SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 10: 1423-1429, 2015

Mutation of the *PIK3CA* gene as a prognostic factor in patients with colorectal cancer

RAFAŁ STEC[1], ALEKSANDRA SEMENIUK-WOJTAŚ[1], RADOSŁAW CHARKIEWICZ[2], LUBOMIR BODNAR[1], JAN KORNILUK[1], MARTA SMOTER[1], LECH CHYCZEWSKI[3], JACEK NIKLIŃSKI[2] and CEZARY SZCZYLIK[1]

[1]Department of Oncology, Military Institute of Medicine, Warsaw 04-141; Departments of [2]Clinical Molecular Biology and [3]Clinical Pathology, Medical University of Bialystok, Bialystok 15-089, Poland

Received July 17, 2014; Accepted May 28, 2015

DOI: 10.3892/ol.2015.3398

Abstract. Colorectal cancer (CRC) is one of the most common cancers worldwide, with ~700,000 mortalities occurring due to CRC in 2012. The treatment options are effective in a small percentage of patients, and it is important to identify specific biomarkers in order to determine patients for whom the available therapies will be beneficial. It has been hypothesised that the *PIK3CA* gene mutation may affect the response to therapy of patients with metastatic CRC. In the present study, primary tumour specimens were collected from 156 patients with CRC who were treated in the Military Institute of Medicine in Warsaw (Warsaw, Poland). Codons 12 and 13 of exon 1 of *KRAS*, exons 11 and 15 of *BRAF* and exons 9 and 20 of *PIK3CA* were analysed for mutation using direct sequencing. The prognostic value of each mutation and the clinical and pathological variables of these tumours were estimated. The results revealed that *PIK3CA* mutations were present in 15 patients (9.6%), of whom seven (46.7%) possessed mutations in codon 9 and eight (53.3%) possessed mutations in codon 20. Mutation in the *PIK3CA* gene was detected in six patients with *KRAS* gene mutations, which accounted for 40% of *PIK3CA*-mutated tumours, and in one patient with *BRAF* mutations, which accounted for 6.6% of *PIK3CA*-mutated tumours. No significant differences were identified between the overall survival (OS) rates of patients with *PIK3CA* mutations (median OS, 56.7 months) and those with wild-type *PIK3CA* genes (median OS, 47.6 months) (P=0.1270). Univariate analysis identified that the following prognostic factors affected the OS rate in the current patient cohort: Gender, female patients survived for 57.5 months compared with 39.3 months for male patients (P=0.0111); and lymph node involvement grade, as survival of patients without lymph node metastases was 61.4 months compared with 45.4 months in patients presenting with metastases (P=0.0122). The findings of the present analysis indicate that *PIK3CA* mutation status is not a prognostic factor in CRC patients. In addition, no statistically significant association exists between tumours with *PIK3CA* mutations and clinical or pathological factors.

Correspondence to: Dr Aleksandra Semeniuk -Wojtaś, Department of Oncology, Military Institute of Medicine, 128 Szaserów Street, Warsaw 04-141, Poland
E-mail: osemeniuk@wp.pl

Key words: colorectal cancer, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha mutations, prognostic factor

Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide, with more than one million newly diagnosed cases reported annually. In total, ~700,000 CRC-associated mortalities occurred worldwide in 2012, accounting for 8% of all cancer mortalities, and making CRC the fourth most common cause of cancer-associated mortality (1). The standard treatment for patients with unresectable metastases includes chemotherapy regimens based on irinotecan, oxaliplatin, fluoropyrimidines, anti-vascular endothelial growth factor (anti-VEGF) therapy (bevacizumab) and anti-epidermal growth factor receptor (anti-EGFR) therapy, such as panitumumab and cetuximab. These treatment options are efficient in a small percentage of patients, and it is important to identify specific biomarkers to determine the patients that are likely to benefit from anti-EGFR therapy (2-4).

