Novel somatic mutations of *PIK3CA* in patients with colorectal cancer

CHITTIBABU VATTE[1], CYRIL CYRUS[1], SHAHANAS CHATHOTH[1], AHMED ALSAYYAH[2], NADA JABER ALDOSSARY[2], HASSAN MOHAMMED ALBISHER[3], RUDAYNAH A. ALALI[4], MOHAMMED ABDULLAH ALRUBAISH[5] and ALI M. AL AMRI[4]

[1]Department of Biochemistry, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam 31441, Kingdom of Saudi Arabia; [2]Department of Pathology, King Fahd Hospital of The University, Imam Abdulrahman Bin Faisal University, Al Khobar 34445, Kingdom of Saudi Arabia; [3]Department of General Surgery, College of Medicine, Imam Abdulrahman Bin Faisal University, Al Khobar 34445, Kingdom of Saudi Arabia; [4]Department of Internal Medicine, King Fahd Hospital of The University, Imam Abdulrahman Bin Faisal University, Al Khobar 34445, Kingdom of Saudi Arabia; [5]Department of Ophthalmology, King Fahd Hospital of The University, Imam Abdulrahman Bin Faisal University, Al Khobar 34445, Kingdom of Saudi Arabia

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Abstract. Colorectal cancer (CRC) is a heterogenous disease with varying genetic and epigenetic backgrounds. The phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) gene encodes for the catalytic subunit p110α of phosphatidylinositol 3-kinase (PI3K). Determining the frequency of *PIK3CA* mutations in CRC in different populations may enhance the current understanding of the pathogenicity of CRC and may aid in the prognosis of affected patients. The present study included 71 male and female patients with clinically confirmed CRC (58 colon cancer cases and 13 rectal cancer cases). Mutations in exon 9 and 20 of the *PIK3CA* gene were identified using Sanger capillary sequencing. A total of 6 patients carried mutations in either exon 9 or 20 of the *PIK3CA* gene. The majority of the colon tumors (83.3%) with the *PIK3CA* mutation presented at an advanced stage of the disease and the mutations were found more frequently among women (66.6%). All missense mutations (E545A and L1001V) and novel mutations (two) were only observed in the samples from female patients. The two novel *PIK3CA* gene mutations that were identified in the present study are a missense mutation leading to a c.3001C>G, p.L1001V amino acid change and a nonsense mutation c.3153 G>A, W1051Ter leading to protein termination in exon 20. The patients with these mutations presented with a poor prognosis. The novel mutations identified in the kinase domain of PIK3CA may induce the hyperactivation of the protein in the PI3K pathway, leading to a poor prognosis. CRC screening is recommended at an early age (>40 years) for all patients, but particularly for females who carry these mutations.

Correspondence to: Mr. Chittibabu Vatte, Department of Biochemistry, College of Medicine, Imam Abdulrahman Bin Faisal University, P.O. Box 1982, Dammam 31441, Kingdom of Saudi Arabia
E-mail: cbvatte@iau.edu.sa

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Introduction

The incidence of colorectal cancer (CRC), which affects the colon, large intestine and rectum, is increasing globally, particularly in developing countries (1). In addition, the incidence and mortality rates due to CRC are expected to increase in the coming decades (2). As per global cancer statistics (2020), the incidence of CRC (in males and females) was 10%, the third highest after breast and lung cancers. From a mortality perspective, CRC is the second leading cause of cancer-related death (9.4%) (3). According to the 2018 Cancer Incidence Report, the most common cancers among Saudi nationals were breast cancer (17.9%) and CRC (12.2%). In CRC, the occurrence of colon cancer (males, 60.2%; females, 64.9%) is higher than that of rectal cancer (4). A report by the World Health Organization, stated that the incidence and mortality rates associated with CRC in Saudi Arabia were estimated to be 14.6 and 15.2%, respectively (5).

The first line of defense in the standard treatment plan for the CRC is surgery, along with chemotherapy and radiotherapy as adjuvant and neoadjuvant settings to reduce the tumor mass and stabilize the tumor (6). However, 25% of CRC cases are not manifested until the advanced stages of the disease. In such instances, surgical treatment alone may not result in a positive prognosis and may lead to death (7). Notably, targeted therapy in the form of immunotherapy enhances the overall survival rate of patients with CRC. Several pathways and checkpoints have been identified, and targeted agents have been developed against specific pathways or proteins to control disease progression. The Food and Drug Administration has approved agents to target specific proteins, such as vascular endothelial

growth factor receptor, epidermal growth factor receptor (EGFR) and programmed cell death protein 1 (8).

It has been well established that genetic and environmental factors increase the risk of developing CRC. However, patients with Crohn's disease and ulcerative colitis are at a greater risk of developing CRC, which increases with age (9). Several studies have demonstrated that chronic inflammation, family history, lifestyle factors (such as a sedentary lifestyle, regular alcohol consumption and smoking) and diet (such as the frequent consumption of red meat and processed meats) are major risk factors for the development of CRC (10). CRC is genetically diverse, involving various pathways and mechanisms in its development. CRC cells consist of several mutations and different gene expression profiles (11). Several genomic aberrations, such as genetic mutations in the BRAF, NRAS and KRAS genes, are associated with resistance to treatment with EGFR antibody. This phenomenon has raised the issue of selecting suitable agents in the treatment of metastatic CRC (12). Several CRC studies have reported genetic alterations in the genes of various pathways, including p53, TGF-β, WNT-β-catenin, EGFR, MAPK and phosphatidylinositol 3 kinase (PI3K)/Akt (13-18).

