Correlation between *KRAS*, *NRAS* and *BRAF* mutations and tumor localizations in patients with primary and metastatic colorectal cancer

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Abstract

Introduction: Detection of abnormalities in the *KRAS*, *NRAS* and *BRAF* genes is extremely important for proper qualification of colorectal cancer (CRC) patients for therapy with anti-EGFR (epidermal growth factor receptor) monoclonal antibodies. However, data about prevalence of mutations in these genes, in different localizations of CRC tumors, are limited.

Material and methods: We examined the frequency of mutations in the *KRAS*, *NRAS* and *BRAF* genes in 500 Caucasian CRC patients (200 women and 300 men, median age 66 years). DNA was isolated from formalin-fixed, paraffin-embedded (FFPE) tissues using a Qiagen QIAamp DNA FFPE-kit. Analysis of mutations was carried out using the *KRAS*/*BRAF*, *NRAS* and *BRAF* Mutation Analysis Kit for Real-Time PCR (EntroGen) with the Cobas 480 real-time PCR apparatus (Roche Diagnostics).

Results: *KRAS* mutations were detected in 190 patients (38%), *NRAS* mutations in 20 patients (4%), and *BRAF* mutations in 24 patients (4.8%). There were no associations between age of CRC patients and frequency of *KRAS*, *NRAS* and *BRAF* gene mutations. These mutations were significantly more often diagnosed in women (55.5%) than in men (41%, $p < 0.005$). Tumors of the rectum and sigmoideum were the most often observed in both groups of CRC patients – with and without *KRAS*, *NRAS* and *BRAF* gene mutations. However, transverse colon, ascending colon and cecum cancers were the most often affected by mutations.

Conclusions: Our study showed that the occurrence of mutations in the *KRAS*, *NRAS* and *BRAF* genes is not accidental and depends on the location of CRC tumors.

Key words: *KRAS*, *NRAS*, *BRAF*, colorectal cancer.

Introduction

According to the American Cancer Society, there were over 135,000 new cases of colorectal cancer (CRC) (95,520 colon cancers and 39,910 rectal cancers) in 2017 in the United States. Due to the large number of new cases, colorectal cancer was in the third place among the cancerous

causes of deaths in men and women, which resulted in more than 50,000 deaths for this reason in the US in 2017, and about 655,000 worldwide. The risk of this type of cancer is slightly higher in men than in women [1–3].

Many aspects are mentioned among the causes of development of CRC. First of all, scientists have acknowledged that the main factor which increases the risk of the disease is inheritance of mutations from first-degree relatives. Almost 30% of patients have at least one relative in the family who suffers from CRC [2]. The second factor that predisposes to CRC is familial adenomatous polyposis (FAP), which appears in around 1% of all CRC cases. Another disease which can predispose to CRC is chronic inflammatory bowel disease, as known as Crohn disease and ulcerative colitis. Authors, among risk factors, also mention diet and lifestyle, type 2 diabetes, cigarette smoking, alcohol abuse, obesity, diet low in fiber, and an excess of consumed fats, carbohydrates as well as red and processed meat. Physical activity and long-term treatment with low doses of aspirin may have preventive value in the development of CRC. These factors have different influence on particular parts of the colon, sigmoideum and rectum [1, 2].

The RAS and RAF family proteins mediate signaling of growth factor receptors via the PI3K-AKT-mTOR and RAS-RAF-MEK-ERK pathways, thereby participating in cell survival and proliferation [4]. Excessive activity of these signaling pathways is often found in various cancers. It is caused mainly by mutations in *RAS* and *BRAF* genes. Based on the deficiency of DNA repair and influence of carcinogens, these oncogenes are often mutated in CRC patients. Right-sided colon cancer is characteristic for women and probably shows microsatellite instability as well as *BRAF* mutations. Left-sided colon cancer is more common in men and shows chromosomal instability as well as *KRAS* mutations. Detection of abnormalities in the *KRAS*, *NRAS* and *BRAF* genes is extremely important for proper qualification of patients for panitumumab and cetuximab therapy, which have been authorized by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) on the basis of several clinical trials, including PRIME (panitumumab) and CRYSTAL (cetuximab) studies [5–11].

In our study, we examined the frequency of mutations in the *KRAS*, *NRAS* and *BRAF* genes in a large group of Caucasian CRC patients. The molecular tests were performed during the routine diagnostic process in qualification of CRC patients for first line chemotherapy with anti-EGFR (epidermal growth factor receptor) antibodies. For the first time, we examined the relationship between the exact location of CRC and the presence of particular mutations.

