South Russian Journal of Cancer 2022, Vol.3, No. 2, P. 52-64
https://doi.org/10.37748/2686-9039-2022-3-2-6
REVIEW

GENES COPY NUMBER VARIATION IN COLORECTAL CANCER PATIENTS AS A MARKER OF THE DISEASE CLINICAL OUTCOME AND RESPONSE TO THERAPY

A. A. Maslov, L. Kh. Chalkhakhyan✉, S. A. Malinin, G. V. Kaminsky, E. A. Mirzoyan

National Medical Research Centre for Oncology, Rostov-on-Don, Russian Federation
✉ gabo80.80@inbox.ru

ABSTRACT

Abnormal gene copies, a special type of genetic polymorphism, is a hallmark of most solid tumors, including colorectal cancer. Abnormal copy number of genes leads to tumor-specific genomic imbalance, which manifests itself already in precancerous precursor lesions. The aim of this review was to systematize the scattered data on changes in gene copy number observed in colorectal cancer and their impact on the outcome of the disease and response to therapy. The data from 58 studies was analyzed on gene copy number changes and their expression in primary carcinomas, cell lines and experimental models. This review examines the spectrum of genetic changes that lead to colorectal cancer, describes the most frequent changes in the number of gene copies at different stages of the disease, and changes in the number of gene copies that can potentially affect the outcome of the disease of individual patients or their response to therapy. In fact, aberrant gene copy number as a form of chromosomal imbalance affects a number of genes that provide a metabolic selective advantage for a tumor cell. Changes in the genes copy number in colorectal cancer patients not only positively correlate with changes in their expression, but also affect the levels of gene transcription at the genome-wide scale. Aberrant gene copy numbers are closely related to disease outcome and response to treatment with 5-fluorouracil, irinotecan, cetuximab and bevacizumab. Nevertheless, the possibility of translating the genes copy number index into clinical practice requires further research.

Keywords:
colorectal cancer, gene copy number, gene expression, biomarkers, overall survival, response to therapy

For correspondence:
Lusegen Kh. Chalkhakhyan – Cand. Sci. (Med.), surgeon at the abdominal oncology department No. 2, National Medical Research Centre for Oncology, Rostov-on-Don, Russian Federation.
Address: 63 14 line str., Rostov-on-Don 344037, Russian Federation
E-mail: gabo80.80@inbox.ru
ORCID: https://orcid.org/0000-0001-8397-4393
SPIN: 6534-5911, AuthorID: 794696

Funding: this work was not funded.
Conflict of interest: authors report no conflict of interest.

For citation:
Maslov A. A., Chalkhakhyan L. Kh., Malinin S. A., Kaminsky G. V., Mirzoyan E. A. Genes copy number variation in colorectal cancer patients as a marker of the disease clinical outcome and response to therapy. South Russian Journal of Cancer. 2022; 3(2): 52-64. (In Russ.).
https://doi.org/10.37748/2686-9039-2022-3-2-6

The article was submitted 28.01.2022; approved after reviewing 07.04.2022; accepted for publication 21.06.2022.

Южно-Российский онкологический журнал 2022, Т. 3, № 2, С. 52-64
https://doi.org/10.37748/2686-9039-2022-3-2-6
ОБЗОР

ПОКАЗАТЕЛЬ КОПИЙНОСТИ ГЕНОВ У БОЛЬНЫХ КОЛОРЕКТАЛЬНЫМ РАКОМ КАК МАРКЕР КЛИНИЧЕСКОГО ИСХОДА ЗАБОЛЕВАНИЯ И ОТВЕТА НА ТЕРАПИЮ

А. А. Маслов, Л. Х. Чалхахян✉, С. А. Малинин, Г. В. Каминский, Э. А. Мирзоян

НМИЦ онкологии, г. Ростов-на-Дону, Российская Федерация
✉ gabo80.80@inbox.ru

РЕЗЮМЕ

Аномальная копийность генов – особый типом генетических полиморфизмов, является отличительной чертой большинства солидных опухолей, включая колоректальный рак.
Аномальная копийность генов приводит к специфическому для опухоли геномному дисбалансу, который проявляется уже в предраковых поражениях-предшественниках. Целью данного обзора стала систематизация разобщенных данных о наблюдаемых при колоректальном раке изменениях копийности генов и их влиянии на исход заболевания и ответ на терапию. Были проанализированы данные 58 исследований по изменению числа копий генов и их экспрессии в первичных карциномах, клеточных линиях и экспериментальных моделях. В данном обзоре рассмотрен спектр генетических изменений, которые приводят к колоректальному раку, описаны наиболее частые изменения количества копий генов на разных стадиях заболевания, и изменения количества копий генов, которые потенциально могут повлиять на исход болезни отдельных пациентов или их ответ на проводимую терапию. Фактически, аберрантная копийность генов как форма хромосомного дисбаланса затрагивает целый ряд генов, обеспечивающих метаболическое избирательное преимущество для опухолевой клетки. Изменения числа копий генов у больных колоректальным раком не только положительно коррелируют с изменениями их экспрессии, но также влияют на уровни транскрипции генов в масштабе всего генома. Аберрантная копийность генов тесно связана с исходом заболевания и ответом на лечение 5-фторурацилом, иринотеканом, цетуксимабом и бевацизумабом. Тем не менее, возможность трансляции показателя копийности генов в клиническую практику требует дальнейших исследований.

Ключевые слова:
колоректальный рак, показатель копийности генов, экспрессия генов, биомаркеры, общая выживаемость, ответ на терапию

Для корреспонденции:
Чалхахян Лусеген Хачатурович – к.м.н., хирург отделения абдоминальной онкологии № 2, ФГБУ «НМИЦ онкологии» Минздрава России, г. Ростов-на-Дону, Российская Федерация.
Адрес: 344037, Российская Федерация, г. Ростов-на-Дону, ул. 14-я линия, д. 63
E-mail: gabo80.80@inbox.ru
ORCID: https://orcid.org/0000-0001-8397-4393
SPIN: 6534-5911, AuthorID: 794696

Финансирование: финансирование данной работы не проводилось.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования:
Маслов А. А., Чалхахян Л. Х., Малинин С. А., Каминский Г. В., Мирзоян Э. А. Показатель копийности генов у больных колоректальным раком как маркер клинического исхода заболевания и ответа на терапию. Южно-Российский онкологический журнал. 2022; 3(2): 52-64. https://doi.org/10.37748/2686-9039-2022-3-2-6

Статья поступила в редакцию 28.01.2022; одобрена после рецензирования 07.04.2022; принята к публикации 21.06.2022.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common oncological diseases in the world. According to WHO, about 1 million new cases are registered every year. In terms of the number of diagnosed cases and the number of deceased patients, this pathology is second only to lung, stomach and breast cancer. Currently, despite the successes achieved in the diagnosis of these tumors, they are often detected at late stages [1].