Previous research has demonstrated the association between genetic mutation profiles and the prognosis of patients with CRC (19). In CRC, the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), *KRAS* and *BRAF* genes may be of prognostic significance (20,21). The *PI3KCA* gene encodes for p110α, a catalytic subunit of PI3K. PI3K functions as a phosphorylating agent for downstream signaling molecules, which play a key role in the pathogenesis of several types of cancer, including CRC, by regulating cell growth and proliferation via the Akt-mTOR signaling pathway (21). *PIK3CA* mutations are observed in various human cancers, including breast, head and neck squamous cell carcinoma, colorectal, endometrial, brain, ovarian, lung, thyroid and cervical cancers, with varying frequencies (22-24). However, additional studies are required to explore the somatic mutations of the genes involved in the development of CRC, including *PIK3CA*, and their association with the pathogenesis of CRC and the prognosis of affected patients. An in-depth understanding of the gene mutation profiles in these pathways, the association of these pathways with CRC development and the identification of patient suitability for the different treatment options are all required in this era of personalized medicine. Hence, in the present study, the mutational spectrum in the *PIK3CA* gene (exons 9 and 20) in the PI3K pathway was investigated to assess the frequency of somatic mutations and their association with clinicopathological parameters in a cohort of patients with CRC.

Patients and methods

Patient samples. A total of 71 formalin-fixed paraffin-embedded (FFPE) tissue samples collected at the King Fahd Hospital of the University (KFHU; Al Khobar, Saudi Arabia) were used in the present study. All the samples were obtained from patients who had received a confirmed diagnosis of CRC (by histological analysis). The inclusion criteria included the following: i) A confirmed diagnosis of CRC with >70% tumor cells in the FFPE block; ii) the absence of a family history of cancer; and iii) an age >18 years. Samples with <70% tumor content in the FFPE block were excluded from the study. Tumor cell percentage was calculated using hematoxylin and eosin staining (this staining was performed at the hospital laboratory as a routine procedure on paraffin-embedded tissues). The present study received Institutional review board approval from Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia (approval no. IRB-2019-01-378). All the samples were anonymized to protect patient confidentiality and clinical and therapeutic data were collected from the hospital laboratory information system in a predesigned format. Written informed consent was obtained from all patients.

DNA isolation and quantification. Paraffin-embedded sections (20-μm-thick) were prepared from the respective FFPE tissues using a microtome. A total of two sections were used for genomic DNA extraction using the QIAMP DNA FFPE Tissue kit (Qiagen GmbH). Briefly, the procedure included the deparaffinization of paraffin-embedded sections by the addition of xylene (Merck KGaA) followed by centrifugation at 19,283 x g for 2 min at 25˚C to remove the supernatant. Absolute ethanol (Merck KGaA) was added to the pellet and centrifuged at 19,283 x g for 2 min at 25˚C to remove the supernatant and the residual ethanol was dried. For the pellet, tissue lysis buffer and proteinase K was added and incubated at 56˚C until complete sample lysis, followed by incubation at 90˚C for 1 h. Another lysis buffer along with absolute ethanol was added to the existing lysate and the solution was transferred to a QIAmp minielute spin column (Qiagen GmbH) and centrifuged at 6,297 x g for 1 min at 25˚C, followed by two washing steps with wash buffer 1 and 2. Finally, an elution buffer was added to the QIAmp minielute spin column to obtain DNA.

The quantity and purity of the isolated DNA was examined using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Inc.). The absorbance at 260 nm was determined to calculate the concentration and purity at a 260/280 ratio. The mean DNA yield was 137.62±85.75 ng/μl and the mean 260/280 ratio was 2.03±0.16.

Capillary sequencing. A total of 100 ng DNA was used to amplify the *PIK3CA* exons 9 and 20 in separate tubes using primers (25) and 2X Phusion U Green Multiplex PCR Master Mix (Thermo Fisher Scientific, Inc.). The master mix was subjected to PCR using an annealing temperature of 54˚C. The resulting PCR amplicons for exon 9 (195 bp) and 20 (338 bp) were confirmed by 2% agarose gel electrophoresis.

The same PCR product was purified using the ExoSAP-IT express PCR product cleanup kit (cat. no. 75001.200.UL; Thermo Fisher Scientific, Inc.) followed by sequencing PCR using forward or reverse primers and BigDye terminator v3.1 cycle sequencing master mix (Thermo Fisher Scientific, Inc.). PGEM DNA was used as the control for the sanger sequencing reaction. The product was purified using BigDye XTerminator purification kit (Thermo Fisher Scientific, Inc.). The purified product was subjected to capillary electrophoresis using Quantstudio sequencer (Thermo Fisher Scientific, Inc.). Primary analysis and quality control of the output sequence was performed using sequence analysis software (Thermo Fisher Scientific, Inc.). Secondary analysis to compare the DNA sequence with the reference *PIK3CA* exon 9 and 20 DNA

Table I. Demographics of the patients in the present study.

