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KRAS MUTATION FREQUENCY AND SPECTRUM IN COLORECTAL CANCER: CORRELATION WITH THE TUMOR LOCALIZATION IN KAZAKHSTANI POPULATION

Abstract. *Aim.* Activating mutation in KRAS oncogene is one of the most significant events in colorectal cancer (CRC) molecular pathogenesis. Along with the success of complex treatment, understanding the CRC genomics due to the extensive use of molecular genetic studies promotes an optimum choice of therapy variants. The aim of this study was to define the frequency and spectrum of KRAS gene mutations in CRC patients depending on the tumor localization for choosing the treatment tactics and predicting the course of the disease.

Method. This retrospective study included 332 CRC patients treated in the Republic of Kazakhstan from 2010 to 2014. Their tumor material was formalin-fixed and waxed and morphologically assessed. KRAS mutation status was established by PCR study.

Results. The mutations were most frequent with rectal cancer (n=82, 55%), followed by left-sided colon cancer (n=43, 28.9%), and right-sided colon cancer (n=24, 16.1%). The mutations were most frequent in codon 12, in particular, G12D – 32.9%, G12V – 24.2%, and G13D – 19.5%.

Conclusion.

1. The obtained results on KRAS mutation frequency correspond to the data published by other researchers.

2. KRAS mutations are more frequent in left-sided colon cancer compared to right-sided colon cancer ($P = 0.001$).

3. There is an upward trend in KRAS mutation frequency with tumor localization in the distal parts of the colon, especially in the rectum.

4. The mutations were most frequent in codon 12, in particular, G12D, G12V, and G13D. G12V mutations were frequent in tumor localization in the rectum.

Introduction. Colorectal cancer (CRC) is a heterogeneous group of tumors which differ in both the mechanisms of carcinogenesis and, therefore, molecular changes, and the prognosis of the disease and the specifics of treatment. Today, choosing tactics for treating patients with metastatic colorectal cancer shall take into account not only clinical factors like tumor spread, patient functional status but also the molecular profile of the disease.

The frequency and spectrum of KRAS gene mutations and their correlation with clinical and morphological features of patients with CRC are widely studied in the literature. Several studies (see the analysis of 551 cases of CRC at diagnosis by Palomba et al.) did not reveal any significant correlation of KRAS gene mutation frequency with the patient's age, gender, tumor localization and depth of invasion, the degree of malignancy, and the presence of regional or distant metastases [1, 2]. Still, there is evidence that KRAS gene mutates more often in rectal tumors than in tumors in the overlying colon. Some researchers have revealed the relation of the KRAS gene mutation in codon 13 with the stage of the tumor process [3-6].

Thus, the prognostic value of KRAS gene mutation in CRC tumor has not been fully proven in the literature and requires further research.

Purpose of this study was to define the frequency and spectrum of KRAS gene mutations in CRC patients depending on the tumor localization for choosing the treatment tactics and predicting the course of the disease.

Method. *Patients and data extraction.* This retrospective study included 332 patients diagnosed with CRC and registered at the Regional Cancer Centers of the Republic of Kazakhstan in 2010-2014. Their KRAS status was established by PCR study of their post-surgery or biopsy samples.

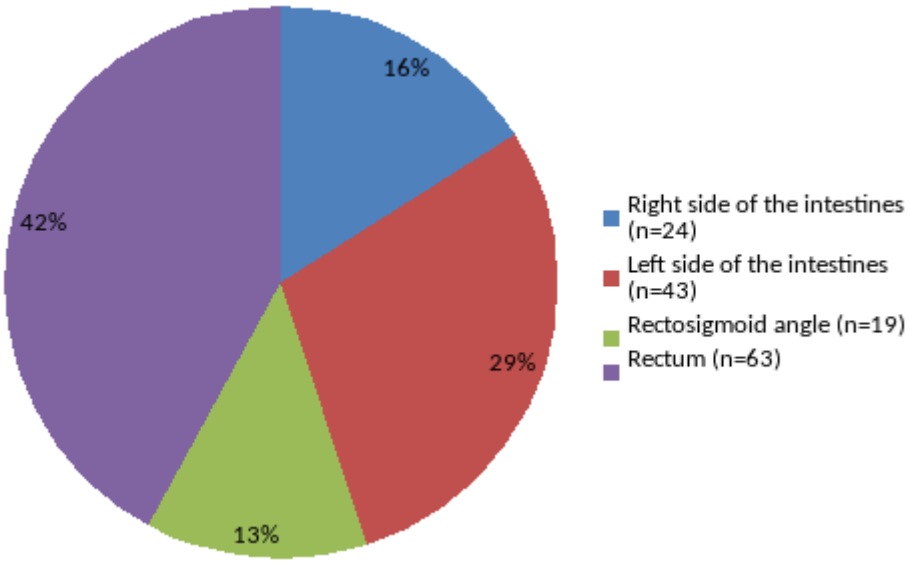
In the study, women (n=182, 54.8%) prevailed over men (n=150, 45.2%). Most of the patients (88%) were aged 44 and above; only 12% were below 44 years. The average age was 56.4 ± 10.5 years (25 to 79 years).