EGFR triggers a downstream signalling cascade through, for example, the Kirsten rat sarcoma viral oncogene homolog-serine/threonine-protein kinase B-Raf and the phosphatidylinositol 3-kinase (PI3K), catalytic subunit α-phosphatase and tensin homolog-Akt pathways, which regulate cell proliferation, survival, apoptosis resistance, invasion and migration (2,3,5-8). The *PIK3CA* gene encodes the p110α subunit of PI3K α and belongs to class IA of the PI3Ks. The PI3K α protein is composed of regulatory subunit p85, which mediates anchorage to EGFR-specific docking sites, and catalytic subunit p110, which generates a second messenger that is responsible for the activation of Akt in response to the activation of growth factors from various ligands. These ligands include epidermal growth factor (EGF) or VEGF. Somatic mutations in cancer cells only occur in *PIK3CA* and *PI3KR1*, which encodes the p85 α subunit (3,6,9). These mutations are concentrated in two key regions of the *PIK3CA* gene, consisting of the helical domain of exon 9 and the kinase domain of exon 20 (7,8,10).

Activating mutations in *PIK3CA* are detected in 7-32% patients, with G>A transversions in exon 9 being the most commonly observed configuration, which may coincide with *KRAS* and *BRAF* mutations. Tumours with the *PIK3CA* gene mutation are characterised by a predominant proximal colonic location (10,11) and by the frequent presence of mucinous differentiation (10).

Mutations of EGFR-dependent signalling molecules confer resistance to EGFR-specific antibody therapy. *KRAS* mutation is the first molecular marker of response to EGFR inhibitors (11). It has been hypothesised that the *PIK3CA* gene mutation may also affect the response to anti-EGFR therapy in patients with metastatic CRC (12,13). Certain studies indicate that *PIK3CA* exon 20 mutations negatively affect the response rate, disease control rate, progression-free survival (PFS) time and overall survival (OS) time, whilst *PIK3CA* exon 9 mutations demonstrate no significant effect on objective response (3,4).

The aim of the present study was to evaluate the importance of mutation in the *PIK3CA* gene as a prognostic factor in CRC. Additionally, the frequency of *PIK3CA* mutations in patients with CRC and the incidence of mutations in particular exons were examined. The association between the *PIK3CA* gene mutation and mutations in other downstream effectors of the EGFR signalling pathway was also analysed, in addition to the association between the *PIK3CA* gene mutation and various clinical or pathological features.

Materials and methods

Patient characteristics. Based on the database of the Military Institute of Medicine (Warsaw, Poland), 156 patients that were consecutively diagnosed with CRC were identified. The patients had been treated with palliative chemotherapy at the Oncology Department of the Military Institute of Medicine between 2006 and 2010. The inclusion criteria were as follows: Confirmed histopathological diagnosis of CRC; aged >18 years; presence of measurable lesions, determined by Response Evaluation Criteria in Solid Tumours, version 1.1 (14); adequate haematological parameters, consisting of a neutrophil count of $\geq 1.5 \times 10^9$, platelet count of $\geq 100 \times 10^9$/l and haemoglobin count of ≥9.0 g/dl; adequate biochemical parameters, comprising a bilirubin level <2 x upper limit of normal (ULN); an aspartate transaminase (AST); alanine transaminase (ALT) level <2.5 x ULN; a glomerular filtration rate (GFR) of >50 ml/min; and, in premenopausal women, an absence of pregnancy. The exclusion criteria were as follows: Renal insufficiency, demonstrated by a GFR <50 ml/min; hepatic insufficiency, demonstrated by AST and ALT levels >2.5 x ULN; and severe concomitant disease, such as unstable cardiac angina. This study was approved by the ethics commmittee of the Military Institute of Medicine and written informed consent was obtained from all patients.

Histopathological examination of tumour specimens. Primary tumour specimens were collected from CRC patients. Formalin-fixed paraffin embedded (FFPE) tissue blocks were cut into serial 5 μm-thick sections for haematoxylin and eosin staining. The presence of tumour tissue was verified by an experienced pathologist. Subsequently, tissue samples from at least three serial sections were macrodissected to ensure that the specimens contained ≥80% tumour cells.

DNA extraction. DNA from FFPE colorectal tumour tissues was isolated from 10-30-μm thick sections subsequent to macrodissection, resulting in the selection of specimens containing ≥80% tumour cells. Tumour samples were extracted with xylene and ethanol to remove paraffin, and placed in 1% SDS/proteinase K (10 mg/ml) at 56˚C overnight. DNA was isolated using the NucliSENS easyMag platform (bioMérieux, Marcy l'Etoile, France) for automated nucleic acid extraction.