CRC is characterized by aberrant behavior of cells that destroy already existing tissues, both locally in the organ of origin and at a distance, in the niches of metastasis. The aberrant behavior of tumor cells is caused by changes in cell biology and affects critical processes such as proliferation, invasion, avoidance of apoptosis and the immune system [2]. These changes in cell biology, in turn, are the result of an evolutionary process whereby gene mutations and copy number changes (CNVs) accumulate and lead to the selective advantage of cell clones carrying these changes.

There are different types of genetic changes in cancer: small nucleotide variations (SNV), small insertions or deletions (Indels), structural variants (SV) and variations in the number of copies of genes (CNV). The role of CNV in oncogenesis has long been underestimated. Fogelstein's pioneering work with collaborators in the early 90s of the 20th century showed that the accumulation of changes in genes involved in key signaling pathways leads to neoplastic changes in normal epithelial cells of the colon, eventually transforming into cancer [3]. Accordingly, an early decisive event in the development of CRC is a violation of the functioning of the WNT signaling pathway, leading to the formation of an adenoma, and this occurs in most cases due to changes in the APC gene. Further, there is an accumulation of mutations in the *KRAS* gene (involved in the MAPK signaling pathway), large deletions on the long arm of chromosome 18 (affecting the TGF-β signaling pathway), and deletion of the short arm of chromosome 17 (17p), where *TP53* is located, which eventually leads to the formation of cancer [4].

Variations in the number of copies of genes (CNV) are a special type of genetic polymorphisms that lead to a change in the number of copies of a certain gene and, consequently, to a change in the expression level of the product of this gene – protein or non-coding RNA [5]. With the advent of comparative genomic hybridization (CGH), it became possible to analyze CNV throughout the genome. This molecular approach confirmed and refined the results obtained by karyotype analysis [6], allowed us to thoroughly characterize the CNVs observed in microsatellite stable CRC – mainly amplifications of chromosomes 7, 8q, 13 and 20q, as well as deletions of 8p, 17p and 18 [7]. In 2012, information on the number of gene copies in 276 CRC samples was entered into the TCGA. It has been confirmed that CNVs in CRC affect regions of chromosomes 1q, 7, 8q, 13q and 20q and 1p, 4, 5q, 8p, 14q, 15q, 17p and 18q [8].

A great contribution to the study of the role of CNV in the malignancy of various tissues, the response to therapy, including radiation, predicting the course of the disease and the survival of patients, was made by employees of the National Medical Research Centre for Oncology in a series of works performed in 2014–2021. Thus, data were obtained indicating the important role of changes in the copyicity of the genes *BAX, CASP3, CASP8, OCT4, C–MYC, SOX2, BCL2, NANOG, CASP9, NFKB1, HV2, ACTB, MKI67, IL-10, GSTP1* and *P53* in gastric tissue malignancy [9], the copyicity of genetic loci was investigated, responsible for the regulation of apoptosis (*BAX, BCL2, C-FLAR, P53, MDM2, BFAR, SEMA3B, RASSF1A, CASP9, CASP3, CASP8*), proliferation (*SOX2, OCT4, NANOG, PIK3* and *MKI67*), oxidative phosphorylation (*HV2*), response to hypoxia (*HIF1A1*), DNA repair (*XRCC1*), destruction of the intercellular matrix (MMP1), maintenance of telomere length (*TERT*), regulation of adhesive intercellular contacts (CTNNB1) and angiogenesis (*VEGFA*), functioning of the EGFR signaling pathway (*KRAS, EGFR, GRB2, SOS1, MAPK1, STAT1, BRAF*) in normal and tumor lung cells in 90 patients with lung adenocarcinoma[10]. There was also a study of the features of the copyicity of the genes *BAX, BCL2, TP53, MDM2, CASP9, CASP3, CASP7, CASP8, PRKCI, SOX2, OCT4, PIK3, PTEN, C–MYC, SOX18, AKT1, NOTCH1, BRCA1, BRCA2, EXO1, SCNN1A, KRAS, EGFR, BRAF, CYP1A1, CYP1A2, CYP1B1, CYP19A, ESR1, ESR2, GPER, STS, SULT1A, SULT1E1* in tumor and normal cells of serous ovarian adenocarcinoma of high and low malignancy [11]. The role of the replication of a number of genes (*RBBP8, BRCA2, H2AX* and *BCL2*) in the response of malignant tumors of the prostate and rectum to radiation therapy has been established [12].

Thus, the important role of the gene copy index as biomarkers of oncological diseases and the effectiveness of their therapy becomes obvious. The NCBI database contains information on a large number of studies on changes in gene copyness in CRC and their association with certain clinical characteristics, however, all the data presented are extremely heterogeneous and require generalization to form a unified understanding of the role of CNV in CRC.

Therefore, the purpose of this review was to systematize the disjointed data on changes in gene replication observed in colorectal cancer and their impact on the outcome of the disease and response to therapy.

Molecular classification of colorectal cancer

In CRC, two main pathways of genomic instability are observed: chromosomal instability (CIN) – 85 % of cases, and microsatellite instability (MSI) – 15 % of cases [4; 13]. CINS are characterized by large chromosomal aberrations, while MSI are characterized by mutations at the level of one nucleotide in repeating regions (microsatellites) [14].

CRC can also be classified based on data on the level of hypermethylation of the promoter (CpG Island Methylator Phenotype; CIMP) into CRC with high and low levels of CIMP. There is a strong association of the MSI phenotype with CIMP due to hypermethylation of the hMLH1 gene [15]. Another classification based on the transcriptome is also proposed, including 4 subtypes of CRC (CMS) [16], which are not completely discrete classes, since there is some degree of overlap reflecting the continuity of CRC transcriptomes [17]. With the exception of CMS1 (MSI CRC), all other 3 CMS groups (CMS2–4) represent to a certain extent a higher/lower degree of CIN-CRC [17]. The transfer of the CMS classification system to preclinical models and clinical practice opens up prospects for targeted therapy [18].