All patients underwent a complete clinical examination, X-ray, CT, ultrasound, MRI of the chest, abdomen, pelvic organs; their CRC diagnosis was confirmed morphologically.

DNA extraction and KRAS mutational analysis. Molecular genetic study of KRAS status was conducted at the Laboratory of Pathomorphology and Molecular Genetics of the Kazakh Institute of Oncology and Radiology. The quality of the obtained materials was assessed morphologically. Depending on the percentage of tumor cells in the sample, the samples were subjected to 3-5 macro dissections and dewaxing for DNA extraction. The samples containing less than 20% of tumor cells were microdissected along the slide zone previously marked by the morphologist to avoid false-negative results. The DNA was extracted using the FFPE DNA extraction kits (QIAGEN, Inc. Valencia, CA). The concentration of the extracted DNA was determined using NanoDrop spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA); DNA quality was assessed using real-time control PCR comparing the results with control DNA. The mutations in KRAS codons 12 and 13 in exon 2 were detected by allele-specific PCR method using BioLink kits.

The statistical processing of data was made using a PC with installed IBM SPSS Statistics 20 package (trial version). Pearson's linear correlation coefficient (r_p) was used to identify the relationship between the variables.

Results. KRAS gene status was determined in tumors localized in different parts of the colon. Of the 332 CRC patients included in the study, 48 (14.5%) patients had right-sided colon cancer (RCC) with tumor localization in the right side of the intestines (cecum, ascending colon, hepatic angle, or transverse colon) vs. 99 (29.8%) cases of left-sided colon cancer (LCC) with tumor localization in the left side of the intestines (splenic angle, descending colon, or sigmoid colon). The remaining 185 (55.7%) patients had a tumor in the rectum, including its rectosigmoid part (figure).



KRAS mutation frequency depending on the tumor localization in CRC (n=332)

Legend:
Blue – Right side of the intestines (n=24)
Red – Left side of the intestines (n=43)
Green – Rectosigmoid angle (n=19)
Violet – Rectum (n=63)

Of all the CRC patients included in the study (n=332), 149 (44.9%) had mutant-type KRAS (mt-KRAS), and 183 (55.1%) had wild-type KRAS (wt-KRAS). Among patients with RCC, the number of mt-KRAS and wt-KRAs cases was the same (n=24, 50%). Among patients with lesions of the left sections of the colon, including the rectum, 125 (44%) patients had mt-KRAS against 159 (56%) with wt-KRAS. KRAS mutations depending on the tumor localization were most frequent with rectal cancer (n=82, 55%) followed by LCC (n=43, 28.9%), and RCC (n=24, 16.1%) (table 1).

Table 1 – KRAS gene status and mutation frequency depending on the tumor localization in colorectal cancer

KRAS status	Right-sided colon cancer (n=48, 100%)	Left-sided colon cancer (n=99, 100%)	Rectal cancer (n=185, 100%)
Wild-type KRAS (n=183)	24 (50±3.1%)	56 (56.6±4.9%)	103 (55.7±3.6%)
Mutation KRAS (n=149)	24 (50±3.0%)	43 (43.4±4.9%)	82 (44.3±3.6%)

For colon tumors (n=67), the KRAS mutation frequency with LCC (n=43, 64.2%±5.8%) was higher than with RCC (n=24, 35.8±5.8%); the difference was statistically significant ($P = 0.001$).

In our study, 120 out of 149 (80.5%) patients with mt-KRAS had mutations *in codon* 12, of which G12D (32.9%) and G12V (24.2%) were the most common. G12D, G12V mutations were especially frequent in rectal cancer (28 out of 49 and 25 out of 36, respectively). G12S and G12C were less frequent (up to 10%). G13D was observed only in 29 (19.5%) of cases (Table 2).

No increase in mutation frequency with the tumor localization in the intestine was observed. The correlation coefficient for the pair "Right intestine – KRAS mutations" was $r_p = –0.042$, $P = 0.06$. The inverse relationship between these variables was very weak (table 2).