KRAS, BRAF and PIK3CA mutation analysis. The detection of mutations in codons 12 and 13 of exon 1 of the *KRAS* gene and exons 11 and 15 of the *BRAF* gene was conducted using a previously described method (15). The analysis of mutations in exons 9 and 20 of the *PIK3CA* gene was performed by direct sequencing, as described by Samuels *et al* (16) and Li *et al* (17), with a number of modifications. The primers for exon 9 were designed to avoid amplification of homologous sequences located at the chromosome 22q11.2 cat-eye syndrome region and on chromosome 16. Genomic DNA obtained from tumour samples was amplified by polymerase chain reaction (PCR) using the following primers: Forward strand exon (FSE)9, 5'-TTGCTTTTTCTGTAAATCATCTGTG-3'; Reverse strand exon (RSE)9, for exon 9 of *PIK3CA*, 5'-CTGCTTTAT TTATTCCAATAG GTATG-3'; FSE20, 5'-ACATCATTT GCTCCAAACTGA-3', RSE20, for exon 20 of *PIK3CA*, 5'-CATAACATGAAATTG CGCATT-3'. PCR was conducted in a total volume of 10 μl, containing 2 μl of the extracted genomic DNA, using 10X PCR buffer, 1.5 mmol/l $MgCl_2$, 0.2 μmol/l of each primer, 0.1 mmol/l of deoxynucleoside triphosphate, and 1 unit of Taq DNA polymerase (Eurx Ltd., Gdańsk, Poland). PCR conditions were as follows: 95˚C for 10 min; 45 cycles of 95˚C for 30 sec, 59˚C for 30 sec and 72˚C for 30 sec; and finally 7 min at 72˚C. The amplification products were purified using the DNA Gel-Out kit (DNA Gdańsk, Gdynia, Poland). Automated sequencing was conducted using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Warsaw, Poland). Sequencing reactions were purified using the ExTerminator kit (DNA Gdańsk), and analysed on a 3500 Genetic Analyzer sequencer (Life Technologies). A wild-type control DNA sample without *PIK3CA* mutation and a known mutation sample (substitution 1633 G>A, E545K within exon 9 and substitution 3140 A>G, H1047R within exon 20) were included in the experiment. The mutation was confirmed by sequencing at least two independent PCR products.

Statistical analysis. The OS time was defined as the time elapsed between the commencement of the first line of palliative chemotherapy, and the date of mortality or of the final follow-up, and was estimated according to the Kaplan-Meier method. The cut-off date for the present analysis was December 2013.

The χ^2 test was used to investigate the association between variables in the two treatment groups with respect to baseline characteristics. The log-rank test was performed in the Kaplan-Meier survival analyses to assess differences between

the groups with regard to OS time. P<0.05 was considered to indicate a statistically significant difference. Multivariate analyses of OS time were performed by Cox proportional-hazard regression using the forward stepwise method, all variables determined to be significant in the univariate analysis were included in the multivariate analysis. Analyses were performed using the statistical package Statistica, version 7.0 (Statsoft, Inc., Tulsa, OK, USA).

Results

Patient characteristics. The characteristics of included patients are summarised in Table I. The cohort comprised 100 women and 56 men, with a median age of 67 years. The majority of patients (91%) underwent primary tumour resection. The primary tumour was located in the colon in 67 patients (42.9%), and in the sigmoid colon or in the rectum in 89 patients (57.1%). Metastases were located in the liver in 77 patients (70.6% of patients with metastases), the lungs in 21 patients (19.3% of patients with metastases), and other organs in 72 patients (66.0% of patients with metastases). Lymph node metastasis was also identified in 50.6% of patients. The majority of patients had a good performance status (Karnofsky status of 80-100).

KRAS, BRAF, PIK3CA gene mutation status. *KRAS* mutations were present in 44 patients (28.2%), of whom 41 patients (93.2%) possessed mutations in codon 12, and three patients (6.8%) possessed mutations in codon 13. *BRAF* mutations were present in 12 patients (7.7%). *PIK3CA* mutations were present in 15 patients (9.6%), of whom seven (46.7%) had mutations in codon 9, and eight (53.3%) had mutations in codon 20. Mutation in the *PIK3CA* gene was detected in six patients who had *KRAS* gene mutations (40% of *PIK3CA* mutated tumours) and in one patient with *BRAF* mutations (6.6% of *PIK3CA* mutated tumours).

