

in multiple malignancies[28]. In the present study, we evaluated two biomarkers with predictive value to identify subgroups of patients who would greatly benefit from 5-FU-based chemotherapy. We found that the double mutation of *PIK3CA* and *TP53* was greatly associated with worse clinical outcomes in 241 stage Ⅱ/Ⅲ CRC patients receiving 5-FU-based adjuvant chemotherapy. In contrast, mutations in *PIK3CA* or *TP53* alone had no effect on the OS of CRC patients.

Although mutations in these two genes have been widely researched, the present study is by far the first to report the predictive role of the combined mutation status of *PIK3CA* and *TP53* in CRC patients. Targeted next-generation sequencing was used to detect gene mutations rather than mutational hotspots in the present study. The frequencies of *PIK3CA* and *TP53* mutations were 35% (85/241) and 65% (156/241), respectively, which are consistent with previously published studies reporting *PIK3CA* and *TP53* mutations in 10%-32% and 40%-60% of CRC patients, respectively, in Western studies[29-32]. The present results are consistent with those of two previous studies, showing that the proximal

Table 4 Association of *PIK3CA* functional domain mutations with overall survival in stage Ⅱ/Ⅲ CRC patients *n* (%)

Domain	Alive	Dead	Multivariate HR (95%CI)	*P* value
Kinase domain				
Wild-type	116 (60.42)	76 (39.58)	1 (Ref.)	
Mutation	23 (46.94)	26 (53.06)	1.56 (1.00-2.44)	0.052
C2 domain				
Wild-type	118 (58.71)	83 (41.29)	1 (Ref.)	
Mutation	21 (52.50)	19 (47.50)	1.13 (0.68-1.86)	0.638
Helical domain				
Wild-type	110 (59.14)	76 (40.86)	1 (Ref.)	
Mutation	29 (52.73)	26 (47.27)	1.30 (0.83-2.05)	0.248
p85 binding domain				
Wild-type	127 (57.73)	93 (42.27)	1 (Ref.)	
Mutation	12 (57.14)	9 (42.86)	1.05 (0.53-2.08)	0.894
Ras binding domain				
Wild-type	123 (58.57)	87 (41.43)	1 (Ref.)	
Mutation	16 (51.61)	15 (48.39)	1.27 (0.73-2.20)	0.404

HR: Hazard ratio; 95%CI: 95% confidence interval.

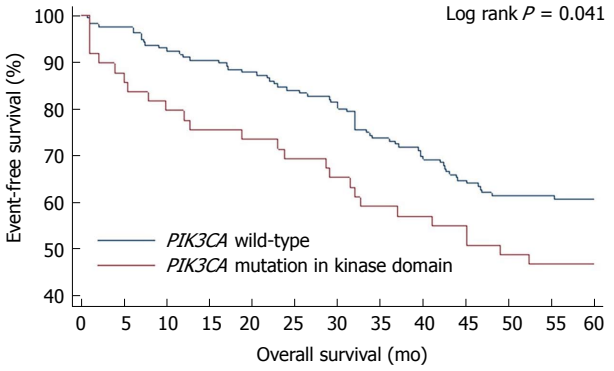

Figure 2 Kaplan-Meier survival analysis for overall survival according to the *PIK3CA* kinase domain mutation status in stage Ⅱ/Ⅲ patients.

colon showed a higher frequency of *PIK3CA* mutations than any other sites[33,34].

Several studies on the predictive roles of the *PIK3CA* mutation for response have been published[35-37]. A meta-analysis showed that the *PIK3CA* mutation in *KRAS* wild-type patients with metastatic CRC could predict responses to anti-EGFR monoclonal antibody therapy[38], while another study identified the *PIK3CA* mutation in exon 20[39]. Moreover, recent studies have suggested that the *PIK3CA* mutation might serve as a predictive biomarker for adjuvant aspirin therapy in CRC[40,41]. Thus, the *PIK3CA* mutation has a plausible role as a predictive marker for response to drugs. In clinical studies, there was no evidence that a mutation in *PIK3CA* was associated with 5-FU-based treatment benefits in colorectal cancer[42]. The present results are consistent with those of a previous study, showing that *PIK3CA* was limited as a marker for predicting responses to 5-FU-based treatment in CRC.

