УДК 616.348/.351-006.6-089.844
https://doi.org/10.20538/1682-0363-2022-4-37-43

Development and characterization of patient-derived xenograft models of colorectal cancer for testing new pharmacological substances

Goncharova A.S., Kolesnikov E.N., Egorov G.Yu., Maksimov A.Yu., Shevchenko A.N., Nepomnyashchaya E.M., Gvaldin D.Yu., Kurbanova L.Z., Khodakova D.V., Kit S.O., Kaymakchi O.Yu., Snezhko A.V.

National Medical Research Center for Oncology
63, 14th Liniya Str., Rostov-on-Don, 344037, Russian Federation

ABSTRACT

The aim of the study was to create a patient-derived xenograft (PDX) model of human colorectal cancer and to determine its histologic and molecular characteristics, such as the status of *KRAS, NRAS,* and *BRAF* genes and the presence of microsatellite instability.

Materials and methods. First generation xenograft models *in vivo* were created using tumors from patients with colorectal cancer (n = 4) and immunodeficient Balb/c Nude mice (n = 20); second, third, and fourth generation models were created in the same mouse line (n = 3 for each generation). A caliper was used to measure subcutaneous xenografts; their size was calculated by the ellipsoid formula. Cryopreservation involved immersing the samples in a freezing medium (80% RPMI 1640, 10% fetal bovine serum, 10% dimethyl sulfoxide (DMSO)) and storing them at –80 °C. The histologic analysis was performed according to the standard technique (preparation of paraffin blocks and staining of microsections with hematoxylin and eosin). Mutations in the *KRAS, NRAS*, and *BRAF* genes were determined by direct Sanger sequencing; microsatellite instability was determined by the fragment analysis at five loci: *Bat-25, Bat-26, NR21, NR24,* and *NR27*.

Results. Stable, transplantable xenografts of colorectal cancer were obtained from two out of four patients. The average waiting time from the implantation to the growth of the first generation xenograft was 28 days. The latency phase after cryopreservation was comparable to that at the creation of the first generation PDX model. The model reproduced the histotype, grade and mutational status of the *KRAS*, *NRAS*, and *BRAF* genes, as well as microsatellite instability of the donor tumor.

Conclusion. The developed model of human colorectal cancer was characterized in terms of growth dynamics, cryopreservation tolerance, and histologic and molecular genetic parameters.

Keywords: xenograft, colorectal cancer, *in vivo* models, PDX model, Balb/c Nude

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The authors state that they received no funding for the study.

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at the National Medical Research Center for Oncology.

For citation: Goncharova A.S., Kolesnikov E.N., Egorov G.Yu., Maksimov A.Yu., Shevchenko A.N., Nepomnyashchaya E.M., Gvaldin D.Yu., Kurbanova L.Z., Khodakova D.V., Kit S.O., Kaymakchi O.Yu., Snezhko A.V. Development and characterization of patient-derived xenograft models of colorectal cancer for testing new pharmacological substances. *Bulletin of Siberian Medicine.* 2022;21(4):37–43. https://doi.org/10.20538/1682-0363-2022-4-37-43.

✉ *Goncharova Anna S.,* fateyeva_a_s@list.ru

Разработка и характеристика ксенотрансплантатов, полученных от пациентов с колоректальным раком, для тестирования новых фармакологических субстанций

Гончарова А.С., Колесников Е.Н., Егоров Г.Ю., Максимов А.Ю., Шевченко А.Н., Непомнящая Е.М., Гвалдин Д.Ю., Курбанова Л.З., Ходакова Д.В., Кит С.О., Каймакчи О.Ю., Снежко А.В.

Национальный медицинский исследовательский центр (НМИЦ) онкологии
Россия, 344037, г. Ростов-на-Дону, ул. 14-я Линия, 63

РЕЗЮМЕ

Цель. Создание модели ксенотрансплантата, полученного от пациента с колоректальным раком (КРР), и определение ее гистологических и молекулярных характеристик, таких как статус генов *KRAS, NRAS BRAF* и наличие микросателлитной нестабильности.