Parameter	Colon cancer	Rectal cancer	P-value
Cases, n (%)	58 (100)	13 (100)	NA
Sex (M: F), n (%)	25 (43.1):33 (56.9)	6 (46.15):7 (53.85)	0.841
Age at diagnosis, years (mean ± SD)	55.24±12.48	50.69±13.19	0.243
PIK3CA mutation	6 (10.34)	0	0.584
Tumor type, n (%)		NA	NA
Cecum cancer	3 (5.2)		
Rectosigmoid cancer	7 (12)		
Sigmoid cancer	11 (19)		
Different parts of colon	37 (63.8)		
Tumor grade, n (%)			0.160
Well-differentiated (grade 1)	28 (48.27)	3 (23)	
Moderately differentiated (grade 2)	23 (39.65)	9 (69.23)	
Poorly differentiated (grade 3)	7 (12.08)	1 (7.69)	
Tumor stage, n (%)			0.0007
Stage I	3 (5.17)	5 (38.5)	
Stage II	24 (41.37)	8 (61.5)	
Stage III	17 (29.31)	0 (0)	
Stage IV	14 (24.13)	0 (0)	
Hospital, n (%)			NA
KFHU	41 (70.6)	2 (15.38)	
Other	17 (29.4)	11 (84.62)	
Treatment strategy (only KFHU)			0.999
Surgical resection	20 (48.7)	1 (50)	
Surgical resection and chemotherapy/radiotherapy	21 (51.2)	1 (50)	
Recurrence at 5 years	13 (33.33)	No Data	NA

PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

sequence (NM_006218.4) was achieved by CodonCode aligner software, version 10.0.2 (CodonCode Corporation). Finally, two novel mutation sequence data were submitted to a data repository of the NCBI GenBank with accession numbers PQ785769 and PQ785770.

Statistical analysis. All data, including demographic, histological, clinical, therapeutic and mutation details were organized in MS excel format. The statistical analysis was carried out using SPSS version 22 (IBM Corp.). The Kolmogorov-Smirnov test was used to determine the normality of the data, which revealed a normal distribution. For categorical data, the Chi-squared test and Fisher's exact test were used and for continuous variable data, the Student's t-test was used. A value of $P<0.05$ was considered to indicate a statistically significant difference.

Results

Demographic data. Among the 71 tissue samples collected, 58 samples were from patients with colon cancer, and 5.2% of the cases were cecum cancer, 12% were rectosigmoid cancer, 19% were sigmoid cancer, and the remaining cases were from different parts of the colon. The remaining 13 tissue samples were from patients with rectal cancer. The diagnosis was confirmed using the hematoxylin and eosin staining technique. The overall frequency for colon (43.1%) and rectal (46.1%) cancer was lower among males. In rectal cancer, the overall mean age at diagnosis was 50.69 years and stratification based on sex revealed 53.17 and 48.57 years in male and female patients, respectively. Similarly, the overall mean age at diagnosis among the patients with colon cancer was 55.24 years. The mean age at diagnosis of the male and female patients with colon cancer was 58.68 and 52.64 years, respectively. The patient demographics are presented in Table I.

Tumor classification. Tumor stage was classified based on the tumor, node and metastasis staging system, which is calculated by assessing the tumor size and growth, number of regional lymph nodes involved and metastasis. Tumor grade is identified based on the morphology of the tumor by comparing healthy and malignant cells under the microscope. In the colon cancer group, well-differentiated tumors were found in 48.27% of the cases, followed by moderately differentiated

Table II. Exon 9 and 20 mutation details, clinical and histopathological details in colon cancer cases.

PIK3CA exon	Type of mutation	Nucleotide change	Amino acid change	Age at diagnosis (years)	Sex	Grade	Stage	Recurrence
9	Missense mutation	c.1634 A>C	E545A	64	Female	2	III	No
20	Silent mutation	c.3063 C>T	Y1021Y	83	Male	1	I	No
		c.3063 C>T	Y1021Y	55	Male	1	III	No
		c.3063 C>T	Y1021Y	52	Female	2	III	No
		c.3063 C>T	Y1021Y	41	Female	2	IV	Yes
		c.3075 C>T	T1025T					
		c.3204 C>T	N1068N					
	Missense mutation[a]	c.3001 C>G	L1001V	57	Female	2	IV	Yes
	Nonsense mutation[a]	c.3153 G>A	W1051Ter					

[a]Novel mutations; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

tumors (39.65%) and poorly differentiated tumors (12%). However, the majority of the tumors in the rectal cancer group were moderately differentiated tumors (69.23%), followed by 23% of the tumors being well-differentiated and only 7.69% of the cases exhibiting poorly differentiated tumors. The cancer stages revealed that the majority of the tumors in the colon cancer group were stage II tumors (41.37%), followed by stage III tumors (29.31%), stage IV tumors (24.13%) and stage I tumors (5.17%). In the rectal cancer group, the highest percentage of tumors (61.5%) were observed in stage II and the remaining (38.4%) cases were in stage I. There were no cases observed in the rectal cancer group with stage III and stage IV disease (Table I).