Formation of CNV during oncogenesis

Disruption in the functioning of the WNT signaling pathway and the acquisition of chromosomal aneuploidy (for example, an additional copy of chromosome 7) can lead to the formation of adenoma, which progresses into carcinoma due to the accumulation of additional genetic and epigenetic changes. Different lesions with different morphology can lead to the development of CRC. These can be ordinary (polypoid, flat) adenomas or toothed polyps. Although the total amount of CNV in adenomas is low compared to carcinomas, it is necessary to take into account the presence of chromosomal aneuploidies and genomic changes in such precancerous lesions, which contributes to achieving relatively high levels of genetic heterogeneity [19]. In addition, differences in CNV patterns can be observed between different morphologies, namely polypoid and non-polypoid adenomas. When comparing a large series of non-polypoid adenomas with polypoid adenomas, it was shown that the former have 5q deletions more often and 1p, 10q, 17p and 18q deletions less often than the latter [20]. Other precursors of CRC, such as dentate polyps [21], progress to a malignant tumor along the MSI pathway and, therefore, do not show common CNVs with tumors arising from polypoid adenomas [22].

Despite the fact that chromosomal aneuploidies can be observed in precancerous lesions, their appearance is more common at later stages of the transition to a malignant neoplasm [23]. Several studies have shown that CNVs are associated with this transition in certain regions of chromosomes – 8q, 13q, 20q, 8p, 15q, 17p and 18q [24].

Colorectal adenomas are a very common finding in the elderly (prevalence 35 %) [4]. However, it is believed that only about 5 % of colon polyps removed during endoscopy could develop into cancer. Indeed, histopathological features associated with the presence of focal cancer in adenomas include a size of ≥ 10 mm, high degree dysplasia and villi histology. The presence of at least one of these histopathological features leads to the progression of adenoma into cancer [4]. However, the accuracy of these indicators for detecting adenomas that can progress to cancer is low [25]. New markers are needed that more accurately reflect the natural course of the disease and more specifically identify adenomas with a high risk of cancer [26].

Strategies and approaches to the analysis of changes in the number of copies of genes in cancer

Molecular cytogenetic methods, including approaches related to FISH and CGH, have improved the analysis of chromosomal aberrations in tumors of various localizations [4]. CGH allowed mapping the genomic imbalance in tumors to an unprecedent-

ed level by comparing genomic DNA isolated from a tumor sample with a reference genome without the need for metaphase chromosome preparations. This made it possible to use formalin-fixed and paraffin-filled material (FFPE blocks) for cytogenetic analyses. The use of CGH has provided evidence that genomic imbalance is responsible for tumor progression from dysplastic lesions to invasive disease. Later, DNA microarrays made it possible to simultaneously measure the number of copies of many polymorphic loci in the genome, which led to the high-resolution detection of LOH, a common phenomenon in oncogenesis [27].

The development of mass parallel sequencing with has led to the development of many tools for analyzing the CNV of the entire exome (WES) or the complete genome (WGS). Consistent analysis of sequencing data was made possible partly thanks to the Genome Analysis Toolkit (GATK) [28]. 4 computational genome sequencing approaches have been described to detect structural variants:

1) paired reading (the distance between the mapped reads and the average size of the genomic insert is compared);

2) split reading (detection of small insertions and deletions by means of alignment analysis on the reference genome);

3) assembly method (a reference-free reconstruction of the entire genome from a set of readings is calculated and compared with the reference genome using several programs)

4) counting the number of reads or the depth of coverage (the most recent approach, which takes into account the number of reads displayed for each region in the genome, and assumes a uniform sequencing process, so the number of reads in a particular region will be proportional to the number of copies of it) [29].

Next, we attempted to compare several methods, further emphasizing the differences between the tools [30–34]. Tools such as GISTIC 2.0 [35], ConVaQ [36] or CNApp [37] allow researchers to

Table 1. Bioinformatic tools and methods for CNV detection using mass parallel sequencing platforms

Name	Sequencing platform	Programming language
CNVkit	WES/WGS	Python
ExomeDepth	WES	R
VarScan2	WES/WGS	Java
ControlFreeC	WES/WGS	C++
ExomeCNV	WES	R
XHMM	WES	C++
CoNIFER	WES	Python
Delly	WGS	C++
XCAVATOR	WGS	Perl, bash, R, Fortran
CNVnator	WGS	C++
CNV-seq	WGS	R, perl
Pindel	WGS	C++
CONTRA	WES	Python/R

Note: WES is whole exome sequencing; WGS is whole genome sequencing.

integrate CNV genomic data with additional molecular and clinical characteristics and uncover new functionality and implications for these genomic events (Table 1).

CNV signatures

To a certain extent, SNV and CNV in the genome of malignant cells represent a trace of uncorrected genetic changes that have accumulated during the life of the tumor. SNV studies have revealed mutational patterns resulting from various types of nucleotide changes in this type of tumor, and defined as mutational signatures [38]. Unlike SNV, only the presence or absence of a specific chromosome in tumor cells has been well described in the literature, but the mechanisms underlying such patterns have not been described. Attempts have been made to identify the signatures of the number of copies of genes taking into account various approaches. Thus, using non-negative matrix factorization models, 6 signatures were extracted to 32 ranked subclasses of breast cancer data obtained by sequencing the entire genome, based on their association with homologous recombination mediated by microhomology [39]. Similarly, 8 gene copy number signatures based on structural features were identified by whole genome sequencing in serous ovarian cancer [40]. These authors showed the correlation of CNV signatures with prognosis and response to treatment, and showed their importance as clinical biomarkers. Finally, "pan-cancer" studies have identified 9 signatures that determine the etiology of structural variants, suggesting that mechanisms based on DNA replication generate different chromosomal structures in different types of tumors, including CRC [41].