Clinicopathological variables and PIK3CA gene mutation status. The evaluation of *PIK3CA* mutation status relative to clinicopathological variables is summarised in Table II. *PIK3CA* gene mutations were present in 15 patients (9.6%). An increased incidence of *PIK3CA* gene mutations was detected in patients with involved lymph nodes, with low-grade or unknown histological differentiation of the tumour, and with tubular cancer. *PIK3CA* gene mutations were also frequently present in patients with advanced disease, T stage III or IV. However, the association between these variables and *PIK3CA* status was not statistically significant.

Prognostic significance of PIK3CA gene mutation status. No significant difference in OS rate was identified between patients with *PIK3CA* mutations and those with wild-type *PIK3CA* genes (P=0.1270; Fig. 1). However, patients with *PIK3CA* mutations tended to demonstrate a decreased OS rate. The median OS in patients with wild-type *PIK3CA* genes was 56.7 months, compared with 47.6 months in patients presenting with mutations.

Clinical and pathological variables identified by univariate analysis as potential prognostic factors for OS rate. The results of the univariate analysis are summarised in Table III. Univariate

Table I. Characteristics of the patients with colorectal cancer enrolled in the present study.

Characteristic	Value
Total, n	156
Age in years, median (range)	67 (25-85)
Gender, n (%)	
Female	100 (64.9)
Male	56 (35.9)
KRAS status, n (%)	
Mutation	44 (28.2)
Codon 12	41 (26.3)
Codon 13	3 (1.9)
Wild-type	112 (71.8)
BRAF status, n (%)	
Mutation	12 (7.7)
Wild-type	144 (92.3)
PIK3CA status, n (%)	
Mutation	15 (9.6)
Codon 9	7 (4.5)
Codon 20	8 (5.1)
Wild-type	141 (90.4)
Primary tumour localisation, n (%)	
Colon	67 (42.9)
Sigmoid/rectum	89 (57.1)
Localisation of metastases (n=109), n (%)	
Liver	77 (70.6)
Lungs	21 (19.3)
Other localisations	72 (66.0)
Karnofsky performance status, n (%)	
100	79 (50.6)
90	63 (40.4)
80	12 (7.7)
70	2 (1.3)
Histological differentiation grade, n (%)	
High/moderate	126 (80.8)
Low/unknown	30 (19.2)
Histological type, n (%)	
Mucinous	7 (4.5)
Mixed	44 (28.2)
Cylindocellular	3 (1.9)
Tubular	70 (44.9)
Unclassified	32 (20.5)
Previous adjuvant chemotherapy, n (%)	55 (35.3)
Lymph node status, n (%)	
N0	35 (22.4)
N1	42 (26.9)
N2a	20 (12.8)
N2b	17 (10.9)
Nx	42 (27.0)
Invasive extent, n (%)	
Tx	10 (6.4)
T1	1 (0.6)
T2	17 (10.9)
T3	105 (67.3)
T4	23 (14.7)

N, lymph node involvement stage; T, tumour stage.

Table II. Comparison of *PIK3CA* gene mutation status and clinicopathological variables (n=156).

Clinicopathological variable	*PIK3CA* status		Statistical test	P-value
	Wild-type	Mutation		
Patients, n	141	15		
Age, years			1026.0[a]	0.8521
Median	67	69		
Range	25-85	37-79		
Gender, n			0.25[c]	0.6165
Male	52	4		
Female	89	11		
Histological differentiation grade, n			2.70[c]	0.1003
High/moderate	30	0		
Low/unknown	111	15		
Primary tumour localisation, n			0.07[b]	0.7914
Sigmoid/rectum	79	9		
Colon	61	6		
Karnofsky performance status, n			0.65[c]	0.4214
≤80	14	0		
>80	127	15		
Primary tumour size, n			0.04[c]	0.8445
T1/T2	17	1		
T3/T4	124	14		
Lymph node involvement grade, n			2.92[b]	0.0873
N0	29	6		
N positive	112	9		
Histological type, n			0.02[b]	0.8835
Tubular	63	7		
Other	78	8		
BRAF status, n			0.12[c]	0.7242
Wild-type	130	14		
Mutation	11	1		
KRAS status, n			1.13[b]	0.2872
Wild-type	103	9		
Mutation	38	6		