Материалы и методы. Для создания первого поколения модели *in vivo* использовали опухоли от пациентов с КРР (n = 4) и иммунодефицитных мышей линии Balb/c Nude (n = 20), для создания второго, третьего и четвертого поколения – мышей этой же линии (n = 3 для каждого поколения). Измерения подкожных ксенотрансплантатов выполняли штангенциркулем, их размеры вычисляли по формуле Шрека для эллипсоида. Криоконсервацию выполняли путем погружения образцов в микс для криоконсервации (80% RPMI 1640, 10% фетальной бычьей сыворотки, 10% диметилсульфоксида) и хранения их на –80 °С. Гистологическое исследование выполняли согласно стандартной методике (приготовление парафиновых блоков и окрашивание микросрезов гематоксилином и эозином). Мутации в генах *KRAS*, *NRAS* и *BRAF* определяли методом прямого секвенирования по Сэнгеру, микросателлитную нестабильность – методом фрагментарного анализа по пяти локусам: *Bat-25*, *Bat-26*, *NR21*, *NR24*, *NR27*.

Результаты. Стабильные перевиваемые ксенотрансплантаты КРР получены от двух пациентов из четырех. Среднее время ожидания между имплантацией и ростом трансплантата первого поколения составило 28 сут. Латентная фаза после криоконсервации была сопоставима с латентной фазой при создании первого поколения пациентоподобной модели. Показано, что в модели воспроизведены гистотип, степень дифференцировки и мутационный статус генов *KRAS*, *NRAS*, *BRAF* и микросаттелитная нестабильность донорской опухоли.

Заключение. Созданная модель КРР человека охарактеризована с учетом динамики роста, способности переносить криоконсервацию, гистологических и молекулярно-генетических параметров.

Ключевые слова: ксенотрансплантат, колоректальный рак, модели *in vivo*, PDX модель, Balb/c Nude

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом НИМЦ онкологии.

Для цитирования: Гончарова А.С., Колесников Е.Н., Егоров Г.Ю., Максимов А.Ю., Шевченко А.Н., Непомнящая Е.М., Гвалдин Д.Ю., Курбанова Л.З., Ходакова Д.В., Кит С.О., Каймакчи О.Ю., Снежко А.В. Разработка и характеристика ксенотрансплантатов, полученных от пациентов с колоректальным раком, для тестирования новых фармакологических субстанций. *Бюллетень сибирской медицины.* 2022;21(4):37–43. https://doi.org/10.20538/1682-0363-2022-4-37-43.

INTRODUCTION

Colorectal cancer (CRC) is one the most common cancers worldwide. It is characterized by high lethality at advanced stages [1]. Mortality from CRC can be reduced by its early detection and an optimal treatment regimen in management of patients with advanced disease.

The treatment strategy for CRC depends on the stage and site of the tumor, as well as on its molecular

characteristics [2]. To date, treatment of patients with CRC involves surgical resection combined with standard adjuvant chemotherapy, and neoadjuvant radiochemotherapy is recommended for patients with locally advanced rectal cancer [2, 3]. A combination of chemotherapy with new targeted drugs, such as epidermal growth factor receptor (EGFR) inhibitors, and immunotherapy improves the median survival of patients [4]. Patients with wild-type *KRAS* genes in tumors have been found to respond favorably to targeted therapies, including anti-EGFR or anti-VEGFR drugs, while patients with high microsatellite instability in tumors (MSI-high tumors) benefit more from immunotherapy [5].

Despite advances in CRC treatment, the search for new anticancer drugs continues around the world. Early stages of development of potentially useful pharmacological substances involve the use of cancer cell line panels as a tool to study the biological mechanisms of action and test the activity of new compounds *in vitro*. However, cell lines fail to reproduce heterogeneity of human tumors both *in vitro* and *in vivo*. On the contrary, patient-derived xenograft (PDX) models better reflect the existing molecular heterogeneity of human cancers and, therefore, are considered more suitable for drug efficacy studies [6].

The use of PDX models as a platform for assessing therapeutic responses in preclinical studies requires standardization of these models, which is especially important for assessments at the molecular level [7].