Regional lymph node involvement was observed in only the colon cancer cases. An average of 3.6 regional lymph nodes were involved in patients with stage III and stage IV tumors. However, in the rectal cancer cases, there was no involvement of the regional lymph nodes as all the cases presented with stage I and stage II disease.

Treatment and follow-up. Out of the 58 colon cancer cases, 70.6% of the patients underwent treatment at KFHU and the remaining cases were diagnosed at KFHU, but treated at other hospitals. Hence, the therapeutic and follow-up data for the patients treated outside KFHU was not obtained. Among the 70.6% of the cases of colon cancer, 48.7% of the patients underwent surgical resection alone and the remaining 51.2% of the patients underwent surgical resection followed by chemotherapy. Out of the 13 rectal cancer cases, only 15.38% of the cases were treated at KFHU and the remaining patients were referred to King Fahd Specialist Hospital, Dammam, Saudi Arabia, which is the major center for cancer treatment in the Eastern Province of Saudi Arabia. Of the rectal cancer cases who were treated at KFHU, 50% underwent surgical resection and 50% were treated by surgical resection followed by chemotherapy and radiotherapy. In the colon cancer cases, 67.24% were followed up after treatment. Among these patients, recurrence within 5 years was observed in 33.33% of the cases. The majority of follow-up data from the rectal cancer group were not available as the patients were treated at another center.

PIK3CA mutation. *PIK3CA* exon 9 and 20 genes were amplified by Sanger Sequencing to determine the known and unknown mutations. From the entire cohort, including mutation analysis of colon and rectal tumors, it was revealed that 6 patients (8.4%) had mutations in either exon 9 or 20. These mutations were observed only in colon cancer tumors (10.3%) with no mutations in rectal cancer tumors. A total of six different types of mutations were identified, with one mutation in exon 9 and the remaining five mutations in exon 20 (Table II and Fig. 1). Out of the total mutations, 50% of the mutations were silent mutations, followed by missense and nonsense mutations (33.3 and 16.6%, respectively). Only one silent mutation was observed in the 4 patients with colon cancer. Mutation status comparison with tumor grade and stage revealed that 66.6% of the mutations were observed in grade II tumors and 50% of the mutations were observed in stage III tumors.

Exon 9 mutation c. 1634A>C was identified in a patient with colon cancer with the primary tumor located in the cecum. This mutation was not observed in the rectal group of tumors. This was a missense mutation leading to an amino acid change at codon 545 from glutamic acid to alanine (E545K). The other hotspot mutations, such as c.1624G>A (E542K) and c.1633G>A (E545K) in exon 9 were absent in the cohort.

Exon 20 mutations were observed in five patients, with the silent mutation c.3063C>T observed in 4 patients who all presented with colon cancer. One patient had the silent mutation c.3063C>T along with two other silent mutations (c.3075C>T and c.3204C>T). The missense mutation c. 3001C>G, which was also observed in a patient with colon cancer, leading to an amino acid change at codon 1001 from leucine to valine (L1001V). In the same patient, downstream of the missense mutation, a nonsense mutation (c.3153G>A) was observed,