[a]Mann-Whitney test; [b]V-square; [c]Yates' χ^2 test. T, tumour stage; N0, no lymph node involvement; N positive, lymph node involvement.

analysis of the present patient cohort identified that gender and lymph node involvement acted as prognostic factors that influenced OS rate, as female patients survived for 57.5 months, compared with 39.3 months for male patients (P=0.0111, and the median OS in patients without lymph node metastases was 61.4 months, compared with 45.4 months in patients presenting with metastases (P=0.0122). The OS rate associated with other clinical parameters, consisting of age, primary tumour localisation, Karnofsky performance status, histological type, histological differentiation grade, primary tumour size and gene mutation status, did not differ significantly between groups.

Clinical and pathological variables identified by multivariate analysis as potential prognostic factors for OS rate. The results of the multivariate analysis are summarised in Table IV. Multivariate analysis identified that lymph node involvement grade [hazard ratio (HR), 1.68; P=0.0467] and male gender (HR, 1.57; P=0.0249) were adverse prognostic factors for OS rates. *KRAS*, *BRAF* and *PIK3CA* gene mutation status was not found to significantly affect OS rate in this analysis.

Discussion

The treatment of cancer is increasingly based on targeted therapy, including morphological identification of tumour histology, tumour staging and identification of target pathways and molecules. It has been established that *KRAS* mutation

Table III. Univariate analysis of OS rate (log-rank test).

Clinical parameter	n	Median OS, months	P-value
Age, years			0.9269
<70	102	59.2	
≥70	54	45.4	
Gender			0.0111[a]
Male	56	39.3	
Female	100	57.5	
Primary tumour localisation			0.9432
Sigmoid/rectum	67	44.6	
Colon	89	49.6	
Karnofsky performance status			0.6373
≤80	14	20.9	
>80	142	49.4	
Lymph node involvement			0.0122[a]
Present	121	45.4	
Absent	35	61.4	
Histological type			0.9808
Tubular	86	48.8	
Other	70	47.8	
Histological differentiation grade			0.1331
High/moderate	126	52.4	
Low/unknown	30	29.3	
Primary tumour size			0.1280
T1/T2	18	60.1	
T3/T4	138	47.4	
PIK3CA status			0.1271
Mutation	15	56.7	
Wild-type	141	47.6	
KRAS status			0.7740
Mutation	44	47.9	
Wild-type	112	48.8	
BRAF status			0.6398
Mutation	12	22.7	
Wild-type	144	49.4	

[a]Statistically significant correlation (P<0.05). OS, overall survival; T, tumour stage.

is a marker of resistance to anti-EGFR therapy in patients with CRC (4,18-21). Despite the exclusion of patients with *KRAS*-mutant tumours, anti-EGFR treatment fails in numerous patients with CRC. A number of studies have demonstrated a negative correlation between *PIK3CA* mutations and clinical outcomes (3,4,8,10,12,22).

The aim of the current study was to evaluate the incidence of *PIK3CA* gene mutation in patients with CRC at all stages, and also to determine the association between mutation of the *PIK3CA* gene and mutations in other downstream effectors of the EGFR signalling pathway. Additionally, the incidence of mutations in exon 9 and exon 20 of the *PIK3CA* gene were examined. The present study also evaluated the role of the *PIK3CA* gene mutation and the select clinical and pathological variables of these tumours as potential prognostic factors.

Activating mutations in the *PIK3CA* gene are identified in 7-32% of CRC patients. In the present study, *PIK3CA* mutations were detected in 9.6% of CRCs, which is consistent with previously published data (3,7,8,10-12,18,23,24). However, in contrast to a number of earlier studies (3,10,11,18,23-25), the present analysis identified a similar frequency of mutations in exons 9 and 20. Overall, 46.7% of patients possessed mutations in codon 9, while 53.3% possessed mutations in codon 20. Pentheroudakis *et al* (25) detected the *PIK3CA* mutation in exon 9 in 54% of cases, and in exon 20 in 13.5% of cases. Double *PIK3CA* gene mutations in exons 9 or 20 were not detected in the present cohort, in contrast to the studies

Table IV. Multivariate analysis of overall survival rate.