Changes in the number of copies of genes and their transcriptional activity

In the work of Ried and co-authors [42], it was found that for each type of tumor there is a specific CNV landscape reflecting genomic imbalance. As mentioned earlier, the following CNV profile is observed in CRC-an increase in copyness in the region of chromosomes 7, 8q, 13, 20q and a decrease in copyness in the region of 8p, 17p and 18. Such observations raise the question of what their effect is on the levels of gene transcription in the affected areas of chromosomes. In fact, among several hypotheses as to why transcription programs are affected by CNVs, the bulk of the literature indicates that CNVs directly affect the expression of most genes in the altered genomic segment; however, the extent to which genes other than oncogenes and tumor suppressors contribute to malignant transformation or preservation of the transformed state remains unclear. The biological consequences of such aneuploidy are not limited to the affected chromosomal region, but may be associated with the effect on the transcriptional activity of genes located in other regions of the genome. Naturally, the third possibility is that these aneuploidies target only a limited number of genes that give a selective advantage to the cancer cell [4].

Cell lines derived from primary carcinomas are widely used to measure the effect of genomic CNVs on gene expression. Analysis of 15 CRC cell lines, including lines with effective and defective repair systems, showed a positive correlation throughout the genome between CNV and the corresponding gene expression [4]. Such correlations have been confirmed for many other types of tumors, for example, prostate cancer and cervical cancer [43].

The correlation of the number of copies of genes and the average level of gene expression is also applicable to primary tumors. In fact, several authors have shown the effect of CNV on gene expression levels in precancerous lesions and carcinomas of various origins [44; 45] In these studies, the authors examined several groups of rectal and colon cancer samples and compared the normal mucosa, and determined that the increased expression was in those genes that are located on chromosomes 7, 13 and 20, that is, chromosomes on which amplifications are observed, while the genes with reduced expression were located on chromosomes 18, 14 and 15, in which CNV deletions are usually observed in CRC. The data obtained by genome-wide sequencing and presented by the Cancer Genome Atlas consortium were used to map somatic structural changes, including CNV, in 600 tumors of various origins, and showed their contribution to altered gene expression in CRC [46].

The positive correlation between CNV and gene expression has led to the discovery of new cancer-related genes. In particular, in CRC, the amplification of chromosome 13 regions and the associated overexpression of multiple genes provided a unique chance to uncover several genes associated with on-

Table 2. Changes in the gene copy index and the outcome of the disease

Chromosome locus	CNV type	Genes	Sample Size, *n*	Clinical significance	Link
1p36.33 – p36.32	Amplification	*SKI*	159	Patients with *SKI* amplification had worse OS and RFS	[52]
5p14.3 – p13.3	Amplification	*RNASEN, C5orf22, GOLPH3, MTMR12, ZFR, SUB1* and *TARS*	111	Amplification was associated with a shorter PFS	[53]
5q12.1 – q12.3	Deletion	*SFRS12IP1, SDCCAG10, CENPK, PPWD1* and *SFRS12*	105	Deletion was associated with a shorter PFS	[53]
5q34	Deletion	*CCNG1*	133	Deletion was associated with a shorter PFS	[53]
6q16.1 – q16.3	Amplification	*KIAA0776, C6orf66, C6orf167, FBXL4, SFRS18, CCNC, ASCC3, ATG5, QRSL1, 6orf203, PDSS2, LACE1, CD164, SMPD2* and *ZBTB24*	111	Amplification was associated with a shorter PFS	[53]
7p11.2	Amplification	*EGFR*	44	Patients with *EGFR* amplification achieved a high percentage of partial remission, while patients without increased *EGFR* replication had progressive disease. In addition, patients with a high *EGFR* copy count had a longer period of time before progression.	[4]
7q22.1	Amplification	*GAEC1*	79	Associated with tumor perforation and later stage T	[53]
17q21 – q21.3	Deletion	*PSMB3, PIP4K2B, CCDC49, RPL23, LASP1, RPL19, FBXL20, MED1, CRKRS, NEUROD2, STARD3, TOP2A, SMARCE1, TMEM99, KRTAP3-3, KRTAP1-1, EIF1, NT5C3L, KLHL11, ACLY2 MLX, EZH1, VPS25, CCDC56, BECN1, PSME3, RUNDC1, RPL27, BRCA1, NBR2, NBR1, DUSP3, TMEM101, LSM12, TMUB2, GPATCH8, CCDC43, EFTUD2, NMT1* and *MAP3K14*	133	Deletion was associated with a shorter PFS	[53]
18p11.32	Deletion	*USP14, THOC1, C18orf56, TYMS, ENOSF1* and *YES1*	111	Deletion was associated with a shorter PFS	[53]
18p11.32 – p11.21	Deletion	*METTL4, NDC80, SMCHD1, EMILIN2, LPIN2, MRCL3, MRLC2, ZFP161, RAB12, KIAA0802, NDUFV2, ANKRD12, TWSG1, RALBP1, PPP4R1, VAPA* and *NAPG*	133	Deletion was associated with a shorter PFS	[53]

Table 2. Changes in the gene copy index and the outcome of the disease

Chromosome locus	CNV type	Genes	Sample Size, *n*	Clinical significance	Link
18p11.21	Deletion	*CHMP1B, MPPE1, IMPA2, TUBB6, AFG3L2, CEP76, PSMG2, PTPN2, SEH1L, CEP192, C18orf19* and *RNMT*	133	Deletion was associated with a shorter PFS	[53]
18q11.2	Deletion	*LAMA3*	133	Deletion was associated with a shorter PFS	[53]
18q21.2	Deletion	*SMAD4*	147	Associated with tumor developement	[4]
18q21.33 – q22	Deletion	*MYO5B, MBD1, CXXC1, C18orf24, ME2, ELAC1, SMAD4, MEX3C, MBD2, POLI, RAB27B, CCDC68, TXNL1, WDR7, FECH, NARS, ATP8B1, ALPK2, MALT1, SEC11C, KIP2A, PF2A1, PF2A14, PF2A1, PF2A1, PF2A1 TNFRSF11A, ZCCHC2, PHLPP, BCL2, KDSR, VPS4B, SERPINB8, TMX3, RTTN, SOCS6, C18orf55* and *CNDP2*	111	Deletion was associated with a shorter PFS	[53]
20q11 – q13.3	Amplification	*BCL2L1, ASXL1, SRC, DNMT3b, Gnas, TOP1, AURKA, PTPRT,* and *NCOA3*	354	Amplification was associated with better OS	[55]
20q11.21 – q13.33	Amplification	*PTK6* and *EEFIA2*	269	Significantly associated with improved overall survival in grade III tumors	[4]
20q13.2	Amplification	*CSE1L, NABC1, ZNF217* and *STK15*	146	An increase in the number of copies is associated with poorer overall survival and faster tumor progression	[4]

Note: RFS – relapse–free survival; OS – overall survival; PFS – progression free survival.

cogenesis, including CDK8, CDX2 and LNX2, for which overexpression was *associated with WNT activity and oncogenic functions [46]. In addition, several other cancer-related genes (AHCY, TPX2, POFUT1, Rpn2, AURKA, TH1L, MTUS1, PPP2CB, ARGLU1, UGGT2, CES2, FUT10, PAOX,* and *PRPF6*) showed a significant linear correlation between the dose of the gene (CNV) and its expression [48; 49].