The inactivation of tumor suppressor genes plays a key role in tumorigenesis[43]. Obviously, the detection of *TP53* status in view of one of the most common tumor suppressor genes is important in the research of cancer[44]. *TP53* could serve as a potential biomarker for prognosis with potential predictive value and clinical utility. However, the controversial results of the available literature make it difficult to achieve a chorus of approval[45-49]. Several studies have shown that the *TP53* status was an independent predictive factor for responses to 5-FU[47,50,51], although other studies showed null association[46]. Many reasons account for these discrepant results, such as lack of reproducibility, underpowered robust statistical analysis, poor study design, and general methodological differences. There is no consensus on whether or not *TP53* emerges as a critical selection criterion to predict chemotherapy efficacy in CRC patients. Interestingly, our results showed that no association was found between clinical outcome and *TP53* status in univariate and multivariate analyses. Based on the results of the Kaplan-Meier curve, patients with wild-type *TP53* had a significantly prolonged survival compared to those harboring the *TP53* mutation.

The effect of a single gene variation as a predictive biomarker is often modest, but sometimes an additive or powerful predictive effect can be achieved by combining multiple gene alterations with the same function. Indeed, gene alteration of *PIK3CA* or *TP53* can result in cell apoptosis and drug-resistance of tumor cells through activating specific signaling pathways. Recent studies have reported that *PIK3CA* expression was correlated with the expression of *MDR-1* (encoding the MDR-associated protein P-glycoprotein)[52,53]. Additionally, the up-regulation of *MRP-1* (encoding the MDR-associated protein) induced by the activation of PI3K could cause the chemoresistance of cells in prostatic carcinomas[54]. This same regulatory mechanism also occurred in *TP53*, as studies have shown that wild-type *TP53* could serve as a negative regulator of both *MDR-1* and *MRP-1*[18,55], implicating potential associations of combined *PIK3CA*

and *TP53* with clinical outcomes to comprehend the value of their combination in predicting the benefit of patients receiving 5-FU-based chemotherapy. Interestingly, the double mutation of *PIK3CA* and *TP53* has previously been correlated with a shorter OS. Remarkably, multivariate analysis showed that this correlation was independent of age, gender, stage, and tumor location, thereby confirming that the combined analysis of *PIK3CA* and *TP53* mutation status could become a marker to identify subgroups of patients who have a poor prognosis and provide valuable information for more clinical therapy projects.

The *PIK3CA* gene is divided into five functional domains: p85 binding domain, Ras binding domain, C2 domain, helical domain, and kinase domain[30]. The main mutation of *PIK3CA* occurs in exons 9 and 20, corresponding to the helical and kinase domains, respectively[56]. Recent studies have shown that patients treated with anti-EGFR monoclonal antibodies (MoAbs) and harboring *PIK3CA* mutations in exon 20 were significantly associated with worsening outcomes in KRAS wild-type mCRC[39,57]. In the present study, the Cox proportional hazard model analysis of the effect of the *PIK3CA* mutation occurring in the kinase domain on clinical outcome reached marginally statistical significance (P = 0.052), whereas the Kaplan-Meier curve achieved statistical significance (Log-rank P = 0.041; Figure 2). The trend for statistical significance was evident for worsening clinical outcomes with mutations occurring in the kinase domain.

Thus far, with regard to research on *PIK3CA* or *TP53* mutations, obviously, the tumor samples of patients with CRC were collected from a single hospital in most studies, and even for many multi-centered clinical trials, patients were enrolled on the basis of epidemiological settings. Similarly, in the present study, we used tumor samples from a single hospital to reduce selection bias. Additionally, genetic heterogeneity is a reality of all tumors and is decreased by limiting the study to stage Ⅱ/Ⅲ patients receiving the same chemotherapy regimens. In addition, considering that a small number of total samples could lead to a less robust statistical analysis, a large sample size (n = 241) warranted adequate statistical power in the present study.

In conclusion, the present study suggests that the double mutation of *PIK3CA* and *TP53* is correlated with a shorter OS of stage Ⅱ/Ⅲ CRC patients receiving 5-FU-based therapy and hence serves as a novel biomarker to identify subgroups of patients who have poor clinical outcome, with potential clinical utility.