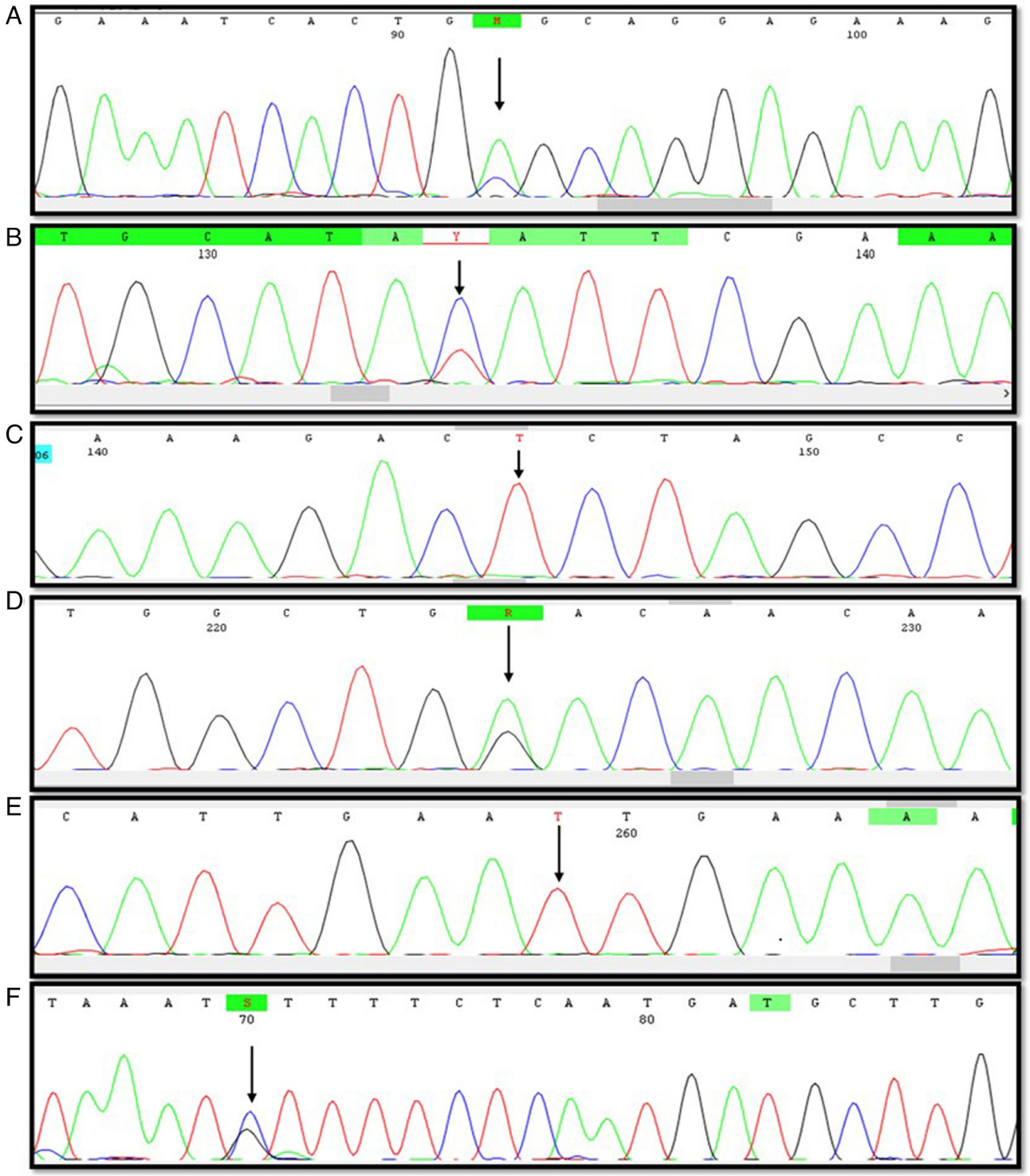

Figure 1. Representative electropherograms of *PIK3CA* exon 9 and 20 mutations identified in the cohort. Nucleotide change (amino acid change): (A) c.1634 A>C (E545A) (B) c.3063 C>T (Y1021Y) (C) c.3075 C>T (T1025T) (D) c.3153 G>A (W1051Ter) (E) c.3204 C>T (N1068N) (F) c.3001 C>G (L1001V).

which created a termination codon sequence at codon 1051 from tryptophan amino acid (W1051Ter). The two mutations, L1001V and W1051Ter, were novel mutations and have not been previously reported. The *PIK3CA* mutation rate was observed more frequently in female patients (66.6%) compared with male patients and the missense mutations were seen only in female patients. The mutation comparison with the prognosis data revealed four patients (66.6%) who had no recurrence of the disease with two patients (33.3%) having a recurrence. The 2 patients who had a recurrence presented with multiple mutations including silent, missense and nonsense mutations.

Discussion

CRC represents the third most common type of cancer globally and in Saudi Arabia it is ranked second following breast

cancer. Universally, among CRC subtypes, colon cancers are the most predominant (60.2%) followed by rectal cancer (39.8%). A similar trend has been observed in Saudi Arabia, with colon and rectal cancers (61 and 39%, respectively) (3,4). However, in the present study, 81.7% of the patients had colon cancer and the remaining had rectal cancer. It was hypothesized that this discrepancy may be due to the single center approach for sample collection and the fact that the majority of the patients were from the Eastern Province of Saudi Arabia. The age range at diagnosis for the majority of male patients with CRC is between 60-64 years and in female patients this is 55-59 years (4). The present study observed a similar pattern in the age range at diagnosis among male (57.61 years) and female (51.93 years) patients with CRC, indicating that females present with the disease at an earlier age than males. The stratified mean age at diagnosis of the patients with colon cancer (55.24 years) and rectal cancer (50.69 years) was in line with the Saudi Cancer Incidence Report 2018 (4). As age is one of the influencing factors for the risk of developing CRC, the majority of CRC cases are diagnosed after the age of 50 years. Therefore, after the age of 40 years, CRC screening is recommended.

CRC is typically an asymptomatic disease and when symptoms such as anemia, rectal bleeding or abdominal pain manifest, the majority of the patients present at an advanced stage of the disease (26). In the present study, ~50% of patients with colon cancer presented at an advanced stage but none of the patients with rectal cancer presented at an advanced stage. Several studies have revealed that the incidence of early-onset CRC is increasing globally (27,28). Improved survival outcomes have been noted in patients whose disease was diagnosed in the early onset of rectal cancer (29). Therefore, patients with rectal cancer in the present study cohort may have improved survival rates compared with the patients with colon cancer. Disease recurrence data within 5 years from the diagnosis date were available for the cases followed-up at KFHU and the data revealed that 33.35% of the patients with colon cancer had disease recurrence. The majority (77%) of the patients with colon cancer and disease recurrence presented with stage IV of the disease. Diagnosis at various stages of the disease influences the treatment options and survival rates. Improvements in the understanding of the pathophysiology of CRC enables physicians to select the optimum treatment options and can increase the survival rate to 3 years (30).