To study the effect of CNV on gene expression, several models of cell lines or animals have been developed. These studies have also shown that CNVs affect the expression not only of genes located on the aneuploid site, but also of many other genes throughout the genome, which in turn affect protein expression [50].

CNV as biomarkers of clinical outcome and response to therapy

Only a few genetic biomarkers are currently used in clinical practice related to CRC. These include mutations in RAS genes, which are commonly used in patients with CRC to prescribe therapy against EGFR. Similarly, the BRAF V600E mutation is a biomarker of poor prognosis in patients with metastatic CRC. Another prognostic marker used in the clinic is the status of MSI [4].

To date, the needs of oncologists in certain areas of CRC treatment are still unsatisfied, in particular, it concerns the prediction of the likelihood of recurrence in patients with stage II colon cancer [51]. In fact, most modern prognostic biomarkers are applied

only to patients with stage IV CRC. CRC still lacks adequate prognostic biomarkers compared to other cancers, such as melanoma, leukemia, breast, ovarian, prostate and lung cancers [4]. Since molecular cytogenetic methodologies, as well as next-generation sequencing methods for CNV assessment can be applied to archived formalin-fixed material (FFPE), the analysis of large series of CRC with well-annotated clinical data has become possible, which allowed the analysis of the prognostic value of certain CNVs. Candidate biomarkers with their respective clinical significance are shown in table 2.

With advanced CRC, an increased number of *EGFR* copies is associated with poor survival and may be an independent prognostic variable [4]. As for *PTEN*, more thorough research is needed here [4]. An increase in the copyicity of the *STRAP* gene was shown in 22 % of cases of stage II and III CRC [52]. This gene is located on chromosome 12 and encodes a protein associated with the serine/threonine kinase receptor. Interestingly, patients who did not receive adjuvant therapy showed a better prognosis with an increase in the copy of the STRAP gene. In another cohort of 354 patients with CRC (stage IV) as an increase in the copyicity of the *SRC, AURKA, TPX2* and *BCL2L1* genes [55].

A decrease in the number of copies of the *CD226* gene located on chromosome 18q, which encodes a glycoprotein expressed on the surface of NK cells, platelets, monocytes and a subpopulation of T cells, is a biomarker of poor prognosis for 5-year overall and relapse-free survival [55]. In the same CDR cohort, a decrease in the number of CDH-7 copies was a biomarker of a good result with respect to 5-year overall and relapse-free survival [55].

Recently, Lee and his colleagues have shown that *GAEC1*, a putative oncogene located on chromosome 7, was amplified in 24 % of a cohort of CRC patients [54]. Moreover, an increase in the number of copies was associated with a worse prognosis due to the increased aggressiveness of the tumor. Another predictive CNV is *SKI*, located on chromosome 1. In a cohort of 533 cases of stage II and III CRC, the number of copies of the SKI gene could be successfully measured in 159 patients [52]. *SKI* amplification was associated with worse overall and relapse-free survival compared to patients without an increase in the number of copies or deletions of this gene [52].

In 2002, a study of an early-stage CRC cohort (*n* = 180) studied the allelic imbalance of chromosomes 8p and 18q in relation to relapse of the disease. Patients with stage A tumors (according to Dukes) showing an allele imbalance in both chromosomal arms were more likely to have a relapse compared to Dukes B patients without an allele imbalance. Focal chromosomal CNVs can also be used in predicting metastases in patients with CRC. Thus, it was shown that both amplifications in the 8q and 20q regions are more often present in tumors with metastases [4].

A number of CNVs are associated with the response to treatment in CRC. An increase in the copyicity of the *TYMS* gene was shown in a sample of patients with CRC resistant to therapy based on 5-fluorouracil (5-FU) [56]. On the contrary, a decrease in the number of copies of the negative prognostic marker *CD226* is associated with better overall survival after therapy based on 5-FU [4]. Bess and his colleagues [52] reported on a study of patients with stage II and III CRC and demonstrated that *STRAP* amplification leads to a worse response to 5-FU-based therapy, which was observed in patients who had a higher rate of relapse and mortality compared to patients without amplification of this gene. Among CRC patients with wild type *KRAS*, only 17 % benefit from monotherapy against *EGFR* [57]. At the same time, in these patients, an increased number of *EGFR* copies is associated with an improved response to irinotecan-cetuximab therapy and a longer time to progression [4]. In 2013, Jiang and his colleagues conducted a meta-analysis of 13 studies involving 1,174 patients with CRC treated with cetuximab or panitumumab. Their results showed that an increase in the number of *EGFR* copies in this sample was associated with an improvement in overall and relapse-free survival [58].

In a comprehensive analysis of CNV in 349 tumors removed from patients participating in the CAIRO and CAIRO2 clinical trials, it was found that changes in certain chromosomal regions, mainly an increase in gene copy in the 6q region and deletions in the 18q region, were associated with a significant difference in progression-free survival between the irinotecan and non-irinotecan treatment groups. him [53]. In addition, van Dijk and his colleagues showed that the loss of a section of chromosome 18q11.2 – q12.1 in patients with CRC is an indicator of a good prognosis, since these patients were characterized by better overall survival and a better response to bevacizumab therapy [59].

CONCLUSION

Thus, this review shows a positive correlation between CNV levels and gene expression in CRC, leading to massive deregulation of cellular signaling pathways. However, modern literature has not allowed us to answer a number of questions: 1) Are all genes affected by such a positive correlation, or do some genes avoid this dependence? 2) Do the transcription networks of genes identified in CRC function in precancerous lesions? That is, it is necessary to find out exactly how CNVs form the transcriptome of tumor cells and why these cells need such specific deregulated transcription networks.