Therefore, the aim of this study was to create a PDX model of human CRC and to determine its molecular characteristics, such as the status of *KRAS*, *NRAS, BRAF*, and *MSI* genes.

MATERIALS AND METHODS

The study included immunodeficient Balb/c Nude mice (29 female mice aged 5–6 weeks) obtained from the SPF-vivarium of the Institute of Cytology and Genetics, SB of RAS (Novosibirsk). The animals were kept in the SPF vivarium at the National Medical Research Center for Oncology. The animals were housed in the IVC system (Tecniplast, Italy) in a room with controlled climate parameters (temperature 21–26 °C, air humidity 50–60%). The animals had free access to food and water which were exposed to autoclave sterilization. All manipulations involving animals were performed in compliance with the Guidelines for the Use of Laboratory Animals.

Subcutaneous PDX models of human CRC were created using tumor samples obtained during surgery from patients receiving treatment at the Department of Abdominal Cancer No.1, National Medical Research Center for Oncology, from February to April 2020. All patients signed an informed consent to the use of biological material.

Subcutaneous PDX models were obtained by implanting a fragment of the donor tumor with a size of 3 × 3 × 3 mm under the skin of the right thigh in recipient animals (n = 5 for a sample obtained from one patient). Mice of the same line were used for the second, third, and fourth generation models (n = 3 for each generation). Implantation was performed under injectable anesthesia with Xyla (20 mg / kg) and Zoletil-100 (50 mg / kg). The animals were euthanized by cervical dislocation.

Subcutaneous xenografts were measured by a caliper (Griff, Russia), and their size was calculated by the ellipsoid formula: $V = a \times b \times c \times \pi / 6$, where V is the tumor volume (mm3), and a, b, and c are measurements of the ellipsoid in three planes (mm).

Isolated tumor nodules were divided into 3 × 3 × 3 mm fragments, placed in a freezing medium (80% RPMI 1640, 10% fetal bovine serum, 10% dimethyl sulfoxide (DMSO)), and then stored in the freezer at –80 °C. Frozen samples were thawed in a 37 °C water bath. Then the samples were placed in a container with the RPMI 1640 medium. After the thawing, the tumor fragments were implanted.

The fragments of donor tumors and xenografts were fixed in 10% formalin for 24 h and then embedded in paraffin. Then the histologic analysis was performed according to the standard technique: paraffin blocks were prepared, and the microsections were stained with hematoxylin and eosin.

Genomic DNA was isolated from PDX using the QIAamp DNA Mini Kit (Qiagen, Germany) and the QIAcube Connect automated nucleic acid purification system (Qiagen, Germany). Mutations in exons 2, 3, and 4 in the *KRAS* and *NRAS* genes and *BRAF* V600 mutations were identified by direct Sanger sequencing (AB3500 Genetic Analyzer, Life Technologies, USA). Microsatellite instability was determined by the fragment analysis (AB3500 Genetic Analyzer, Life Technologies, USA) at five loci: *Bat-25, Bat-26, NR21, NR24,* and *NR27*.

RESULTS

Tumor samples for the PDX model of human CRC were obtained from four patients during surgery for

colon tumors (sigmoid / transverse colon resection). Freshly resected tumor fragments from each patient were transported from the operating unit to the SPF vivarium in a sterile container with the RPMI 1640 medium and implanted into immunodeficient Balb/c Nude mice (n = 5 for a sample taken from one patient) within an hour.

Table 1 presents the characteristics of patients and the corresponding assessment of xenotransplantation results.