CRC is a heterogenous disease with different genetic and epigenetic backgrounds. The development of CRC is caused by an accumulation of somatic mutations in multiple genes and epigenetic events (31). The PI3K/Akt/mTOR pathway plays a crucial role in these processes, such as cell growth, cell cycle progression and survival. In this pathway, PI3K is composed of a catalytic subunit and a regulatory subunit, and is initially encoded by the *PIK3CA* gene. The *PIK3CA* gene mutations were observed frequently in several cancers (32,33). The majority of somatic mutations in *PIK3CA* are confined to exon 9 and 20 (34). Hence, the present study focused on the hotspot mutations situated in exon 9 and 20 of the *PIK3CA* gene in the CRC cohort. The frequency of *PIK3CA* somatic mutations in the CRC cohort were 5.7-32%. Studies with a large CRC sample size, such as The Cancer Genome Atlas study (11), Dana Farber Cancer Institute study (35) and The Memorial Sloan Kettering Cancer Center study (36), reported *PIK3CA* mutations to be 24.7, 21.3 and 20.8%, respectively (37). Other studies with smaller sample sizes reported varying mutation rates. For example, a study conducted in the USA reported a mutation rate of 32% (38), followed by another USA study with a rate of 18.1% (39), an Indian study with a rate of 5.7% (40), an Italian study with 8% (41) and a Chinese study with 18.94% (42).

In the present study, the majority of the patients were from the Eastern Province of Saudi Arabia, and it was observed that 8.4% of these patients presented with the *PIK3CA* mutation. These variations among different studies are possibly due to the difference in the ethnicity of population and different methodologies used to detect the mutations. *PIK3CA* mutation status association with sex revealed mixed results. Of note, one study reported that the *PIK3CA* mutation frequency in colon cancer was higher in female patients (43) and another study reported there was no association between sex and the *PIK3CA* mutation (44). In the present study, the overall CRC frequency was higher in female patients (66.6%). Among the patients who presented with the mutation, all the missense and novel mutations were observed in female patients.

Various studies have found an association between the *PIK3CA* mutation status with the clinicopathological parameters, such as disease stage, survival, recurrence and therapeutic response (45-49). The present study revealed that the majority (83.3%) of colon tumors with the *PIK3CA* mutation presented with an advanced stage of the disease (stage III or IV). These results are in line with those of another study, which reported that all *PIK3CA* mutations were observed in high pathological stages and poorly differentiated CRC tumors (45). *PIK3CA* mutations have been shown to be associated with a poor prognosis of patients with the disease in previous studies (46-48); in the present study, the patient who presented with the *PIK3CA* missense mutation/nonsense mutation had a poor prognosis. The present study reported on two novel mutations in the *PIK3CA* gene, namely one missense mutation leading to an L1001V amino acid change and a nonsense mutation leading to termination (W1051Ter). Both of these novel mutations are situated on exon 20 which is the kinase domain of the protein. The kinase region mutations help to achieve gain of function and change the transforming capacity of the tumor (49).

In conclusion, *PIK3CA* somatic mutations are critical in understanding the pathogenicity of solid cancers, particularly CRC. The novel mutations identified in the kinase domain of PIK3CA may induce the hyperactivation of the protein in the PI3K pathway leading to poor prognosis. Based on the present study findings, CRC screening is recommended at an early age (>40 years) for all patients, but particularly for female patients who carry these mutations. The majority of the tumors if detected early may present at a low grade and early stage, and the response to treatment options in terms of prognosis may be improved. However, the small sample size of the present study is a limitation to a strong genetic association. Hence, larger sample studies are warranted to understand the association between *PIK3CA* mutations with disease prognosis and therapeutic response.

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Availability of data and materials

The sequence data for the two novel mutations generated in the present study may be found in NCBI GenBank under accession numbers PQ785769 and PQ785770. The data generated in the present study are available in the National Centre for Biotechnology information database (https://www.ncbi.nlm.nih.gov/search/all/?term=PRJNA1230587) with the accession no. PRJNA1230587.

Authors' contributions

CV was involved in the conceptualization of the study, in the writing of the original draft of the manuscript, in the study methodology (sequencing), in the writing, reviewing and editing of the manuscript and in the formal analysis. AMAA, AA, NJA, HMA, MAA and RAA were involved in the conception and design of the study, provision of resources, data collection, analysis, interpretation, drafting the article and critical revision of the article. CC and SC were involved in the study methodology (sequencing), in the formal analysis, and in the drafting and critical revision of the article. SC and CC confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethical approval and consent to participate