Material and methods

The study group included 500 patients (200 women and 300 men) with CRC including cancers in the small intestine (ICD-10: C17), colon (ICD-10: C18), rectosigmoid flexure (ICD-10: C19), rectum (ICD-10: C20) and anus (ICD-10: C21). The median age for men and women was the same: 66 years. 447 patients had locally advanced disease, while 53 patients had metastases at the time of diagnosis (with available material from the metastases). Patients were characterized in terms of age, gender and tumor localization. In the studied population, rectal and sigmoid cancers were the most common (61% of all CRC patients). Detailed characteristics of our group are presented in Table I.

DNA was isolated from formalin-fixed, paraffin-embedded (FFPE) tissues using the Qiagen QIAamp DNA FFPE-kit with the CE-IVD certificate. Tissue was collected and mutations were searched at the time of the diagnosis of colon and rectum cancer. The DNA was isolated from a paraffin block containing at least 50% of tumor cells. The percentage and presence of cancer cells were confirmed in the pathomorphological examination. FFPE samples were collected in 2012–2018. Analysis of mutations in the *KRAS*, *NRAS* and *BRAF* genes was carried out using three kits of the *KRAS*/*BRAF*, *NRAS* and *BRAF* Mutation Analysis Kit for Real-Time PCR (EntroGen, CE-IVD), on Cobas 480 real-time PCR apparatus (Roche Diagnostics). The tests examined the most common mutations in codons 12, 13, 59, 61, 117 and 146 in *KRAS* and *NRAS* genes, as well as in codon 600 of the *BRAF* gene. The tests can detect a mutation load of less than 1%. This sensitivity greatly depends on the extent of fragmentation and quality of the isolated DNA.

No attempt was made to find mutations in whole blood due to the availability of only FFPE tissues.

Statistical analysis was performed using the χ^2 test to determine the relationship between different tumor localization and the occurrence of mutations. Results were statistically significant when the p value was below 0.05.

The study was approved by the Local Ethical Committee of the Medical University of Lublin (no. KE-0254/218/2015).

Results

Frequency of *KRAS*, *NRAS* and *BRAF* mutations in colorectal cancer

KRAS mutations were detected in 190 patients (38%), *NRAS* mutations in 20 patients (4%), and *BRAF* mutations in 24 patients (4.8%). The most common substitution in the *KRAS* gene

Table I. Patient characteristics

			***KRAS* mutation status**							***NRAS* mutation status**					***BRAF* mutation status**
				Codon							**Codon**				**Codon 600**
Population characteristics	**Population, *n* (%)**	**Lack of mutations, *n* (%)**	**Presence of mutations, *n* (%)**	**12**	**13**	**59**	**61**	**117**	**146**	**Presence of mutations, *n* (%)**	**12**	**13**	**61**	**146**	**Presence of mutations, *n* (%)**
All	500 (100)	266 (100)	190 (100)	144	28	1	7	2	8	20 (100)	4	2	13	1	24 (100)
Gender															
Women	200 (40)	89 (33.5)	92 (48.4)	71	12	0	3	2	4	6 (30)	2	0	4	0	**13 (54)**
Men	**300 (60)**	**177 (66.5)**	**98 (51.6)**	73	16	1	4	0	4	**14 (70)**	2	2	9	1	11 (46)
Age (years)															
≥ 66	**267 (53.4)**	**144 (54.1)**	**102 (53.7)**	76	15	0	5	1	5	8 (40)	2	0	5	1	**13 (54)**
< 66	233 (46.6)	122 (45.9)	88 (46.3)	68	13	1	2	1	3	**12 (60)**	2	2	8	0	11 (46)
Tumor localization															
Cecum	37 (7.4)	14 (5.3)	18 (9.4)	12	2	0	2	1	1	0 (0)	0	0	0	0	5 (21)
Ascending colon	39 (7.8)	12 (4.5)	22 (11.6)	15	4	0	2	0	1	2 (10)	1	0	1	0	3 (12.5)
Transverse colon	17 (3.4)	5 (1.9)	8 (4.2)	5	1	0	1	0	1	0 (0)	0	0	0	0	4 (16.7)
Hepatic flexure	9 (1.8)	4 (1.5)	4 (2.1)	3	1	0	0	0	0	0 (0)	0	0	0	0	1 (4.2)
Splenic flexure	22 (4.4)	17 (6.4)	3 (1.6)	2	1	0	0	0	0	1 (5)	0	0	1	0	1 (4.2)
Descending colon	10 (2)	4 (1.5)	5 (2.6)	5	0	0	0	0	0	1 (5)	1	0	0	0	0 (0)
Sigmoid colon	102 (20.4)	63 (23.7)	33 (17.5)	25	6	1	1	0	0	3 (15)	0	1	2	0	3 (12.5)
Rectosigmoid flexure	53 (10.6)	34 (12.7)	16 (8.4)	12	2	0	0	0	2	2 (10)	0	0	2	0	1 (4.2)
Rectum	**150 (30)**	**84 (31.5)**	**53 (27.9)**	43	7	0	0	1	2	**9 (45)**	1	1	6	1	**4 (16.7)**
Small intestine	4 (0.8)	1 (0.4)	2 (1.1)	1	0	0	1	0	0	1 (5)	0	0	1	0	0 (0)
Ileocecal valve	4 (0.8)	2 (0.8)	2 (1.1)	2	0	0	0	0	0	0 (0)	0	0	0	0	0 (0)
Metastases	53 (10.6)	26 (9.8)	24 (12.5)	19	4	0	0	0	1	1 (5)	1	0	0	0	2 (8.3)