Table 1

Clinical characteristics of patients and assessment of the results of tumor xenotransplantation into immunodeficient Balb/c Nude mice

Procedure number	Sampling method	Tumor site	TNM stage	Histology	Implantation results	Latency phase duration. days
PDX-1	Surgical resection	Transverse colon	$T_{4в}N_{1c}M_0$	Moderately differentiated adenocarcinoma	2/5	25 (20–30)
PDX-2	Surgical resection	Sigmoid colon	$T_3N_{1b}M_0$	Moderately differentiated adenocarcinoma	3/5	31 (24–45)
PDX-3	Surgical resection	Sigmoid colon	$T_3N_0M_0$	Moderately differentiated adenocarcinoma	0/5	–
PDX-4	Surgical resection	Sigmoid colon	$T_3N_{1b}M_0$	Moderately differentiated adenocarcinoma	0/5	–

Stable, transplantable PDX models of CRC were obtained from two out of four patients. The average waiting time from the implantation to the growth of the first generation PDX (P1) was 28 days (the range of 20–45 days). Engraftment and growth rate of freshly implanted tumor fragments varied (Fig. 1).

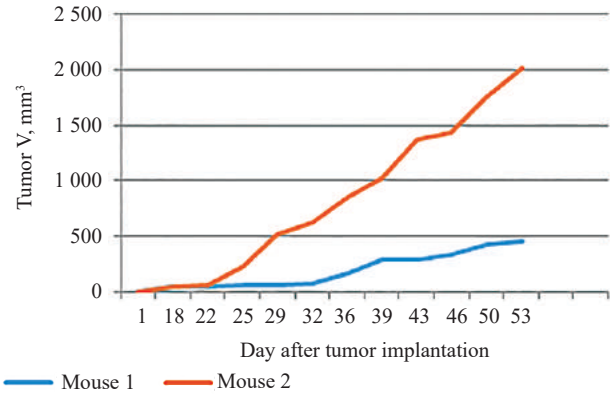


Fig. 1. Growth dynamics of the first generation PDX-1 model (P1)

If xenotransplantation did not result in the growth of subcutaneous tumor nodules within 60 days, the procedure was regarded as unsatisfactory, and the corresponding observations (PDX-3 and PDX-4) were stopped. Two successfully implanted PDX models (PDX-1 and PDX-2) were serially passaged to generate second (P2, n = 3) and third generation (P3, n = 3) models.

To assess the effect of cryopreservation on engraftment and growth rate, subcutaneous tumor nodules of the third generation PDX-1 were isolated, fragmented, cryopreserved according to the standard procedure, and stored at –80 °C. After recovery of PDX-1 from cryopreservation, two of the three samples showed linear growth forming the fourth generation (P4) (Fig. 2).

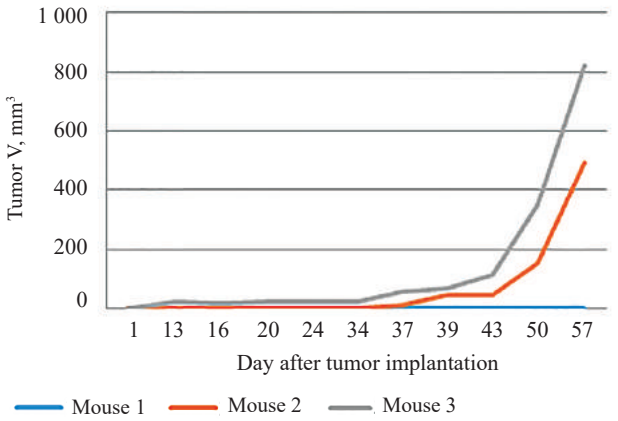
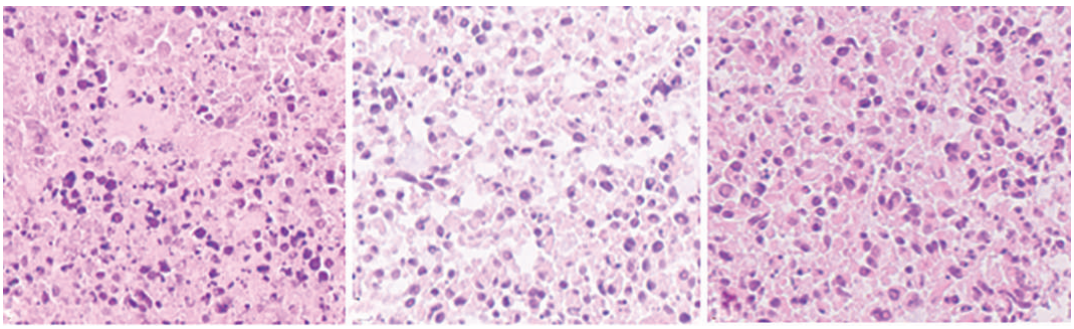


Fig. 2. Growth dynamics of PDX-1 thawed after cryopreservation, fourth generation (P4)

The latency phase of the fourth generation PDX-1 (P4) after cryopreservation was slightly longer than that of the first generation (P1).