Table 2 – Correlation of KRAS gene mutations with tumor localization in colorectal cancer

KRAS mutations	Location												Mutations in codons
	Right-sided colon cancer				Left-sided colon cancer				Rectal cancer				
	n	%	r_p	ρ - value	n	%	r_p	ρ - value	n	%	r_p	ρ - value	
G12A	5	20.8	-0.025	0.05	7	16.3	0.013	0.06	8	9.7	0.006	0.06	20 (13.4%)
G12C	2	8.3	-0.034	0.06	1	2.3	-0.002	0.06	4	4.9	0.026	0.06	7 (4.7%)
G12D	9	37.5	-0.027	0.06	12	27.9	-0.011	0.06	28	34.1	0.029	0.06	49 (32.9%)
G12S	0	0	-0.053	0.06	5	11.6	0.023	0.05	3	3.7	0.016	0.06	8 (5.4%)
G12V	3	12.5	-0.068	0.06	8	18.6	-0.008	0.06	25	30.5	0.056	0.06	36 (24.2%)
G13D	5	20.8	-0.038	0.06	10	23.3	0.016	0.05	14	17.1	0.012	0.06	29 (19.5%)
Total mutations	24 (16.1%)	100	-0.042	0.06	43 (28.9%)	100	0.006	0.06	82 (55.0%)	100	0.025	0.06	149

Discussion and Conclusion. Of the 332 CRC patients included in the study, 149 (44.9%) had mt-KRAS, and 183 (55.1%) had wt-KRAS. Our results correspond to the results obtained in extensive multicenter studies which confirm the 30-50% mutation frequency of KRAS in colon tumors [1, 3, 5-7]. Thus, our results on the frequency of KRAS gene mutation are consistent with data published by other researchers.

The literature sources reported about 97-99% of KRAS gene mutations in codons 12 and 13 compared to 1-3% in other codons. Therefore, we focused our research on these codons as they mutated most often. A mutation was detected at any location of the tumor in the colon, but its frequency varied. We compared the KRAS mutation frequency in rectal cancer with other colon sections, including its right and

left sides. In our study, KRAS mutations in rectal cancer (n=82, 55.0%) were more frequent than in colon cancers of other localizations (n=67, 44.9%). Also, we revealed a statistically significant prevalence of KRAS gene mutations in LCC (n=43, 28.9%) vs. RCC (n=24, the least frequency – 16.1%) (P = 0.001). The mutation frequency was also growing with tumor localization in the lower section of the colon, and especially in the rectum (table 1).

The mutations were most frequent in codon 12, in particular, G12D – 32.9%, G12V – 24.2%, and G13D – 19.5%. However, in our study, a high G12V mutation rate was observed in rectal cancer (25 mutations out of 36) (table 2).

The obtained data on mutations in codons 12 and 13 suggests different etiology of carcinogenesis in different parts of the colon.

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ИЗУЧЕНИЕ ЧАСТОТЫ И СПЕКТРА МУТАЦИИ ГЕНА KRAS У БОЛЬНЫХ С КОЛОРЕКТАЛЬНЫМ РАКОМ (КРР) В ЗАВИСИМОСТИ ОТ ЛОКАЛИЗАЦИЕЙ ОПУХОЛИ В МАСШТАБЕ РЕСПУБЛИКИ КАЗАХСТАН

Аннотация. Одним из наиболее значимых событий в молекулярном патогенезе КРР является активирующая мутация в онкогене KRAS. Наряду с успехами комплексного лечения, понимание геномики КРР, благодаря широкому использованию молекулярно-генетических исследований, предоставило возможность оптимального выбора терапевтических опций.

Целью настоящего исследования было определение частоты и спектра мутаций гена KRAS у больных КРР в зависимости от локализации опухоли для определения выбора тактики лечения и прогнозирования течения заболевания.

Материалы и методы. Нами было изучен опухолевый материал 332 пациентов с диагнозом КРР, фиксированный в формалине, заключенный в парафин, проходивших лечение в онкологических диспансерах, онкологических центрах и в Казахском научно-исследовательском институте онкологии и радиологии (КазНИИОиР) за период с 2010 по 2014 годы. После морфологической оценки качества исследуемого материала в лаборатории молекулярной генетики было проведено молекулярно-генетическое исследование по определению мутации гена KRAS методом ПЦР.

Результаты. По полученным нами результатам, мы можем судить, чтонаибольшее количество мутаций гена KRAS было обнаружено при поражении прямой кишки – у 82(55%) из 149 пациентов. Далее по частоте встречаемости мутации занимали левые отделы толстой кишки – у 43(28,9%) пациентов. При поражении правых отделов толстой кишки мутация гена KRAS встречалась в 24(16,1%) случае. Наиболее частые мутации были в 12 кодоне, а именно G12D - 32,9%, G12V - 24,2% и G13D -19,5 %.

Выводы.

1. Полученные нами результаты по частоте встречаемости мутации генаKRAS согласуются с данными, опубликованными в литературных источниках другими исследователями.

2. Мутаций гена KRAS встречается чаще при первичной локализации опухоли в левых отделах по сравнению с правой локализации, (p= 0,001).

3. Прослеживается тенденция к увеличению числа мутации генаKRASс
возрастанием частоты поражения дистальных отделов толстой кишки и
особенно прямой кишки.

4. Наиболее частые мутации были в 12 кодоне, а именно G12D, G12V и G13D. Высокая частота мутацииG12V наблюдалась при локализации опухоли в прямой кишке (25 мутации из 36).

Ключевые слова: колоректальный рак; молекулярно-генетические исследования; мутация KRAS; дикий тип.

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