Clinical parameter comparison	Multivariate analysis, HR (95% CI)	P-value
Lymph node involvement	1.68 (1.01-2.82)	0.0467
Gender	1.57 (1.06-2.32)	0.0249
PIK3CA status	NS	>0.05
KRAS status	NS	>0.05
BRAF status	NS	>0.05

NS, not significant; HR, hazard ratio; CI, confidence interval.

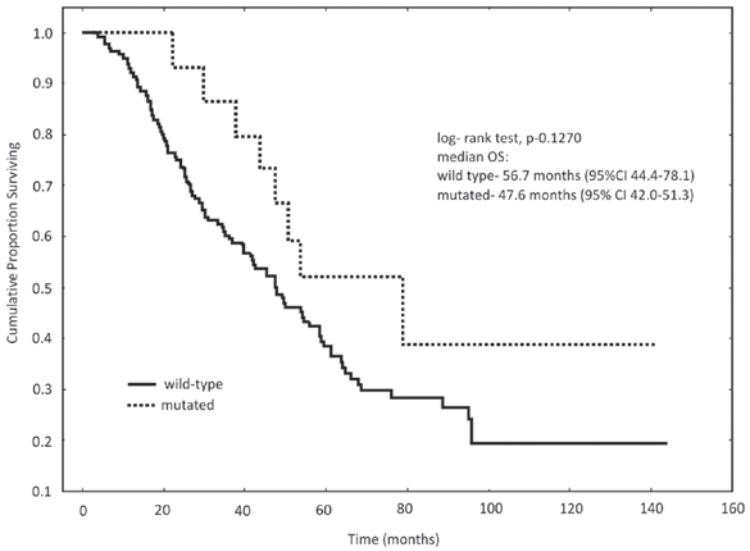

Figure 1. OS rates in patients with *PIK3CA* gene mutations, relative to those with wild-type *PIK3CA*. OS, overall survival.

conducted by Rock *et al* (18) and Sartore-Bianchi *et al* (26). In the present study, mutation in the *PIK3CA* gene coincided with *KRAS* gene mutations in six patients, comprising 40% of *PIK3CA* mutated tumours, and with *BRAF* mutations in one patient, comprising 6.6% of *PIK3CA* mutated tumours. Similar associations have been reported in previous studies (10,12,24,26).

Tumours with *PIK3CA* mutations were characterised by a predominantly distal colonic location, the frequent presence of tubular differentiation and a low grade of histological differentiation. These results are in contrast with previously published studies, with certain studies identifying no clinical features that were associated with *PIK3CA* gene mutations (7,22), while others identified an association between *PIK3CA* gene mutation and tumour mucinous differentiation and proximal colon location (10,24). Notably, patients with more advanced disease, T stage III or IV, or those demonstrating the involvement of lymph nodes presented with an increased rate of *PIK3CA* gene mutations. Similar associations have been previously reported (7,27). The difference in the clinicopathological characteristics of the tumour between the mutation statuses of particular exons was not estimated due to the small number of tumours demonstrating *PIK3CA* mutations in the present patient population.

In the present study, the results of the univariate and multivariate analyses into the role of clinical and pathological variables revealed a positive, statistically significant association between female gender and uninvolved lymph nodes on the overall patient survival.

The present study did not confirm a prognostic role for *PIK3CA* mutation status in CRC patients, in contrast to the results obtained by Rosty *et al* (10) and Therkildsen *et al* (28), who observed a shorter survival time in patients with *PIK3CA* mutations. The current results are consistent with findings reported by Cathomas (24), Zhu *et al* (7) and Karapetis *et al* (29), that *PIK3CA* exhibited no prognostic impact. Ogino *et al* (30) also reported that tumour *PIK3CA* mutation status is not associated with stage III colon cancer prognosis. Compared with carriers of wild-type *PIK3CA*, patients with a *PIK3CA*-mutated tumour had a shorter OS rate. However, this trend was not statistically significant. Similar data have been reported in previous studies (11,18,26). Studies have reported differences between exon 9 and 20 mutations with regard to their effects on PFS and OS, noting that *PIK3CA* exon 20 mutations were significantly associated with poorer PFS and OS (3,4). The biological effects of mutations in exons 9 and 20 of the *PIK3CA* gene were not compared in the present study due to the small number of patients with mutant

PIK3CA. It has previously been reported that the coexistence of *PIK3CA* exon 9 and 20 mutations is associated with poor prognosis in CRC patients (31).