The histologic characteristics of the primary tumor were preserved during serial passage, and they were reproduced in the fourth generation PDX-1 after cryopreservation. The preparations were described as moderately differentiated adenocarcinoma (G2). The tumor showed necrotic foci with pronounced infiltrative growth and areas with high mitotic activity. Slight lymphocytic infiltration was determined in the preparations obtained from the donor tumor material (Fig. 3).



a *b* *c*

Fig. 3. Histologic samples of the patient tumor and corresponding PDX models: *a* – patient tumor; *b* – first generation PDX model; *c* – fourth generation PDX model (after cryopreservation). Staining with hematoxylin and eosin, ×400

Molecular and genetic tests showed no mutations in exons 2, 3, and 4 in the *KRAS* and *NRAS* genes and no *BRAF* V600 mutations. Microsatellite stability was also determined both in the sample of the donor tumor material and in the corresponding third (P3) and fourth generation (P4) PDX models.

DISCUSSION

In this study, we performed four subcutaneous implantations of tumor fragments from patients with CRC into immunodeficient Balb/c Nude mice, resulting in two stable, transplantable PDX-derived cell lines, which complied with earlier published engraftment-characterizing parameters [7, 8]. Successful engraftment of tumor fragments in a PDX model is not universal for all types of tumors; however, according to the literature, PDX models of human CRC have relatively high engraftment rates ranging from 50 to 70% [7–9].

A. Katsiampoura et al. (2017) demonstrated that surgically obtained samples had higher engraftment rates compared with biopsy samples, about 70 and 35%, respectively [8]. In this regard, we chose a surgical tumor sampling method to create PDX models. In addition, a small size of biopsy samples complicates the choice of the implantation site and creation of a series of xenografts with simultaneous sample duplication for biobanking [10, 11]. The growth dynamics of PDX models after cryopreservation (P4) showed that the duration of a latency phase was generally comparable to that during the creation of the first generation PDX model (P1).

We demonstrated the ability of PDX to reproduce the morphological features of the disease, namely the histotype and grade of the tumor. In addition, the study of sequentially created generations P1, P2, P3, and P4 allowed to conclude that PDX passaging did not significantly affect the ability to reproduce the histological subtype, at least at early stages of PDX creation, which is consistent with the literature data [7]. Some researchers demonstrated in larger-scale studies that differentiation of donor tumor cells did not affect the establishment of the PDX cell line [8].

PDX models can reproduce both morphological characteristics of tumors and molecular and genetic heterogeneity which is a fundamental feature of the human disease [12]. We noted in this study that wild-type *KRAS*, *NRAS,* and *BRAF* genes were preserved in serial passaging, and microsatellite stability was established in samples of the third generation PDX model (P3), which complied with the molecular and genetic characteristics of the donor tumor. This makes the resulting model suitable for testing both new pharmacological substances with a cytotoxic effect and monoclonal antibody drugs.

Other studies have found that clinically significant genetic mutations (*KRAS*, *BRAF*, and *PIK3CA*) do not affect the development of a PDX model, but the authors believe that three genes are not enough to fully reproduce the diversity of CRC biology. On the contrary, M. Cybulska et al. (2018) in a large-scale study on the stratification of PDX models of human CRC investigated transcriptomic and mutation profiles of primary tumors and xenografts derived from them using a panel of 409 cancer-associated genes. The differences were found in both genetic and transcriptomic profiles of donor tumors and PDX models, which might result from subclonal evolution at an early stage of PDX model development or technical errors. In this regard, the authors concluded that standardization of the PDX model requires taking into account more stable parameters, such as the presence of targets and a response to standard anticancer therapy [7].