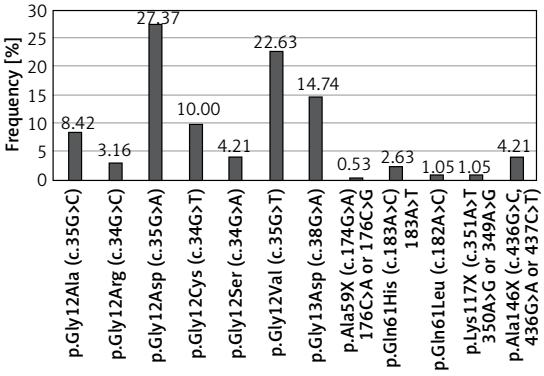

Figure 1. Frequency of mutations in particular codons of the *KRAS* gene

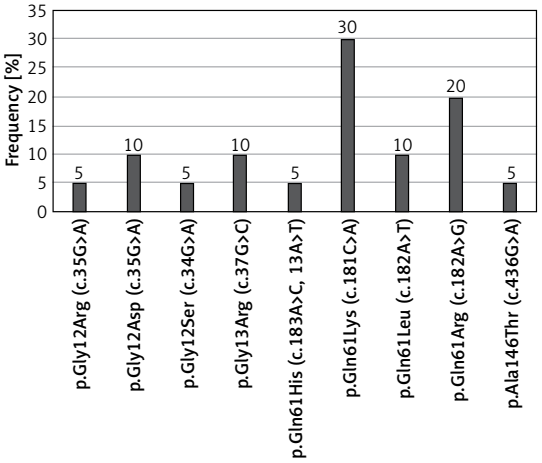

Figure 2. Frequency of mutations in particular codons of the *NRAS* gene

was p.Gly12Asp (27.37% of all *KRAS* mutations; 52/190). 90.53% of *KRAS* mutations occurred in codon 12 or 13 (Figure 1). The most common substitution in the *NRAS* gene was Gln61Lys (30% of all *NRAS* mutations; 6/20). 65% of *NRAS* mutations were found in codon 61 (Figure 2). Among all *BRAF* mutations, only Val600Glu was found.

Association between age, gender, tumor localization and mutation status

There were no associations between age of CRC patients and frequency of *KRAS*, *NRAS* and *BRAF* gene mutations. These mutations were significantly more often diagnosed in women (55.5% of female patients; 111/200) than in men (41% of male patients; 123/300, $p < 0.005$). The frequency of *KRAS* mutations and *BRAF* mutation was significantly higher in female than in male patients ($\chi^2 = 8.266$, $p = 0.0044$ and $\chi^2 = 4.14$, $p = 0.042$, respectively), while the frequency of *NRAS* mutations was similar in both sexes ($\chi^2 = 0.1$, $p = 0.75$) (Tables I and II).

Rectal and sigmoid cancers were the most often diagnosed tumors in both groups of patients: with and without *KRAS*, *NRAS* or *BRAF* gene mutations (Table I). However, only 28% of patients with *KRAS* mutations (53/190) and up to 45% of patients with *NRAS* mutation (9/20) had rectal cancer. The most common tumor localization in patients with *BRAF* mutations was the cecum (21% of patients with this mutation; 5/24) (Table I). Mutations were most often found in tumors of the transverse colon (70.6% of all patients with transverse colon cancer, 12/17, $p < 0.05$ in comparison to other CRC localizations) and the ascending colon (73% of all patients with ascending colon cancer, 27/39, $p < 0.005$ in comparison to other CRC localizations) as well as in cecum cancer (62.2% of patients with cecum cancer, 23/37, $p = 0.0516$ in comparison to other CRC localizations). Patients with cancers of the sigmoideum (38.2% of all sigmoid cancer, 39/102, $p = 0.052$ in comparison to other CRC localizations) and splenic flexure (22.7% of all splenic flexure cancer, 5/22, $p < 0.05$ in comparison to other CRC localizations) had mutations confirmed the least frequently. Mutations were significantly more often found in patients with colon cancer including cancer of the cecum (56.7% of mutated tumors) than in patients with sigmoid and rectal cancers (42.6% of mutated tumors, $p = 0.002$, $\chi^2 = 9.57$) (Table II). The mutation significantly more frequently occurred on the right side of the large intestine (65% of this localizations of tumor, 63/97) than on the left side of the large intestine (40.8% of this localization of tumor, 141/366). The incidence of *KRAS* and *BRAF* genes varied depending on the CRC localization in the right or left parts of the large intestine. In contrast, mutations in the *NRAS* gene occurred at a similar frequency in these two CRC localizations (Table III).