Institutional review board approval (IRB-2019-01-378) was received from Imam Abdulrahman Bin Faisal University. The procedures in the present study adhere to the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, van de Velde CJH and Watanabe T: Colorectal cancer. Nat Rev Dis Primers 1: 15065, 2015.
2. Hossain MS, Karuniawati H, Jairoun AA, Urbi Z, Ooi J, John A, Lim YC, Kibria KMK, Mohiuddin AKM, Ming LC, *et al*: Colorectal cancer: A review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies. Cancers (Basel) 14: 1732, 2022.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
4. Saudi Health Council, National Cancer Center and Saudi Cancer Registry: Cancer incidence report from Saudi Arabia 2018. Saudi Health Council, 2022. https://shc.gov.sa/Arabic/NCC/Activities/AnnualReports/2018.pdf. Accessed July 23, 2023.
5. World Health Organization (WHO): Cancer Saudi Arabia 2020 country profile. WHO, Geneva, 2020. https://cdn.who.int/media/docs/default-source/country-profiles/cancer/sau-2020.pdf?sfvrsn=37936f36_2&download=true. Accessed July 23, 2023.
6. Messersmith WA: NCCN guidelines updates: Management of metastatic colorectal cancer. J Natl Compr Canc Netw 17: 599-601, 2019.
7. Keum N and Giovannucci E: Global burden of colorectal cancer: Emerging trends, risk factors and prevention strategies. Nat Rev Gastroenterol Hepatol 16: 713-732, 2019.
8. Xie YH, Chen YX and Fang JY: Comprehensive review of targeted therapy for colorectal cancer. Signal Transduct Target Ther 5: 22, 2020.
9. Triantafillidis JK, Nasioulas G and Kosmidis PA: Colorectal cancer and inflammatory bowel disease: Epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies. Anticancer Res 29: 2727-2737, 2009.
10. Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, Jemal A, Schymura MJ, Lansdorp-Vogelaar I, Seeff LC, *et al*: Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer 116: 544-573, 2010.
11. Cancer Genome Atlas Network: Comprehensive molecular characterization of human colon and rectal cancer. Nature 487: 330-337, 2012.
12. Sepulveda AR, Hamilton SR, Allegra CJ, Grody W, Cushman-Vokoun AM, Funkhouser WK, Kopetz SE, Lieu C, Lindor NM, Minsky BD, *et al*: Molecular biomarkers for the evaluation of colorectal cancer: Guideline from the American society for clinical pathology, college of American pathologists, association for molecular pathology, and American society of clinical oncology. Arch Pathol Lab Med 141: 625-657, 2017.
13. Krasinskas AM: EGFR signaling in colorectal carcinoma. Patholog Res Int 2011: 932932, 2011.
14. Novellasdemunt L, Antas P and Li VS: Targeting Wnt signaling in colorectal cancer. A review in the theme: Cell signaling: Proteins, pathways and mechanisms. Am J Physiol Cell Physiol 309: C511-C521, 2015.
15. Malapelle U, Pisapia P, Sgariglia R, Vigliar E, Biglietto M, Carlomagno C, Giuffrè G, Bellevicine C and Troncone G: Less frequently mutated genes in colorectal cancer: Evidences from next-generation sequencing of 653 routine cases. J Clin Pathol 69: 767-771, 2016.
16. Inamura K: Colorectal Cancers: An update on their molecular pathology. Cancers (Basel) 10: 26, 2018.
17. Zhou R, Huang Y, Cheng B, Wang Y and Xiong B: TGFBR1*6A is a potential modifier of migration and invasion in colorectal cancer cells. Oncol Lett 15: 3971-3976, 2018.
18. Koveitypour Z, Panahi F, Vakilian M, Peymani M, Seyed Forootan F, Nasr Esfahani MH and Ghaedi K: Signaling pathways involved in colorectal cancer progression. Cell Biosci 9: 97, 2019.
19. Kwak MS, Cha JM, Yoon JY, Jeon JW, Shin HP, Chang HJ, Kim HK, Joo KR and Lee JI: Prognostic value of KRAS codon 13 gene mutation for overall survival in colorectal cancer: Direct and indirect comparison meta-analysis. Medicine (Baltimore) 96: e7882, 2017.
20. De Roock W, De Vriendt V, Normanno N, Ciardiello F and Tejpar S: KRAS, BRAF, PIK3CA, and PTEN mutations: Implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol 12: 594-603, 2011.
21. Hamada T, Nowak JA and Ogino S: PIK3CA mutation and colorectal cancer precision medicine. Oncotarget 8: 22305-22306, 2017.
22. Samuels Y and Waldman T: Oncogenic mutations of PIK3CA in human cancers. Curr Top Microbiol Immunol 347: 21-41, 2010.
23. Al-Amri AM, Vatte C, Cyrus C, Chathoth S, Hashim TM, Mohamed YS, Al Ali R, Alsaid A and Al Ali A: Novel mutations of PIK3CA gene in head and neck squamous cell carcinoma. Cancer Biomark 16: 377-383, 2016.