From the point of view of the translation of the CNV indicator into clinical practice, further research is required. In the end, an in-depth understanding of the role of CNV in CRC will allow stratifying patients based on biological and genetic characteristics to improve the prognosis of the disease and determine therapeutic strategies.

Reference

1. Kit OI, Gevorkyan YuA, Soldatkina NV, Timoshkina NN, Kharagezov DA, Kaymakchi DO, et al. Prognostic factors in colorectal cancer. Koloproktologia. 2021;20(2):42–49. (In Russ.). https://doi.org/10.33878/2073-7556-2021-20-2-42-49
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646–674. https://doi.org/10.1016/j.cell.2011.02.013
3. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990 Jun 1;61(5):759–767. https://doi.org/10.1016/0092-8674(90)90186-i
4. Ried T, Meijer GA, Harrison DJ, Grech G, Franch-Expósito S, Briffa R, et al. The landscape of genomic copy number alterations in colorectal cancer and their consequences on gene expression levels and disease outcome. Mol Aspects Med. 2019 Oct;69:48–61. https://doi.org/10.1016/j.mam.2019.07.007
5. Kosheleva NG, Gusareva MA, Udalenkova IA, Fatkina NB, Legostaev VM, Shlyakhova OV et al. Genes copy number variation index in cell-free dna of blood plasma as a marker for low invasive efficiency assessment of rectal tumors radiotherapy. Modern Problems of Science and Education. 2020;(6):167. (In Russ.). https://doi.org/10.17513/spno.30396
6. Bardi G, Johansson B, Pandis N, Bak-Jensen E, Orndal C, Heim S, et al. Cytogenetic aberrations in colorectal adenocarcinomas and their correlation with clinicopathologic features. Cancer. 1993 Jan 15;71(2):306–314. https://doi.org/10.1002/1097-0142(19930115)71:2<306::aid-cncr2820710207>3.0.co;2-c
7. Camps J, Armengol G, del Rey J, Lozano JJ, Vauhkonen H, Prat E, et al. Genome-wide differences between microsatellite stable and unstable colorectal tumors. Carcinogenesis. 2006 Mar;27(3):419–428. https://doi.org/10.1093/carcin/bgi244
8. Takahashi Y, Sheridan P, Niida A, Sawada G, Uchi R, Mizuno H, et al. The AURKA/TPX2 axis drives colon tumorigenesis cooperatively with MYC. Ann Oncol. 2015 May;26(5):935–942. https://doi.org/10.1093/annonc/mdv034
9. Kit OI, Vodolazhsky DI, Kutilin DS, Gudueva EN. Changes in the number of copies of genetic loci in gastric cancer. Mol Biol (Mosk). 2015 Aug;49(4):658–597. https://doi.org/10.7868/S0026898415040096
10. Kutilin DS, Airapetova TG, Anistratov PA, Pyltsin SP, Leiman IA, Karnaukhov NS, et al COPY Number variation in tumor cells and extracellular dna in patients with lung adenocarcinoma. Bulletin of Experimental Biology and Medicine. 2019;167(6):731–738. (In Russ.).
11. Kutilin DS, Tsandekova MR, Porkhanova NV. Features of the replication of some genes in tumor cells in patients with serous ovarian adenocarcinoma. Bulletin of Experimental Biology and Medicine. 2020;170(9):325–333. (In Russ.).
12. Kutilin DS, Zenkovich MS, Gusareva MA, Faenson AV, Karnaukhova EA, Rozenko LYa, et al. Gene replication as a factor of resistance of prostate tumor cells to learning. Modern Problems of Science and Education. 2020;(4):82. (In Russ.). https://doi.org/10.17513/spno.29866
13. Tryakin AA, Khakimova GG, Zabotina TN, Borunova AA, Malikhova OA. Modern views on immunological biomarkers of colon cancer. Malignant tumours. 2018;8(4):50–58. (In Russ.). https://doi.org/10.18027/2224-5057-2018-8-4-50-58
14. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010 Jun;138(6):2073–2087. https://doi.org/10.1053/j.gastro.2009.12.064
15. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012 Jul 18;487(7407):330–337. https://doi.org/10.1038/nature11252

16. Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015 Nov;21(11):1350–1356. https://doi.org/10.1038/nm.3967
17. Ma X, Ezer D, Adryan B, Stevens TJ. Canonical and single-cell Hi-C reveal distinct chromatin interaction sub-networks of mammalian transcription factors. Genome Biol. 2018 Oct 25;19(1):174. https://doi.org/10.1186/s13059-018-1558-2
18. Sveen A, Bruun J, Eide PW, Eilertsen IA, Ramirez L, Murumägi A, et al. Colorectal Cancer Consensus Molecular Subtypes Translated to Preclinical Models Uncover Potentially Targetable Cancer Cell Dependencies. Clin Cancer Res. 2018 Feb 15;24(4):794–806. https://doi.org/10.1158/1078-0432.CCR-17-1234
19. Cross W, Kovac M, Mustonen V, Temko D, Davis H, Baker A-M, et al. The evolutionary landscape of colorectal tumorigenesis. Nat Ecol Evol. 2018 Oct;2(10):1661–1672. https://doi.org/10.1038/s41559-018-0642-z
20. Voorham QJM, Carvalho B, Spiertz AJ, van Grieken NCT, Mongera S, Rondagh EJA, et al. Chromosome 5q loss in colorectal flat adenomas. Clin Cancer Res. 2012 Sep 1;18(17):4560–4569. https://doi.org/10.1158/1078-0432.CCR-11-2385
21. IJspeert JEG, Medema JP, Dekker E. Colorectal neoplasia pathways: state of the art. Gastrointest Endosc Clin N Am. 2015 Apr;25(2):169–182. https://doi.org/10.1016/j.giec.2014.11.004
22. Bettington M, Walker N, Rosty C, Brown I, Clouston A, McKeone D, et al. Clinicopathological and molecular features of sessile serrated adenomas with dysplasia or carcinoma. Gut. 2017 Jan;66(1):97–106. https://doi.org/10.1136/gutjnl-2015-310456
23. Saito T, Niida A, Uchi R, Hirata H, Komatsu H, Sakimura S, et al. A temporal shift of the evolutionary principle shaping intratumor heterogeneity in colorectal cancer. Nat Commun. 2018 Jul 23;9(1):2884. https://doi.org/10.1038/s41467-018-05226-0
24. Carvalho B, Postma C, Mongera S, Hopmans E, Diskin S, van de Wiel MA, et al. Multiple putative oncogenes at the chromosome 20q amplicon contribute to colorectal adenoma to carcinoma progression. Gut. 2009 Jan;58(1):79–89. https://doi.org/10.1136/gut.2007.143065
25. Løberg M, Kalager M, Holme Ø, Hoff G, Adami H-O, Bretthauer M. Long-term colorectal-cancer mortality after adenoma removal. N Engl J Med. 2014 Aug 28;371(9):799–807. https://doi.org/10.1056/NEJMoa1315870
26. Carvalho B, Diosdado B, Terhaar Sive Droste JS, Bolijn AS, Komor MA, de Wit M, et al. Evaluation of Cancer-Associated DNA Copy Number Events in Colorectal (Advanced) Adenomas. Cancer Prev Res (Phila). 2018 Jul;11(7):403–412. https://doi.org/10.1158/1940-6207.CAPR-17-0317
27. Torabi K, Erola P, Alvarez-Mora MI, Díaz-Gay M, Ferrer Q, Castells A, et al. Quantitative analysis of somatically acquired and constitutive uniparental disomy in gastrointestinal cancers. Int J Cancer. 2019 Feb 1;144(3):513–524 https://doi.org/10.1002/ijc.31936
28. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010 Sep;20(9):1297–1303. https://doi.org/10.1101/gr.107524.110
29. Magi A, Pippucci T, Sidore C. XCAVATOR: accurate detection and genotyping of copy number variants from second and third generation whole-genome sequencing experiments. BMC Genomics. 2017 Sep 21;18(1):747. https://doi.org/10.1186/s12864-017-4137-0
30. Alkodsi A, Louhimo R, Hautaniemi S. Comparative analysis of methods for identifying somatic copy number alterations from deep sequencing data. Brief Bioinform. 2015 Mar;16(2):242–254 https://doi.org/10.1093/bib/bbu004
31. Kadalayil L, Rafiq S, Rose-Zerilli MJJ, Pengelly RJ, Parker H, Oscier D, et al. Exome sequence read depth methods for identifying copy number changes. Brief Bioinform. 2015 May;16(3):380–392. https://doi.org/10.1093/bib/bbu027
32. Nam J-Y, Kim NKD, Kim SC, Joung J-G, Xi R, Lee S, et al. Evaluation of somatic copy number estimation tools for whole-exome sequencing data. Brief Bioinform. 2016 Mar;17(2):185–192. https://doi.org/10.1093/bib/bbv055
33. Trost B, Walker S, Wang Z, Thiruvahindrapuram B, MacDonald JR, Sung WWL, et al. A Comprehensive Workflow for Read Depth-Based Identification of Copy-Number Variation from Whole-Genome Sequence Data. Am J Hum Genet. 2018 Jan 4;102(1):142–155. https://doi.org/10.1016/j.ajhg.2017.12.007
34. Zare F, Dow M, Monteleone N, Hosny A, Nabavi S. An evaluation of copy number variation detection tools for cancer using whole exome sequencing data. BMC Bioinformatics. 2017 May 31;18(1):286. https://doi.org/10.1186/s12859-017-1705-x
35. Mermel CH, Schumacher SE, Hill B, Meyerson ML, Beroukhim R, Getz G. GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. Genome Biol. 2011;12(4):R41. https://doi.org/10.1186/gb-2011-12-4-r41
36. Larsen SJ, do Canto LM, Rogatto SR, Baumbach J. CoNVaQ: a web tool for copy number variation-based association studies. BMC Genomics. 2018 May 18;19(1):369. https://doi.org/10.1186/s12864-018-4732-8