The differences in the occurrence of individual CRC in men and women with and without mutations in the *KRAS*, *NRAS* and *BRAF* genes were not significant. However, mutations in the *KRAS* gene in the small intestine and in the ileocecal valve were found only in female patients (Figure 3). Male patients with *NRAS* mutations suffered primarily from rectal cancer (57.1% of all men with *NRAS* mutations) and cancer of the rectosigmoid flexure (14.3% of all men with *NRAS* mutations). Single cases of sigmoid, ascending colon and small intestine cancers were found in men with *NRAS* mutations, whereas 50% of female patients with *NRAS* mutations suffered from colon cancer. Two cases of sigmoid and one case of rectal cancer were found in female patients with *NRAS* mutations. 45.5% of men and only 23.1% of women with *BRAF* mutation had rectal or sigmoid cancers, whereas colon (38.5% of all women with *BRAF* mutations) and cecum (23.1% of all women with *BRAF* mutations) cancers predominated in female patients with *BRAF* mutations.

Table II. Relationship between occurrence of mutations in the *KRAS*, *NRAS* and *BRAF* genes and sex, age, and tumor localization in colorectal cancer (CRC) patients

Patient characteristics	*n*	%	Mutation status	*n*	%
All	500	100	Lack of mutation	266	53.2
			Presence of mutation	234	46.8
Age (years)					
≥ 66	267	53.4	Lack of mutation	144	53.9
			Presence of mutation	123	46.1
< 66	233	46.6	Lack of mutation	122	52.4
			Presence of mutation	111	47.6
p, χ^2			0.72, 0.24		
Gender					
Women	200	40	Lack of mutation	89	44.5
			Presence of mutation	111	55.5
Men	300	60	Lack of mutation	177	59.0
			Presence of mutation	123	41.0
p, χ^2			**0.00145, 10.134**		
Tumor localization					
Small intestine	4	0.8	Lack of mutation	1	25.00
			Presence of mutation	3	75.00
Other localizations	496	99.2	Lack of mutation	265	53.43
			Presence of mutation	231	46.57
p, χ^2			0.2564, 1.288		
Ileocecal valve	4	0.8	Lack of mutation	2	50.00
			Presence of mutation	2	50.00
Other localizations	496	99.2	Lack of mutation	264	53.23
			Presence of mutation	232	46.77
p, χ^2			0.8962, 0.017		
Cecum	37	7.4	Lack of mutation	14	37.84
			Presence of mutation	23	62.16
Other localizations	463	92.6	Lack of mutation	252	54.43
			Presence of mutation	211	45.57
p, χ^2			0.0516, 3.787		
Ascending colon	39	7.8	Lack of mutation	12	30.77
			Presence of mutation	27	69.23
Other localizations	461	92.2	Lack of mutation	254	55.10
			Presence of mutation	207	44.90
p, χ^2			**0.0034, 8.548**		
Hepatic flexure	9	1.80	Lack of mutation	4	44.44
			Presence of mutation	5	55.56
Other localizations	491	98.2	Lack of mutation	262	53.36
			Presence of mutation	229	46.64
p, χ^2			0.5953, 0.282		
Transverse colon	17	3.40	Lack of mutation	5	29.41
			Presence of mutation	12	70.59
Other localizations	483	96.6	Lack of mutation	261	54.04
			Presence of mutation	222	45.96
p, χ^2			**0.0455, 4.000**		

Table II. Cont.