The results of the present study indicate that aberrations in *PIK3CA* did not contribute significant prognostic information. The role of the *PIK3CA* mutation status remains unclear; therefore future prospective multi-centre trials involving CRC patients are essential in order to fully assess the clinical relevance of the *PIK3CA* mutation status.

In summary, activating mutations in the *PIK3CA* gene were present in 9.6% of colorectal carcinomas, and coincided with mutations in other downstream effectors of the EGFR signalling pathway. The results from this analysis of CRC patients of all disease stages indicates that the *PIK3CA* mutation status is not a prognostic factor in these patients. In addition, there is no statistically significant association between *PIK3CA* mutation and clinicopathological factors.

References

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. http://globocan.iarc.fr. Accessed April 30, 2014.
2. Baldus SE, Schaefer KL, Engers R, Hartleb D, Stoecklein NH and Gabbert HE: Prevalence and heterogeneity of *KRAS*, *BRAF*, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases. Clin Cancer Res 16: 790-799, 2010.
3. Custodio A and Feliu J: Prognostic and predictive biomarkers for epidermal growth factor receptor-targeted therapy in colorectal cancer: Beyond *KRAS* mutations. Crit Rev Oncol Hematol 85: 45-81, 2013.
4. Yang ZY, Wu XY, Huang YF, Di MY, Zheng DY, Chen JZ, Ding H, Mao C and Tang JL: Promising biomarkers for predicting the outcomes of patients with *KRAS* wild-type metastatic colorectal cancer treated with anti-epidermal growth factor receptor monoclonal antibodies: A systematic review with meta-analysis. Int J Cancer 133: 1914-1925, 2013.
5. Soeda H, Shimodaira H, Watanabe M, Suzuki T, Gamoh M, Mori T, Komine K, Iwama N, Kato S and Ishioka C: Clinical usefulness of *KRAS*, *BRAF*, and PIK3CA mutations as predictive markers of cetuximab efficacy in irinotecan- and oxaliplatin-refractory Japanese patients with metastatic colorectal cancer. Int J Clin Oncol 18: 670-677, 2013.
6. German S, Aslam HM, Saleem S, Raees A, Anum T, Alvi AA and Haseeb A: Carcinogenesis of PIK3CA. Hered Cancer Clin Pract 11: 5, 2013.
7. Zhu K, Yan H, Wang R, Zhu H, Meng X, Xu X, Dou X and Chen D: Mutations of *KRAS* and PIK3CA as independent predictors of distant metastases in colorectal cancer. Med Oncol 31: 16, 2014.
8. Stintzing S and Lenz HJ: A small cog in a big wheel: PIK3CA mutations in colorectal cancer. J Natl Cancer Inst 105: 1775-1776, 2013.
9. Ikenoue T, Kanai F, Hikiba Y, *et al*: Functional analysis of PIK3CA gene mutations in human colorectal cancer. Cancer Res 65: 4562-4567, 2005.
10. Rosty C, Young JP, Walsh MD, *et al*: PIK3CA activating mutation in colorectal carcinoma: Associations with molecular features and survival. PLoS ONE 8: e65479, 2013.
11. Shen Y, Wang J, Han X, Yang H, Wang S, Lin D and Shi Y: Effectors of epidermal growth factor receptor pathway: The genetic profiling of *KRAS*, *BRAF*, PIK3CA, NRAS mutations in colorectal cancer characteristics and personalized medicine. PLoS ONE 8: 12, 2013.
12. Soeda H, Shimodaira H, Watanabe M, Suzuki T, Gamo M, Takahashi M, Komine K, Kato S and Ishioka C: *KRAS* mutation in patients with metastatic colorectal cancer does not preclude benefit from oxaliplatin-or irinotecan-based treatment. Mol Clin Oncol 2: 356-362, 2014.
13. Huang L, Liu Z, Deng D, Tan A, Liao M, Mo Z and Yang X: Anti-epidermal growth factor receptor monoclonal antibody-based therapy for metastatic colorectal cancer: A meta-analysis of the effect of PIK3CA mutations in *KRAS* wild-type patients. Arch Med Sci 10: 1-9, 2014.