24. Vatte C, Al Amri AM, Cyrus C, Chathoth S, Alsayyah A, Ahmad A, Akhtar MS, Alrashidi NF, Jayaseeli N, Al Wadani H, *et al*: Helical and kinase domain mutations of PIK3CA, and their association with hormone receptor expression in breast cancer. Oncol Lett 18: 2427-2433, 2019.
25. Qiu W, Tong GX, Manolidis S, Close LG, Assaad AM and Su GH: Novel mutant-enriched sequencing identified high frequency of PIK3CA mutations in pharyngeal cancer. Int J Cancer 122: 1189-1194, 2008.
26. Xi Y and Xu P: Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol 14: 101174, 2021.
27. Lu XQ, Li Y, Wang W, Feng WT, Shi OM and Wang Q: International incidence trends in early- and late-onset colorectal cancer: A population-based study. Int J Colorectal Dis 35: 1077-1086, 2020.
28. Saad El Din K, Loree JM, Sayre EC, Gill S, Brown CJ, Dau H and De Vera MA: Trends in the epidemiology of young-onset colorectal cancer: A worldwide systematic review. BMC Cancer 20: 288, 2020.
29. Zaborowski AM, Murphy B, Creavin B, Rogers AC, Kennelly R, Hanly A, Martin ST, O'Connell PR, Sheahan K and Winter DC: Clinicopathological features and oncological outcomes of patients with young-onset rectal cancer. Br J Surg 107: 606-612, 2020.
30. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM and Wallace MB: Colorectal cancer. Lancet 394: 1467-1480, 2019.
31. Ogino S, Chan AT, Fuchs CS and Giovannucci E: Molecular pathological epidemiology of colorectal neoplasia: An emerging transdisciplinary and interdisciplinary field. Gut 60: 397-411, 2011.
32. Reddy D, Ghosh P and Kumavath R: Strophanthidin attenuates MAPK, PI3K/AKT/mTOR, and Wnt/β-catenin signaling pathways in human cancers. Front Oncol 9: 1469, 2019.
33. Wang Y, Li J and Che G: Clinical significance of PIK3CA gene in non-small-cell lung cancer: A systematic review and meta-analysis. Biomed Res Int 2020: 3608241, 2020.
34. Kumar R, Kumar R, Goel H, Kumar S, Ningombam SS, Haider I, Agrawal U, Deo S, Gogia A, Batra A, *et al*: Whole exome sequencing identifies novel variants of PIK3CA and validation of hotspot mutation by droplet digital PCR in breast cancer among Indian population. Cancer Cell Int 23: 236, 2023.
35. Giannakis M, Mu XJ, Shukla SA, Qian ZR, Cohen O, Nishihara R, Bahl S, Cao Y, Amin-Mansour A, Yamauchi M, *et al*: Genomic correlates of immune-cell infiltrates in colorectal carcinoma. Cell Rep 15: 857-865, 2016.
36. Yaeger R, Chatila WK, Lipsyc MD, Hechtman JF, Cercek A, Sanchez-Vega F, Jayakumaran G, Middha S, Zehir A, Donoghue MTA, *et al*: Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. Cancer Cell 33: 125-136.e3, 2018.
37. Voutsadakis IA: The landscape of PIK3CA mutations in colorectal cancer. Clin Colorectal Cancer 20: 201-215, 2021.
38. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, *et al*: High frequency of mutations of the PIK3CA gene in human cancers. Science 304: 554, 2004.
39. Gong J, Cho M, Sy M, Salgia R and Fakih M: Molecular profiling of metastatic colorectal tumors using next-generation sequencing: A single-institution experience. Oncotarget 8: 42198-42213, 2017.
40. Shetty O, Vengurlekar V, Kapoor A, Kamble V, Gurav M, Bhargava P, Srinivas S, Ramaswamy A, Ramadwar M, Saklani AP, *et al*: The prevalence of BRAF, PIK3CA, and RAS mutations in indian patients with colorectal cancer. South Asian J Cancer 11: 190-194, 2022.
41. Reggiani Bonetti L, Barresi V, Maiorana A, Manfredini S, Caprera C and Bettelli S: Clinical impact and prognostic role of KRAS/BRAF/PIK3CA mutations in stage I colorectal cancer. Dis Markers 2018: 2959801, 2018.
42. Li X, Yang T, Li CS, Song Y, Lou H, Guan D and Jin L: Surface enhanced raman spectroscopy (SERS) for the multiplex detection of Braf, Kras, and Pik3ca mutations in plasma of colorectal cancer patients. Theranostics 8: 1678-1689, 2018.
43. Jhawer M, Goel S, Wilson AJ, Montagna C, Ling YH, Byun DS, Nasser S, Arango D, Shin J, Klampfer L, *et al*: PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. Cancer Res 68: 1953-1961, 2008.
44. Sartore-Bianchi A, Martini M, Molinari F, Veronese S, Nichelatti M, Artale S, Di Nicolantonio F, Saletti P, De Dosso S, Mazzucchelli L, *et al*: PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res 69: 1851-1857, 2009.
45. Barault L, Veyrie N, Jooste V, Lecorre D, Chapusot C, Ferraz JM, Lièvre A, Cortet M, Bouvier AM, Rat P, *et al*: Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. Int J Cancer 122: 2255-2259, 2008.
46. Ogino S, Nosho K, Kirkner GJ, Shima K, Irahara N, Kure S, Chan AT, Engelman JA, Kraft P, Cantley LC, *et al*: PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol 27: 1477-1484, 2009.
47. Foltran L, De Maglio G, Pella N, Ermacora P, Aprile G, Masiero E, Giovannoni M, Iaiza E, Cardellino GG, Lutrino SE, *et al*: Prognostic role of KRAS, NRAS, BRAF and PIK3CA mutations in advanced colorectal cancer. Future Oncol 11: 629-640, 2015.
48. Phipps AI, Ahnen DJ, Cheng I, Newcomb PA, Win AK and Burnett T: PIK3CA somatic mutation status in relation to patient and tumor factors in racial/ethnic minorities with colorectal cancer. Cancer Epidemiol Biomarkers Prev 24: 1046-1051, 2015.
49. Dirican E, Akkiprik M and Özer A: Mutation distributions and clinical correlations of PIK3CA gene mutations in breast cancer. Tumour Biol 37: 7033-7045, 2016.