Patient characteristics	*n*	%	Mutation status	*n*	%
Splenic flexure	22	4.4	Lack of mutation	17	77.27
			Presence of mutation	5	22.73
Other localizations	478	95.6	Lack of mutation	249	52.09
			Presence of mutation	229	47.91
p, χ^2			**0.0206, 5.356**		
Descending colon	10	2	Lack of mutation	4	40.00
			Presence of mutation	6	60.00
Other localizations	490	98	Lack of mutation	262	53.47
			Presence of mutation	228	46.53
p, χ^2			0.3981, 0.714		
Sigmoid colon	102	20.4	Lack of mutation	63	61.76
			Presence of mutation	39	38.24
Other localizations	398	79.6	Lack of mutation	203	51.00
			Presence of mutation	195	49.00
p, χ^2			0.05202, 3.775		
Rectosigmoid flexure	53	10.60	Lack of mutation	34	64.15
			Presence of mutation	19	35.85
Other localizations	447	89.4	Lack of mutation	232	51.90
			Presence of mutation	215	48.10
p, χ^2			0.0910, 2.856		
Rectum	150	30	Lack of mutation	84	56.00
			Presence of mutation	66	44.00
Other localizations	350	70	Lack of mutation	182	52.00
			Presence of mutation	168	48.00
p, χ^2			0.4113, 0.675		
Metastases	53	10.60	Lack of mutation	26	49.05
			Presence of mutation	27	50.95
Other localizations	447	89.4	Lack of mutation	240	53.69
			Presence of mutation	207	46.31
p, χ^2			0.5224, 0.409		
Right side of large intestine	97	20.95	Lack of mutation	34	35.06
			Presence of mutation	63	64.94
Left side of large intestine	366	79.05	Lack of mutation	205	59.25
			Presence of mutation	141	40.75
p, χ^2			**0.00002385, 17.854**		

Discussion

Frequency of *KRAS*, *NRAS* and *BRAF* gene mutations was assessed in previous clinical trials that evaluated the efficacy of anti-EGFR antibodies in the first and third line of treatment in CRC patients [4, 11–15].

The lack of efficacy of cetuximab combined with first line chemotherapy with 5-fluorouracil and oxaliplatin in patients with *KRAS* gene mutations was demonstrated in the OPUS study (the study group consisted of 314 patients). *KRAS* codons 12 and 13 mutations were found in 43.2% of CRC patients and the Val600Glu *BRAF* mutation in 3.5% of CRC patients. Efficacy of cetuximab was observed only in patients with the wild-type *KRAS* gene (codons 12 and 13). Rare *RAS* mutations were examined in archival material a few years later using the BEAMing technique. 26.3% of patients without *KRAS* codons 12 and 13 mutations had rare *RAS* mutations, including *KRAS* mutations in codon 59 or 61 (5.9% of patients), in codon 117 or 146 (9.3% of patients) as well as *NRAS* mutations in codon 12 or 13 (6.8% of patients), in codon 59 or 61 (in 5.1% of patients) and in codon 117 or 146 (0.8% of patients). Patients with rare *RAS* mutations also did not benefit from cetuximab therapy [15, 16].

Table III. Relationship between occurrence of mutations in the *KRAS*, *NRAS* and *BRAF* genes and tumor localization in colorectal cancer (CRC) patients. *Lack of any examined mutations (wild type)

Gene status					
***KRAS* gene status**	***n***	**%**		***n***	**%**
Right side of large intestine	84	20.84	Lack of mutations*	34	40.48
			Presence of mutations	50	59.52
Left side of large intestine	319	79.16	Lack of mutations*	205	64.26
			Presence of mutations	114	35.74
p, χ^2			0.00007871, 15.589		
***NRAS* gene status**					
Right side of large intestine	36	14	Lack of mutations*	34	94.45
			Presence of mutations	2	5.55
Left side of large intestine	221	86	Lack of mutations*	205	92.76
			Presence of mutations	16	7.24
p, χ^2			0.7133, 0.135		
***BRAF* gene status**					
Right side of large intestine	45	17.24	Lack of mutations*	34	75.56
			Presence of mutations	11	24.44
Left side of large intestine	216	82.76	Lack of mutations*	205	94.91
			Presence of mutations	11	5.09
p, χ^2			0.0000213, 18.069		