ARTICLE HIGHLIGHTS

Research background

5-fluorouracil (5-FU) remains one of the most effective and commonly used chemotherapeutic agents in both adjuvant and palliative settings for advanced colorectal cancer (CRC). However, many CRC patients treated with 5-FU-based adjuvant chemotherapy not only fail to show an objective response to chemotherapy treatment but also suffer from side effects. Therefore, predictive markers are in urgent demand to identify whether patients can benefit from adjuvant chemotherapy.

Research motivation

Multiple studies have indicated that *PIK3CA* and *TP53* mutation status was correlated with drug resistance of tumor cells. By analyzing the associations between mutation status of these two genes and overall survival (OS), we may be able to identify subgroups of patients who have a poor prognosis, which can help clinicians make suitable treatment of patients.

Research objectives

The objectives of this study were to detect gene mutations of *PIK3CA* and *TP53* by using targeted next-generation sequencing (NGS) in a large cohort of CRC patients, and to investigate the predictive value of the mutational status of *PIK3CA* and *TP53*, alone or in combination.

Research methods

A total of 315 patients with histologically proven CRC between 2007 and 2011 were retrospectively analyzed. Formalin-fixed paraffin-embedded lesion samples of the patients with curatively resected CRC were collected from the pathology department. Ten-μm-thick sections from FFPE tumor samples were used for DNA extraction with a QIAamp DNA FFPE Tissue kit (Qiagen). Targeted NGS was performed using the Ion Torrent platform to characterize the mutational spectrum of *PIK3CA* and *TP53* genes. The distribution of gene mutation according to clinicopathologic variables was analyzed using Chi-square tests. The associations between mutation status of *PIK3CA* and *TP53* and OS were evaluated using Cox proportional hazards models adjusted for clinicopathologic variables. The Kaplan-Meier method was performed to generate a survival curve, with significance evaluated using a log-rank test.

Research results

Among the 315 patients, the incidence of *PIK3CA* and *TP53* mutations was 38.4% (n = 121) and 65.1% (n = 205), respectively. A significant difference was observed in the distribution of *PIK3CA* mutations according to tumor location (P = 0.036). The frequency of *PIK3CA* mutations in the proximal, distal, and rectum location was 50% (39/78), 38.7% (29/75), and 32.7% (53/162), respectively. The *PIK3CA* and/or *TP53* mutation was detected in 177 out of 241 patients with stage Ⅱ/Ⅲ CRC receiving 5-FU-based adjuvant chemotherapy, of whom 54 had *PIK3CA* and *TP53* double mutations. In both univariate and multivariate analyses, neither *PIK3CA* nor *TP53* mutation was significantly correlated with OS. In Kaplan-Meier survival curve, patients with *TP53* mutation had a worse clinical outcome than patients with wild-type *TP53* (Log-rank P = 0.046). Compared with patients without *PIK3CA* and *TP53* mutations, those with double *PIK3CA*-*TP53* mutations had a significantly poorer OS (univariate HR = 2.21; 95%CI: 1.15-4.24; multivariate HR = 2.02; 95%CI: 1.04-3.91). In contrast, the presence of a single gene mutation, either *PIK3CA* or *TP53*, was not significantly associated with OS. The Kaplan-Meier curve showed that shorter OS was detected in patients harboring double *PIK3CA* and *TP53* mutations (Log-rank P = 0.034). In Kaplan-Meier survival curve, patients harboring the *PIK3CA* mutation located in the kinase domain experienced a significantly shorter OS when compared with wild-type status (Log-rank P = 0.041).

Research conclusions

This study is by far the first to report the predictive role of the combined mutation status of *PIK3CA* and *TP53* in CRC patients receiving 5-FU-based adjuvant chemotherapy. Our data revealed that the double mutation of *PIK3CA* and *TP53* is correlated with a shorter OS of stage Ⅱ/Ⅲ CRC patients receiving 5-FU-based therapy and hence serves as a novel biomarker to identify subgroups of patients who have poor clinical outcome, with potential clinical utility.

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