CONCLUSION

To overcome certain shortcomings associated with drug testing on traditional tumor models, we attempted to develop and standardize PDX models of human CRC to reproduce a wide range of biological and clinical properties of human tumors. One of the PDX models obtained during the study was characterized in terms of growth dynamics, cryopreservation tolerance, histologic parameters, and molecular and genetic criteria for choosing a treatment strategy.

REFERENCES

1. Gevorkyan Yu.A., Kolesnikov V.E., Soldatkina N.V., Kharagezov D.A., Dashkov A.V., Kaymakchi D.O., et al. Minimally invasive surgery in treatment of patients with metastatic colorectal cancer. *Yuzhno-Rossiyskiy Onkologicheskiy Zhurnal*. 2020;1(2):22–27 (in Russ.). DOI: *10.37748/2687-0533-2020-1-2-3.*
2. Baran B., Ozupek N.M., Tetik N.Y., Acar E., Bekcioglu O., Baskin Y. Difference between left-sided and right-sided colorectal cancer: a focused review of literature. *Gastroenterology Research*. 2018;1(4):264–273. DOI: 10.14740/gr1062w.
3. Kit O.I., Gevorkyan Yu.A., Soldatkina N.V., Kolesnikov V.E., Kharagezov D.A. Laparoscopic combined surgical interventions for metastatic colorectal cancer. *Koloproktologiya.* 2015;4:19–23 (in Russ.).
4. Punt C.J.A., Koopman M., Vermeulen L. From tumour heterogeneity to advances in precision treatment of colorectal cancer. *Nature reviews Clinical Oncology*. 2017;14(4):235–246. DOI: 10.1038/nrclinonc.2016.171.
5. Wang F., Bai L., Liu T.S., Yu Y.Y., He M.M., Liu K.Y. et al. Right-and left-sided colorectal cancers respond differently to cetuximab. *Cancer Communications*. 2015;34(9):384–393. DOI: 10.1186/s40880-015-0022-x.
6. Ireson C.R., Alavijeh M.S., Palmer A.M., Fowler E.R., Jones H. The role of mouse tumour models in the discovery and development of anticancer drugs. *British Journal of Cancer*. 2019;121(2):101–108. DOI: 10.1038/s41416-019-0495-5.
7. Cybulska M., Olesinski T., Goryca K., Paczkowska K., Statkiewicz M., Kopczynski M. et al. Challenges in stratifying the molecular variability of patient-derived colon tumor xenografts. *BioMed Research International*. 2018;2018:2954208. DOI: 10.1155/2018/2954208.
8. Katsiampoura A., Raghav K., Jiang Z.Q., Menter D.G., Varkaris A., Morelli M.P. et al. Modeling of patient-derived xenografts in colorectal cancer. *Molecular Cancer Therapeutics*. 2017;16(7):1435–1442. DOI: 10.1158/1535-7163.MCT-16-0721.
9. Conte N., Mason J.C., Halmagui C., Neuhauser S., Mosaku A., Yordanova G. et al. PDX finder: a portal for patient-derived tumor xenograft model discovery. *Nucleic Acids Research.* 2019;47(D1):D1073–D1079. DOI: 10.1093/nar/gky984.
10. Jung J., Seol H.S., Chang S. The generation and application of patient-derived xenograft model for cancer research. *Cancer Research and Treatment: Official Journal of Korean Cancer Association.* 2018;50(1):1–10. DOI: 10.4143/crt.2017.307.
11. Ivanics T., John R., Bergquist J.R., Liu G., Kim M.P., Kang Y. et al. Patient-derived xenograft cryopreservation and reanimation outcomes are dependent on cryoprotectant type. *Laboratory Investigation.* 2018;98(7):947–956. DOI: 10.1038/s41374-018-0042-7.
12. Yoshida G.J. Applications of patient-derived tumor xenograft models and tumor organoids. *Journal of Hematology & Oncology*. 2020;13(1):4. DOI: 10.1186/s13045-019-0829-z.