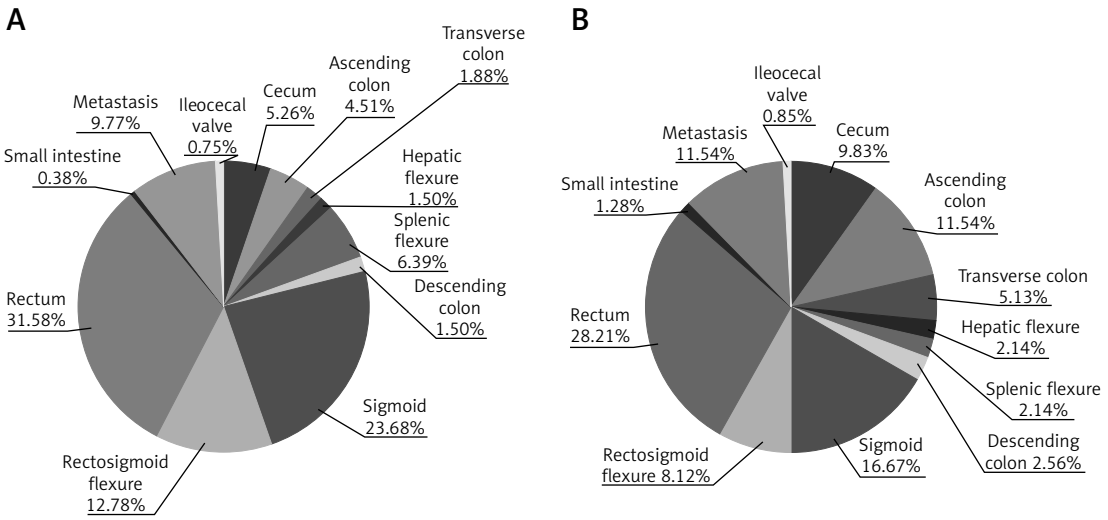

Figure 3. Differences in colorectal cancer (CRC) localization in patients without *KRAS*, *NRAS* or *BRAF* gene mutations (**A**) and in patients with mutations in the *KRAS*, *NRAS* or *BRAF* genes (**B**)

The efficacy of first line chemotherapy based on irinotecan and 5-fluorouracil with or without cetuximab in patients without *KRAS* mutations (codons 12 and 13) was examined in the CRYSTAL study. *KRAS* gene mutations in codon 12 or 13 were found in 37.3% and the Val600Glu mutation in the *BRAF* gene in 6.6% of CRC patients (the study group consisted of 1063 patients). The benefit of cetuximab was not the same in all patients with the wild-type *KRAS* gene (codons 12 and 13). Therefore, rare *RAS* mutations in patients enrolled in thee CRYSTAL trial were examined. 14.7% of 430 patients with wild-type *KRAS* codons 12 and 13 had rare *RAS* gene mutations. Mutations in codons 59 and 61 of the *KRAS* gene were present in 3.3% of patients and mutations in codons 117 and 146 were present in 5.6% of patients. *NRAS* gene mutations were found in codons 12 and 13 in 3.5% of patients, in codons 59 and 61 in 2.8% of patients, and in codons 117 and 146 in 0.9% of patients. Effectiveness of cetuximab in patients with rare *RAS* mutations was unsatisfactory [9, 17].

The PRIME clinical trial compared the efficacy and safety of panitumumab, 5-fluorouracil and oxaliplatin with chemotherapy alone in the first-line

treatment of 1096 CRC patients. 67% of patients had *KRAS* codon 12 or 13 mutations. *KRAS* codon 61 was mutated in 4% of patients and codon 117 or 146 was mutated in 6% of patients. Mutations in the *NRAS* gene in codons 12 and 13 were found in 3% of patients and in codon 61 in 4% of patients. There were no mutations in codon 117 or 146 of the *NRAS* gene. Mutations in the *BRAF* gene occurred in 8% of CRC patients. The effectiveness of panitumumab was closely related to the absence of mutations in *RAS* and *BRAF* genes [8].

In the PEAK study (221 patients with known status of examined genes), the effectiveness of panitumumab monotherapy in third line treatment of CRC patients was examined. The following mutations were found in CRC patients from the PEAK trial: in codon 12 or 13 of the *KRAS* gene in 43.1% of patients, in codon 59 or 61 of the *KRAS* gene in 4.8% of patients, in codon 117 or 146 of the *KRAS* gene in 5% of patients, in codon 12 or 13 of the *NRAS* gene in 4.2% of patients, in codon 59 or 61 of the *NRAS* gene in 3% of patients and in codons 117 and 146 of the *NRAS* gene in 1.1% of patients. The occurrence of the mutations was closely related to the lack of efficacy of panitumumab [18].

The incidence of examined mutations in our patients is lower than in the cited studies. Mainly, the frequency of mutations in the *KRAS* and *BRAF* genes is lower than in the CRYSTAL and PRIME studies. This is probably due to the lower sensitivity of real-time PCR technique used in the routine diagnosis of *RAS* and *BRAF* mutations in CRC patients in our study. The results of our study indicated that the most frequent mutation in the *KRAS* gene was Gly12Asp and in the *BRAF* gene was Val600Glu, according to the results of genetic tests carried out in clinical trials. Mutation in codon 61 was the most frequent mutation in the *NRAS* gene in our patients, which does not match with the results of other studies. The research should be supplemented by demonstrating that tumor heterogeneity and/or low sensitivity of diagnostic tests may have contributed to the fact that patients with wild-type RAS did not respond to anti-EGFR therapy due to the presence of mutations. The weakness of our study was the lack of information on how the patients were treated.