37. Franch-Exposito S, Bassaganyas L, Vila-Casadesús M, Hernández-Illán E, Esteban-Fabró R, Díaz-Gay M, et al. CNApp, a tool for the quantification of copy number alterations and integrative analysis revealing clinical implications. Elife. 2020 Jan 15;9:e50267. https://doi.org/10.7554/eLife.50267
38. Nesic K, Wakefield M, Kondrashova O, Scott CL, McNeish IA. Targeting DNA repair: the genome as a potential biomarker. J Pathol. 2018 Apr;244(5):586–597. https://doi.org/10.1002/path.5025
39. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature. 2016 Jun 2;534(7605):47–54. https://doi.org/10.1038/nature17676
40. Macintyre G, Goranova TE, De Silva D, Ennis D, Piskorz AM, Eldridge M, et al. Copy number signatures and mutational processes in ovarian carcinoma. Nat Genet. 2018 Sep;50(9):1262–1270. https://doi.org/10.1038/s41588-018-0179-8
41. Li Y, Roberts ND, Weischenfeldt J, Wala JA, Shapira O, Schumacher SE, et al. Patterns of structural variation in human cancer. bioRxiv. 2017 Jan 1;181339. https://doi.org/10.1101/181339
42. Ried T, Hu Y, Difilippantonio MJ, Ghadimi BM, Grade M, Camps J. The consequences of chromosomal aneuploidy on the transcriptome of cancer cells. Biochim Biophys Acta. 2012 Jul;1819(7):784–793. https://doi.org/10.1016/j.bbagrm.2012.02.020
43. Yan D, Yi S, Chiu WC, Qin LG, Kin WH, Kwok Hung CT, et al. Integrated analysis of chromosome copy number variation and gene expression in cervical carcinoma. Oncotarget. 2017 Dec 12;8(65):108912–108922. https://doi.org/10.18632/oncotarget.22403
44. Fehrmann RSN, Karjalainen JM, Krajewska M, Westra H-J, Maloney D, Simeonov A, et al. Gene expression analysis identifies global gene dosage sensitivity in cancer. Nat Genet. 2015 Feb;47(2):115–125. https://doi.org/10.1038/ng.3173
45. Ortiz-Estevez M, De Las Rivas J, Fontanillo C, Rubio A. Segmentation of genomic and transcriptomic microarrays data reveals major correlation between DNA copy number aberrations and gene-loci expression. Genomics. 2011 Feb;97(2):86–93. https://doi.org/10.1016/j.ygeno.2010.10.008
46. Alaei-Mahabadi B, Bhadury J, Karlsson JW, Nilsson JA, Larsson E. Global analysis of somatic structural genomic alterations and their impact on gene expression in diverse human cancers. Proc Natl Acad Sci U S A. 2016 Nov 29;113(48):13768–13773. https://doi.org/10.1073/pnas.1606220113
47. Camps J, Pitt JJ, Emons G, Hummon AB, Case CM, Grade M, et al. Genetic amplification of the NOTCH modulator LNX2 upregulates the WNT/β-catenin pathway in colorectal cancer. Cancer Res. 2013 Mar 15;73(6):2003–2013. https://doi.org/10.1158/0008-5472.CAN-12-3159
48. Loo LWM, Tiirikainen M, Cheng I, Lum-Jones A, Seifried A, Church JM, et al. Integrated analysis of genome-wide copy number alterations and gene expression in microsatellite stable, CpG island methylator phenotype-negative colon cancer. Genes Chromosomes Cancer. 2013 May;52(5):450–466. https://doi.org/10.1002/gcc.22043
49. Ali Hassan NZ, Mokhtar NM, Kok Sin T, Mohamed Rose I, Sagap I, Harun R, et al. Integrated analysis of copy number variation and genome-wide expression profiling in colorectal cancer tissues. PLoS One. 2014;9(4):e92553. https://doi.org/10.1371/journal.pone.0092553
50. Wangsa D, Braun R, Stuelten CH, Brown M, Bauer KM, Emons G, et al. Induced Chromosomal Aneuploidy Results in Global and Consistent Deregulation of the Transcriptome of Cancer Cells. Neoplasia. 2019 Jul;21(7):721–729. https://doi.org/10.1016/j.neo.2019.04.009
51. Dimitriou N, Arandjelović O, Harrison DJ, Caie PD. A principled machine learning framework improves accuracy of stage II colorectal cancer prognosis. NPJ Digit Med. 2018;1:52. https://doi.org/10.1038/s41746-018-0057-x
52. Buess M, Terracciano L, Reuter J, Ballabeni P, Boulay J-L, Laffer U, et al. Amplification of SKI is a prognostic marker in early colorectal cancer. Neoplasia. 2004 Jun;6(3):207–212. https://doi.org/10.1593/neo.03442
53. Haan JC, Labots M, Rausch C, Koopman M, Tol J, Mekenkamp LJM, et al. Genomic landscape of metastatic colorectal cancer. Nat Commun. 2014 Nov 14;5:5457. https://doi.org/10.1038/ncomms6457
54. Lee KT-W, Gopalan V, Islam F, Wahab R, Mamoori A, Lu C-T, et al. GAEC1 mutations and copy number aberration is associated with biological aggressiveness of colorectal cancer. Eur J Cell Biol. 2018 Apr;97(3):230–241. https://doi.org/10.1016/j.ejcb.2018.03.002
55. Ptashkin RN, Pagan C, Yaeger R, Middha S, Shia J, O'Rourke KP, et al. Chromosome 20q Amplification Defines a Subtype of Microsatellite Stable, Left-Sided Colon Cancers with Wild-type RAS/RAF and Better Overall Survival. Mol Cancer Res. 2017 Jun;15(6):708–713. https://doi.org/10.1158/1541-7786.MCR-16-0352
56. Watson RG, Muhale F, Thorne LB, Yu J, O'Neil BH, Hoskins JM, et al. Amplification of thymidylate synthetase in metastatic colorectal cancer patients pretreated with 5-fluorouracil-based chemotherapy. Eur J Cancer. 2010 Dec;46(18):3358–3364. https://doi.org/10.1016/j.ejca.2010.07.011

57. Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 2011 Nov;1(6):508–523. https://doi.org/10.1158/2159-8290.CD-11-0109

58. Jiang Z, Li C, Li F, Wang X. EGFR gene copy number as a prognostic marker in colorectal cancer patients treated with cetuximab or panitumumab: a systematic review and meta analysis. PLoS One. 2013;8(2):e56205.
https://doi.org/10.1371/journal.pone.0056205

59. Van Dijk E, Biesma HD, Cordes M, Smeets D, Neerincx M, Das S, et al. Loss of Chromosome 18q11.2-q12.1 Is Predictive for Survival in Patients With Metastatic Colorectal Cancer Treated With Bevacizumab. J Clin Oncol. 2018 Jul 10;36(20):2052–2060.
https://doi.org/10.1200/JCO.2017.77.1782

Information about authors:

Andrey A. Maslov – Dr. Sci. (Med.), professor, chief doctor National Medical Research Centre for Oncology, Rostov-on-Don, Russian Federation. ORCID: https://orcid.org/0000-0001-7328-8074, SPIN: 5963-5915, AuthorID: 817983

Lusegen Kh. Chalkhakhyan ✉ – Cand. Sci. (Med.), surgeon at the abdominal oncology department No.2, National Medical Research Centre for Oncology, Rostov-on-Don, Russian Federation. ORCID: https://orcid.org/0000-0001-8397-4393, SPIN: 6534-5911, AuthorID: 794696

Sergey A. Malinin – Cand. Sci. (Med.), oncologist at the abdominal oncology department No. 2, National Medical Research Centre for Oncology, Rostov-on-Don, Russian Federation. ORCID: https://orcid.org/0000-0002-1220-7143, SPIN: 7229-1610, AuthorID: 794691

Gennadii V. Kaminsky – Cand. Sci. (Med.), surgeon at the abdominal oncology department No.2, National Medical Research Centre for Oncology, Rostov-on-Don, Russian Federation. ORCID: https://orcid.org/0000-0003-4905-4977, SPIN: 3308-4107, AuthorID: 794670

Ellada A. Mirzoyan – PhD student National Medical Research Centre for Oncology, Rostov-on-Don, Russian Federation. ORCID: https://orcid.org/0000-0002-0328-9714, SPIN: 2506-8605, AuthorID: 1002948, ResearcherID: AAZ-2780-2021, Scopus Author ID: 57221118516

Contribution of the authors:

Maslov A. A. – scientific editing, data analysis;
Chalkhakhyan L. Kh., Malinin S. A., Kaminsky G. V., Mirzoyan E. A. – writing the text, technical editing, design of the list of references.