14. Eisenhauer EA, Therasse P, Bogaerts J, *et al*: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45: 228-247, 2009.
15. Stec R, Bodnar L, Charkiewicz R, *et al*: K-Ras gene mutation status as a prognostic and predictive factor in patients with colorectal cancer undergoing irinotecan- or oxaliplatin-based chemotherapy. Cancer Biol Ther 13: 1235-1243, 2012.
16. Samuels Y, Wang Z, Bardelli A, *et al*: High frequency of mutations of the PIK3CA gene in human cancers. Science 304: 554, 2004.
17. Li VS, Wong CW, Chan TL, Chan AS, Zhao W, Chu KM, So S, Chen X, Yuen ST and Leung SY: Mutations of PIK3CA in gastric adenocarcinoma. BMC Cancer 5: 29, 2005.
18. De Roock W, Claes B, Bernasconi D, *et al*: Effects of *KRAS*, *BRAF*, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: A retrospective consortium analysis. Lancet Oncol 11: 753-762, 2010.
19. Razis E, Pentheroudakis G, Rigakos G, *et al*: EGFR gene gain and PTEN protein expression are favorable prognostic factors in patients with *KRAS* wild-type metastatic colorectal cancer treated with cetuximab. J Cancer Res Clin Oncol 140: 737-748, 2014.
20. Tejpar S and Piessevaux H: Personalized medicine in metastatic colorectal cancer treated with anti-epidermal growth factor receptor agents: A future opportunity? Asia Pac J Clin Oncol 10: 2-10, 2014.
21. Domagała P, Hybiak J, Sulżyc-Bielicka V, Cybulski C, Ryś J and Domagała W: *KRAS* mutation testing in colorectal cancer as an example of the pathologist's role in personalized targeted therapy: A practical approach. Pol J Pathol 63: 145-164, 2012.
22. Ogino S, Nosho K, Kirkner GJ, *et al*: PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol 27: 1477-1484, 2009.
23. Herreros-Villanueva M, Gomez-Manero N, Muñiz P, García-Girón C and Coma del Corral MJ: PIK3CA mutations in *KRAS* and *BRAF* wild type colorectal cancer patients. A study of Spanish population. Mol Biol Rep 38: 1347-1351, 2011.
24. Cathomas G: PIK3CA in Colorectal Cancer. Front Oncol 4: 35, 2014.
25. Pentheroudakis G, Kotoula V, De Roock W, *et al*: Biomarkers of benefit from cetuximab-based therapy in metastatic colorectal cancer: Interaction of EGFR ligand expression with RAS/RAF, PIK3CA genotypes. BMC Cancer 13: 49, 2013.
26. Sartore-Bianchi A, Martini M, Molinari F, *et al*: PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res 69: 1851-1857, 2009.
27. Prenen H, De Schutter J, Jacobs B, De Roock W, Biesmans B, Claes B, Lambrechts D, Van Cutsem E and Tejpar S: PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. Clin Cancer Res 15: 3184-3188, 2009.
28. Therkildsen C, Bergmann TK, Henrichsen-Schnack T, Ladelund S and Nilbert M: The predictive value of *KRAS*, NRAS, *BRAF*, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis. Acta Oncol 53: 852-864, 2014.
29. Karapetis CS, Jonker D, Daneshmand M, *et al*; NCIC Clinical Trials Group and the Australasian Gastro-Intestinal Trials Group: PIK3CA, *BRAF*, and PTEN status and benefit from cetuximab in the treatment of advanced colorectal cancer--results from NCIC CTG/AGITG CO.17. Clin Cancer Res 20: 744-753, 2014.
30. Ogino S, Liao X, Imamura Y, *et al*; Alliance for Clinical Trials in Oncology: Predictive and prognostic analysis of PIK3CA mutation in stage III colon cancer intergroup trial. J Natl Cancer Inst 105: 1789-1798, 2013.
31. Liao X, Morikawa T, Lochhead P, *et al*: Prognostic role of PIK3CA mutation in colorectal cancer: Cohort study and literature review. Clin Cancer Res 18: 2257-2268, 2012.