Kodaz *et al*. studied the relationship between the prevalence of *KRAS* mutations and the clinicopathological characteristics of colorectal cancer. The study group included 189 patients with CRC diagnosis. 47.6% of patients had a mutation in the *KRAS* gene. The study also showed that the most common *KRAS* mutations occurred in codon 12 (73.3% of all *KRAS* examined mutations) and the most common substitution was Gly12Asp (42.4% of all *KRAS* examined mutations). The authors found that a high percentage of young CRC patients (< 40 years) had the wild-type *KRAS* gene. They also suggested that *KRAS* point mutations in colorectal cancer exhibited a heterogeneous distribution in terms of tumor localization. In the cited study, there was no significant difference in *KRAS* mutation frequency according to tumor localization. Moreover, the authors found no association between *KRAS* mutation occurrence and gender [19].

Kawazoe *et al*. searched for *KRAS*, *NRAS*, *BRAF* and *PIK3CA* gene mutations in the material from 246 patients with metastatic CRC. 50% of patients had wild-type examined genes. Mutations in codons 12 and 13 of *KRAS* gene were found in 34.1% of patients, while mutations in codons 61 and 146 were detected in 10 cases (3.8%). *NRAS* gene mutations occurred in 11 patients (4.2%) and Val600Glu mutation in the *BRAF* gene occurred in 14 people (5.4%). The authors stated that primary rectal tumors tended to be more frequently *RAS*-mutated and *BRAF* mutant tumors were more likely to develop in the right colon. They observed no significant association between *RAS* gene status and other clinicopathological features such as age, sex, primary lesion localization, histology, or site of metastases, which is similar to the results of the study by Morris *et al*. [20, 21].

In our results, mutations were found to be associated with sex and anatomical location of the tumor. We observed the highest percentage of tumors with *KRAS*, *NRAS* and *BRAF* gene mutations in colon cancers. Moreover, the highest percentage of tumors with mutations were found in the right side of the large intestine. A higher percentage of female patients had *KRAS*, *NRAS* or *BRAF* mutations than male patients, which was also observed by Ng *et al*. in their research [22].

KRAS mutations were reported to be more frequent in right colon tumors by Bleeker *et al*. [23] and Loree *et al*. [24], but in left colon tumors by Zulhabri *et al*. [25]. Watanabe *et al*. [26] and Sinicrope *et al*. [27] found that *KRAS* codon 12 or 13 mutations were significantly more frequent in the right colon. Yamauchi *et al*. [28] reported that *KRAS* mutations were more common in cecum tumors. Brink *et al*. [29] reported that *KRAS* codon 13 mutation was more common among females with rectal tumors.

Moretto *et al*. [11] conducted a study of 75 CRC patients with wild-type *RAS* and *BRAF* genes. They found that patients with tumors located on the right side of the large intestine more often did not respond to therapy based on cetuximab or panitumumab compared to patients with tumors on the left side of the large intestine. If we assume that mutations occur more often on the right side of the large intestine, this relationship may result from the problems with detection of *RAS* and *BRAF* gene mutations due to tumor heterogeneity or low sensitivity of molecular tests.

In conclusion, our study showed that the occurrence of mutations in the *KRAS*, *NRAS* and *BRAF* genes is not accidental and depends on the location of CRC tumors. In case of failure of treatment with anti-EGFR antibodies in patients with tumor localization suggesting a higher probability of mutation presence, an insightful molecular examination is necessary.

Availability of data and materials

https://figshare.com/s/c7de96cf9b0220-7409c6

https://figshare.com/s/c6824d9cef9bbf-b2272e

Patient consent for publication

All patients gave us a statement of patient consent to use their tissues.

Conflict of interest

The authors declare no conflict of interest.