Acknowledgments

The authors would like to thank Olesya A. Ossovskaya, translator at the National Medical Research Center for Oncology, for the translation of the manuscript in English.

Authors contribution

Goncharova A.S. – conception and design. Kolesnikov E.N. – carrying out of surgeries. Egorov G.Yu. – collection of patient clinical data. Maksimov A.Yu. – collection and analysis of data, carrying out of surgeries. Shevchenko A.N. – collection and analysis of data. Nepomnyashchaya E.M. – carrying out of the histologic study. Gvaldin D.Yu. – compilation of a list of references. Kurbanova L.Z. – drafting and technical editing of the manuscript. Khodakova D.V. – drafting of the manuscript. Kit S.O., Snezhko A.V. – editing of the manuscript. Kaymakchi O.Yu. – analysis and interpretation of the data.

Authors information

Goncharova Anna S. – Cand. Sci. (Biology), Head of the Experimental Laboratory Center, National Medical Research Center for Oncology, Rostov-on-Don, fateyeva_a_s@list.ru, http://orcid.org/0000-0003-0676-0871

Kolesnikov Evgeniy N. – Cand. Sci. (Med.), Head of the Department of Abdominal Cancer No.1, National Medical Research Center for Oncology, Rostov-on-Don, onko-sekretar@mail.ru, http://orcid.org/0000-0001-9749-709X

Egorov Georgiy Yu. – Post-Graduate Student, National Medical Research Center for Oncology, Rostov-on-Don, egorovgu@gmail.com, http://orcid.org/0000-0002-1413-6406

Maksimov Aleksey Yu. – Dr. Sci. (Med.)., Professor, Deputy Director General for Prospective Scientific Research, National Medical Research Center for Oncology, Rostov-on-Don, onko-sekretar@mail.ru, http://orcid.org/0000-0002-1397-837X

Shevchenko Aleksey N. – Dr. Sci. (Med.)., Professor, Head of the Department of Oncourology, Urologist, National Medical Research Center for Oncology, Rostov-on-Don, alexshew@mail.ru, http://orcid.org/0000-0002-9468-134X

Nepomnyashchaya Evgenia M. – Dr. Sci. (Med.)., Professor, Pathologist, Department of Anatomical Pathology, National Medical Research Center for Oncology, Rostov-on-Don, rnioi-patology@yandex.ru, http://orcid.org/0000-0003-0521-8837

Gvaldin Dmitriy Yu. – Cand. Sci. (Biology), Researcher, National Medical Research Center for Oncology, Rostov-on-Don, 89dmitry@mail.ru, http://orcid.org/0000-0001-8633-2660

Kurbanova Luiza Z. – Junior Researcher, National Medical Research Center for Oncology, Rostov-on-Don, luizacurbanowa@mail.ru, http://orcid.org/0000-0003-3436-1325

Khodakova Darya V. – Junior Researcher, National Medical Research Center for Oncology, Rostov-on-Don, coctail.moxuto@yandex.ru, http://orcid.org/0000-0003-3753-4463

Kit Sergey O. – Junior Researcher, National Medical Research Center for Oncology, Rostov-on-Don, onko-sekretar@mail.ru, http://orcid.org/0000-0002-1566-8906

Kaymakchi Oleg Yu. – Dr. Sci. (Med.)., Surgeon, Department of Abdominal Cancer No.2, National Medical Research Center for Oncology, Rostov-on-Don, onko-sekretar@mail.ru, http://orcid.org/0000-0002-7556-9897

Snezhko Aleksandr V. – Dr. Sci. (Med.), Surgeon, Department of Abdominal Cancer No.1 with a Group of X-ray Endovascular Methods of Diagnosis and Treatment, National Medical Research Center for Oncology, Rostov-on-Don, snezko.sania@yandex.ru, http://orcid.org/0000–0003–3998–8004

(✉) **Goncharova Anna S.,** fateyeva_a_s@list.ru

Received 03.02.2022;
approved after peer review 27.02.2022;
accepted 09.06.2022