References

1. American Cancer Society. Colorectal cancer facts and figures 2017-2019. American Cancer Society, Atlanta 2017.
2. Colussi D, Brandi G, Bazzoli F, Ricciardiello L. Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. Int J Mol Sci 2013; 14: 16365-85.
3. Lamami Y, Mesediyeva R, Arikan S, et al. Preliminary report: one of the PD-1 gene variants may be a valuable marker for colorectal cancer. Arch Med Sci 2018; 3: e34-e40.
4. Lovly C, Horn L, Pao W. KRAS. My Cancer Genome. https://www.mycancergenome.org/content/disease/colorectal-cancer/kras/?tab=0, 2015 (accessed: 7 December 2017).
5. Kim SE, Paik HY, Yoon H, Lee JE, Kim N, Sung MK. Sex- and gender-specific disparities in colorectal cancer risk. World J Gastroenterol 2015; 21: 5167-75.
6. https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125084s0228lbl.pdf/ (accessed: 22 February 2018).
7. https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125147s080lbl.pdf/ (accessed: 22 February 2018).
8. Douillard JY, Siena S, Cassidy J, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol 2010; 28: 4697-705.
9. Van Cutsem E, Köhne CH, Láng I, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol 2011; 29: 2011-9.
10. Benson AB, Venook AP, Cederquist L, et al. Colon cancer. Version 1.2017. J Natl Compr Canc Netw 2017; 15: 370-98.
11. Moretto R, Cremolini C, Rossini D, Pietrantonio F, Battaglin F. Location of primary tumor and benefit from anti-epidermal growth factor receptor monoclonal antibodies in patients with RAS and BRAF wild-type metastatic colorectal cancer. Oncologist 2016; 21: 988-994.
12. Pang XL, Li QX, Ma ZP, et al. Association between clinicopathological features and survival in patients with primary and paired metastatic colorectal cancer and KRAS mutation. Onco Targets Ther 2017; 10: 2645-54.
13. Li W, Qiu T, Ling Y, Guo L, Li L, Ying J. Molecular pathological epidemiology of colorectal cancer in Chinese patients with KRAS and BRAF mutations. Oncotarget 2015; 37: 39607-13.
14. Irahara N, Baba Y, Nosho K, et al. NRAS mutations are rare in colorectal cancer. Diagn Mol Pathol 2010; 19: 157-63.
15. Bokemeyer C, Bondarenko I, Hartmann JT, et al. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. Ann Oncol 2011; 22: 1535-46.
16. Bokemeyer C, Köhne CH, Ciardiello F, et al. FOLFOX4 plus cetuximab treatment and RAS mutations in colorectal cancer. Eur J Cancer 2015; 51: 1243-52.
17. Van Cutsem E, Lenz HJ, Köhne CH, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. J Clin Oncol 2015; 33: 692-700.
18. Patterson SD, Peeters M, Siena S, et al. Comprehensive analysis of KRAS and NRAS mutations as predictive biomarkers for single agent panitumumab (pmab) response in a randomized, phase III metastatic colorectal cancer (mCRC) study (20020408). J Clin Oncol 2013; 31: 3617.
19. Kodaz H, Hacibekiroblu I, Erdogan B, et al. Association between specific KRAS mutations and the clinicopathological characteristics of colorectal tumors. Mol Clin Oncol 2015; 3: 179-84.
20. Kawazoe A, Shitara K, Fukuoka S, et al. A retrospective observational study of clinicopathological features of KRAS, NRAS, BRAF and PIK3CA mutations in Japanese patients with metastatic colorectal cancer. BMC Cancer 2015; 15: 258.
21. Morris VK, San Lucas FA, Overman MJ, et al. Clinicopathologic characteristics and gene expression analyses of non-KRAS 12/13, RAS-mutated metastatic colorectal cancer. Ann Oncol 2014; 25: 2008-14.
22. Yan-Seen Ng J, Lu CT, King-Yin Lam A. BRAF mutation: Current and future clinical. Histol Histopathol 2019; 34: 469-77.
23. Bleeker WA, Hayes VM, Karrenbeld A, et al. Impact of KRAS and TP53 mutations on survival in patients with left- and right-sided Dukes' C colon cancer. Am J Gastroenterol 2000; 95: 2953-7.
24. Loree JM, Pereira A, Lam M, et al. Classifying colorectal cancer by tumor location rather than sidedness highlights a continuum in mutation profiles and consensus molecular subtypes. Clin Cancer Res 2017; 24: 1-11.
25. Zulhabri O, Rahman J, Ismail S, Isa MR, Wan Zurinah WN. Predominance of G to A codon 12 mutation K-ras gene in Dukes' B colorectal cancer. Singapore Med J 2012; 53: 26-31.
26. Watanabe T, Yoshino T, Uetake H, et al. KRAS mutational status in Japanese patients with colorectal cancer: results from a nationwide, multicentre, cross-sectional study. Jpn J Clin Oncol 2013; 43: 706-12.
27. Sinicrope FA, Mahoney MR, Yoon HH, et al. Analysis of molecular markers by anatomic tumor site in stage III colon carcinomas from adjuvant chemotherapy Tri-

al NCCTG N0147 (Alliance). Clin Cancer Res 2015; 21: 5294-304.
28. Yamauchi M, Morikawa T, Kuchiba A, et al. Assessment of colorectal cancer molecular features along bowel subsites challenges the conception of distinct dichotomy of proximal versus distal colorectum. Gut 2012; 61: 847-54.
29. Brink M, de Goeij AF, Weijenberg MP, et al. K-ras oncogene mutations in sporadic colorectal cancer in The Netherlands Cohort Study. Carcinogenesis 2003; 24: 703-10.